SILK-HYALURONIC ACID COMPOSITIONS FOR TISSUE FILLING, TISSUE SPACING, AND TISSUE BULKING

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is an international application claiming the benefit of U.S. Provisional Application No. 63/041,678, filed on June 19, 2020, U.S. Provisional Application No. 63/041,616, filed on June 19, 2020, and U.S. Provisional Application No. 63/041,581, filed on June 19, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

Silk is a natural polymer produced by a variety of insects and spiders. Silkworm fibroin comprises a filament core protein, silk fibroin, and a glue-like coating consisting of a non-filamentous protein, sericin. Silk has been historically studied for use in the medical field. Hyaluronic acid (hyaluronan) is a glycosaminoglycan that is distributed throughout the body and is found in connective and epithelial tissues. Due to its biocompatibility and structural benefits, it is a useful component in medical devices and implantable materials.

Soft tissues of the human body owe their structures in part to an extracellular matrix that includes collagen, elastin, and glycosaminoglycan. Soft tissue defects may occur, which distort, deform, or otherwise alters soft tissue structures. Such structure may be restored through the use of tissue fillers that may be deposited at the defect site remedy the defect. For example, tissue fillers may be placed at the site of a facial wrinkle to remedy the wrinkle.

However, new tissue fillers are needed in the field that remedy a number of tissue defects while providing tunable properties, which may allow for tailoring of the tissue filler to the specific tissue defect.

SUMMARY OF THE INVENTION

In some embodiments, the disclosure relates to a biocompatible tissue filler comprising silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG), wherein a portion of the HA is

modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol, wherein the linker moieties are attached to the HA at one end of the linker. In some embodiments, a portion of the silk fibroin or silk fibroin fragments are modified or crosslinked. In some embodiments, a portion of the silk fibroin or silk fibroin fragments are crosslinked to HA. In some embodiments, a portion of the silk fibroin or silk fibroin fragments are crosslinked to silk fibroin or silk fibroin fragments. In some embodiments, the silk fibroin or silk fibroin fragments are substantially devoid of sericin.

In some embodiments, a portion of silk fibroin or silk fibroin fragments have an average weight average molecular weight selected from about 12 kDa, about 13 kDa, about 14 kDa, about 15 kDa, about 16 kDa, about 48 kDa, and about 100 kDa. In some embodiments, the silk fibroin or silk fibroin fragments have a polydispersity of between 1 and about 5.0. In some embodiments, the silk fibroin or silk fibroin fragments have a polydispersity of between about 1.5 and about 3.0. In some embodiments, a portion of the silk fibroin or silk fibroin fragments have low molecular weight, medium molecular weight, or high molecular weight.

In some embodiments, the tissue filler has a degree of modification (MoD) of about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15%. In some embodiments, modification or crosslinking is obtained using as crosslinker a diepoxy-PEG, a polyglycidyl-PEG, a diglycidyl-PEG, a diepoxy-PPG, a polyglycidyl-PPG, a diglycidyl-PPG, or any combinations thereof. In some embodiments, modification or cross-linking is obtained using polyethylene glycol diglycidyl ether having a MW of about 200 Da, about 500 Da, 1000 Da, about 2,000 Da, or about 6000 Da. In some embodiments, modification or cross-linking is obtained using polypropylene glycol diglycidyl ether having a MW of about 380 Da, or about 640 Da.

In some embodiments, the tissue filler further includes lidocaine. In some embodiments, the concentration of lidocaine in the tissue filler is about 0.3%.

In some embodiments, the tissue filler is a gel. In some embodiments, the tissue filler is a hydrogel. In some embodiments, the tissue filler further includes water. In some embodiments, the tissue filler is monophasic. In some embodiments, the total

concentration of HA and silk in the tissue filler is about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, or about 30 mg/mL. In some embodiments, the ratio of HA to silk fibroin or silk fibroin fragments in the tissue filler is about 92/8, about 93/7, about 94/6, about 95/5, about 96/4, about 97/3, about 18/12, about 27/3, about 29.4/0.6, about 99/1, about 92.5/7.5, or about 90/10. In some embodiments, the tissue filler is a dermal filler. In some embodiments, the tissue filler is biodegradable. In some embodiments, the tissue filler is injectable. In some embodiments, the tissue filler is injectable through 30 G or 27 G needles. In some embodiments, the tissue filler has a storage modulus (G') of from about 5 Pa to about 500 Pa. In some embodiments, the tissue filler has a storage modulus (G') of about 5 Pa, about 6 pa, about 7 Pa, about 8 Pa, about 9 Pa, about 10 Pa, about 11 Pa, about 12 Pa, about 13 Pa, about 14 Pa, about 15 Pa, about 16 Pa, about 17 Pa, about 18 Pa, about 19 Pa, about 20 Pa, about 21 Pa, about 22 Pa, about 23 Pa, about 24 Pa, about 25 Pa, about 26 Pa, about 27 Pa, about 28 Pa, about 29 Pa, about 30 Pa, about 31 Pa, about 32 Pa, about 33 Pa, about 34 Pa, about 35 Pa, about 36 Pa, about 37 Pa, about 38 Pa, about 39 Pa, about 40 Pa, about 41 Pa, about 42 Pa, about 43 Pa, about 44 Pa, about 45 Pa, about 46 Pa, about 47 Pa, about 48 Pa, about 49 Pa, about 50 Pa, about 51 Pa, about 52 Pa, about 53 Pa, about 54 Pa, about 55 Pa, about 56 Pa, about 57 Pa, about 58 Pa, about 59 Pa, about 60 Pa, about 61 Pa, about 62 Pa, about 63 Pa, about 64 Pa, about 65 Pa, about 66 Pa, about 67 Pa, about 68 Pa, about 69 Pa, about 70 Pa, about 71 Pa, about 72 Pa, about 73 Pa, about 74 Pa, about 75 Pa, about 76 Pa, about 77 Pa, about 78 Pa, about 79 Pa, about 80 Pa, about 81 Pa, about 82 Pa, about 83 Pa, about 84 Pa, about 85 Pa, about 86 Pa, about 87 Pa, about 88 Pa, about 89 Pa, about 90 Pa, about 91 Pa, about 92 Pa, about 93 Pa, about 94 Pa, about 95 Pa, about 96 Pa, about 97 Pa, about 98 Pa, about 99 Pa, about 100 Pa, about 101 Pa, about 102 Pa, about 103 Pa, about 104 Pa, about 105 Pa, about 106 Pa, about 107 Pa, about 108 Pa, about 109 Pa, about 110 Pa, about 111 Pa, about 112 Pa, about 113 Pa, about 114 Pa, about 115 Pa, about 116 Pa, about 117 Pa, about 118 Pa, about 119 Pa, about 120 Pa, about 121 Pa, about 122 Pa, about 123 Pa, about 124 Pa, or about 125 Pa. In some embodiments, G' is measured by means of an oscillatory stress of about 1 Hz, about 5 Hz, or about 10 Hz. In some embodiments, the

tissue filler has a complex viscosity from about 1 Pa·s to about 10 Pa·s. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 1 Hz, about 5 Hz, or about 10 Hz.

In some embodiments, the disclosure relates to a method of treating a condition in a subject in need thereof, including administering to the subject a therapeutically effective amount of any tissue filler described herein, for example a biocompatible tissue filler including silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG), wherein a portion of the HA is modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol, wherein the linker moieties are attached to the HA at one end of the linker. In some embodiments, the condition is a skin condition. In some embodiments, the skin condition is selected from the group consisting of skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle.

In some embodiments, the disclosure relates to a method of cosmetic treatment in a subject in need thereof, including administering to the subject an effective amount of any tissue filler described herein, for example a biocompatible tissue filler including silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG), wherein a portion of the HA is modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol, wherein the linker moieties are attached to the HA at one end of the linker.

In some embodiments, a tissue filler is administered into a dermal region of the subject. In some embodiments, the methods described herein include an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In some embodiments, the methods described herein include a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection.

In some embodiments, the methods described herein include using tissue fillers that resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least

about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

In some embodiments, the methods described herein include administration of tissue fillers resulting in a reduced inflammatory response compared to the inflammatory response induced by a control tissue filler comprising a substantially similar HA, wherein the control tissue filler does not include silk fibroin or silk fibroin fragments. In some embodiments, administration of the tissue filler to the subject results in a reduced inflammatory response compared to the inflammatory response induced by a control tissue filler comprising a substantially similar HA, wherein the control tissue filler does not include silk fibroin or silk fibroin fragments and/or PEG or PPG. In some embodiments, administration of any tissue filler to the subject results in increased collagen production compared to the collagen production induced by a control tissue filler comprising a substantially similar HA, wherein the control tissue filler does not include silk fibroin or silk fibroin fragments, or wherein the control tissue filler does not include silk fibroin or silk fibroin fragments and/or PEG or PPG.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an active agent selected from the group consisting of an enzyme inhibitor, an anesthetic agent, a medicinal neurotoxin, an antioxidant, an anti-infective agent, an anti-inflammatory agent, an ultraviolet (UV) light blocking agent, a dye, a hormone, an immunosuppressant, and an anti-inflammatory agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the glycosaminoglycan is hyaluronic acid (HA). In some embodiments, the %w/w amount of crosslinked HA relative to the total amount of HA is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%,

about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of cross-linking of the crosslinked HA is between about 1% and about 100%. In some embodiments, the degree of cross-linking of the crosslinked HA is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of cross-linking of the crosslinked HA is between about 1% and about 15%. In some embodiments, the degree of cross-linking of the crosslinked HA is one or more of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, and about 15%.

In some embodiments, the crosslinked HA comprises a cross-linking moiety comprising a polyethylene glycol (PEG) chain. In some embodiments, the cross-linking agent and/or the cross-linking precursor comprises an epoxy group. In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent selected from the group consisting of a polyepoxy linker, a diepoxy linker, a polyepoxy-PEG, a diepoxy-PEG, a polyglycidyl-PEG, a diglycidyl-PEG, a poly acrylate PEG, a diacrylate PEG, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, cross-linking is obtained using a polyfunctional epoxy compound selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), ethylene glycol diglycidyl ether (EGDGE), 1,6-hexanediol diglycidyl ether, polyethylene glycol diglycidyl ether, polypropylene glycol diglycidyl ether, polytetramethylene glycol diglycidyl ether, neopentyl glycol diglycidyl ether, polyglycerol polyglycidyl ether, diglycerol polyglycidyl ether, glycerol polyglycidyl ether, tri-methylolpropane polyglycidyl ether, pentaerythritol polyglycidyl ether, and sorbitol polyglycidyl ether. In some embodiments, cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group consisting of polyethylene glycol diglycidyl ether, diepoxy PEG, PEG diglycidyl ether, polyoxyethylene bis-glycidyl ether, PEGDE, and PEGDGE. In some embodiments, cross-linking is obtained using polyethylene glycol diglycidyl ether having an average M_n of about 500, about 1000, about 2000, or about 6000. In some embodiments, cross-linking is obtained using polyethylene glycol diglycidyl ether having from 2 to 25 ethylene glycol groups. In some embodiments, cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group consisting of a polyepoxy silk fibroin linker, a diepoxy silk fibroin linker, a polyepoxy silk fibroin fragment linker, a diepoxy silk fibroin fragment linker, a polyglycidyl silk fibroin linker,

a diglycidyl silk fibroin linker, a polyglycidyl silk fibroin fragment linker, and a diglycidyl silk fibroin fragment linker.

In some embodiments, the invention relates to a tissue filler further comprising an organic compound and/or an inorganic compound. In some embodiments, the inorganic compound comprises calcium hydroxyapatite. In some embodiments, the calcium hydroxyapatite is formulated as particles having a diameter between about 1 μm and about 100 μm, between about 1 μm and about 10 μm, between about 2 μm and about 12 μm, between about 3 μm and about 10 μm, between about 4 μm and about 15 μm, between about 8 μm and about 12 μm, between about 5 μm and about 10 μm, between about 6 μm and about 12 μm, between about 7 μm and about 20 μm, between about 9 μm and about 18 μm, or between about 10 μm and about 25 μm. In some embodiments, the concentration of calcium hydroxyapatite is between about 0.001% and about 5%. In some embodiments, the concentration of calcium hydroxyapatite is about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.011%, about 0.012%, about 0.013%, about 0.014%, about 0.015%, about 0.016%, about 0.017%, about 0.018%, about 0.019%, or about 0.02%. In some embodiments, the concentration of calcium hydroxyapatite is about 0.05%, about 0.1%, about 0.15%, about 0.2%, about 0.25%, about 0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 1.05%, about 1.1%, about 1.15%, about 1.2%, about 1.25%, about 1.3%, about 1.35%, about 1.4%, about 1.45%, about 1.5%, about 1.55%, about 1.6%, about 1.65%, about 1.7%, about 1.75%, about 1.8%, about 1.85%, about 1.9%, about 1.95%, or about 2%.

In some embodiments, the organic compound comprises an amino acid selected from the group consisting of glycine, L-proline, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

In some embodiments, the invention relates to a tissue filler comprising HA, wherein the HA is obtained from Streptococcus bacteria, or from Bacillus subtilis bacteria.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the anesthetic agent is lidocaine. In some embodiments, the concentration of anesthetic agent in the tissue filler is from about 0.001% to about 5%. In some embodiments, the concentration of lidocaine in the tissue filler is about 0.3%.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent; wherein the tissue filler is a gel. In some embodiments, the tissue filler is a hydrogel. In some embodiments, the tissue filler further comprises water. In some embodiments, the total concentration of HA in the tissue filler is from about 10 mg/mL to about 50 mg/mL. In some embodiments, the total concentration of HA in the tissue filler is about 15 mg/mL, about 16 mg/mL, 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, or about 30 mg/mL. In some embodiments, the concentration of cross linked HA in the tissue filler is from about 10 mg/mL to about 50 mg/mL. In some embodiments, the concentration of cross linked HA in the tissue filler is about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24

mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, or about 30 mg/mL.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent; the tissue filler comprising silk protein or silk protein fragments (SPF). In some embodiments, the silk protein is silk fibroin. In some embodiments, the silk protein is silk fibroin substantially devoid of sericin. In some embodiments, the SPF have an average weight average molecular weight ranging from about 1 kDa to about 250 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 5 kDa to about 150 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 6 kDa to about 17 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 17 kDa to about 39 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 39 kDa to about 80 kDa. In some embodiments, the SPF have low molecular weight. In some embodiments, the SPF have medium molecular weight. In some embodiments, the SPF have high molecular weight. In some embodiments, the silk protein fragments (SPF) have a polydispersity of between about 1.5 and about 3.0. In some embodiments, the SPF have a degree of crystallinity of up to 60%. In some embodiments, a portion of the SPF are crosslinked. In some embodiments, the %w/w amount of crosslinked SPF relative to the total amount of SPF is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%,

about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of cross-linking of the crosslinked SPF is between about 1% and about 100%. In some embodiments, the degree of cross-linking of the crosslinked SPF is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of cross-linking of the crosslinked SPF is between about 1% and about 15%. In some embodiments, the degree of cross-linking of the crosslinked SPF is one or more of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, and about 15%.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate,

chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent; the tissue filler comprising silk protein or silk protein fragments (SPF), wherein a portion of the SPF are crosslinked. In some embodiments, the crosslinked SPF comprises a cross-linking moiety comprising an alkane or alkyl chain, and/or an ether group. In some embodiments, the crosslinked SPF comprises a cross-linking moiety comprising a polyethylene glycol (PEG) chain. In some embodiments, the crosslinked SPF comprises a cross-linking moiety comprising a secondary alcohol. In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the cross-linking agent and/or the cross-linking precursor comprises an epoxy group. In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent selected from the group consisting of a polyepoxy linker, a diepoxy linker, a polyepoxy-PEG, a diepoxy-PEG, a polyglycidyl-PEG, a diglycidyl-PEG, a poly acrylate PEG, a diacrylate PEG, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, cross-linking is obtained using a polyfunctional epoxy compound selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), ethylene glycol diglycidyl ether (EGDGE), 1,6-hexanediol diglycidyl ether, polyethylene glycol diglycidyl ether, polypropylene glycol diglycidyl ether, polytetramethylene glycol diglycidyl ether, neopentyl glycol diglycidyl ether, polyglycerol polyglycidyl ether, diglycerol polyglycidyl ether, glycerol polyglycidyl ether, tri-methylolpropane polyglycidyl ether, pentaerythritol polyglycidyl ether, and sorbitol polyglycidyl ether. In some embodiments, cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group

consisting of polyethylene glycol diglycidyl ether, diepoxy PEG, PEG diglycidyl ether, polyoxyethylene bis-glycidyl ether, PEGDE, and PEGDGE. In some embodiments, cross-linking is obtained using polyethylene glycol diglycidyl ether having an average M_n of about 500, about 1000, about 2000, or about 6000. In some embodiments, cross-linking is obtained using polyethylene glycol diglycidyl ether having from 2 to 25 ethylene glycol groups. In some embodiments, cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group consisting of a polyepoxy silk fibroin linker, a diepoxy silk fibroin linker, a polyepoxy silk fibroin fragment linker, a diepoxy silk fibroin fragment linker, a polyglycidyl silk fibroin linker, a diglycidyl silk fibroin linker, a polyglycidyl silk fibroin fragment linker, and a diglycidyl silk fibroin fragment linker. In some embodiments, a portion of SPF is cross linked to HA. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, the tissue filler is a gel. In some embodiments, the tissue filler is a hydrogel. In some embodiments, the tissue filler further comprises water. In some embodiments, the total concentration of SPF in the tissue filler is from about 0.1 mg/mL to about 15 mg/mL. In some embodiments, the total concentration of SPF in the tissue filler is about 0.1 mg/mL, about 0.5 mg/mL, about 1 mg/mL, about 1.5 mg/mL, about 2 mg/mL, about 2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 6.5 mg/mL, about 7 mg/mL, about 7.5 mg/mL, about 8 mg/mL, about 8.5 mg/mL, about 9 mg/mL, about 9.5 mg/mL, about 10 mg/mL, about 10.5 mg/mL, about 11 mg/mL, about 11.5 mg/mL, about 12 mg/mL, about 12.5 mg/mL, about 13 mg/mL, about 13.5 mg/mL, about 14 mg/mL, about 14.5 mg/mL, or about 15 mg/mL. In some embodiments, the concentration of cross linked SPF in the tissue filler is from about 0.1 mg/mL to about 15 mg/mL. In some embodiments, the concentration of cross linked SPF in the tissue filler is about 0.1 mg/mL, about 0.5 mg/mL, about 1 mg/mL, about 1.5 mg/mL, about 2 mg/mL, about 2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 6.5 mg/mL, about 7 mg/mL, about 7.5 mg/mL, about 8 mg/mL, about 8.5 mg/mL, about 9 mg/mL, about 9.5 mg/mL, about 10 mg/mL, about 10.5 mg/mL, about 11 mg/mL, about 11.5 mg/mL, about 12 mg/mL, about

12.5 mg/mL, about 13 mg/mL, about 13.5 mg/mL, about 14 mg/mL, about 14.5 mg/mL, or about 15 mg/mL.

In one embodiment, the invention relates to a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent; the tissue filler optionally comprising silk protein or silk protein fragments (SPF), wherein a portion of the SPF are crosslinked. In some embodiments, the tissue filler is a dermal filler. In some embodiments, the tissue filler is biodegradable. In some embodiments, the tissue filler is injectable. In some embodiments, the tissue filler has a storage modulus (G') of from about 25 Pa to about 1500 Pa. In some embodiments, the tissue filler has a storage modulus (G') of about 25 Pa, about 26 Pa, about 27 Pa, about 28 Pa, about 29 Pa, about 30 Pa, about 31 Pa, about 32 Pa, about 33 Pa, about 34 Pa, about 35 Pa, about 36 Pa, about 37 Pa, about 38 Pa, about 39 Pa, about 40 Pa, about 41 Pa, about 42 Pa, about 43 Pa, about 44 Pa, about 45 Pa, about 46 Pa, about 47 Pa, about 48 Pa, about 49 Pa, about 50 Pa, about 51 Pa, about 52 Pa, about 53 Pa, about 54 Pa, about 55 Pa, about 56 Pa, about 57 Pa, about 58 Pa, about 59 Pa, about 60 Pa, about 61 Pa, about 62 Pa, about 63 Pa, about 64 Pa, about 65 Pa, about 66 Pa, about 67 Pa, about 68 Pa, about 69 Pa, about 70 Pa, about 71 Pa, about 72 Pa, about 73 Pa, about 74 Pa, about 75 Pa, about 76 Pa, about 77 Pa, about 78 Pa, about 79 Pa, about 80 Pa, about 81 Pa, about 82 Pa, about 83 Pa, about 84 Pa, about 85 Pa, about 86 Pa, about 87 Pa, about 88 Pa, about 89 Pa, about 90 Pa, about 91 Pa, about 92 Pa, about 93 Pa, about 94 Pa, about 95 Pa, about 96 Pa, about 97 Pa, about 98 Pa, about 99 Pa, about 100 Pa, about 101 Pa, about 102 Pa, about 103 Pa, about 104 Pa, about 105 Pa, about 106 Pa, about 107 Pa, about 108 Pa, about 109 Pa, about 110 Pa, about 111 Pa, about 112 Pa, about 113 Pa, about 114 Pa, about 115 Pa, about 116 Pa, about 117 Pa, about 118 Pa, about 119 Pa, about 120 Pa, about 121 Pa, about 122 Pa, about 123 Pa, about 124 Pa, or about 125 Pa. In some embodiments, herein

G' is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 1 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 5 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 10 Hz. In some embodiments, the tissue filler has a complex viscosity from about 1 Pa·s to about 10 Pa·s. In some embodiments, the tissue filler has a complex viscosity of about 1 Pa·s, about 1.5 Pa·s, about 2 Pa·s, about 2.5 Pa·s, about 3 Pa·s, about 3.5 Pa·s, about 4 Pa·s, about 4.5 Pa·s, about 5 Pa·s, about 5.5 Pa·s, about 6 Pa·s, about 6.5 Pa·s, about 7 Pa·s, about 7.5 Pa·s, about 8 Pa·s, about 8.5 Pa·s, about 9 Pa·s, about 9.5 Pa·s, or about 10 Pa·s. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 1 Hz. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 5 Hz.

In one embodiment, the invention relates to a method of treating a condition in a subject in need thereof, and/or a method of cosmetic treatment in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a biocompatible tissue filler comprising: a glycosaminoglycan selected from the group consisting of hyaluronic acid (HA), carboxymethyl cellulose (CMC), starch, alginate, chondroitin-4-sulfate, chondroitin-6-sulfate, xanthan gum, chitosan, pectin, agar, carrageenan, and guar gum; and an anesthetic agent; wherein a portion of the glycosaminoglycan is crosslinked by cross-linking moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol; and wherein cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent; the tissue filler optionally comprising silk protein or silk protein fragments (SPF), wherein a portion of the SPF are crosslinked. In some embodiments, the condition is a skin condition. In some embodiments, the skin condition is selected from the group consisting of skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle. In some embodiments the tissue filler is administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder,

correcting a defect or imperfection of a body part, region or area. In some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection. In some embodiments, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some embodiments, administration of the tissue filler to the subject results in a reduced inflammatory response compared to the inflammatory response induced by a control tissue filler comprising a polysaccharide and lidocaine, wherein the control tissue filler does not include silk protein fragments (SPF). In some embodiments, administration of the tissue filler to the subject results in increased collagen production compared to the collagen production induced by a control tissue filler comprising a polysaccharide and lidocaine, wherein the control tissue filler does not include silk protein fragments (SPF).

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide. In some embodiments, the polysaccharide is hyaluronic acid (HA). In an embodiment, the invention includes tissue fillers that may be prepared from silk and hyaluronic acid.

In some embodiments, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) with an average molecular weight ranging from about 1 kDa to about 250 kDa. In some embodiments, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) with an average molecular weight ranging from about 5 kDa to about 150 kDa. In some embodiments, the SPF have an average molecular weight ranging from about 6 kDa to about 17 kDa. In some embodiments, the SPF have an average molecular weight ranging from about 17 kDa to about 39 kDa. In some embodiments, the SPF have an average molecular weight ranging from about 39 kDa to about 80 kDa. In some embodiments, the SPF have an average molecular weight ranging from about 80 kDa to about 150 kDa.

In some embodiments, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) which are up to about 0% to 100% crosslinked

with SPF. In some embodiments, the SPF were crosslinked to SPF using cross-linking agents such as BDDE, or one of the other cross-linking agents described herein. In some embodiments, the degree of cross-linking is up to about 100%.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein up to about 0% to 100% of the SPF are crosslinked to SPF, and the SPF were crosslinked to SPF using a cross-linking agent such as BDDE, or one of the other cross-linking agents described herein, and the SPF degree of cross-linking is up to about 100%.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein up to 100% of HA is crosslinked to HA using a cross-linking agent such as BDDE, or one of the other cross-linking agents described herein. In some embodiments, up to about 100% of the SPF are crosslinked to SPF, wherein the SPF were crosslinked to SPF using a cross-linking agent such as BDDE, or one of the other cross-linking agents described herein, and the SPF degree of cross-linking is up to about 100%.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein 0% to 100% of HA is non-crosslinked. In some embodiments, up to about 100% of the SPF are crosslinked, wherein the SPF were crosslinked using a cross-linking agent such as BDDE, or one of the other cross-linking agents described herein, and the SPF degree of cross-linking is up to about 100%. In some embodiments, all of the HA is non-crosslinked.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein 0% to 100% of SPF is crosslinked to HA. In some embodiments, the SPF and HA were crosslinked using a cross-linking agent such as BDDE, or one of the cross-linking agents described herein. In some embodiments, the degree of SPF-HA cross-linking is up to about 100%. In some embodiments, up to 100% of HA is crosslinked to HA. In some embodiments, HA was crosslinked to HA using a

cross-linking agent such as BDDE, or one of the cross-linking agents described herein. In some embodiments, at least 0.1% of HA is non-crosslinked. In some embodiments, all of the HA is non-crosslinked.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein at least 0.1% of HA is non-crosslinked. In some embodiments, up to about 100% of the SPF are crosslinked, wherein the SPF were crosslinked using a cross-linking agent such as BDDE, or one of the other cross-linking agents described herein, and the SPF degree of cross-linking is up to about 100%. In some embodiments, all of the HA is non-crosslinked.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and hyaluronic acid (HA), wherein at least 0.1% of SPF is crosslinked to HA. In some embodiments, the SPF and HA were crosslinked using a cross-linking agent such as BDDE, or one of the cross-linking agents described herein. In some embodiments, the degree of SPF-HA cross-linking is up to about 100%. In some embodiments, up to 100% of HA is crosslinked to HA. In some embodiments, HA was crosslinked to HA using a cross-linking agent such as BDDE, or one of the cross-linking agents described herein. In some embodiments, at least 0.1% of HA is non-crosslinked. In some embodiments, all of the HA is non-crosslinked.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, wherein the SPF are substantially devoid of sericin.

In one embodiment, the invention relates to a biocompatible gel tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide.

In one embodiment, the invention relates to a biocompatible hydrogel tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, a polysaccharide, and water.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, wherein SPF have a degree of crystallinity of about 0% to about 60%.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, and further including an active agent. In some embodiments, the active agent can be an enzyme inhibitor, an anesthetic agent, a medicinal neurotoxin, an antioxidant, an anti-infective agent, vasodilators, a reflective agent, an anti-inflammatory agent, an ultraviolet (UV) light blocking agent, a dye, a hormone, an immunosuppressant, or an anti-inflammatory agent. In one embodiment, the anesthetic agent is lidocaine.

In one embodiment, the invention relates to an injectable biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide.

In one embodiment, the invention relates to a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide. In some embodiments, G' is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In one embodiment, G' is measured by means of an oscillatory stress of about 1 Hz.

In one embodiment, the invention relates to a method of making a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the method including providing an SPF solution, and adding to the solution a gelation enhancer, which may be any proton donating species.

In one embodiment, the invention relates to a method of making a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the method including providing an SPF solution, and subjecting the solution to mechanical excitation.

In one embodiment, the invention relates to a method of treating a condition in a subject in need thereof, the method including administering to the subject a therapeutically effective amount of a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide. In some embodiments, the condition is a skin condition. In some embodiments, the skin condition can be skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, sunken temple, a thin lip, a retro-orbital defect, a facial fold, or a wrinkle.

In one embodiment, the invention relates to a method of cosmetic treatment in a subject in need thereof, the method including administering to the subject an effective amount of a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide.

In some embodiments, the methods of the invention include administering a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, into a dermal region of a subject.

In one embodiment, a method of the invention including administering a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, can be an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area.

In one embodiment, a method of the invention including administering a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, can be a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection.

In one embodiment, a biocompatible tissue filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, administered according to a method of the invention, resists biodegradation, bioabsorption, and/or bioresorption, for at least about 3 days after administration.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some

embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water.

In some embodiments, the %w/w amount of crosslinked SPF relative to the total amount of SPF is up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the degree of cross-linking of SPF is up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%,

about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the %w/w amount of crosslinked HA relative to the total amount of HA is up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the degree of cross-linking of HA is up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%,

about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the cross-linking agent and/or the cross-linking precursor comprise an epoxy group. In some embodiments, the SPF are substantially devoid of sericin.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-

linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the cross-linking agent and/or the cross-linking precursor comprise an epoxy group. In some embodiments, the SPF are substantially devoid of sericin.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent selected from the group consisting of 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, the SPF are substantially devoid of sericin.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent selected from the group consisting of 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, the SPF are substantially devoid of sericin.

In one embodiment, the invention relates to a biocompatible tissue filler gel, e.g., a dermal filler gel, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a

portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the gel further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler gel, e.g., a dermal filler gel, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the gel further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler hydrogel, e.g., a dermal filler hydrogel, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments,

cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the hydrogel further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler hydrogel, e.g., a dermal filler hydrogel, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the hydrogel further comprises water.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical

bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF have a degree of crystallinity of up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, or more than 60%.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF have a degree of crystallinity of up to about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about

26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, or more than 60%.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the tissue filler further comprises an active agent. In some embodiments, the active agent is selected from the group consisting of an enzyme inhibitor, an anesthetic agent, a medicinal neurotoxin, an antioxidant, an anti-infective agents, an anti-inflammatory agent, an ultraviolet (UV) light blocking agent, a dye, a hormone, an immunosuppressant, and an anti-inflammatory agent. In some embodiments, the anesthetic agent is lidocaine.

In one embodiment, the invention relates to a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some

embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the tissue filler further comprises an active agent. In some embodiments, the active agent is selected from the group consisting of an enzyme inhibitor, an anesthetic agent, a medicinal neurotoxin, an antioxidant, an anti-infective agent, an anti-inflammatory agent, an ultraviolet (UV) light blocking agent, a dye, a hormone, an immunosuppressant, and an anti-inflammatory agent. In some embodiments, the anesthetic agent is lidocaine.

In one embodiment, the invention relates to a biocompatible injectable tissue filler, e.g., an injectable dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA).

In one embodiment, the invention relates to a biocompatible injectable tissue filler, e.g., an injectable dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having

low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA).

In one embodiment, the invention relates to a biocompatible tissue filler having a storage modulus (G') of from about 50 Pa to about 1500 Pa, e.g., a dermal filler having a storage modulus (G') of from about 50 Pa to about 1500 Pa, the filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, G' is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 1 Hz.

In one embodiment, the invention relates to a biocompatible tissue filler having a storage modulus (G') of from about 50 Pa to about 1500 Pa, e.g., a dermal filler having a storage modulus (G') of from about 50 Pa to about 1500 Pa, the filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, cross-linking includes chemical bond cross-linking. In some embodiments, a portion of cross-linking is zero-length cross-linking. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, G' is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 1 Hz.

In some embodiments, the invention relates to a method of making a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the method including providing a composition comprising SPF and a polysaccharide, and adding to the solution a cross-linking agent, a cross-linking precursor, an activating agent, or a gelation enhancer, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of

cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, the tissue filler further comprises water.

In some embodiments, the invention relates to a method of making a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the method including providing a composition comprising SPF and a polysaccharide, and adding to the solution a cross-linking agent, a cross-linking precursor, an activating agent, or a gelation enhancer, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water.

In some embodiments, the invention relates to a method of treating a condition in a subject in need thereof, e.g., a skin condition, the method comprising administering to the subject a therapeutically effective amount of a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of

the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water. In some embodiments, the skin condition is selected from the group consisting of skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle. In some embodiments, the tissue filler is administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection. In some embodiments, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

In some embodiments, the invention relates to a method of treating a condition in a subject in need thereof, e.g., a skin condition, the method comprising administering to the subject a therapeutically effective amount of a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the

tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water. In some embodiments, the skin condition is selected from the group consisting of skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle. In some embodiments, the tissue filler is administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection. In some embodiments, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

In some embodiments, the invention relates to a method of cosmetic treatment in a subject in need thereof, the method comprising administering to the subject an effective amount of a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, about 5 kDa to about 150 kDa, from about 6 kDa to about 17 kDa, from about 17 kDa to about 39 kDa, or from about 39 kDa to about 80 kDa. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking

moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water. In some embodiments, the tissue filler is administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection. In some embodiments, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

In some embodiments, the invention relates to a method of cosmetic treatment in a subject in need thereof, the method comprising administering to the subject an effective amount of a biocompatible tissue filler, e.g., a dermal filler, including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, and a polysaccharide, the SPF having low molecular weight, medium molecular weight, and/or high molecular weight. In some embodiments, the tissue filler is biodegradable. In some embodiments, a portion of SPF are crosslinked. In some embodiments, a portion of the SPF are crosslinked to polysaccharide. In some embodiments, a portion of the SPF are crosslinked to SPF. In some embodiments, a portion of the polysaccharide is crosslinked to polysaccharide. In some embodiments, the tissue filler further includes cross-linking moieties, e.g., epoxy derived cross-linking moieties. In some embodiments, a portion of cross-linking is auto-cross-linking. In some embodiments, the portion of crosslinked SPF is up to about 100%. In some embodiments, the portion of crosslinked polysaccharide is up to about 100%. In some embodiments, the polysaccharide is hyaluronic acid (HA). In some embodiments, the SPF are substantially devoid of sericin. In some embodiments, tissue filler further comprises water. In some embodiments, the tissue filler is

administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection. In some embodiments, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.

In some embodiments, the invention relates to a biocompatible tissue filler, comprising hyaluronic acid (HA) and an anesthetic agent, wherein a portion of the HA is modified by one or more linker moieties comprising one or more of an alkane or alkyl chain, an ether group, and a secondary alcohol, wherein the linker moieties are attached to the HA at one end of the linker. In some embodiments, modification is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent. In some embodiments, the HA in the tissue filler has a degree of modification (MoD) of about 10.0%, about 10.1%, about 10.2%, about 10.3%, about 10.4%, about 10.5%, about 10.6%, about 10.7%, about 10.8%, about 10.9%, about 11.0%, about 11.1%, about 11.2%, about 11.3%, about 11.4%, about 11.5%, about 11.6%, about 11.7%, about 11.8%, about 11.9%, about 12.0%, about 12.1%, about 12.2%, about 12.3%, about 12.4%, about 12.5%, about 12.6%, about 12.7%, about 12.8%, about 12.9%, about 13.0%, about 13.1%, about 13.2%, about 13.3%, about 13.4%, about 13.5%, about 13.6%, about 13.7%, about 13.8%, about 13.9%, about 14.0%, about 14.1%, about 14.2%, about 14.3%, about 14.4%, about 14.5%, about 14.6%, about 14.7%, about 14.8%, about 14.9%, about 15.0%, about 15.1%, about 15.2%, about 15.3%, about 15.4%, about 15.5%, about 15.6%, about 15.7%, about 15.8%, about 15.9%, about 16.0%, about 16.1%, about 16.2%, about 16.3%, about 16.4%, about 16.5%, about 16.6%, about 16.7%, about 16.8%, about 16.9%, about 17.0%, about 17.1%, about 17.2%, about 17.3%, about 17.4%, about 17.5%, about 17.6%, about 17.7%, about 17.8%, about 17.9%, about 18.0%, about 18.1%, about 18.2%, about 18.3%, about 18.4%, about 18.5%, about 18.6%, about 18.7%, about 18.8%, about 18.9%, about 19.0%, about 19.1%, about 19.2%, about 19.3%, about 19.4%, about 19.5%, about 19.6%, about 19.7%,

about 19.8%, about 19.9%, or about 20.0%. In some embodiments, the %w/w amount of modified HA relative to the total amount of HA in the tissue filler is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the modified HA includes crosslinked HA, wherein the degree of cross-linking of the crosslinked HA is between about 1% and about 100%. In some embodiments, the degree of cross-linking of the crosslinked HA is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%,

about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of cross-linking of the crosslinked HA is between about 1% and about 15%.

In some embodiments, the modified or crosslinked HA comprises a linker or cross-linking moiety comprising a polyethylene glycol (PEG) chain. In some embodiments, the cross-linking agent and/or the cross-linking precursor comprises an epoxy group. In some embodiments, modification or cross-linking is obtained using a cross-linking agent, a cross-linking precursor, or an activating agent selected from the group consisting of a polyepoxy linker, a diepoxy linker, a polyepoxy-PEG, a diepoxy-PEG, a polyglycidyl-PEG, a diglycidyl-PEG, a poly acrylate PEG, a diacrylate PEG, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, modification or cross-linking is obtained using a polyfunctional epoxy compound selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), ethylene glycol diglycidyl ether (EGDGE), 1,6-hexanediol diglycidyl ether, polyethylene glycol diglycidyl ether, polypropylene glycol diglycidyl ether, polytetramethylene glycol diglycidyl ether, neopentyl glycol diglycidyl ether, polyglycerol polyglycidyl ether, diglycerol polyglycidyl ether, glycerol polyglycidyl ether, tri-methylolpropane polyglycidyl ether, pentaerythritol polyglycidyl ether, and sorbitol polyglycidyl ether. In some embodiments, modification or cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group consisting of polyethylene glycol diglycidyl ether, diepoxy PEG, PEG diglycidyl ether, polyoxyethylene bis-glycidyl ether, PEGDE, and PEGDGE. In some embodiments, modification or cross-linking is obtained using polyethylene glycol diglycidyl ether having an average M_n of about 500, about 1000, about 2000, or about 6000. In some embodiments, modification or cross-linking is obtained using polyethylene glycol diglycidyl ether having from about 2 to about 25 ethylene glycol groups. In some

embodiments, modification or cross-linking is obtained using a cross-linking agent and/or a cross-linking precursor selected from the group consisting of a polyepoxy silk fibroin linker, a diepoxy silk fibroin linker, a polyepoxy silk fibroin fragment linker, a diepoxy silk fibroin fragment linker, a polyglycidyl silk fibroin linker, a diglycidyl silk fibroin linker, a polyglycidyl silk fibroin fragment linker, and a diglycidyl silk fibroin fragment linker.

In some embodiments, the tissue filler further includes an organic compound and/or an inorganic compound. In some embodiments, the inorganic compound comprises calcium hydroxyapatite. In some embodiments, the calcium hydroxyapatite is formulated as particles having a diameter between about 1 μm and about 100 μm, between about 1 μm and about 10 μm, between about 2 μm and about 12 μm, between about 3 μm and about 10 μm, between about 4 μm and about 15 μm, between about 8 μm and about 12 μm, between about 5 μm and about 10 μm, between about 6 μm and about 12 μm, between about 7 μm and about 20 μm, between about 9 μm and about 18 μm, or between about 10 μm and about 25 μm. In some embodiments, the concentration of calcium hydroxyapatite is between about 0.001% and about 5%. In some embodiments, the concentration of calcium hydroxyapatite is about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.011%, about 0.012%, about 0.013%, about 0.014%, about 0.015%, about 0.016%, about 0.017%, about 0.018%, about 0.019%, or about 0.02%. In some embodiments, the concentration of calcium hydroxyapatite is about 0.05%, about 0.1%, about 0.15%, about 0.2%, about 0.25%, about 0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 1.05%, about 1.1%, about 1.15%, about 1.2%, about 1.25%, about 1.3%, about 1.35%, about 1.4%, about 1.45%, about 1.5%, about 1.55%, about 1.6%, about 1.65%, about 1.7%, about 1.75%, about 1.8%, about 1.85%, about 1.9%, about 1.95%, or about 2%. In some embodiments, the organic compound comprises an amino acid selected from the group consisting of glycine, L-proline, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

In some embodiments, the HA is obtained from Streptococcus bacteria, or from Bacillus subtilis bacteria. In some embodiments, the active agent is lidocaine. In some embodiments, the concentration of active agent in the tissue filler is from about 0.001% to about 5%. In some embodiments, the concentration of lidocaine in the tissue filler is about 0.3%.

In some embodiments, the tissue filler disclosed herein is a gel. In some embodiments, the tissue filler is a hydrogel. In some embodiments, the tissue filler further comprises water. In some embodiments, the total concentration of HA in the tissue filler is from about 10 mg/mL to about 50 mg/mL. In some embodiments, the total concentration of HA in the tissue filler is about 15 mg/mL, about 16 mg/mL, 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, or about 30 mg/mL. In some embodiments, the concentration of modified or cross linked HA in the tissue filler is from about 10 mg/mL to about 50 mg/mL. In some embodiments, the concentration of modified or cross linked HA in the tissue filler is about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, or about 30 mg/mL.

In some embodiments, the tissue filler disclosed further includes silk protein or silk protein fragments (SPF). In some embodiments, the silk protein is silk fibroin. In some embodiments, the silk protein is silk fibroin substantially devoid of sericin. In some embodiments, the SPF have an average weight average molecular weight ranging from about 1 kDa to about 250 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 5 kDa to about 150 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 6 kDa to about 17 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 17 kDa to about 39 kDa. In some embodiments, the SPF have an average weight average molecular weight ranging from about 39 kDa to about 80 kDa. In some embodiments, the SPF have low molecular weight. In some embodiments, the SPF have medium molecular weight. In some

embodiments, the SPF have high molecular weight. In some embodiments, the silk protein fragments (SPF) have a polydispersity of between about 1.5 and about 3.0. In some embodiments, the SPF have a degree of crystallinity of up to 60%.

In some embodiments, the invention relates to a tissue filler including HA and SPF, wherein a portion of the SPF are modified or crosslinked. In some embodiments, the %w/w amount of modified or crosslinked SPF relative to the total amount of SPF is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of modification or cross-linking of the modified or crosslinked SPF is between about 1% and about 100%. In some embodiments, the degree of modification or cross-linking of the modified or crosslinked SPF is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about

68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. In some embodiments, the degree of modification or cross-linking of the modified or crosslinked SPF is between about 1% and about 15%. In some embodiments, the degree of modification or cross-linking of the modified or crosslinked SPF is one or more of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, and about 15%.

In some embodiments, the modified or crosslinked SPF comprises a linker or cross-linking moiety comprising an alkane or alkyl chain, and/or an ether group, wherein the linker or cross-linking moiety is attached to the SPF at one end of the linker or cross-linking moiety. In some embodiments, the modified or crosslinked SPF comprises a linker or cross-linking moiety comprising a polyethylene glycol (PEG) chain. In some embodiments, the modified or crosslinked SPF comprises a linker or cross-linking moiety comprising a secondary alcohol. In some embodiments, modification or cross-linking is obtained using a modification or cross-linking agent, a modification or cross-linking precursor, or an activating agent. In some embodiments, the modification or cross-linking agent and/or the modification or cross-linking precursor comprises an epoxy group. In some embodiments, modification or cross-linking is obtained using a modification or cross-linking agent, a modification or cross-linking precursor, or an activating agent selected from the group consisting of a polyepoxy linker, a diepoxy linker, a polyepoxy-PEG, a diepoxy-PEG, a polyglycidyl-PEG, a diglycidyl-PEG, a poly acrylate PEG, a diacrylate PEG, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCDI), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, a carbodiimide, and any combinations thereof. In some embodiments, modification or cross-linking is obtained using a polyfunctional

epoxy compound selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), ethylene glycol diglycidyl ether (EGDGE), 1,6-hexanediol diglycidyl ether, polyethylene glycol diglycidyl ether, polypropylene glycol diglycidyl ether, polytetramethylene glycol diglycidyl ether, neopentyl glycol diglycidyl ether, polyglycerol polyglycidyl ether, diglycerol polyglycidyl ether, glycerol polyglycidyl ether, tri-methylolpropane polyglycidyl ether, pentaerythritol polyglycidyl ether, and sorbitol polyglycidyl ether.

In some embodiments, modification or cross-linking is obtained using a modification or cross-linking agent and/or a modification or cross-linking precursor selected from the group consisting of polyethylene glycol diglycidyl ether, diepoxy PEG, PEG diglycidyl ether, polyoxyethylene bis-glycidyl ether, PEGDE, and PEGDGE. In some embodiments, the modification or cross-linking is obtained using polyethylene glycol diglycidyl ether having an average M_n of about 500, about 1000, about 2000, or about 6000. In some embodiments, modification or cross-linking is obtained using polyethylene glycol diglycidyl ether having from about 2 to about 25 ethylene glycol groups. In some embodiments, modification or cross-linking is obtained using a modification or cross-linking agent and/or a modification or cross-linking precursor selected from the group consisting of a polyepoxy silk fibroin linker, a diepoxy silk fibroin linker, a polyepoxy silk fibroin fragment linker, a diepoxy silk fibroin fragment linker, a polyglycidyl silk fibroin linker, a diglycidyl silk fibroin linker, a polyglycidyl silk fibroin fragment linker, and a diglycidyl silk fibroin fragment linker.

In some embodiments, the invention relates to a tissue filler including HA and SPF, wherein a portion of SPF is cross linked to HA. In some embodiments, the invention relates to a tissue filler including HA and SPF, wherein a portion of the SPF are crosslinked to SPF. In some embodiments, the tissue filler is a gel. In some embodiments, the tissue filler is a hydrogel. In some embodiments, the tissue filler further comprises water. In some embodiments, the total concentration of SPF in the tissue filler is from about 0.1 mg/mL to about 15 mg/mL. In some embodiments, the total concentration of SPF in the tissue filler is about 0.1 mg/mL, about 0.5 mg/mL, about 1 mg/mL, about 1.5 mg/mL, about 2 mg/mL, about 2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 6.5

mg/mL, about 7 mg/mL, about 7.5 mg/mL, about 8 mg/mL, about 8.5 mg/mL, about 9 mg/mL, about 9.5 mg/mL, about 10 mg/mL, about 10.5 mg/mL, about 11 mg/mL, about 11.5 mg/mL, about 12 mg/mL, about 12.5 mg/mL, about 13 mg/mL, about 13.5 mg/mL, about 14 mg/mL, about 14.5 mg/mL, or about 15 mg/mL. In some embodiments, the concentration of modified or cross linked SPF in the tissue filler is from about 0.1 mg/mL to about 15 mg/mL. In some embodiments, the concentration of modified or cross linked SPF in the tissue filler is about 0.1 mg/mL, about 0.5 mg/mL, about 1 mg/mL, about 1.5 mg/mL, about 2 mg/mL, about 2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 6.5 mg/mL, about 7 mg/mL, about 7.5 mg/mL, about 8 mg/mL, about 8.5 mg/mL, about 9 mg/mL, about 9.5 mg/mL, about 10 mg/mL, about 10.5 mg/mL, about 11 mg/mL, about 11.5 mg/mL, about 12 mg/mL, about 12.5 mg/mL, about 13 mg/mL, about 13.5 mg/mL, about 14 mg/mL, about 14.5 mg/mL, or about 15 mg/mL.

In some embodiments, the invention relates to a tissue filler including modified or crosslinked HA, and/or modified or crosslinked SPF, wherein the tissue filler is a dermal filler. In some embodiments, the tissue filler is biodegradable. In some embodiments, the tissue filler is injectable. In some embodiments, the tissue filler has a storage modulus (G') of from about 25 Pa to about 1500 Pa. In some embodiments, the tissue filler has a storage modulus (G') of about 25 Pa, about 26 Pa, about 27 Pa, about 28 Pa, about 29 Pa, about 30 Pa, about 31 Pa, about 32 Pa, about 33 Pa, about 34 Pa, about 35 Pa, about 36 Pa, about 37 Pa, about 38 Pa, about 39 Pa, about 40 Pa, about 41 Pa, about 42 Pa, about 43 Pa, about 44 Pa, about 45 Pa, about 46 Pa, about 47 Pa, about 48 Pa, about 49 Pa, about 50 Pa, about 51 Pa, about 52 Pa, about 53 Pa, about 54 Pa, about 55 Pa, about 56 Pa, about 57 Pa, about 58 Pa, about 59 Pa, about 60 Pa, about 61 Pa, about 62 Pa, about 63 Pa, about 64 Pa, about 65 Pa, about 66 Pa, about 67 Pa, about 68 Pa, about 69 Pa, about 70 Pa, about 71 Pa, about 72 Pa, about 73 Pa, about 74 Pa, about 75 Pa, about 76 Pa, about 77 Pa, about 78 Pa, about 79 Pa, about 80 Pa, about 81 Pa, about 82 Pa, about 83 Pa, about 84 Pa, about 85 Pa, about 86 Pa, about 87 Pa, about 88 Pa, about 89 Pa, about 90 Pa, about 91 Pa, about 92 Pa, about 93 Pa, about 94 Pa, about 95 Pa, about 96 Pa, about 97 Pa, about 98 Pa, about 99 Pa, about 100 Pa, about 101 Pa, about 102 Pa, about 103 Pa, about 104 Pa, about 105 Pa, about 106 Pa, about 107 Pa, about 108 Pa,

about 109 Pa, about 110 Pa, about 111 Pa, about 112 Pa, about 113 Pa, about 114 Pa, about 115 Pa, about 116 Pa, about 117 Pa, about 118 Pa, about 119 Pa, about 120 Pa, about 121 Pa, about 122 Pa, about 123 Pa, about 124 Pa, or about 125 Pa. In some embodiments, G' is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 1 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 5 Hz. In some embodiments, G' is measured by means of an oscillatory stress of about 10 Hz. In some embodiments, the tissue filler has a complex viscosity from about 1 Pa·s to about 10 Pa·s. In some embodiments, the tissue filler has a complex viscosity of about 1 Pa·s, about 1.5 Pa·s, about 2 Pa·s, about 2.5 Pa·s, about 3 Pa·s, about 3.5 Pa·s, about 4 Pa·s, about 4.5 Pa·s, about 5 Pa·s, about 5.5 Pa·s, about 6 Pa·s, about 6.5 Pa·s, about 7 Pa·s, about 7.5 Pa·s, about 8 Pa·s, about 8.5 Pa·s, about 9 Pa·s, about 9.5 Pa·s, or about 10 Pa·s. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 0.1 to about 10 Hz. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 1 Hz. In some embodiments, the complex viscosity is measured by means of an oscillatory stress of about 5 Hz.

In some embodiments, the invention relates to a method of treating a condition in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a tissue filler including modified or crosslinked HA, and/or modified or crosslinked SPF. In some embodiments, the condition is a skin condition. In some embodiments, the skin condition is selected from the group consisting of skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle.

In some embodiments, the invention relates to a method of cosmetic treatment in a subject in need thereof, comprising administering to the subject an effective amount of a tissue filler including modified or crosslinked HA, and/or modified or crosslinked SPF. In some embodiments, the tissue filler is administered into a dermal region of the subject. In some embodiments, the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area. In

some embodiments, the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection.

In some embodiments of the methods described herein, the tissue filler resists biodegradation, bioerosion, bioabsorption, and/or bioresorption, for at least about 3 days, about 7 days, about 14 days, about 21 days, about 28 days, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some embodiments of the methods described herein, administration of the tissue filler to the subject results in a reduced inflammatory response compared to the inflammatory response induced by a control tissue filler comprising a polysaccharide and lidocaine, wherein the control tissue filler does not include silk protein fragments (SPF).

In some embodiments of the methods described herein, administration of the tissue filler to the subject results in increased collagen production compared to the collagen production induced by a control tissue filler comprising a polysaccharide and lidocaine, wherein the control tissue filler does not include silk protein fragments (SPF).

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, comprising SPF nano- or microparticles. In some embodiments, the particles are integrated into the gel. In some embodiments, the particles are covalently integrated into the gel. In some embodiments, the particles are non-covalently integrated into the gel. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising any nano- and/or microparticles particles known in the art. In some embodiments, the nano- and/or microparticles comprise caprolactone. In some embodiments, the nano- and/or microparticles comprise cellulose. In some embodiments, the nano- and/or microparticles are integrated into the gel. In some embodiments, the nano- and/or microparticles are

covalently attached. In some embodiments, the nano- and/or microparticles are non-covalently attached. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising nanofibers or microfibers integrated into the gel. In some embodiments, the nanofibers or microfibers are covalently attached. In some embodiments, the nanofibers or microfibers are non-covalently attached. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein. In some embodiments, the nanofibers or microfibers comprise SPF described herein. In some embodiments, the nanofibers or microfibers comprise caprolactone. In some embodiments, the nanofibers or microfibers comprise cellulose.

In some embodiments, the disclosure provides a gel, for example and without limitation a hydrogel, and without limitation for use in any methods of use described herein, the gel and/or hydrogel comprising SPF nano- or microparticles. In some embodiments, the gel and/or hydrogel may or may not include HA as described herein. In some embodiments, the gel and/or hydrogel matrix does not include SPF as described herein, except for the SPF nano- or microparticles embedded in the matrix. In some embodiments, the gel and/or hydrogel is any gel or hydrogel known in the art. In some embodiments, the particles are integrated into the gel. In some embodiments, the particles are covalently integrated into the gel. In some embodiments, the particles are non-covalently integrated into the gel. In some embodiments, the gel or hydrogel include lidocaine or any other anesthetic as described herein. In some embodiments, the gel or hydrogel do not include an anesthetic as described herein.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, configured to deliver another molecule, compound, drug, and the like. In some embodiments, the molecule, compound,

drug, or the like, comprises free silk and/or free SPF as described herein. In some embodiments, free silk and/or free SPF boosts collagen expression. In some embodiments, the molecule, compound, drug, or the like, comprises retinol. In some embodiments, the molecule, compound, drug, or the like, comprises a vitamin, including without limitation vitamin C. In some embodiments, the molecule, compound, drug, or the like, comprises and inflammatory agent. In some embodiments, the molecule, compound, drug, or the like, comprises an anti-inflammatory agent. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate epithelial cell regeneration. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate wound healing. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate pain management. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents able to provide sustained release. In some embodiments, the molecule, compound, drug, or the like, comprises one or more lubricant agents.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising an imaging agent. In some embodiments, the imaging agent is selected from iodine, DOPA, and imaging nanoparticles. In some embodiments, the imaging agent is selected from a paramagnetic imaging agent and a superparamagnetic imaging agent. In some embodiments, the imaging agent is selected from NP-based magnetic resonance imaging (MRI) contrast agents, positron emission tomography (PET)/single photon emission computed tomography (SPECT) imaging agents, ultrasonically active particles, and optically active (e.g., luminescent, fluorescent, infrared) particles. In some embodiments, the imaging agent is a SPECT imaging agent, a PET imaging agent, an optical imaging agent, an MRI or MRS imaging agent, an ultrasound imaging agent, a multimodal imaging agent, an X-ray imaging agent, or a CT imaging agent.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without

limitation a gel, and all methods of use described herein, for use to deliver drugs relevant to a specific area, including without limitation an area of injection.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising micro particles or micro capsules. In some embodiments, microparticles or micro capsules further comprise a drug.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, wherein the composition or tissue filler is radio opaque.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising a substantially solid silk composition comprising SPF described herein, having an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight, and a polydispersity between 1 and about 5. In some embodiments, the SPF have a polydispersity between 1 and about 1.5. In some embodiments, the SPF have a polydispersity between about 1.5 and about 2.0. In some embodiments, the SPF have a polydispersity between about 1.5 and about 3.0. In some embodiments, the SPF have a polydispersity between about 2.0 and about 2.5. In some embodiments, the SPF have a polydispersity between about 2.5 and about 3.0. In some embodiments, the composition further comprises about 0.01% (w/w) to about 10% (w/w) sericin relative to the SPF. In some embodiments, the SPF are formulated into particles. In some embodiments, the particles have a size of between about 1 μm and about 1000 μm. In some embodiments, the SPF in the substantially solid silk composition are obtained from a precursor solution comprising SPF fragments having an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight, and a polydispersity between 1 and about 5. In some embodiments, the SPF in the precursor solution have a polydispersity between 1 and about 1.5. In some embodiments, the SPF in the precursor solution have a polydispersity

between about 1.5 and about 2.0. In some embodiments, the SPF in the precursor solution have a polydispersity between about 1.5 and about 3.0. In some embodiments, the SPF in the precursor solution have a polydispersity between about 2.0 and about 2.5. In some embodiments, the SPF in the precursor solution have a polydispersity between about 2.5 and about 3.0. In some embodiments, the precursor solution further comprises about 0.01% (w/w) to about 10% (w/w) sericin relative to the SPF in the precursor solution. In some embodiments, the SPF in the precursor solution do not spontaneously or gradually gelate and do not visibly change in color or turbidity when in the precursor solution for at least 10 days prior to obtaining the silk fibroin fragments in the substantially solid silk composition. In some embodiments, the SPF in the substantially solid silk composition are obtained from the precursor solution by a process selected from a lyophilization process, a thin film evaporation process, a salting-out process, and a PVA-assisted method. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 0.01 wt. % to about 10.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 0.01 wt. % to about 1.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 1.0 wt. % to about 2.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 2.0 wt. % to about 3.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 3.0 wt. % to about 4.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 4.0 wt. % to about 5.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 5.0 wt. % to about 6.0 wt. % relative to the total weight.

In one aspect, the disclosure includes a method of treatment or prevention of a disorder, disease, or condition alleviated by administering a treatment to a subject in need thereof. In some embodiments, the method comprises administering to the subject a composition of the disclosure. In some embodiments, the composition comprises a tissue

filler of the disclosure. In some embodiments, the composition is administered by injection.

Any disease, disorder, or condition that can be alleviated by administering a treatment, such as radiation, cryotherapy, or drug treatment, is contemplated by the disclosure. Non-limiting examples of diseases, disorders, and conditions include cervical cancer, rectal cancer, pulmonary tumors, mediastinum lymphoma, breast cancer, uterine cancer, Benign prostatic hyperplasia (BPH), menorrhagia, uterine fibroids, and prostate adenocarcinomas. See, for example, US 8,257,723, US 7,744,913, US 20170056689, US 20160338793, US 7,771,339, CA 2,498,166, and US 6,746,465, all of which are incorporated by reference herein in their entireties.

Non-limiting examples of treatment include cryosurgery; radiation therapy including, but not limited to, external beam radiotherapy (e.g., 3D conformal or Intensity Modulated Radiotherapy), interstitial prostate brachytherapy (e.g., using permanent or temporary seeds, or using High Dose Rate remote after loading), external radiation therapy using gamma irradiation, high energy photon beam therapy, proton beam therapy, neutron beam therapy, heavy particle beam therapy, brachytherapy, thermal radiation, or any combination thereof; and drug treatment (local) such as alcohol tissue ablation or hyperosmolar ablation using NaCl crystals or hyperosmolar solution or physical tissue manipulation (e.g. dissection). Another embodiment is the use of these techniques for brachytherapy radiation treatments for prostate cancer or gynecological cancers. Brachytherapy includes the placement of a radioactive isotope within or near the tumor, target organ, or other tissue. For example, a brachytherapy technique is placement of permanent I-125 radioactive seeds into the prostate for treatment of prostate cancer. Applications for gynecology include embodiments involving displacing a tissue from another tissue that is to be targeted by radiation.

In some embodiments, the composition is administered between a first tissue and a second tissue. In some embodiments, the composition is administered into a space between a first tissue and a second tissue. In some embodiments, the first tissue is displaced relative to the second tissue. In some embodiments, the first tissue is irradiated. In some embodiments, the first tissue receives a substantially similar radiation dose compared to the radiation dose the first tissue would receive in the absence of the

composition. In some embodiments, the second tissue is irradiated. In some embodiments, the second tissue receives a lower radiation dose compared to the radiation dose the second tissue would receive in the absence of the composition. In some embodiments, the second tissue receives substantially no radiation dose.

Some embodiments also provide methods for treating a tissue of a body by radiation. In one embodiment, the method comprises the steps of injecting an effective amount of a composition described herein into a space between a first tissue (e.g., prostate) of a body and a second tissue (e.g., rectum), which can be a critically sensitive organ; and treating the first tissue by radiation whereby the composition within the space reduces passage of radiation into the second tissue.

In one aspect, the present disclosure describes a method of displacing a tissue to protect the tissue against the effects of a treatment, such as radiation or cryotherapy. One embodiment involves using a composition described herein to displace the tissue relative to a tissue that is to receive the treatment. Another embodiment involves introducing a composition described herein to radiate a first tissue and displace a second tissue. In some embodiments, the first tissue is close to the second tissue. In another embodiment, the method comprises the steps of injecting a composition described herein into a space between tissues; and may further include irradiating one of the tissues so that the other tissue receives less radiation than it would have in the absence of the composition.

Tissue is a broad term that encompasses a portion of a body: for example, a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body, e.g., a rectum, ovary, prostate, nerve, cartilage, bone, brain, or portion thereof. In some embodiments, the first tissue and the second tissue each independently comprises a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body.

In some embodiments, the terms “first tissue” and “second tissue” denote two tissue types (for example, prostate-rectum, uterus-rectum, uterus-small bowels, urinary bladder-uterus, ovary-bowels, uterus-urinary bladder, liver-gallbladder, lung-mediastinum, mediastinum-lung, mammary gland-thoracic wall, esophagus-spine, thyroid—blood vessels, thyroid-pharynx and larynx, small bowels and large bowels-retroperitoneum, kidney-liver, pancreas-stomach, pancreas-spine, stomach—liver,

stomach—spine, etc.) or different tissue regions of the same tissue type. It will be appreciated that in the latter case, the two tissue regions can be naturally adjacent and attached by fibroconjunctive tissue (e.g., lobes of a lung) and can be separated by the introduction of an incision. In some embodiments, the first tissue comprises a tumor tissue, and the second tissue comprises an organ. In some embodiments, the first tissue comprises an organ, and the second tissue comprises an organ. In some embodiments, the first tissue comprises a prostate and the second tissue comprises a rectum. In some embodiments, the first tissue comprises a portion of prostate and the second tissue comprises a portion of rectum. In some embodiments, the first tissue comprises a posterior vaginal wall/uterine cervix, and the second tissue comprises a rectum. In some embodiments, the first tissue comprises a rectum and the second tissue comprises a prostate. In some embodiments, the first tissue comprises a lung and the second tissue comprises a mediastinum. In some embodiments, the first tissue comprises a breast and the second tissue comprises an abdominal wall. See, for example, US 20160338793, which is incorporated by reference herein in its entirety.

In one embodiment, an injection of a composition described herein into Denonvilliers' space can change the radiation dose that the rectum receives when the prostate is exposed to radiation. "Denonvilliers' space" is a region located between the rectum and prostate. See, for example, de Castro Abreu et al., 2014, International J. Urology 21:416-418, which is incorporated by reference herein in its entirety. In some embodiments, the composition is administered into Denonvilliers' space.

In one aspect, the present disclosure describes a method of displacing a first tissue to protect the first tissue against the effects of a treatment in a subject in need thereof. In some embodiments, the method comprises administering to the subject a composition of the disclosure. In some embodiment, the method comprising displacing the first tissue relative to a second tissue. In some embodiment, the method further comprising injecting the composition into a space between the first tissue and the second tissue. In some embodiment, the method the space is Denonvilliers' space. In some embodiment, the method comprises injecting the composition between the first tissue and the second tissue to create a space between the tissues. In some embodiments, the second tissue is irradiated.

In some embodiments, the first tissue receives less of the dose of radioactivity compared to the amount of the dose of radioactivity the first tissue would receive in the absence of the composition. In some embodiments, the first tissue and the second tissue each independently comprise a tissue selected from a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body. In some embodiments, the first tissue comprises an organ, and the second tissue comprises a tumor tissue. In some embodiments, the first tissue comprises an organ, and the second tissue comprises an organ. In some embodiments, the first tissue comprises a rectum, and the second tissue comprises a prostate.

In some embodiments, the present invention includes methods for displacing a sensitive body tissue relative to another body tissue that is the target of a treatment protocol, to effectively reduce side effects on/in the sensitive tissue induced by or resulting from a treatment directed to the target tissue. In one embodiment, the method comprises injecting a composition described herein into a space between the sensitive body tissue (e.g., rectum) and the target body tissue (e.g., prostate); and conducting a treatment protocol on the target body tissue whereby the sensitive body tissue is less affected by the treatment as a result of the presence of the composition.

In one aspect of the disclosure, the composition described herein is biodegradable. In some embodiments, the composition is biodegradable by hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the composition is biodegradable by enzymatic degradation. In some embodiments, the enzyme is hyaluronidase. Biodegradation may be measured by palpitation or other observations to detect the change in volume of the composition after its introduction into a patient. In some embodiments, a suitable length for biodegradation to occur is between one day and twelve months after introduction of the composition into the body. In some embodiments, the composition may remain in place for other periods, including from one week to three months and two to eight weeks. In some embodiments, the composition described herein can be biodegraded in less than about two months after implantation, as is preferable for the case of displacing rectal tissue from the prostate gland. The time for biodegradability for a specific use may be determined by the time required to complete a course of radiation, which may vary for different radiological

applications and different requirements for administering the full course of radiological therapy, as would be understood by one of ordinary skill in the art. In some embodiments, the composition is removed by biodegradation in the subject.

In one aspect, the present disclosure describes methods of removing a composition of the disclosure from a subject. In a non-limiting example, a composition administered to a tissue can later be removed by causing the composition to degrade. In one embodiment, the composition is removed by degradation. In one embodiment, the composition is removed by biodegradation in the subject. In one aspect, the methods described herein further comprise a step wherein the composition is removed by biodegradation in the subject. In some embodiments, the removal step comprises administering to the subject a composition that causes biodegradation. In some embodiments, the biodegradation is hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the removal step comprises administering to the subject a composition comprising an enzyme. In some embodiments, the composition is biodegradable by hyaluronidase enzymatic degradation.

In one aspect of the disclosure, the composition described herein is radiopaque. As used herein, the term “radiopaque” is used to describe a material that is not transparent to X-rays or other forms of radiation. In some embodiments, the composition protects a tissue by blocking radiation being administered to another tissue. In some embodiments, the composition blocks about 10%, about 20%, about 30%, about 40%, about 50%, about 60&, about 70% about 80%, about 90%, or about 100% of the radiation. In some embodiments, the tissue receives about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% about 80%, about 90$%, or about 100% less radiation than it would have in the absence of the composition described herein.

In one aspect of the disclosure, a device for delivering a composition described herein to a body is described. In some embodiments, the device is loaded with a composition described herein, and the composition is introduced into the body, preferably so that the distance between a first and a second tissue in the body is thereby increased. A further step may include administering a dose of radiation to a tissue, preferably so that the second tissue receives less radiation than it would have received if the distance between the first and second tissue had not been increased. A further step may also be

administration of cryogenic treatment to the first or second tissue or a tissue nearby. The radiation may alternatively be directed to a third tissue so that the first tissue or the second tissue received a lower amount of radiation as a result of its separation from the other tissue(s). The first tissue and the second tissue may be adjacent to each other in the body, or may be separate from each other by other tissues. In many cases, such separation does not reduce the beneficial effects of achieving separation between the first and second tissue.

As would be understood by one of ordinary skill in the art, composition volumes for separating tissues are dependent on the configuration of the tissues to be treated and the tissues to be separated from each other. In many cases, a volume of about 20 cubic centimeters (cc's or mls) is suitable. In other embodiments, as little as 1 cc might be needed. Other volumes are in the range of 5-1000 cc, and all ranges therebetween, e.g., 5-400 cc, 10-30 cc, 15-25, cc, 10-150 cc, 20-200 cc, 15-500 cc, 50-1000 cc, and 30-200 cc. In some embodiments, the compositions described herein are administered in two doses at different times so as to allow the tissues to stretch and accommodate the compositions and thereby receive a larger volumes of composition than would otherwise be readily possible.

An example of a delivery device is a syringe. The compositions described herein can be loaded into the syringe and injected through a needle into a body. Another example is a device that accepts, e.g., a folded, deswelled, or rolled composition and provides a propelling mechanism to propel the compositions through a needle or catheter into a body. Propulsion may be by, e.g., a handle, a plunger, gas, or liquid force.

Another embodiment is a kit for introducing a composition described herein into a body. The kit may include a composition and a device for delivering the composition to the body. Embodiments include instructions for use. Embodiments include anesthetics mixed with the composition or separate therefrom. Embodiments include kits wherein the delivery device is a syringe, and other embodiments include a needle for the syringe, and may include a needle for administering the composition and/or the anesthetic.

Instructions may be included with a kit. Instructions may include words that direct a user in a use of a kit. Instructions may be fully or partially included with the kit, including as an insert, on a label, on a package, in a brochure, a seminar handout, a

seminar display, an internet teaching course, or on an internet or intranet web site. For example, a label on a kit could reference an internet address having instructions. Instructions may include explanations of embodiments set forth herein. Instructions may include dose histograms, and explanations of suitable composition volumes for use.

In some embodiments, the methods of the disclosure further include the administration of an anesthetic. In some embodiments, the anesthetic is administered prior to the administration of the composition described herein. In some embodiments, the anesthetics are local anesthetics, particularly 1% lidocaine for use in applying a compositions described herein to a body. The lidocaine may be used to perform a nerve block. In one embodiment, the needle for anesthetic application is a short 22-gauge needle and a 7 cm 22-gauge spinal needle. In one embodiment, the needle for delivering a filler via syringe injection is an 8-gauge spinal needle that is 3.5 cm length. Kits can include anesthetics.

In one aspect, the disclosure includes a method of treatment or prevention of a disorder, disease, or condition in a subject in need thereof. In some embodiments, the method comprises administering to the subject a composition of the disclosure. In some embodiments, the composition is injected into a tissue. In some embodiments, the composition comprises a tissue filler described herein.

In some embodiments, the tissue is associated with the disorder, disease, or condition, as would be understood by one of ordinary skill in the art. For example, a tissue can be associated with a disorder, disease, or condition when administering a composition of the disclosure into the tissue results in the alleviation, treatment, prevention, or amelioration of the disorder, disease, or condition.

Any type of tissue is contemplated by the disclosure. Tissue is a broad term that encompasses a portion of a body: for example, a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body, e.g., a rectum, ovary, prostate, nerve, cartilage, bone, brain, or portion thereof. See, for example, US 8,257,723, which is incorporated by reference herein in its entirety.

In some embodiments, the tissue is an organ. In some embodiments, the tissue is a portion of an organ. Non-limiting examples of a tissue include the urethra, the urethral sphincter, the lower esophageal sphincter, the diaphragm, the rectum, a vocal cord, the

larynx, and skin. In some embodiments, the tissue comprises a portion of a wall of an internal organ. In some embodiments, the tissue is a portion of the urethra or the urethral sphincter. In some embodiments, the tissue is a portion of the lower esophageal sphincter or the diaphragm. In some embodiments, the tissue is a portion of the urethral sphincter. In some embodiments, the tissue is a portion of the rectum. In some embodiments, the tissue is a portion of a vocal cord or larynx. In some embodiments, the tissue is a portion of skin.

In some embodiments, augmentation, bulking or otherwise decreasing the distensibility of the tissue results in the treatment or prevention of the disorder, disease, or condition. In some embodiments, the administration of the composition leads to bulking of the tissue. In some embodiments, the disorder, disease, or condition is treated or prevented by the bulking of the tissue.

In some embodiments, the composition is administered into a wall of a tissue, as would be understood by one of ordinary skill in the art. In some embodiments, the tissue comprises a portion of a wall of an internal organ. In some embodiments, the composition is administered into a region of a rectal wall. In some embodiments, the region of the rectal wall is in the vicinity of the anal sphincter. In some embodiments, the composition is administered into the wall of the internal sphincter. In some embodiments, the composition is administered into the internal sphincter.

Any disorder, disease, or condition that can be alleviated, treated, prevented, or ameliorated using the compositions of the disclosure is contemplated by the present disclosure. Non-limiting examples of disorders, diseases, or conditions include urinary incontinence, gastroesophageal reflux disease (GERD), vesicoureteral reflux, skin deficiencies, fecal incontinence, dental tissue defects, vocal cord tissue defects, larynx defects, and other non-dermal soft tissue defects. See, for example, US 9,295,648, US 8,932,637, US 8,882,654, US 9,308,301, US 7,780,980, CA 2,133,756, US 6,060,053, US 8,394,400, US 8,821,857, and US 6,660,301, all of which are incorporated by reference herein in their entireties.

In one aspect, the present disclosure describes a method of treating urinary incontinence. Urinary incontinence is a prevalent problem that affects people of all ages and levels of physical health, both in the community at large and in healthcare settings.

Medically, urinary incontinence predisposes a patient to urinary tract infections, pressure ulcers, perineal rashes, and urosepsis. Socially and psychologically, urinary incontinence is associated with embarrassment, social stigmatization, depression, and especially for the elderly, an increased risk of institutionalization (Herzo et al., Ann. Rev. Gerontal. Geriatrics, 9:74 (1989)). Examples of types of urinary incontinence include, but are not limited to, stress incontinence, intrinsic sphincter deficiency (ISD), urge incontinence, overflow incontinence, and enuresis. See, for example, US 9,295,648, US 9,308,301, US 7,780,980, CA 2,133,756, US 6,060,053, US 8,394,400, and US 6,660,301, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with urinary incontinence. In some embodiments, the tissue is the urethra or urethral sphincter. In some embodiments, the tissue is a portion of the urethra or the urethral sphincter. In some embodiments, the administration of the composition leads to bulking of the urethra or the urethral sphincter, or a portion thereof, to treat or prevent urinary incontinence.

In one aspect, the present disclosure describes a method of treating gastroesophageal reflux disease (GERD). GERD describes a backflow of acidic and enzymatic liquid from the stomach to the esophagus. It causes burning sensations behind the sternum that may be accompanied by regurgitation of gastric acid into the mouth or even the lung. Complications of GERD which define the severity of the disease include esophageal tissue erosion, and esophageal ulcer wherein normal epithelium is replaced by a pathological tissue. See, for example, US 9,295,648, US 9,308,301, and US 6,660,301, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with gastroesophageal reflux disease. In some embodiments, the tissue is the lower esophageal sphincter or the diaphragm. In some embodiments, the tissue is a portion of the lower esophageal sphincter or the diaphragm. In some embodiments, the administration of the composition leads to bulking of the urethra or the urethral sphincter, or a portion thereof, to treat or prevent gastroesophageal reflux disease.

In one aspect, the present disclosure describes a method of treating vesicoureteral reflux (urinary reflux disease). Urinary reflux disease, or "vesicoureteral reflux" in its medical term, simply means that urine goes backwards in the ureters during urination. The disease often occurs in young children. The ureter is the tube which connects the kidneys with the bladder. Urine is supposed to go in one direction: from the kidneys to the bladder. When urine goes up from the bladder to the kidneys, it can result in health problems for the person. See, for example, US 9,295,648, US 6,060,053, and US 8,394,400, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with vesicoureteral reflux. In some embodiments, the tissue is the urethral sphincter. In some embodiments, the tissue is a portion of the urethral sphincter. In some embodiments, the administration of the composition leads to bulking of the urethral sphincter, or a portion thereof, to treat or prevent vesicoureteral reflux.

In one aspect, the present disclosure describes a method of treating fecal incontinence. Fecal incontinence, which is most common in the elderly, is the loss of voluntary control to retain stool in the rectum. In most cases, fecal incontinence is the result of an impaired involuntary internal anal sphincter. The internal sphincter may be incompetent due to laxity or discontinuity. Discontinuity, or disruption of the internal anal sphincter, can be caused by a number of different muscle injuries. See, for example, US 8,882,654, US 9,308,301, and US 8,394,400, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with fecal incontinence. In some embodiments, the tissue is the rectum. In some embodiments, the tissue is a portion of the rectum. In some embodiments, the composition is administered into a region of a rectal wall. In some embodiments, the region of the rectal wall is in the vicinity of the anal sphincter. In some embodiments, the composition is administered into the internal sphincter. In some embodiments, the administration of the composition leads to bulking of the rectum, rectal wall, or internal sphincter, or a portion thereof, to treat or prevent fecal incontinence.

In one aspect, the present disclosure describes a method of treating a vocal cord tissue defect or larynx defect. Non-limiting examples of vocal cord tissue defects or larynx defects include glottic incompetence, unilateral vocal cord paralysis, bilateral vocal cord paralysis, paralytic dysphonia, nonparalytic dysphonia, spasmodic dysphonia or a combination thereof. In other embodiments, the methods of the disclosure may also be used to manage or treat diseases, disorders or other abnormalities that result in the vocal cords closing improperly, such as an incomplete paralysis of the vocal cord ("paresis"), generally weakened vocal cords, for instance, with old age ("presbylaryngis"), and/or scarring of the vocal cords (e.g., from previous surgery or radiotherapy). See, for example, US 9,295,648, and US 8,821,857, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with a vocal cord tissue defect or larynx defect. In some embodiments, the tissue is a vocal cord or larynx. In some embodiments, the tissue is portion of a vocal cord or larynx. In some embodiments, the administration of the composition leads to bulking of a vocal cord or larynx, or a portion thereof, to treat or prevent a vocal cord tissue defect or larynx defect.

In one aspect, the present disclosure describes a method of treating a skin deficiency. Damage to the skin due to aging, environmental exposure to the sun and other elements, weight loss, child bearing, disease such as acne and cancer, and surgery often results in skin contour deficiencies and other skin anomalies. Non-limiting examples of skin deficiencies include acne and cancer. In some embodiments, the skin deficiency is a skin contour deficiency. Examples of skin contour deficiencies include, but are not limited to, frown lines, worry lines, wrinkles, crow's feet, marionette lines, stretch marks, and internal or external scars resulted from injury, wound, bite, surgery, and accident. See, for example, US 9,295,648, US 8,932,637, US 8,821,857, and US 6,660,301, all of which are incorporated by reference herein in their entireties.

In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into a tissue associated with a skin deficiency. In some embodiments, the tissue is skin. In

some embodiments, the tissue is portion of skin. In some embodiments, the administration of the composition leads to bulking of skin, or a portion thereof, to treat or prevent a skin deficiency.

In one aspect, the present disclosure describes a method of causing dermal augmentation in a subject in need thereof. In some embodiments, the method comprises administering to the subject a composition of the disclosure. In some embodiments, the composition is injected into the skin or into a portion of the skin. In some embodiments, the dermal augmentation method of the present disclosure is especially suitable for the treatment of skin contour deficiencies.

In one aspect, the present disclosure describes a method of causing tissue bulking in a subject. In some embodiments, the method comprises administering to a subject in need thereof a composition of the disclosure. In some embodiments, the composition is injected into an area of the subject in need of tissue bulking. In some embodiments, the tissue bulking treats or prevents a disorder, disease, or condition in the subject.

In one aspect of the disclosure, the composition described herein is biodegradable. In some embodiments, the composition is biodegradable by hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the composition is biodegradable by enzymatic degradation. In some embodiments, the enzyme is hyaluronidase. Biodegradation may be measured by palpitation or other observations to detect the change in volume of the composition after its introduction into a patient. In some embodiments, a suitable length for biodegradation to occur is between one day and twelve months after introduction of the composition into the body. In some embodiments, the composition may remain in place for other periods, including from one week to three months and two to eight weeks. In some embodiments, the composition described herein can be biodegraded in less than about two months after implantation. In some embodiments, the composition is removed by biodegradation in the subject.

In one aspect, the present disclosure describes methods of tissue debulking. In a non-limiting example, a tissue that is bulked with a biodegradable composition of the disclosure can be debulked by causing the composition to degrade. In one aspect, the methods described herein further comprise a tissue debulking step. In some

embodiments, the debulking step comprises administering to the subject a composition that causes biodegradation. In some embodiments, the composition causes hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the debulking step comprises administering to the subject a composition comprising an enzyme. In some embodiments, the enzyme is a hyaluronidase.

In one aspect of the disclosure, the composition described herein is radiopaque. As used herein, the term "radiopaque" is used to describe a material that is not transparent to X-rays or other forms of radiation. In some embodiments, the composition protects a tissue by blocking radiation being administered to another tissue. In some embodiments, the composition blocks about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% about 80%, about 90%, or about 100% of the radiation. In some embodiments, the tissue receives about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% about 80%, about 90%, or about 100% less radiation than it would have in the absence of the composition described herein.

As would be understood by one of ordinary skill in the art, composition volumes for administering within the methods described herein are dependent on the configuration of the tissues to be treated and the tissues to be separated from each other. In many cases, a volume of about 20 cubic centimeters (cc's or mls) is suitable. In other embodiments, as little as 1 cc might be needed. Other volumes are in the range of 5-1000 cc, and all ranges therebetween, e.g., 5-400 cc, 10-30 cc, 15-25, cc, 10-150 cc, 20-200 cc, 15-500 cc, 50-1000 cc, and 30-200 cc. In some embodiments, the compositions described herein are administered in two doses at different times so as to allow the tissues to stretch and accommodate the filler and thereby receive a larger volumes of composition than would otherwise be readily possible.

An example of a delivery device is a syringe. The compositions described herein can be loaded into the syringe and injected through a needle into a body. Another example is a device that accepts, e.g., a folded, deswelled, or rolled filler and provides a propelling mechanism to propel the compositions through a needle or catheter into a body. Propulsion may be by, e.g., a handle, a plunger, gas, or liquid force.

Another embodiment is a kit for introducing a compositions described herein into a body. The kit may include a compositions and a device for delivering the filler to the body. Embodiments include instructions for use. Embodiments include anesthetics mixed with the compositions or separate therefrom. Embodiments include kits wherein the delivery device is a syringe, and other embodiments include a needle for the syringe, and may include a needle for administering the compositions and/or the anesthetic.

Instructions may be included with a kit. Instructions may include words that direct a user in a use of a kit. Instructions may be fully or partially included with the kit, including as an insert, on a label, on a package, in a brochure, a seminar handout, a seminar display, an internet teaching course, or on an internet or intranet web site. For example, a label on a kit could reference an internet address having instructions. Instructions may include explanations of embodiments set forth herein. Instructions may include dose histograms, and explanations of suitable filler volumes for use.

In some embodiments, the methods of the disclosure further include the administration of an anesthetic. In some embodiments, the anesthetic is administered prior to the administration of the composition described herein. In some embodiments, the anesthetics are local anesthetics, particularly 1% lidocaine for use in applying a compositions described herein to a body. The lidocaine may be used to perform a nerve block. In one embodiment, the needle for anesthetic application is a short 22-gauge needle and a 7 cm 22-gauge spinal needle. In one embodiment, the needle for delivering a filler via syringe injection is an 8-gauge spinal needle that is 3.5 cm length. Kits can include anesthetics.

In some embodiments, the disclosure provides compositions useful for reducing inflammation. In some embodiments, the composition further comprises an anti-inflammatory agent. Non-limiting examples of anti-inflammatory agents include cyclosporine, hydrocortisone, hydrocortisone acetate, dexamethasone, dexamethasone 21-phosphate, fluocinolone, medrysone, prednisolone, prednisolone 21-phosphate, prednisolone acetate, fluoromethalone, betamethasone, and triamcinolone. In some embodiments, the anti-inflammatory agent is cyclosporine.

In some embodiments, the disclosure provides compositions useful for wound healing. In some embodiments, the composition further comprises a wound healing agent.

Examples of wound healing agents include antibiotics, disinfectants, wound healing agents and the like. Examples of active drug include fucic acid, centelia asiatica, mucyrosin, neomycin, bacitracin, gentamicin, (FGF), hepatic fibroblast growth factor (FGF), hepatic fibroblast growth factor (FGF), hepatic fibroblast growth factor (FGF), hepatocyte growth factor Growth promoting agents such as growth factor (HGF) and indicator cell growth factor (EGF), and the like, preferably, fucic acid or its pharmaceutically acceptable salt, Acrinol, and triclosan.

In one aspect of the disclosure, the composition described herein is biodegradable. In some embodiments, the biodegradability is effected by hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the composition is biodegradable by enzymatic degradation. In some embodiments, the enzymatic degradation is hyaluronidase enzymatic degradation. Biodegradation may be measured by palpitation or other observations to detect the change in volume of the composition after its introduction into a patient. In some embodiments, a suitable length for biodegradation to occur is between one day and twelve months after introduction of the composition into the body. In some embodiments, the composition may remain in place for other periods, including from one week to three months and two to eight weeks. In some embodiments, the composition described herein can be biodegraded in less than about two months after implantation. In some embodiments, the composition is removed by biodegradation in the subject. In some embodiments, the composition is biodegradable in vivo.

In one aspect of the disclosure, the composition further comprises a lubricant. Non-limiting examples of a lubricant include glycerin, polyethylene glycol 400 (PEG 400), and propylene glycol. In some embodiments, the lubricant is a sustained lubricant. In some embodiments, the lubricant comprises silk fibroin or silk fibroin fragments or a portion of silk fibroin or silk fibroin fragments.

In some embodiments, the silk fibroin-based protein fragment composition further comprises a thickening agent or gelling agent selected from the group of hydroxyethyl cellulose, hydroxypropyl methylcellulose, cyclodextrin, dextran, gelatin, carboxymethyl cellulose, propylene glycol, polyethylene glycol, polysorbate 80, polyvinyl alcohol, povidone, sucrose, fructose, maltose, carrageenan, chitosan, alginate, hyaluronic acid,

gum arabic, galactomannans, pectin, and combinations thereof. Without the thickening agent, O/W emulsions are unstable to creaming once radius of the emulsion droplets is greater than 0.5 μm.

In some embodiments, the silk fibroin-based protein fragment composition comprises about 0.01 wt. % to about 10.0 wt. % of the thickening/gelling agent. In some embodiments, the silk fibroin-based protein fragment composition comprises about 0.2 wt. % to about 2.0 wt. % of the thickening/gelling agent. In some embodiments, the silk fibroin-based protein fragment composition comprises the thickening/gelling agent at an amount selected from the group of about 0.01 wt. %, about 0.1 wt. %, about 0.2 wt. %, about 0.3 wt. %, about 0.4 wt. %, about 0.5 wt. %, about 0.6 wt. %, about 0.7 wt. %, about 0.8 wt. %, about 0.9 wt. %, about 1.0 wt. %, about 1.1 wt. %, about 1.2 wt. %, about 1.3 wt. %, about 1.4 wt. %, about 1.5 wt. %, about 1.6 wt. %, about 1.7 wt. %, about 1.8 wt. %, about 1.9 wt. %, about 2.0 wt. %, about 2.1 wt. %, about 2.2 wt. %, about 2.3 wt. %, about 2.4 wt. %, about 2.5 wt. %, about 2.6 wt. %, about 2.7 wt. %, about 2.8 wt. %, about 2.9 wt. %, about 3.0 wt. %, about 3.1 wt. %, about 3.2 wt. %, about 3.3 wt. %, about 3.4 wt. %, about 3.5 wt. %, about 3.6 wt. %, about 3.7 wt. %, about 3.8 wt. %, about 3.9 wt. %, about 4.0 wt. %, about 4.1 wt. %, about 4.2 wt. %, about 4.3 wt. %, about 4.4 wt. %, about 4.5 wt. %, about 4.6 wt. %, about 4.7 wt. %, about 4.8 wt. %, about 4.9 wt. %, about 5.0 wt. %, about 5.1 wt. %, about 5.2 wt. %, about 5.3 wt. %, about 5.4 wt. %, about 5.5 wt. %, about 5.6 wt. %, about 5.7 wt. %, about 5.8 wt. %, about 5.9 wt. %, about 6.0 wt. %, about 6.1 wt. %, about 6.2 wt. %, about 6.3 wt. %, about 6.4 wt. %, about 6.5 wt. %, about 6.6 wt. %, about 6.7 wt. %, about 6.8 wt. %, about 6.9 wt. %, about 7.0 wt. %, about 7.1 wt. %, about 7.2 wt. %, about 7.3 wt. %, about 7.4 wt. %, about 7.5 wt. %, about 7.6 wt. %, about 7.7 wt. %, about 7.8 wt. %, about 7.9 wt. %, about 8.0 wt. %, about 8.1 wt. %, about 8.2 wt. %, about 8.3 wt. %, about 8.4 wt. %, about 8.5 wt. %, about 8.6 wt. %, about 8.7 wt. %, about 8.8 wt. %, about 8.9 wt. %, about 9.0 wt. %, about 9.1 wt. %, about 9.2 wt. %, about 9.3 wt. %, about 9.4 wt. %, about 9.5 wt. %, about 9.6 wt. %, about 9.7 wt. %, about 9.8 wt. %, about 9.9 wt. %, and about 10.0 wt. % by the basis of the silk fibroin-based protein fragment composition.

In some embodiments, the thickening/gelling agent is hyaluronic acid at about 0.2 wt. % by the total weight of the silk fibroin-based protein fragment composition.

In some embodiments, when producing a silk gel, an acid is used to help facilitate gelation. In an embodiment, when producing a silk gel that includes a neutral or a basic molecule and/or therapeutic agent, an acid can be added to facilitate gelation. In an embodiment, when producing a silk gel, increasing the pH (making the gel more basic) increases the shelf stability of the gel. In an embodiment, when producing a silk gel, increasing the pH (making the gel more basic) allows for a greater quantity of an acidic molecule to be loaded into the gel.

In some embodiments, the silk gel comprises multi-lamellar liquid crystal gel network formed by the silk fibroin protein-based fragments and the natural emulsifier described herein. The multi-lamellar liquid crystals are biomimetic and serve as barrier and water-retention functions. The multi-lamellar liquid crystal networks can be formed in oil-in-water emulsions by combining a high HLB primary emulsifier (e.g., hydrophilic surfactant) and a second low-to-medium HLB co-emulsifier (e.g., a hydrophobic surfactant). The high HLB primary emulsifier reduces interfacial tension and facilitates the formation of small oil droplets in the outer aqueous phase. The low HLB co-emulsifier forms a gel network. This network structure stabilizes the emulsion by preventing creaming and coalescence of the oil droplets as well as by building viscosity.

In some embodiments, the multi-lamellar liquid crystalline gel network of the emulsion further comprise a thickener selected from the group of acrylic acid polymer, carrageenan, xanthan gum, guar gum, and magnesium aluminum silicate, and combinations thereof. In some embodiments, the thickener is carrageenan, xanthan gum and guar gum. In some embodiments, the thickener is presented in the emulsion at an amount ranging from about 0.05 wt. % to about 0.5 wt. % by the total weight of the emulsion.

In some embodiments, the silk fibroin-based protein fragments are present in the silk gel at a weight amount ranging from about 0.001 wt. % to about 10.0 wt. % by the total weight of the silk gel. In some embodiments, the silk fibroin-based protein fragments are present in the silk gel at a weight amount ranging from about 0.001 wt. % to about 5.0 wt. % by the total weight of the silk gel. In some embodiments, the silk fibroin-based

protein fragments are present in the silk gel at a weight amount ranging from about 0.001 wt. % to about 1.0 wt. % by the total weight of the silk gel. In some embodiments, the silk fibroin-based protein fragments are present in the silk gel at a weight amount ranging from about 10 wt. % by the total weight of the silk gel.

In one aspect, the present disclosure describes a method of treatment or prevention of a disorder, disease, or condition in a subject in need thereof. In some embodiments, the method comprising administering to the subject a composition of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The presently disclosed embodiments will be further explained with reference to the attached drawings. The drawings shown are not necessarily to scale, with emphasis instead generally being placed upon illustrating the principles of the presently disclosed embodiments.

Fig. 1 is a flow chart showing various embodiments for producing pure silk fibroin-based protein fragments (SPFs) of the present disclosure.

Fig. 2 is a flow chart showing various parameters that can be modified during the process of producing SPFs of the present disclosure during the extraction and the dissolution steps.

Fig. 3 is a table summarizing the LiBr and Sodium Carbonate (Na_2CO_3) concentration in silk protein solutions of the present disclosure.

Fig. 4 is a table summarizing the LiBr and Na_2CO_3 concentration in silk protein solutions of the present disclosure.

Fig. 5 is a table summarizing the Molecular Weights of silk protein solutions of the present disclosure.

Figs. 6 and 7 are graphs representing the effect of extraction volume on % mass loss.

Fig. 8 is a table summarizing the Molecular Weights of silk dissolved from different concentrations of LiBr and from different extraction and dissolution sizes.

Fig. 9 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 100 °C LiBr and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 10 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, boiling LiBr and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 11 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 °C LiBr and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 12 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C LiBr and 80 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 13 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C LiBr and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 14 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 100 °C LiBr and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 15 is a graph summarizing the effect of Extraction Time on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 140 °C LiBr and 140 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 16 is a graph summarizing the effect of Extraction Temperature on Molecular Weight of silk processed under the conditions of 60 minute Extraction Time, 100 °C LiBr and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 17 is a graph summarizing the effect of LiBr Temperature on Molecular Weight of silk processed under the conditions of 60 minute Extraction Time, 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 18 is a graph summarizing the effect of LiBr Temperature on Molecular Weight of silk processed under the conditions of 30 minute Extraction Time, 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Fig. 19 is a graph summarizing the effect of Oven/Dissolution Temperature on Molecular Weight of silk processed under the conditions of 100 °C Extraction

Temperature, 30 minute Extraction Time, and 100 °C Lithium Bromide (Oven/Dissolution Time was varied).

Fig. 20 is a graph summarizing the effect of Oven/Dissolution Temperature on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 minute Extraction Time, and 100 °C Lithium Bromide. (Oven/Dissolution Time was varied).

Fig. 21 is a graph summarizing the effect of Oven/Dissolution Temperature on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 minute Extraction Time, and 140 °C Lithium Bromide (Oven/Dissolution Time was varied).

Fig. 22 is a graph summarizing the effect of Oven/Dissolution Temperature on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 30 minute Extraction Time, and 140 °C Lithium Bromide (Oven/Dissolution Time was varied).

Fig. 23 is a graph summarizing the effect of Oven/Dissolution Temperature on Molecular Weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 minute Extraction Time, and 80 °C Lithium Bromide (Oven/Dissolution Time was varied).

Fig. 24 is a graph summarizing the Molecular Weights of silk processed under varying conditions including Extraction Time, Extraction Temperature, Lithium Bromide (LiBr) Temperature, Oven Temperature for Dissolution, Oven Time for Dissolution.

Fig. 25 is a graph summarizing the Molecular Weights of silk processed under conditions in which Oven/Dissolution Temperature is equal to LiBr Temperature.

Fig. 26 is a picture of silk/HA formulations in water or phosphate-buffered saline (PBS) at various concentrations, which demonstrate that silk/HA formulations result in homogenous, opaque solutions. The first unmarked vial is a control vial (22 mg/mL HA in water).

Fig. 27 is a picture of aqueous silk/HA formulations deposited in syringes, which demonstrate that silk/HA formulations result in homogenous, opaque solutions. The control is a solution of 22 mg/mL HA in water.

Fig. 28 is a chart depicting the degradation profile of silk-HA and HA hydrogels.

Fig. 29 is a picture of an intradermal area in a guinea pig injected with a control dermal filler (commercially available HA filler including lidocaine); the increased degree of inflammation is reflected by the extent of granulomatous areas. The commercially available filler is noted as blue/gray material. Granulomatous inflammation associated with the material can be observed at 7 days.

Fig. 30 is a picture of an intradermal area in a guinea pig injected with a control dermal filler (commercially available HA filler including lidocaine); the commercially available product is noted as blue/gray material. At 30 days, inflammation with fibrosis can be observed.

Fig. 31 is a picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (24 mg/ml HA, 9.6 mg/ml silk, BDDE cross linked); the reduced granulomatous areas as compared to the control injection indicates negligible acute inflammatory response, and a better biodegradability of the silk-HA filler compared to the control. There is very little inflammation at 7 days. The inflammation is focal and at times hard to find. No implant material is noted.

Fig. 32 is a picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (24 mg/ml HA, 9.6 mg/ml silk, BDDE cross linked); at 30 days the inflammation is extremely difficult to find and minimal. No implant material is noted.

Fig. 33 is a picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (24 mg/ml HA, 0.48 mg/ml silk, BDDE cross linked); the filler results in focal mild inflammation in the 7 days. The inflammation is chronic. This inflammation required close evaluation to identify since it was focal and minimal. No implant material is observed.

Fig. 34 is a picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (24 mg/ml HA, 0.48 mg/ml silk, BDDE cross linked); the 30-day image demonstrates even less inflammation. It was even more difficult to identify as compared to the 7 day implants. No implant material is observed.

Fig. 35 is a chart depicting turbidity measurement of a silk-HA hydrogel. Black curve (a): standard transmittance; Red curve (b): transmittance plus forward scatter.

Fig. 36 is a chart depicting turbidity measurement of HA hydrogel without silk. Black curve (a): standard transmittance; Red curve (b): transmittance plus forward scatter.

Fig. 37 is a representative histology picture of an intradermal area in a guinea pig injected with a control dermal filler.

Fig. 38 is a representative histology picture of an intradermal area in a guinea pig injected with an HA dermal filler of the invention (24 mg/ml HA, PEGDE cross linked, Sample C4 – Table 25).

Fig. 39 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample L – Table 25).

Fig. 40 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (23.76 mg/ml HA, 0.24 mg/ml silk, PEGDE cross linked, Sample M – Table 25).

Fig. 41 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample N – Table 25).

Fig. 42 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample O – Table 25).

Fig. 43 is a graph showing 7-day post-implantation histology results for gel degradation (Table 25 formulations - BDDE crosslinked formulations are mostly degraded; scoring: 0 - normal; 1 - minimal; 2 - mild; 3 - moderate; and 4 - severe).

Fig. 44 is a graph showing 7-day post-implantation histology results for gel migration (Table 25 formulations; scoring: 0 - normal; 1 - minimal; 2 - mild; 3 - moderate; and 4 - severe).

Fig. 45 is a graph showing 7-day post-implantation histology results for inflammation (Table 25 formulations - no tissue necrosis was observed, no blood clotting was observed, and minimal collagen deposition was observed on the control formulation and some of the test formulations; scoring: 0 - normal; 1 - minimal; 2 - mild; 3 - moderate; and 4 - severe).

Fig. 46 is a graph showing 7-day post-implantation histology results for macrophages (Table 25 formulations; scoring: 0 - normal; 1 - minimal; 2 - mild; 3 - moderate; and 4 - severe).

Figs. 47A and 47B show the G' of hydrogels with various silk concentrations before and after dialysis. Fig. 47A: mixed HA crosslinked at 100 gm/ml, and Fig. 47B: single MW HA crosslinked at 25 mg/ml.

Figs. 48A and 48B show the swelling ratio of hydrogel with various silk concentrations during dialysis. Fig. 48A: mixed HA crosslinked at 100 mg/ml, and Fig. 48B: single MW HA crosslinked at 25 mg/ml.

Figs. 49A and 49B show the calibration curves for medium and low molecular weight silk solutions, respectively.

Figs. 50A and 50B show the absorbance spectra of diluted silk-HA gels with unknown silk concentration; the theoretical silk concentration (mg/ml) is shown for each silk-HA gel sample in Table 26.

Fig. 51 shows turbidity measurement of HA hydrogel without silk (red; higher transmittance across the entire wavelength interval) and with 3 mg/ml silk (blue; lower transmittance across the entire wavelength interval); a higher % transmittance indicates a less turbid sample, with less optical opacity.

Fig. 52 illustrates the signature ions of the PEG crosslinked silk fibroin fragments (LC MS/MS spectrum shows signature ions of the silk crosslinked with PEG).

Fig. 53A-B illustrates the semi-quantitative evaluation (the lower scoring the better; a total score of 6.9 for the control group and a total score of 3.8 for the test group); 7-day histology images: Juvederm® (Fig. 53A) and silk dermal filler (Fig. 53B).

Fig. 54 shows a silk dermal filler in 1-ml syringe showing turbid hydrogel with fine silk fibers suspended.

Figs. 55A-C illustrate the testing results for G', MoD and injection force. Storage modulus G' (Fig. 55A), degree of modification MoD (Fig. 55B), and injection force (Fig. 55C, 30 gauge needle) of silk-HA hydrogels, are represented as a function of the ratio of silk to the total amount of silk and HA in the formulation (% silk = 100*(silk concentration)/(combined concentration of silk and HA)). HA concentration = 24.7 mg/ml for all formulations, and PEG is present at ~30% w/w. Plotted are the average ±

standard deviation of three samples for Fig. 55A and Fig. 55C. In Fig. 55B, multiple hydrogel samples were combined for each measurement.

Fig. 56 illustrates the testing results for storage modulus G' and injection force IF of more than 100 dermal filler candidates. (Blue dots), IF measured through a 30G x 1/2 needle (Orange dots), IF measured through a 27G x 1/2 needle. The HA and silk total concentrations range from 15 mg/mL to 26 mg/mL.

Fig. 57 illustrates the absorption spectra of HA hydrogels formulated with (solid line) and without silk (dotted line) and a competitor hydrogel product (Juvéderm® Ultra Plus XC, dashed line). Plotted are the average of three measurements for each hydrogel.

Fig. 58A illustrates the in vitro hydrogel reversibility for AS-V1 (white) or Juvéderm® Ultra Plus XC (black). Approximately 1 g of each hydrogel was digested with 150 U hyaluronidase at 37 °C for 30 minutes, and the weight of the remaining gels was measured. This process was repeated three more times for a total of 600 U of hyaluronidase over 120 minutes. The degree of hydrogel degradation is represented by a weight ratio (%) of the remaining hydrogel to the original hydrogel. Plotted is the average ± standard deviation of three samples at each time point.

Fig. 58B illustrates the in vivo hydrogel reversibility for AS-V1 (white) or Juvéderm® Ultra Plus XC (black). Approximately 0.1 mL of each injected hydrogel site was digested with 0.1 mL hyaluronidase and observed for 30 min to determine reversing based on remaining bolus. The number of additional reversibility injections is represented by the number of additional hyaluronidase injections. In 61% and 47% of instances AS-V1 and Juvéderm® Ultra Plus XC only required one reversibility injection respectively.

Fig. 59 illustrates the results of Draize skin irritancy test results for guinea pigs injected with AS-V1 (white) or Juvéderm® Ultra Plus XC (black). Six animals were tested at each timepoint (days 1-5 post-injection); each animal received 3 injections of 0.1 mL AS-V1 and 3 of Juvéderm® Ultra Plus XC spaced ~1 cm apart in the dorsal dermis. Data plotted are the daily average scores ± standard deviation; the maximum possible score is 8.

Figs. 60A-D illustrate the testing results for the post-injection bruising in guinea pigs injected with AS-V1 (top circle, indicated in blue) or Juvéderm® Ultra Plus XC (bottom circle, indicated in red). Figs. 60A and 60B show the testing results 3-days post

injection. Figs. 60C and 60D show the testing results 4-days post injection. Six animals were tested at each timepoint (days 3 and 4 post-injection); each animal received 3 injections of 0.1 mL AS-V1 and 3 of Juvéderm® Ultra Plus XC spaced 1 cm apart in the dorsal dermis. Representative bruising images from two animals (Fig. 60A and Fig. 60B, or Fig. 60C and Fig. 60D) are shown.

Figs. 61A-D illustrate the animal testing results for inflammation (Fig. 61A), in vivo hydrogel reversibility (degradation, Figs. 61B and 61D), and hydrogel migration (Figs. 61C and 61E) post-injection with AS-V1 (solid lines) or Juvéderm® Ultra Plus XC (dashed lines). Six animals were tested at each timepoint (7 days, 30 days, 3 months, 6 months and 12 months post-injection); each animal received 3 injections of 0.1 mL AS-V1 and 3 of Juvéderm® Ultra Plus XC spaced about 1 cm apart in the dorsal dermis. Tissue sections from guinea pig dorsal dermis were stained with hematoxylin and eosin and representative sections scored by a blinded pathologist. Data plotted are the average assessment scores ± standard deviation at each timepoint. For inflammation, the maximum possible score is 28, and for hydrogel degradation and migration the maximum possible scores are 4. Fig. 61F illustrates the testing results for inflammation response with AS-V1 (solid lines) or Juvéderm Ultra Plus XC (dashed lines). Six animals were tested at each timepoint (7 days, 30 days, 90 days, 180 days and 365 days post-injection); each animal received 3 injections of 0.1 ml AS-V1 and 3 of Juvéderm Ultra Plus XC spaced ~1 cm apart in the dorsal dermis. Tissue sections from guinea pig dorsal dermis were stained with hematoxylin and eosin and representative sections scored by a blinded pathologist. Data plotted are the average assessment scores ± standard deviation at each timepoint. For inflammation, the maximum possible score is 28.

Figs. 62A-J illustrate the representative histology slides for GLP Guinea pig study comparing AS-V1 (test) top row (A, C, E, G, and I) and Juvéderm Ultra Plus XC (control) bottom row (B, D, F, H, and J). Samples A and B represent test and control at 7 days respectively, samples C and D represent test and control at 30 days respectively, samples D and F represent test and control at 90 days respectively, samples G and H represent test and control at 180 days respectively, and samples I and J represent test and control at 365 days respectively.

Figs. 63A-D illustrate the representative histology of dermal tissues at 3 months (Figs. 63A, C) or 6 months (Figs. 63B, D) post-injection with AS-V1 (Figs. 63 A, B) or Juvéderm® Ultra Plus XC (Figs. 63 C, D). Tissue sections from guinea pig dorsal dermis were stained with hematoxylin and eosin. Representative sections were from six animals injected with 0.1 mL AS-V1 or Juvéderm® Ultra Plus XC. Magnification 25x.

Fig. 64 illustrates the NMR spectra of an exemplary HA used in the methods and gels of the disclosure, NMR spectrum with assigned labels; the peak labeled "**a**" is assigned and normalized as 3, and the integration of peaks from 3.30 to 4.05 is 11.

Fig. 65 illustrates the NMR spectra of an exemplary gel of the disclosure, including the calculation of gel MoD based on peak integration.

Figs. 66A-66C illustrate Low-MW silk solid resulted from lyophilization described herein at different stages of grinding. Fig. 66A illustrate the coarse particles of the Low-MW silk solid immediate after removal from the lyophilization bottle. Fig. 66B illustrates the reduced size particle midway through grinding. Fig. 66C illustrates the fine particles with even size distribution at the completion grinding.

Fig. 67 illustrates solid particles of Mid-MW silk solid.

Fig. 68 illustrates example of two different particle size solid silk particles formed during thin film evaporation described herein.

Figs. 69A and 69B illustrate examples of microparticles prepared by a solution precipitation process described herein.

Fig. 70 illustrates milled silk powder for uses described herein.

Fig. 71 illustrates SMA Dermal Filler Injection Force (IF) vs. Storage Modulus (G').

Fig. 72 illustrates SMA Dermal Filler Injection Force (IF) vs. Loss Modulus (G").

Fig. 73 illustrates SMA Dermal Filler Storage Modulus (G') vs. Tan(δ).

Fig. 74 illustrates SMA Dermal Filler Injection Force (IF) vs. Complex Viscosity (η*).

Fig. 75 illustrates SMA Dermal Filler Storage Modulus (G') vs. Loss Modulus (G").

Fig. 76 illustrates SMA Dermal Filler Storage Modulus (G') vs. Silk + HA Concentration.

While the above-identified drawings set forth presently disclosed embodiments, other embodiments are also contemplated, as noted in the discussion. This disclosure presents illustrative embodiments by way of representation and not limitation. Numerous other modifications and embodiments can be devised by those skilled in the art which fall within the scope and spirit of the principles of the presently disclosed embodiments.

DETAILED DESCRIPTION

Dermal fillers have revolutionized soft tissue augmentation, becoming increasingly popular in recent years for the correction of moderate to severe skin wrinkles and folds due to the increased demands of an aging United States (US) population that desires less-invasive cosmetic procedures. In fact, over the past thirty years, dermal fillers have become a significant part of both medical and cosmetic dermatology. Medically, dermal fillers are used to correct debilitating scars, morphological asymmetry and facial lipoatrophy in patients under treatment for HIV infection. Cosmetically, dermal fillers are used to minimize skin creases and lift depressed scars throughout the upper, mid, and lower face, eliminating fine forehead lines and crow's feet. Dermal fillers reverse these effects by restoring volume and lift, by correcting the descent of the malar fat pad, and softening nasolabial folds. As the use of dermal fillers has increased in popularity, and because no one product is applicable for all indications, the number of available dermal filler products has also increased, with approval by the FDA of 5 new products for soft tissue augmentation in just the past ~5 years. Initially, autologous tissue and animal-derived collagens were available for use; now, dermal filler options include biopolymers and synthetic implants. Dermal fillers fall, without limitation, into three types: temporary (non-permanent), semi-permanent, and permanent. Collagen, hyaluronic acid (HA) and other biologically-based and biodegradable fillers are temporary, with effects lasting from a few months to two years; semi-permanent fillers have effects lasting a few years and include biodegradable poly-L-lactic acid and calcium hydroxyapatite-based products; permanent filler products can last five or more years and include non-biodegradable polymethylmethacrylate (PMMA), polyacrylamide hydrogel, and liquid silicone.

Unfortunately, after decades of research and development, limitations still exist with current dermal fillers. Adverse reactions have been reported to result from injection

of currently available dermal filler products in some patients. These include immediate pain, hypersensitivity, and anaphylaxis, early post-injection swelling, erythema, infection, overcorrection, and necrosis, late post-injection herpes (HSV) activation, bluish skin discoloration (described as the Tyndall effect), nodule or granuloma formation, and permanent post-injection scarring. In general, the more permanent filler products are responsible for the more severe of these reactions, while the more temporary products, such as HA-based fillers, lead to less severe reactions. Meanwhile, the public is likely to prefer a product that both gives longer-lasting results and avoids these often hard-to-address complications. One strategy for reaching this goal is the modification of hyaluronic acid (HA)-based hydrogels to increase their longevity. HA, which is found naturally in the skin, has a high turnover rate in the body, making it a challenge to use HA as a long-lasting dermal filler. To improve its clinical persistence, the stability of HA in dermal fillers can be enhanced via the crosslinking of HA chains. Crosslinking restricts the access of degrading factors such as the enzyme hyaluronidase and reactive oxygen species (e.g., superoxide) to individual HA chains, protecting them from degradation. Moreover, HA crosslinked via one particular method – the VyCross™ technology –has recently been associated with an increase in occurrence of delayed-onset firm lesions, one of the more severe adverse reactions seen with dermal fillers. For using as dermal fillers, it is desirable that the hydrogel materials exhibit appropriate viscoelasticity and resistance to deformation ("stiffer" materials with higher G'), ease of flow during injection (low IF), and longevity or resistance to degradation *in vivo* (typically achieved with a higher MoD).

For these reasons, other strategies for modifying and optimizing HA-based hydrogels are under study; these are expected to have greater potential for avoiding adverse events while maintaining durability. The use of silk fibroin protein boasts many advantages: with a unique structure that affords it remarkable strength and toughness compared to other biomaterials, and has an inherent ability to adopt different structural conformations, the fibroin units can self-assemble into dozens of different higher-ordered polymers without the need for solvents, plasticizers, or catalysts that often have deleterious effects on living organisms. Looking beyond the addition of silk fibroin to HA-based hydrogels, the use of polyethylene glycol (PEG), a polymer with proven

biocompatibility, affords additional benefits in controlling the mechanical properties of silk-HA dermal filler gels. For decades, PEG has been used itself or as a modification for other carriers/coatings to deliver bioactive agents, enhancing the biocompatibility, hydrophilicity, stability, and biodegradability of nanocarriers, and often effectively reducing the toxicity of bioactives and carriers. This disclosure provides novel silk based tissue and/or dermal filler formulations and products to provide new treatment options that avoid adverse event issues seen recently in the dermal filler market. The silk-containing tissue and/or dermal fillers described herein with different characteristics can be made that would individually meet the needs of a host of different aesthetic and medical indications while maintaining the biocompatibility profiles.

Although silk-HA composites have been studied for various uses as scaffolds in tissue engineering, the exploration of their use as tissue and/or dermal filler agents expands the possible uses of silk-HA hydrogels, and represents the foundation of a new approach to the formulation of tissue and/or dermal fillers with considerable promise. The present disclosure describes the establishment of a novel platform – the activated silk hydrogel platform – for the formulation of silk integrated HA hydrogels that vary in storage modulus (G') – important for the development of tissue and/or dermal filler products for different indications – while maintaining characteristics that promote product longevity (high MoD). In fact, the lead candidate (AS-V1) showed promising in vitro and in vivo performance, demonstrating suitable properties for intradermal tissue filler applications, with a high MoD at operable IF and desirable G' (See Examples 32-35 infra).

The incorporation of silk into HA-based dermal fillers provides an advantageous choice on multiple fronts. The incorporation of silk protein may help avoid some of the adverse effects that occur with current dermal filler products. For example, AS-V1 demonstrated increased absorbance of UV to blue visible light as compared to a commercially-available product, indicating that it is less likely to result in Tyndall-type bluing of patient skin, and may thus be more applicable for superficial aesthetic corrections. Lesion/nodule formation has been observed with some filler products, potentially as a result of a high degree of crosslinking or of using multiple sizes (molecular weights) of HA, such as occurs in the VyCross™ technologies. This may be

avoided with silk-containing hydrogels as a single-sized HA is used, and MoD can be easily modulated.

Moreover, AS-V1 performs equivalently to or better than the current market leader in safety and efficacy testing. Biocompatibility testing confirmed expectations built upon the demonstrated safety of all three gel components for in vivo use: (1) HA as a natural component of the skin's viscoelastic extracellular matrix; (2) silk that has been used in different biomedical applications throughout history, including for dermal tissue reconstruction; and (3) PEG as a biocompatible polymer (See Examples 32-35 infra). In fact, AS-V1 satisfied all criteria in ISO 10993 biocompatibility studies, and in in vivo studies caused minimal post-injection irritation and bruising, and inflammation at levels similar to or lower than those seen with a commercial product. In vivo hydrogel performance characteristics of longevity, degradation, migration and reversibility were also similar between AS-V1 and a commercial product. In particular, the AS-V1 dermal filler meets desired longevity criteria, with gel volume remaining at 12 months post-injection comparable to Juvéderm Ultra Plus XC (Figs. 61D-E and Figs. 62A-J *infra*), a commercial product known to last 12 months as a nasolabial fold treatment. Further, the silk-HA gel incorporated into the skin's collagen matrix more smoothly than did Juvéderm Ultra Plus XC (Fig.s 63A-D *infra*); this may be the result of viscosity differences between the two gels and/or of the inclusion of silk protein, hypotheses that will be tested in future studies.

The strategy of incorporating silk into HA-based dermal fillers is advantageous on multiple fronts, from the versatility of the developed formulation platform that carries the potential to generate a suite of dermal filler products appropriate for a variety of aesthetic and medical indications, to the superior biocompatibility of the resulting gels.

The key advantages that result from incorporating silk into HA-based dermal fillers are as follows: (1) with different target applications, tissue and/or dermal filler products require different mechanical properties, longevity, and reversibility profiles. Because silk fibroin can self-assemble into dozens of different highly-ordered polymers/structural conformations and is naturally resilient to changes in temperature, moisture, and pH, the physicochemical and mechanical properties of the hydrogel, including its ability to bind water (potential for swelling), can be controlled through

varying concentrations of silk in combination with a single, smaller HA chain instead of mixing different HA forms or varying concentrations of crosslinker. This points to the ability the platform described herein to generate a variety of silk-HA dermal filler formulations; (2) Because the silk-HA hydrogels have properties indicating the potential to avoid the Tyndall effect, have a similar reversibility profile to currently available HA-based products, and incorporate non-toxic, biocompatible purified silk fibroin protein and PEG crosslinker, the likelihood of their use causing adverse events is relatively low.

The activated silk hydrogel platform described herein leverages the unique ability of silk fibroin to self-assemble into dozens of different highly-ordered polymers/structural conformations and its natural resilience to changes in temperature, moisture, and pH. Via this platform, a hydrogel's biophysical properties, including its ability to bind water (potential for swelling), and its interactions with the skin, can be controlled through varying concentrations of silk in combination with a single, smaller HA chain instead of mixing different HA forms or varying concentrations of crosslinker. In fact, the Activated Silk Hydrogel platform has already been leveraged to generate a library of products with a variety of structural characteristics (Fig. 56 *infra*) from which gel properties crucially important for performance in patients, such as mechanical properties and longevity, can be optimized for different target applications.

SPF Definitions and Properties

As used herein, "silk protein fragments" (SPF) include one or more of: "silk fibroin fragments" as defined herein; "recombinant silk fragments" as defined herein; "spider silk fragments" as defined herein; "silk fibroin-like protein fragments" as defined herein; and/or "chemically modified silk fragments" as defined herein. SPF may have any molecular weight values or ranges described herein, and any polydispersity values or ranges described herein. As used herein, in some embodiments the term "silk protein fragment" also refers to a silk protein that comprises or consists of at least two identical repetitive units which are each independently selected from naturally-occurring silk polypeptides or of variations thereof, amino acid sequences of naturally-occurring silk polypeptides, or of combinations of both.

SPF Molecular Weight and Polydispersity

In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 1 to about 5 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 5 to about 10 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 10 to about 15 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 15 to about 20 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 14 to about 30 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 20 to about 25 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 25 to about 30 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 30 to about 35 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 35 to about 40 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 39 to about 54 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 40 to about 45 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 45 to about 50 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 50 to about 55 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 55 to about 60 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 60 to about 65 kDa. In an embodiment, a composition of the present disclosure includes SPF having an

average weight average molecular weight selected from between about 65 to about 70 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 70 to about 75 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 75 to about 80 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 80 to about 85 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 85 to about 90 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 90 to about 95 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 95 to about 100 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 100 to about 105 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 105 to about 110 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 110 to about 115 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 115 to about 120 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 120 to about 125 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 125 to about 130 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 130 to about 135 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 135 to about 140 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 140 to about 145

kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 145 to about 150 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 150 to about 155 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 155 to about 160 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 160 to about 165 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 165 to about 170 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 170 to about 175 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 175 to about 180 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 180 to about 185 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 185 to about 190 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 190 to about 195 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 195 to about 200 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 200 to about 205 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 205 to about 210 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 210 to about 215 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 215 to about 220 kDa. In an embodiment, a composition of the present disclosure includes SPF having an

average weight average molecular weight selected from between about 220 to about 225 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 225 to about 230 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 230 to about 235 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 235 to about 240 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 240 to about 245 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 245 to about 250 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 250 to about 255 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 255 to about 260 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 260 to about 265 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 265 to about 270 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 270 to about 275 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 275 to about 280 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 280 to about 285 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 285 to about 290 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 290 to about 295 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 295 to about 300

kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 300 to about 305 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 305 to about 310 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 310 to about 315 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 315 to about 320 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 320 to about 325 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 325 to about 330 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 330 to about 335 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 335 to about 340 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 340 to about 345 kDa. In an embodiment, a composition of the present disclosure includes SPF having an average weight average molecular weight selected from between about 345 to about 350 kDa.

In some embodiments, compositions of the present disclosure include SPF compositions selected from compositions #1001 to #2450, having weight average molecular weights selected from about 1 kDa to about 145 kDa, and a polydispersity selected from between 1 and about 5 (including, without limitation, a polydispersity of 1), between 1 and about 1.5 (including, without limitation, a polydispersity of 1), between about 1.5 and about 2, between about 1.5 and about 3, between about 2 and about 2.5, between about 2.5 and about 3, between about 3 and about 3.5, between about 3.5 and about 4, between about 4 and about 4.5, and between about 4.5 and about 5:

PDI (about) / MW (about)	1-5	1-1.5	1.5-2	1.5-3	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5
1 kDa	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010
2 kDa	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020
3 kDa	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030
4 kDa	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040
5 kDa	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050
6 kDa	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060
7 kDa	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070
8 kDa	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080
9 kDa	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090
10 kDa	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100
11 kDa	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110
12 kDa	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120
13 kDa	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130
14 kDa	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140
15 kDa	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150
16 kDa	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160
17 kDa	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170
18 kDa	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180
19 kDa	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190
20 kDa	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200
21 kDa	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210
22 kDa	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220
23 kDa	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230
24 kDa	1231	1232	1233	1234	1235	1236	1237	1238	1239	1240
25 kDa	1241	1242	1243	1244	1245	1246	1247	1248	1249	1250
26 kDa	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260
27 kDa	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270
28 kDa	1271	1272	1273	1274	1275	1276	1277	1278	1279	1280
29 kDa	1281	1282	1283	1284	1285	1286	1287	1288	1289	1290
30 kDa	1291	1292	1293	1294	1295	1296	1297	1298	1299	1300
31 kDa	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310
32 kDa	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320
33 kDa	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330
34 kDa	1331	1332	1333	1334	1335	1336	1337	1338	1339	1340
35 kDa	1341	1342	1343	1344	1345	1346	1347	1348	1349	1350
36 kDa	1351	1352	1353	1354	1355	1356	1357	1358	1359	1360
37 kDa	1361	1362	1363	1364	1365	1366	1367	1368	1369	1370

38 kDa	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380
39 kDa	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390
40 kDa	1391	1392	1393	1394	1395	1396	1397	1398	1399	1400
41 kDa	1401	1402	1403	1404	1405	1406	1407	1408	1409	1410
42 kDa	1411	1412	1413	1414	1415	1416	1417	1418	1419	1420
43 kDa	1421	1422	1423	1424	1425	1426	1427	1428	1429	1430
44 kDa	1431	1432	1433	1434	1435	1436	1437	1438	1439	1440
45 kDa	1441	1442	1443	1444	1445	1446	1447	1448	1449	1450
46 kDa	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460
47 kDa	1461	1462	1463	1464	1465	1466	1467	1468	1469	1470
48 kDa	1471	1472	1473	1474	1475	1476	1477	1478	1479	1480
49 kDa	1481	1482	1483	1484	1485	1486	1487	1488	1489	1490
50 kDa	1491	1492	1493	1494	1495	1496	1497	1498	1499	1500
51 kDa	1501	1502	1503	1504	1505	1506	1507	1508	1509	1510
52 kDa	1511	1512	1513	1514	1515	1516	1517	1518	1519	1520
53 kDa	1521	1522	1523	1524	1525	1526	1527	1528	1529	1530
54 kDa	1531	1532	1533	1534	1535	1536	1537	1538	1539	1540
55 kDa	1541	1542	1543	1544	1545	1546	1547	1548	1549	1550
56 kDa	1551	1552	1553	1554	1555	1556	1557	1558	1559	1560
57 kDa	1561	1562	1563	1564	1565	1566	1567	1568	1569	1570
58 kDa	1571	1572	1573	1574	1575	1576	1577	1578	1579	1580
59 kDa	1581	1582	1583	1584	1585	1586	1587	1588	1589	1590
60 kDa	1591	1592	1593	1594	1595	1596	1597	1598	1599	1600
61 kDa	1601	1602	1603	1604	1605	1606	1607	1608	1609	1610
62 kDa	1611	1612	1613	1614	1615	1616	1617	1618	1619	1620
63 kDa	1621	1622	1623	1624	1625	1626	1627	1628	1629	1630
64 kDa	1631	1632	1633	1634	1635	1636	1637	1638	1639	1640
65 kDa	1641	1642	1643	1644	1645	1646	1647	1648	1649	1650
66 kDa	1651	1652	1653	1654	1655	1656	1657	1658	1659	1660
67 kDa	1661	1662	1663	1664	1665	1666	1667	1668	1669	1670
68 kDa	1671	1672	1673	1674	1675	1676	1677	1678	1679	1680
69 kDa	1681	1682	1683	1684	1685	1686	1687	1688	1689	1690
70 kDa	1691	1692	1693	1694	1695	1696	1697	1698	1699	1700
71 kDa	1701	1702	1703	1704	1705	1706	1707	1708	1709	1710
72 kDa	1711	1712	1713	1714	1715	1716	1717	1718	1719	1720
73 kDa	1721	1722	1723	1724	1725	1726	1727	1728	1729	1730
74 kDa	1731	1732	1733	1734	1735	1736	1737	1738	1739	1740
75 kDa	1741	1742	1743	1744	1745	1746	1747	1748	1749	1750
76 kDa	1751	1752	1753	1754	1755	1756	1757	1758	1759	1760
77 kDa	1761	1762	1763	1764	1765	1766	1767	1768	1769	1770
78 kDa	1771	1772	1773	1774	1775	1776	1777	1778	1779	1780
79 kDa	1781	1782	1783	1784	1785	1786	1787	1788	1789	1790
80 kDa	1791	1792	1793	1794	1795	1796	1797	1798	1799	1800

81 kDa	1801	1802	1803	1804	1805	1806	1807	1808	1809	1810
82 kDa	1811	1812	1813	1814	1815	1816	1817	1818	1819	1820
83 kDa	1821	1822	1823	1824	1825	1826	1827	1828	1829	1830
84 kDa	1831	1832	1833	1834	1835	1836	1837	1838	1839	1840
85 kDa	1841	1842	1843	1844	1845	1846	1847	1848	1849	1850
86 kDa	1851	1852	1853	1854	1855	1856	1857	1858	1859	1860
87 kDa	1861	1862	1863	1864	1865	1866	1867	1868	1869	1870
88 kDa	1871	1872	1873	1874	1875	1876	1877	1878	1879	1880
89 kDa	1881	1882	1883	1884	1885	1886	1887	1888	1889	1890
90 kDa	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
91 kDa	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910
92 kDa	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920
93 kDa	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930
94 kDa	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940
95 kDa	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950
96 kDa	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960
97 kDa	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970
98 kDa	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980
99 kDa	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
100 kDa	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
101 kDa	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
102 kDa	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
103 kDa	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030
104 kDa	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040
105 kDa	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050
106 kDa	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060
107 kDa	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070
108 kDa	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080
109 kDa	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090
110 kDa	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
111 kDa	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110
112 kDa	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120
113 kDa	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130
114 kDa	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140
115 kDa	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150
116 kDa	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160
117 kDa	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170
118 kDa	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180
119 kDa	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190
120 kDa	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200
121 kDa	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210
122 kDa	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220
123 kDa	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230

124 kDa	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240
125 kDa	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250
126 kDa	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260
127 kDa	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270
128 kDa	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280
129 kDa	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290
130 kDa	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300
131 kDa	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310
132 kDa	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320
133 kDa	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330
134 kDa	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340
135 kDa	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350
136 kDa	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360
137 kDa	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370
138 kDa	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380
139 kDa	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390
140 kDa	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400
141 kDa	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410
142 kDa	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420
143 kDa	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430
144 kDa	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440
145 kDa	2441	2442	2443	2444	2445	2446	2447	2448	2449	2450

As used herein, “low molecular weight,” “low MW,” or “low-MW” SPF may include SPF having a weight average molecular weight, or average weight average molecular weight selected from between about 5 kDa to about 38 kDa, about 14 kDa to about 30 kDa, or about 6 kDa to about 17 kDa. In some embodiments, a target low molecular weight for certain SPF may be weight average molecular weight of about 5 kDa, about 6 kDa, about 7 kDa, about 8 kDa, about 9 kDa, about 10 kDa, about 11 kDa, about 12 kDa, about 13 kDa, about 14 kDa, about 15 kDa, about 16 kDa, about 17 kDa, about 18 kDa, about 19 kDa, about 20 kDa, about 21 kDa, about 22 kDa, about 23 kDa, about 24 kDa, about 25 kDa, about 26 kDa, about 27 kDa, about 28 kDa, about 29 kDa, about 30 kDa, about 31 kDa, about 32 kDa, about 33 kDa, about 34 kDa, about 35 kDa, about 36 kDa, about 37 kDa, or about 38 kDa.

As used herein, “medium molecular weight,” “medium MW,” or “mid-MW” SPF may include SPF having a weight average molecular weight, or average weight average molecular weight selected from between about 31 kDa to about 55 kDa, or about 39 kDa

to about 54 kDa. In some embodiments, a target medium molecular weight for certain SPF may be weight average molecular weight of about 31 kDa, about 32 kDa, about 33 kDa, about 34 kDa, about 35 kDa, about 36 kDa, about 37 kDa, about 38 kDa, about 39 kDa, about 40 kDa, about 41 kDa, about 42 kDa, about 43 kDa, about 44 kDa, about 45 kDa, about 46 kDa, about 47 kDa, about 48 kDa, about 49 kDa, about 50 kDa, about 51 kDa, about 52 kDa, about 53 kDa, about 54 kDa, or about 55 kDa.

As used herein, "high molecular weight," "high MW," or "high-MW" SPF may include SPF having a weight average molecular weight, or average weight average molecular weight selected from between about 55 kDa to about 150 kDa. In some embodiments, a target high molecular weight for certain SPF may be about 55 kDa, about 56 kDa, about 57 kDa, about 58 kDa, about 59 kDa, about 60 kDa, about 61 kDa, about 62 kDa, about 63 kDa, about 64 kDa, about 65 kDa, about 66 kDa, about 67 kDa, about 68 kDa, about 69 kDa, about 70 kDa, about 71 kDa, about 72 kDa, about 73 kDa, about 74 kDa, about 75 kDa, about 76 kDa, about 77 kDa, about 78 kDa, about 79 kDa, or about 80 kDa.

In some embodiments, the molecular weights described herein (e.g., low molecular weight silk, medium molecular weight silk, high molecular weight silk) may be converted to the approximate number of amino acids contained within the respective SPF, as would be understood by a person having ordinary skill in the art. For example, the average weight of an amino acid may be about 110 daltons (i.e., 110 g/mol). Therefore, in some embodiments, dividing the molecular weight of a linear protein by 110 daltons may be used to approximate the number of amino acid residues contained therein.

In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between 1 to about 5.0, including, without limitation, a polydispersity of 1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 1.5 to about 3.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between 1 to about 1.5, including, without limitation, a polydispersity of 1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 1.5 to about 2.0. In an embodiment, SPF in a composition of

the present disclosure have a polydispersity selected from between about 2.0 to about 2.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 2.5 to about 3.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 3.0 to about 3.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 3.5 to about 4.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 4.0 to about 4.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity selected from between about 4.5 to about 5.0.

In an embodiment, SPF in a composition of the present disclosure have a polydispersity of 1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.2. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.3. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.4. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.6. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.7. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.8. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 1.9. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.2. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.3. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.4. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.6. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.7. In an embodiment, SPF in a

composition of the present disclosure have a polydispersity of about 2.8. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 2.9. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.2. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.3. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.4. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.6. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.7. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.8. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 3.9. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.0. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.1. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.2. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.3. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.4. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.5. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.6. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.7. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.8. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 4.9. In an embodiment, SPF in a composition of the present disclosure have a polydispersity of about 5.0.

In some embodiments, in compositions described herein having combinations of low, medium, and/or high molecular weight SPF, such low, medium, and/or high molecular weight SPF may have the same or different polydispersities.

Silk Fibroin Fragments

Methods of making silk fibroin or silk fibroin protein fragments and their applications in various fields are known and are described for example in U.S. Patents Nos. 9,187,538, 9,511,012, 9,517,191, 9,522,107, 9,522,108, 9,545,369, and 10,166,177, 10,287,728 and 10,301,768, all of which are incorporated herein in their entireties. Raw silk from silkworm *Bombyx mori* is composed of two primary proteins: silk fibroin (approximately 75%) and sericin (approximately 25%). Silk fibroin is a fibrous protein with a semi-crystalline structure that provides stiffness and strength. As used herein, the term "silk fibroin" means the fibers of the cocoon of *Bombyx mori* having a weight average molecular weight of about 370,000 Da. The crude silkworm fiber consists of a double thread of fibroin. The adhesive substance holding these double fibers together is sericin. The silk fibroin is composed of a heavy chain having a weight average molecular weight of about 350,000 Da (H chain), and a light chain having a weight average molecular weight about 25,000 Da (L chain). Silk fibroin is an amphiphilic polymer with large hydrophobic domains occupying the major component of the polymer, which has a high molecular weight. The hydrophobic regions are interrupted by small hydrophilic spacers, and the N- and C-termini of the chains are also highly hydrophilic. The hydrophobic domains of the H-chain contain a repetitive hexapeptide sequence of Gly-Ala-Gly-Ala-Gly-Ser and repeats of Gly-Ala/Ser/Tyr dipeptides, which can form stable anti-parallel-sheet crystallites. The amino acid sequence of the L-chain is non-repetitive, so the L-chain is more hydrophilic and relatively elastic. The hydrophilic (Tyr, Ser) and hydrophobic (Gly, Ala) chain segments in silk fibroin molecules are arranged alternatively such that allows self-assembling of silk fibroin molecules.

Provided herein are methods for producing pure and highly scalable silk fibroin-protein fragment mixture solutions that may be used across multiple industries for a variety of applications. Without wishing to be bound by any particular theory, it is believed that these methods are equally applicable to fragmentation of any SPF described herein, including without limitation recombinant silk proteins, and fragmentation of silk-like or fibroin-like proteins.

As used herein, the term "fibroin" includes silk worm fibroin and insect or spider silk protein. In an embodiment, fibroin is obtained from *Bombyx mori*. Raw silk from

Bombyx mori is composed of two primary proteins: silk fibroin (approximately 75%) and sericin (approximately 25%). Silk fibroin is a fibrous protein with a semi-crystalline structure that provides stiffness and strength. As used herein, the term "silk fibroin" means the fibers of the cocoon of *Bombyx mori* having a weight average molecular weight of about 370,000 Da. Conversion of these insoluble silk fibroin fibrils into water-soluble silk fibroin protein fragments requires the addition of a concentrated neutral salt (e.g., 8-10 M lithium bromide), which interferes with inter- and intramolecular ionic and hydrogen bonding that would otherwise render the fibroin protein insoluble in water. Methods of making silk fibroin protein fragments, and/or compositions thereof, are known and are described for example in U.S. Patents Nos. 9,187,538, 9,511,012, 9,517,191, 9,522,107, 9,522,108, 9,545,369, and 10,166,177.

The raw silk cocoons from the silkworm *Bombyx mori* was cut into pieces. The pieces silk cocoons were processed in an aqueous solution of Na_2CO_3 at about 100 °C for about 60 minutes to remove sericin (degumming). The volume of the water used equals about 0.4 x raw silk weight and the amount of Na_2CO_3 is about 0.848 x the weight of the raw silk cocoon pieces. The resulting degummed silk cocoon pieces were rinsed with deionized water three times at about 60 °C (20 minutes per rinse). The volume of rinse water for each cycle was 0.2 L x the weight of the raw silk cocoon pieces. The excess water from the degummed silk cocoon pieces was removed. After the DI water washing step, the wet degummed silk cocoon pieces were dried at room temperature. The degummed silk cocoon pieces were mixed with a LiBr solution, and the mixture was heated to about 100 °C. The warmed mixture was placed in a dry oven and was heated at about 100 °C for about 60 minutes to achieve complete dissolution of the native silk protein. The resulting silk fibroin solution was filtered and dialyzed using Tangential Flow Filtration (TFF) and a 10 kDa membrane against deionized water for 72 hours. The resulting silk fibroin aqueous solution has a concentration of about 8.5 wt. %. Then, 8.5 % silk solution was diluted with water to result in a 1.0 % w/v silk solution. TFF can then be used to further concentrate the pure silk solution to a concentration of 20.0 % w/w silk to water.

Dialyzing the silk through a series of water changes is a manual and time intensive process, which could be accelerated by changing certain parameters, for

example diluting the silk solution prior to dialysis. The dialysis process could be scaled for manufacturing by using semi-automated equipment, for example a tangential flow filtration system.

In some embodiments, the silk solutions are prepared under various preparation condition parameters such as: 90 °C 30 min, 90 °C 60 min, 100 °C 30 min, and 100 °C 60 min. Briefly, 9.3 M LiBr was prepared and allowed to sit at room temperature for at least 30 minutes. 5 mL of LiBr solution was added to 1.25 g of silk and placed in the 60 °C oven. Samples from each set were removed at 4, 6, 8, 12, 24, 168 and 192 hours.

In some embodiments, the silk solutions are prepared under various preparation condition parameters such as: 90 °C 30 min, 90 °C 60 min, 100 °C 30 min, and 100 °C 60 min. Briefly, 9.3 M LiBr solution was heated to one of four temperatures: 60 °C, 80 °C, 100 °C or boiling. 5 mL of hot LiBr solution was added to 1.25 g of silk and placed in the 60 °C oven. Samples from each set were removed at 1, 4 and 6 hours.

In some embodiments, the silk solutions are prepared under various preparation condition parameters such as: Four different silk extraction combinations were used: 90 °C 30 min, 90 °C 60 min, 100 °C 30 min, and 100 °C 60 min. Briefly, 9.3 M LiBr solution was heated to one of four temperatures: 60 °C, 80 °C, 100 °C or boiling. 5 mL of hot LiBr solution was added to 1.25 g of silk and placed in the oven at the same temperature of the LiBr. Samples from each set were removed at 1, 4 and 6 hours. 1 mL of each sample was added to 7.5 mL of 9.3 M LiBr and refrigerated for viscosity testing.

In some embodiments, SPF are obtained by dissolving raw unscoured, partially scoured, or scoured silkworm fibers with a neutral lithium bromide salt. The raw silkworm silks are processed under selected temperature and other conditions in order to remove any sericin and achieve the desired weight average molecular weight (M_W) and polydispersity (PD) of the fragment mixture. Selection of process parameters may be altered to achieve distinct final silk protein fragment characteristics depending upon the intended use. The resulting final fragment solution is silk fibroin protein fragments and water with parts per million (ppm) to non-detectable levels of process contaminants, levels acceptable in the pharmaceutical, medical and consumer eye care markets. The concentration, size and polydispersity of SPF may further be altered depending upon the desired use and performance requirements.

Fig. 1 is a flow chart showing various embodiments for producing pure silk fibroin protein fragments (SPFs) of the present disclosure. It should be understood that not all of the steps illustrated are necessarily required to fabricate all silk solutions of the present disclosure. As illustrated in Fig. 1, step A, cocoons (heat-treated or non-heat-treated), silk fibers, silk powder, spider silk or recombinant spider silk can be used as the silk source. If starting from raw silk cocoons from *Bombyx mori*, the cocoons can be cut into small pieces, for example pieces of approximately equal size, step B1. The raw silk is then extracted and rinsed to remove any sericin, step C1a. This results in substantially sericin free raw silk. In an embodiment, water is heated to a temperature between 84 °C and 100 °C (ideally boiling) and then Na_2CO_3 (sodium carbonate) is added to the boiling water until the Na_2CO_3 is completely dissolved. The raw silk is added to the boiling water/Na_2CO_3 (100 °C) and submerged for approximately 15 - 90 minutes, where boiling for a longer time results in smaller silk protein fragments. In an embodiment, the water volume equals about 0.4 x raw silk weight and the Na_2CO_3 volume equals about 0.848 x raw silk weight. In an embodiment, the water volume equals 0.1 x raw silk weight and the Na_2CO_3 volume is maintained at 2.12 g/L.

Subsequently, the water dissolved Na_2CO_3 solution is drained and excess water/Na_2CO_3 is removed from the silk fibroin fibers (e.g., ring out the fibroin extract by hand, spin cycle using a machine, etc.). The resulting silk fibroin extract is rinsed with warm to hot water to remove any remaining adsorbed sericin or contaminate, typically at a temperature range of about 40 °C to about 80 °C, changing the volume of water at least once (repeated for as many times as required). The resulting silk fibroin extract is a substantially sericin-depleted silk fibroin. In an embodiment, the resulting silk fibroin extract is rinsed with water at a temperature of about 60 °C. In an embodiment, the volume of rinse water for each cycle equals 0.1 L to 0.2 L x raw silk weight. It may be advantageous to agitate, turn or circulate the rinse water to maximize the rinse effect. After rinsing, excess water is removed from the extracted silk fibroin fibers (e.g., ring out fibroin extract by hand or using a machine). Alternatively, methods known to one skilled in the art such as pressure, temperature, or other reagents or combinations thereof may be used for the purpose of sericin extraction. Alternatively, the silk gland (100% sericin free

silk protein) can be removed directly from a worm. This would result in liquid silk protein, without any alteration of the protein structure, free of sericin.

The extracted fibroin fibers are then allowed to dry completely. Once dry, the extracted silk fibroin is dissolved using a solvent added to the silk fibroin at a temperature between ambient and boiling, step C1b. In an embodiment, the solvent is a solution of Lithium bromide (LiBr) (boiling for LiBr is 140 °C). Alternatively, the extracted fibroin fibers are not dried but wet and placed in the solvent; solvent concentration can then be varied to achieve similar concentrations as to when adding dried silk to the solvent. The final concentration of LiBr solvent can range from 0.1 M to 9.3 M. Complete dissolution of the extracted fibroin fibers can be achieved by varying the treatment time and temperature along with the concentration of dissolving solvent. Other solvents may be used including, but not limited to, phosphate phosphoric acid, calcium nitrate, calcium chloride solution or other concentrated aqueous solutions of inorganic salts. To ensure complete dissolution, the silk fibers should be fully immersed within the already heated solvent solution and then maintained at a temperature ranging from about 60 °C to about 140 °C for 1-168 hrs. In an embodiment, the silk fibers should be fully immersed within the solvent solution and then placed into a dry oven at a temperature of about 100 °C for about 1 hour.

The temperature at which the silk fibroin extract is added to the LiBr solution (or vice versa) has an effect on the time required to completely dissolve the fibroin and on the resulting molecular weight and polydispersity of the final SPF mixture solution. In an embodiment, silk solvent solution concentration is less than or equal to 20% w/v. In addition, agitation during introduction or dissolution may be used to facilitate dissolution at varying temperatures and concentrations. The temperature of the LiBr solution will provide control over the silk protein fragment mixture molecular weight and polydispersity created. In an embodiment, a higher temperature will more quickly dissolve the silk offering enhanced process scalability and mass production of silk solution. In an embodiment, using a LiBr solution heated to a temperature from 80 °C to 140 °C reduces the time required in an oven in order to achieve full dissolution. Varying time and temperature at or above 60 °C of the dissolution solvent will alter and control

the MW and polydispersity of the SPF mixture solutions formed from the original molecular weight of the native silk fibroin protein.

Alternatively, whole cocoons may be placed directly into a solvent, such as LiBr, bypassing extraction, step B2. This requires subsequent filtration of silk worm particles from the silk and solvent solution and sericin removal using methods know in the art for separating hydrophobic and hydrophilic proteins such as a column separation and/or chromatography, ion exchange, chemical precipitation with salt and/or pH, and or enzymatic digestion and filtration or extraction, all methods are common examples and without limitation for standard protein separation methods, step C2. Non-heat treated cocoons with the silkworm removed, may alternatively be placed into a solvent such as LiBr, bypassing extraction. The methods described above may be used for sericin separation, with the advantage that non-heat treated cocoons will contain significantly less worm debris.

Dialysis may be used to remove the dissolution solvent from the resulting dissolved fibroin protein fragment solution by dialyzing the solution against a volume of water, step E1. Pre-filtration prior to dialysis is helpful to remove any debris (i.e., silk worm remnants) from the silk and LiBr solution, step D. In one example, a 3 μm or 5 μm filter is used with a flow-rate of 200-300 mL/min to filter a 0.1% to 1.0% silk-LiBr solution prior to dialysis and potential concentration if desired. A method disclosed herein, as described above, is to use time and/or temperature to decrease the concentration from 9.3 M LiBr to a range from 0.1 M to 9.3 M to facilitate filtration and downstream dialysis, particularly when considering creating a scalable process method. Alternatively, without the use of additional time or temperate, a 9.3 M LiBr-silk protein fragment solution may be diluted with water to facilitate debris filtration and dialysis. The result of dissolution at the desired time and temperate filtration is a translucent particle-free room temperature shelf-stable silk protein fragment-LiBr solution of a known MW and polydispersity. It is advantageous to change the dialysis water regularly until the solvent has been removed (e.g., change water after 1 hour, 4 hours, and then every 12 hours for a total of 6 water changes). The total number of water volume changes may be varied based on the resulting concentration of solvent used for silk protein

dissolution and fragmentation. After dialysis, the final silk solution maybe further filtered to remove any remaining debris (i.e., silk worm remnants).

Alternatively, Tangential Flow Filtration (TFF), which is a rapid and efficient method for the separation and purification of biomolecules, may be used to remove the solvent from the resulting dissolved fibroin solution, step E2. TFF offers a highly pure aqueous silk protein fragment solution and enables scalability of the process in order to produce large volumes of the solution in a controlled and repeatable manner. The silk and LiBr solution may be diluted prior to TFF (20 % down to 0.1 % silk in either water or LiBr). Pre-filtration as described above prior to TFF processing may maintain filter efficiency and potentially avoids the creation of silk gel boundary layers on the filter's surface as the result of the presence of debris particles. Pre-filtration prior to TFF is also helpful to remove any remaining debris (i.e., silk worm remnants) from the silk and LiBr solution that may cause spontaneous or long-term gelation of the resulting water only solution, step D. TFF, recirculating or single pass, may be used for the creation of water-silk protein fragment solutions ranging from 0.1 % silk to 30.0 % silk (more preferably, 0.1 % - 6.0 % silk). Different cutoff size TFF membranes may be required based upon the desired concentration, molecular weight and polydispersity of the silk protein fragment mixture in solution. Membranes ranging from 1-100 kDa may be necessary for varying molecular weight silk solutions created for example by varying the length of extraction boil time or the time and temperate in dissolution solvent (e.g., LiBr). In an embodiment, a TFF 5 or 10 kDa membrane is used to purify the silk protein fragment mixture solution and to create the final desired silk-to-water ratio. As well, TFF single pass, TFF, and other methods known in the art, such as a falling film evaporator, may be used to concentrate the solution following removal of the dissolution solvent (e.g., LiBr) (with resulting desired concentration ranging from 0.1% to 30 % silk). This can be used as an alternative to standard HFIP concentration methods known in the art to create a water-based solution. A larger pore membrane could also be utilized to filter out small silk protein fragments and to create a solution of higher molecular weight silk with and/or without tighter polydispersity values.

An assay for LiBr and Na_2CO_3 detection can be performed using an HPLC system equipped with evaporative light scattering detector (ELSD). The calculation was

performed by linear regression of the resulting peak areas for the analyte plotted against concentration. More than one sample of a number of formulations of the present disclosure was used for sample preparation and analysis. Generally, four samples of different formulations were weighed directly in a 10 mL volumetric flask. The samples were suspended in 5 mL of 20 mM ammonium formate (pH 3.0) and kept at 2-8 °C for 2 hours with occasional shaking to extract analytes from the film. After 2 hours the solution was diluted with 20 mM ammonium formate (pH 3.0). The sample solution from the volumetric flask was transferred into HPLC vials and injected into the HPLC-ELSD system for the estimation of sodium carbonate and lithium bromide.

The analytical method developed for the quantitation of Na_2CO_3 and LiBr in silk protein formulations was found to be linear in the range 10 - 165 μg/mL, with RSD for injection precision as 2% and 1% for area and 0.38% and 0.19% for retention time for sodium carbonate and lithium bromide respectively. The analytical method can be applied for the quantitative determination of sodium carbonate and lithium bromide in silk protein formulations.

Fig. 2 is a flow chart showing various parameters that can be modified during the process of producing a silk protein fragment solution of the present disclosure during the extraction and the dissolution steps. Select method parameters may be altered to achieve distinct final solution characteristics depending upon the intended use, e.g., molecular weight and polydispersity. It should be understood that not all of the steps illustrated are necessarily required to fabricate all silk solutions of the present disclosure.

In an embodiment, silk protein fragment solutions useful for a wide variety of applications are prepared according to the following steps: forming pieces of silk cocoons from the *Bombyx mori* silkworm; extracting the pieces at about 100 °C in a Na_2CO_3 water solution for about 60 minutes, wherein a volume of the water equals about 0.4 × raw silk weight and the amount of Na_2CO_3 is about 0.848 × the weight of the pieces to form a silk fibroin extract; triple rinsing the silk fibroin extract at about 60 °C for about 20 minutes per rinse in a volume of rinse water, wherein the rinse water for each cycle equals about 0.2 L × the weight of the pieces; removing excess water from the silk fibroin extract; drying the silk fibroin extract; dissolving the dry silk fibroin extract in a LiBr solution, wherein the LiBr solution is first heated to about 100 °C to create a silk and LiBr solution

and maintained; placing the silk and LiBr solution in a dry oven at about 100 °C for about 60 minutes to achieve complete dissolution and further fragmentation of the native silk protein structure into mixture with desired molecular weight and polydispersity; filtering the solution to remove any remaining debris from the silkworm; diluting the solution with water to result in a 1.0 wt. % silk solution; and removing solvent from the solution using Tangential Flow Filtration (TFF). In an embodiment, a 10 kDa membrane is utilized to purify the silk solution and create the final desired silk-to-water ratio. TFF can then be used to further concentrate the silk solution to a concentration of 2.0 wt. % silk in water.

Without wishing to be bound by any particular theory, varying extraction (i.e., time and temperature), LiBr (i.e., temperature of LiBr solution when added to silk fibroin extract or vice versa) and dissolution (i.e., time and temperature) parameters results in solvent and silk solutions with different viscosities, homogeneities, and colors. Also without wishing to be bound by any particular theory, increasing the temperature for extraction, lengthening the extraction time, using a higher temperature LiBr solution at emersion and over time when dissolving the silk and increasing the time at temperature (e.g., in an oven as shown here, or an alternative heat source) all resulted in less viscous and more homogeneous solvent and silk solutions.

The extraction step could be completed in a larger vessel, for example an industrial washing machine where temperatures at or in between 60 °C to 100 °C can be maintained. The rinsing step could also be completed in the industrial washing machine, eliminating the manual rinse cycles. Dissolution of the silk in LiBr solution could occur in a vessel other than a convection oven, for example a stirred tank reactor. Dialyzing the silk through a series of water changes is a manual and time intensive process, which could be accelerated by changing certain parameters, for example diluting the silk solution prior to dialysis. The dialysis process could be scaled for manufacturing by using semi-automated equipment, for example a tangential flow filtration system.

Varying extraction (i.e., time and temperature), LiBr (i.e., temperature of LiBr solution when added to silk fibroin extract or vice versa) and dissolution (i.e., time and temperature) parameters results in solvent and silk solutions with different viscosities, homogeneities, and colors. Increasing the temperature for extraction, lengthening the extraction time, using a higher temperature LiBr solution at emersion and over time when

dissolving the silk and increasing the time at temperature (e.g., in an oven as shown here, or an alternative heat source) all resulted in less viscous and more homogeneous solvent and silk solutions. While almost all parameters resulted in a viable silk solution, methods that allow complete dissolution to be achieved in fewer than 4 to 6 hours are preferred for process scalability.

In an embodiment, solutions of silk fibroin protein fragments having a weight average selected from between about 6 kDa to about 17 kDa are prepared according to following steps: degumming a silk source by adding the silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes; removing sericin from the solution to produce a silk fibroin extract comprising non- detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 60 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in an oven having a temperature of about 140 °C for a period of at most 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk protein fragments, the aqueous solution comprising: fragments having a weight average molecular weight selected from between about 6 kDa to about 17 kDa, and a polydispersity of between 1 and about 5, or between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of silk fibroin protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of silk fibroin protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The aqueous solution of silk fibroin protein fragments may be lyophilized. In some embodiments, the silk fibroin protein fragment solution may be further processed into various forms including gel, powder, and nanofiber.

In an embodiment, solutions of silk fibroin protein fragments having a weight average molecular weight selected from between about 17 kDa to about 39 kDa are prepared according to the following steps: adding a silk source to a boiling (100 °C)

aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes so as to result in degumming; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 80 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in a dry oven having a temperature in the range between about 60 °C to about 100 °C for a period of at most 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk fibroin protein fragments, wherein the aqueous solution of silk fibroin protein fragments comprises lithium bromide residuals of between about 10 ppm and about 300 ppm, wherein the aqueous solution of silk protein fragments comprises sodium carbonate residuals of between about 10 ppm and about 100 ppm, wherein the aqueous solution of silk fibroin protein fragments comprises fragments having a weight average molecular weight selected from between about 17 kDa to about 39 kDa, and a polydispersity of between 1 and about 5, or between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of silk fibroin protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high- performance liquid chromatography lithium bromide assay. The aqueous solution of silk fibroin protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay.

In some embodiments, a method for preparing an aqueous solution of silk fibroin protein fragments having an average weight average molecular weight selected from between about 6 kDa to about 17 kDa includes the steps of: degumming a silk source by adding the silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 60 °C to about 140

°C; maintaining the solution of silk fibroin-lithium bromide in an oven having a temperature of about 140 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk protein fragments, the aqueous solution comprising: fragments having an average weight average molecular weight selected from between about 6 kDa to about 17 kDa, and a polydispersity of between 1 and about 5, or between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay . The aqueous solution of pure silk fibroin protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5 % to about 10.0 % to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide. A film may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The film may comprise from about 1.0 wt. % to about 50,0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative

thereof. The gel may have a silk content of at least 2 % and a vitamin content of at least 20 %.

In some embodiments, a method for preparing an aqueous solution of silk fibroin protein fragments having an average weight average molecular weight selected from between about 17 kDa to about 39 kDa includes the steps of: adding a silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes so as to result in degumming; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 80 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in a dry oven having a temperature in the range between about 60 °C to about 100 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of pure silk fibroin protein fragments, wherein the aqueous solution of pure silk fibroin protein fragments comprises lithium bromide residuals of between about 10 ppm and about 300 ppm, wherein the aqueous solution of silk protein fragments comprises sodium carbonate residuals of between about 10 ppm and about 100 ppm, wherein the aqueous solution of pure silk fibroin protein fragments comprises fragments having an average weight average molecular weight selected from between about 17 kDa to about 39 kDa, and a polydispersity of between 1 and about 5, or between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding a vitamin to

the aqueous solution of pure silk fibroin protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide. A film may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The film may comprise from about 1 ,0 wt. % to about 50.0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

In an embodiment, solutions of silk fibroin protein fragments having a weight average molecular weight selected from between about 39 kDa to about 80 kDa are prepared according to the following steps: adding a silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of about 30 minutes so as to result in degumming; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 80 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in a dry oven having a temperature in the range between about 60 °C to about 100 °C for a period of at most 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk fibroin protein fragments, wherein the aqueous solution of silk fibroin protein fragments comprises lithium bromide residuals of between about 10 ppm and about 300 ppm, sodium carbonate residuals of

between about 10 ppm and about 100 ppm, fragments having a weight average molecular weight selected from between about 39 kDa to about 80 kDa, and a polydispersity of between 1 and about 5, or between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of silk fibroin protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of silk fibroin protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. In some embodiments, the method may further comprise adding an active agent (e.g., therapeutic agent) to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding an active agent selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin protein fragments may be lyophilized. The method may further comprise adding an alpha-hydroxy acid to the aqueous solution of pure silk fibroin protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin protein fragments. A film may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The film may comprise from about 1.0 wt. % to about 50.0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2 wt. % and a vitamin content of at least 20 wt. %.

Molecular weight of the silk protein fragments may be controlled based upon the specific parameters utilized during the extraction step, including extraction time and

temperature; specific parameters utilized during the dissolution step, including the LiBr temperature at the time of submersion of the silk in to the lithium bromide and time that the solution is maintained at specific temperatures; and specific parameters utilized during the filtration step. By controlling process parameters using the disclosed methods, it is possible to create silk fibroin protein fragment solutions with polydispersity equal to or lower than 2.5 at a variety of different molecular weight selected from between 5 kDa to 200 kDa, or between 10 kDa and 80 kDa. By altering process parameters to achieve silk solutions with different molecular weights, a range of fragment mixture end products, with desired polydispersity of equal to or less than 2.5 may be targeted based upon the desired performance requirements. For example, a higher molecular weight silk film containing an ophthalmic drug may have a controlled slow release rate compared to a lower molecular weight film making it ideal for a delivery vehicle in eye care products. Additionally, the silk fibroin protein fragment solutions with a polydispersity of greater than 2.5 can be achieved. Further, two solutions with different average molecular weights and polydispersity can be mixed to create combination solutions. Alternatively, a liquid silk gland (100% sericin free silk protein) that has been removed directly from a worm could be used in combination with any of the silk fibroin protein fragment solutions of the present disclosure. Molecular weight of the pure silk fibroin protein fragment composition was determined using High Pressure Liquid Chromatography (HPLC) with a Refractive Index Detector (RID). Polydispersity was calculated using Cirrus GPC Online GPC/SEC Software Version 3.3 (Agilent).

Differences in the processing parameters can result in regenerated silk fibroins that vary in molecular weight, and peptide chain size distribution (polydispersity, PD). This, in turn, influences the regenerated silk fibroin performance, including mechanical strength, water solubility etc.

Parameters were varied during the processing of raw silk cocoons into the silk solution. Varying these parameters affected the MW of the resulting silk solution. Parameters manipulated included (i) time and temperature of extraction, (ii) temperature of LiBr, (iii) temperature of dissolution oven, and (iv) dissolution time. Experiments were carried out to determine the effect of varying the extraction time. Tables 1-7 summarize the results. Below is a summary:

- A sericin extraction time of 30 minutes resulted in larger molecular weight than a sericin extraction time of 60 minutes
- Molecular weight decreases with time in the oven
- 140 °C LiBr and oven resulted in the low end of the confidence interval to be below a molecular weight of 9500 Da
- 30 min extraction at the 1 hour and 4 hour time points have undigested silk
- 30 min extraction at the 1 hour time point resulted in a significantly high molecular weight with the low end of the confidence interval being 35,000 Da
- The range of molecular weight reached for the high end of the confidence interval was 18000 to 216000 Da (important for offering solutions with specified upper limit).

Table 1. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 100 °C Lithium Bromide (LiBr) and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Boil Time	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30	1	57247	12780	35093	93387	1.63
60	1	31520	1387	11633	85407	2.71
30	4	40973	2632	14268	117658	2.87
60	4	25082	1248	10520	59803	2.38
30	6	25604	1405	10252	63943	2.50
60	6	20980	1262	10073	43695	2.08

Table 2. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, boiling Lithium Bromide (LiBr) and 60 °C Oven Dissolution for 4 hr.

Sample	Boil Time	Average Mw	Std dev	Confidence Interval		PD
30 min, 4 hr	30	49656	4580	17306	142478	2.87
60 min, 4 hr	60	30042	1536	11183	80705	2.69

Table 3. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 °C Lithium Bromide (LiBr) and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30 min, 1 hr	30	1	58436		22201	153809	2.63
60 min, 1 hr	60	1	31700		11931	84224	2.66
30 min, 4 hr	30	4	61956.5	13337	21463	178847	2.89
60 min, 4 hr	60	4	25578.5	2446	9979	65564	2.56

Table 4. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C Lithium Bromide (LiBr) and 80 °C Oven Dissolution for 6 hr.

Sample	Boil Time	Average Mw	Std dev	Confidence Interval		PD
30 min, 6 hr	30	63510		18693	215775	3.40
60 min, 6 hr	60	25164	238	9637	65706	2.61

Table 5. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C Lithium Bromide (LiBr) and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30 min, 4 hr	30	4	59202	14028	19073	183760	3.10
60 min, 4 hr	60	4	26312.5	637	10266	67442	2.56
30 min, 6 hr	30	6	46824		18076	121293	2.59
60 min, 6 hr	60	6	26353		10168	68302	2.59

Table 6. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 140 °C Lithium Bromide (LiBr) and 140 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30 min, 4 hr	30	4	9024.5	1102	4493	18127	2.00865
60 min, 4 hr	60	4	15548		6954	34762	2.2358
30 min, 6 hr	30	6	13021		5987	28319	2.1749
60 min, 6 hr	60	6	10888		5364	22100	2.0298

Experiments were carried out to determine the effect of varying the extraction temperature. **Table 7** summarizes the results. Below is a summary:

- Sericin extraction at 90 °C resulted in higher MW than sericin extraction at 100 °C extraction
- Both 90 °C and 100 °C show decreasing MW over time in the oven.

Table 7. The effect of extraction temperature (90 °C vs. 100 °C) on molecular weight of silk processed under the conditions of 60 min. Extraction Temperature, 100 °C Lithium Bromide (LiBr) and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Std dev	Confidence Interval		PD
90 °C, 4 hr	60	4	37308	4204	13368	104119	2.79
100 °C, 4 hr	60	4	25082	1248	10520	59804	2.38
90 °C, 6 hr	60	6	34224	1135	12717	92100	2.69
100 °C, 6 hr	60	6	20980	1262	10073	43694	2.08

Experiments were carried out to determine the effect of varying the Lithium Bromide (LiBr) temperature when added to silk. Tables 8-9 summarize the results. Below is a summary:

- No impact on molecular weight or confidence interval (all CI ~10500-6500 Da)
- Studies illustrated that the temperature of LiBr-silk dissolution, as LiBr is added and begins dissolving, rapidly drops below the original LiBr temperature due to the majority of the mass being silk at room temperature

Table 8. The effect of Lithium Bromide (LiBr) temperature on molecular weight of silk processed under the conditions of 60 min. Extraction Time., 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	LiBr Temp (°C)	Oven Time	Average Mw	Std dev	Confidence Interval		PD
60 °C LiBr, 1 hr	60	1	31700		11931	84223	2.66
100 °C LiBr, 1 hr	100	1	27907	200	10735	72552	2.60
RT LiBr, 4 hr	RT	4	29217	1082	10789	79119	2.71

60 °C LiBr, 4 hr	60	4	25578	2445	9978	65564	2.56
80 °C LiBr, 4 hr	80	4	26312	637	10265	67441	2.56
100 °C LiBr, 4 hr	100	4	27681	1729	11279	67931	2.45
Boil LiBr, 4 hr	Boil	4	30042	1535	11183	80704	2.69
RT LiBr, 6 hr	RT	6	26543	1893	10783	65332	2.46
80 °C LiBr, 6 hr	80	6	26353		10167	68301	2.59
100 °C LiBr, 6 hr	100	6	27150	916	11020	66889	2.46

Table 9. The effect of Lithium Bromide (LiBr) temperature on molecular weight of silk processed under the conditions of 30 min. Extraction Time, 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	LiBr Temp (°C)	Oven Time	Average Mw	Std dev	Confidence Interval		PD
60 °C LiBr, 4 hr	60	4	61956	13336	21463	178847	2.89
80 °C LiBr, 4 hr	80	4	59202	14027	19073	183760	3.10
100 °C LiBr, 4 hr	100	4	47853		19757	115899	2.42
80 °C LiBr, 6 hr	80	6	46824		18075	121292	2.59
100 °C LiBr, 6 hr	100	6	55421	8991	19152	160366	2.89

Experiments were carried out to determine the effect of v oven/dissolution temperature. Tables 10-14 summarize the results. Below is a summary:

- Oven temperature has less of an effect on 60 min extracted silk than 30 min extracted silk. Without wishing to be bound by theory, it is believed that the 30 min silk is less degraded during extraction and therefore the oven temperature has more of an effect on the larger MW, less degraded portion of the silk.

- For 60 °C vs. 140 °C oven the 30 min extracted silk showed a very significant effect of lower MW at higher oven temp, while 60 min extracted silk had an effect but much less
- The 140 °C oven resulted in a low end in the confidence interval at ~6000 Da.

Table 10. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 30 min. Extraction Time, and 100 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time	Oven Temp (°C)	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30	60	4	47853		19758	115900	2.42
30	100	4	40973	2632	14268	117658	2.87
30	60	6	55421	8992	19153	160366	2.89
30	100	6	25604	1405	10252	63943	2.50

Table 11. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 100 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time (minutes)	Oven Temp	Oven Time	Average Mw	Std dev	Confidence Interval		PD
60	60	1	27908	200	10735	72552	2.60
60	100	1	31520	1387	11633	85407	2.71
60	60	4	27681	1730	11279	72552	2.62
60	100	4	25082	1248	10520	59803	2.38
60	60	6	27150	916	11020	66889	2.46
60	100	6	20980	1262	10073	43695	2.08

Table 12. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 140 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time (minutes)	Oven Temp(°C)	Oven Time	Average	Std dev	Confidence Interval		PD
60	60	4	30042	1536	11183	80705	2.69
60	140	4	15548		7255	33322	2.14

Table 13. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 30 min. Extraction Time, and 140 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time (minutes)	Oven Temp (°C)	Oven Time	Average Mw	Std dev	Confidence Interval		PD
30	60	4	49656	4580	17306	142478	2.87
30	140	4	9025	1102	4493	18127	2.01
30	60	6	59383	11640	17641	199889	3.37
30	140	6	13021		5987	28319	2.17

Table 14. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 80 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time (minutes)	Oven Temp (°C)	Oven Time	Average Mw	Std dev	Confidence Interval		PD
60	60	4	26313	637	10266	67442	2.56
60	80	4	30308	4293	12279	74806	2.47
60	60	6	26353		10168	68302	2.59
60	80	6	25164	238	9637	65706	2.61

The raw silk cocoons from the silkworm *Bombyx mori* were cut into pieces. The pieces of raw silk cocoons were boiled in an aqueous solution of Na_2CO_3 (about 100 °C) for a period of time between about 30 minutes to about 60 minutes to remove sericin (degumming). The volume of the water used equals about 0.4 x raw silk weight and the amount of Na_2CO_3 is about 0.848 x the weight of the raw silk cocoon pieces. The resulting degummed silk cocoon pieces were rinsed with deionized water three times at about 60 °C (20 minutes per rinse). The volume of rinse water for each cycle was 0.2 L x the weight of the raw silk cocoon pieces. The excess water from the degummed silk cocoon pieces was removed. After the DI water washing step, the wet degummed silk cocoon pieces were dried at room temperature. The degummed silk cocoon pieces were mixed with a LiBr solution, and the mixture was heated to about 100 °C. The warmed mixture was placed in a dry oven and was heated at a temperature ranging from about 60

°C to about 140 °C for about 60 minutes to achieve complete dissolution of the native silk protein. The resulting solution was allowed to cool to room temperature and then was dialyzed to remove LiBr salts using a 3,500 Da MWCO membrane. Multiple exchanges were performed in Di water until Br^- ions were less than 1 ppm as determined in the hydrolyzed fibroin solution read on an Oakton Bromide (Br^-) double-junction ion-selective electrode.

The resulting silk fibroin aqueous solution has a concentration of about 8.0 % w/v containing pure silk fibroin protein fragments having an average weight average molecular weight selected from between about 6 kDa to about 16 kDa, about 17 kDa to about 39 kDa, and about 39 kDa to about 80 kDa and a polydispersity of between about 1.5 and about 3.0. The 8.0 % w/v was diluted with DI water to provide a 1.0 % w/v, 2.0 % w/v, 3.0 % w/v, 4.0 % w/v, 5.0 % w/v by the coating solution.

A variety of % silk concentrations have been produced through the use of Tangential Flow Filtration (TFF). In all cases a 1 % silk solution was used as the input feed. A range of 750-18,000 mL of 1% silk solution was used as the starting volume. Solution is diafiltered in the TFF to remove lithium bromide. Once below a specified level of residual LiBr, solution undergoes ultrafiltration to increase the concentration through removal of water. See examples below.

Six (6) silk solutions were utilized in standard silk structures with the following results:

Solution #1 is a silk concentration of 5.9 wt. %, average MW of 19.8 kDa and 2.2 PDI (made with a 60 min boil extraction, 100 °C LiBr dissolution for 1 hour).

Solution #2 is a silk concentration of 6.4 wt. % (made with a 30 min boil extraction, 60 °C LiBr dissolution for 4 hrs).

Solution #3 is a silk concentration of 6.17 wt. % (made with a 30 min boil extraction 100 °C LiBr dissolution for 1 hour).

Solution #4 is a silk concentration of 7.30 wt. %: A 7.30 % silk solution was produced beginning with 30 minute extraction batches of 100 g silk cocoons per batch. Extracted silk fibers were then dissolved using 100 °C 9.3 M LiBr in a 100 °C oven for 1 hour. 100 g of silk fibers were dissolved per batch to create 20% silk in LiBr. Dissolved silk in LiBr was then diluted to 1% silk and filtered through a 5 μm filter to remove large

debris. 15,500 mL of 1 %, filtered silk solution was used as the starting volume/diafiltration volume for TFF. Once LiBr was removed, the solution was ultrafiltered to a volume around 1300 mL. 1262 mL of 7.30 % silk was then collected. Water was added to the feed to help remove the remaining solution and 547 mL of 3.91 % silk was then collected.

Solution #5 is a silk concentration of 6.44 wt. %: A 6.44 wt. % silk solution was produced beginning with 60 minute extraction batches of a mix of 25, 33, 50, 75 and 100 g silk cocoons per batch. Extracted silk fibers were then dissolved using 100 °C 9.3 M LiBr in a 100 °C oven for 1 hour. 35, 42, 50 and 71 g per batch of silk fibers were dissolved to create 20 % silk in LiBr and combined. Dissolved silk in LiBr was then diluted to 1 % silk and filtered through a 5 µm filter to remove large debris. 17,000 mL of 1 %, filtered silk solution was used as the starting volume/diafiltration volume for TFF. Once LiBr was removed, the solution was ultrafiltered to a volume around 3000 mL. 1490 mL of 6.44 % silk was then collected. Water was added to the feed to help remove the remaining solution and 1454 mL of 4.88 % silk was then collected.

Solution #6 is a silk concentration of 2.70 wt. %: A 2.70 % silk solution was produced beginning with 60-minute extraction batches of 25 g silk cocoons per batch. Extracted silk fibers were then dissolved using 100 °C 9.3 M LiBr in a 100 °C oven for 1 hour. 35.48 g of silk fibers were dissolved per batch to create 20 % silk in LiBr. Dissolved silk in LiBr was then diluted to 1% silk and filtered through a 5 µm filter to remove large debris. 1000 mL of 1%, filtered silk solution was used as the starting volume/diafiltration volume for TFF. Once LiBr was removed, the solution was ultrafiltered to a volume around 300 mL. 312 mL of 2.7 % silk was then collected.

The preparation of silk fibroin solutions with higher molecular weights is given in Table 15.

Table 15. Preparation and properties of silk fibroin solutions.

Sample Name	Extraction Time (mins)	Extraction Temp (°C)	LiBr Temp (°C)	Oven/Sol'n Temp	Average weight average molecular weight (kDa)	Average polydispersity

Group A TFF	60	100	100	100 °C oven	34.7	2.94
Group A DIS	60	100	100	100 °C oven	44.7	3.17
Group B TFF	60	100	100	100 °C sol'n	41.6	3.07
Group B DIS	60	100	100	100 °C sol'n	44.0	3.12
Group D DIS	30	90	60	60 °C sol'n	129.7	2.56
Group D FIL	30	90	60	60 °C sol'n	144.2	2.73
Group E DIS	15	100	RT	60 °C sol'n	108.8	2.78
Group E FIL	15	100	RT	60 °C sol'n	94.8	2.62

Silk aqueous coating composition for application to fabrics are given in **Tables 16 and 17** below.

Table 16. Silk Solution Characteristics					
	Molecular Weight:	57 kDa			
	Polydispersity:	1.6			
	% Silk	5.0%	3.0%	1.0%	0.5%
Process Parameters					
	Extraction				
	Boil Time:	30 minutes			
	Boil Temperature:	100 °C			
	Rinse Temperature:	60 °C			
	Dissolution				
	LiBr Temperature:	100			
	Oven Temperature:	100 °C			
	Oven Time:	60 minutes			

Table 17. Silk Solution Characteristics					
	Molecular Weight:	25 kDa			
	Polydispersity:	2.4			
	% Silk	5.0%	3.0%	1.0%	0.5%

Process Parameters					
	Extraction				
	Boil Time:	60 minutes			
	Boil Temperature:	100 °C			
	Rinse Temperature:	60 °C			
	Dissolution				
	LiBr Temperature:	100 °C			
	Oven Temperature:	100 °C			
	Oven Time:	60 minutes			

Three (3) silk solutions were utilized in film making with the following results:

Solution #1 is a silk concentration of 5.9 %, average MW of 19.8 kDa and 2.2 PD (made with a 60 min boil extraction, 100 °C LiBr dissolution for 1 hr).

Solution #2 is a silk concentration of 6.4 % (made with a 30 min boil extraction, 60 °C LiBr dissolution for 4 hrs).

Solution #3 is a silk concentration of 6.17 % (made with a 30 min boil extraction, 100 °C LiBr dissolution for 1 hour).

Films were made in accordance with Rockwood et al. (Nature Protocols; Vol. 6; No. 10; published on-line Sep. 22, 2011; doi:10.1038/nprot.2011.379). 4 mL of 1% or 2% (wt/vol) aqueous silk solution was added into 100 mm Petri dish (Volume of silk can be varied for thicker or thinner films and is not critical) and allowed to dry overnight uncovered. The bottom of a vacuum desiccator was filled with water. Dry films were placed in the desiccator and vacuum applied, allowing the films to water anneal for 4 hours prior to removal from the dish. Films cast from solution #1 did not result in a structurally continuous film; the film was cracked in several pieces. These pieces of film dissolved in water in spite of the water annealing treatment.

Silk solutions of various molecular weights and/or combinations of molecular weights can be optimized for gel applications. The following provides an example of this process but it not intended to be limiting in application or formulation. Three (3) silk solutions were utilized in gel making with the following results:

Solution #1 is a silk concentration of 5.9 %, average MW of 19.8 kDa and 2.2 PD (made with a 60 min boil extraction, 100 °C LiBr dissolution for 1 hr).

Solution #2 is a silk concentration of 6.4 % (made with a 30 min boil extraction, 60 °C LiBr dissolution for 4 hrs).

Solution #3 is a silk concentration of 6.17 % (made with a 30 min boil extraction, 100 °C LiBr dissolution for 1 hour).

"Egel" is an electrogelation process as described in Rockwood of al. Briefly, 10 ml of aqueous silk solution is added to a 50 ml conical tube and a pair of platinum wire electrodes immersed into the silk solution. A 20 volt potential was applied to the platinum electrodes for 5 minutes, the power supply turned off and the gel collected. Solution #1 did not form an EGEL over the 5 minutes of applied electric current.

Solutions #2 and #3 were gelled in accordance with the published horseradish peroxidase (HRP) protocol. Behavior seemed typical of published solutions.

Materials and Methods: the following equipment and material are used in determination of Silk Molecular weight: Agilent 1100 with chemstation software ver. 10.01; Refractive Index Detector (RID); analytical balance; volumetric flasks (1000 mL, 10 mL and 5 mL); HPLC grade water; ACS grade sodium chloride; ACS grade sodium phosphate dibasic heptahydrate; phosphoric acid; dextran MW Standards-Nominal Molecular Weights of 5 kDa, 11.6 kDa, 23.8 kDa, 48.6 kDa, and 148 kDa; 50 mL PET or polypropylene disposable centrifuge tubes; graduated pipettes; amber glass HPLC vials with Teflon caps; Phenomenex PolySep GFC P-4000 column (size: 7.8 mm x 300 mm).

Procedural Steps:

A) Preparation of 1 L Mobile Phase (0.1 M Sodium Chloride solution in 0.0125 M Sodium phosphate buffer)

Take a 250 mL clean and dry beaker, place it on the balance and tare the weight. Add about 3.3509 g of sodium phosphate dibasic heptahydrate to the beaker. Note down the exact weight of sodium phosphate dibasic weighed. Dissolve the weighed sodium phosphate by adding 100 mL of HPLC water into the beaker. Take care not to spill any of the content of the beaker. Transfer the solution carefully into a clean and dry 1000 mL volumetric flask. Rinse the beaker and transfer the rinse into the volumetric flask. Repeat the rinse 4-5 times. In a separate clean and dry 250 mL beaker weigh exactly about

5.8440 g of sodium chloride. Dissolve the weighed sodium chloride in 50 mL of water and transfer the solution to the sodium phosphate solution in the volumetric flask. Rinse the beaker and transfer the rinse into the volumetric flask. Adjust the pH of the solution to 7.0 ± 0.2 with phosphoric acid. Make up the volume in volumetric flask with HPLC water to 1000 mL and shake it vigorously to homogeneously mix the solution. Filter the solution through 0.45 µm polyamide membrane filter. Transfer the solution to a clean and dry solvent bottle and label the bottle. The volume of the solution can be varied to the requirement by correspondingly varying the amount of sodium phosphate dibasic heptahydrate and sodium chloride.

B) Preparation of Dextran Molecular Weight Standard solutions

At least five different molecular weight standards are used for each batch of samples that are run so that the expected value of the sample to be tested is bracketed by the value of the standard used. Label six 20 mL scintillation glass vials respective to the molecular weight standards. Weigh accurately about 5 mg of each of dextran molecular weight standards and record the weights. Dissolve the dextran molecular weight standards in 5 mL of mobile phase to make a 1 mg/mL standard solution.

C) Preparation of Sample solutions

When preparing sample solutions, if there are limitations on how much sample is available, the preparations may be scaled as long as the ratios are maintained. Depending on sample type and silk protein content in sample weigh enough sample in a 50 mL disposable centrifuge tube on an analytical balance to make a 1 mg/mL sample solution for analysis. Dissolve the sample in equivalent volume of mobile phase make a 1 mg/mL solution. Tightly cap the tubes and mix the samples (in solution). Leave the sample solution for 30 minutes at room temperature. Gently mix the sample solution again for 1 minute and centrifuge at 4000 RPM for 10 minutes.

D) HPLC analysis of the samples

Transfer 1.0 mL of all the standards and sample solutions into individual HPLC vials. Inject the molecular weight standards (one injection each) and each sample in duplicate. Analyze all the standards and sample solutions using the following HPLC conditions:

Column	PolySep GFC P-4000 (7.8 x 300 mm)
Column Temperature	25 °C
Detector	Refractive Index Detector (Temperature @ 35 °C)
Injection Volume	25.0 µL
Mobile Phase	0.1 M Sodium Chloride solution in 0.0125 M sodium phosphate buffer
Flow Rate	1.0 mL/min
Run Time	20.0 min

E) Data analysis and calculations - Calculation of Average Molecular Weight using Cirrus Software

Upload the chromatography data files of the standards and the analytical samples into Cirrus SEC data collection and molecular weight analysis software. Calculate the weight average molecular weight (M_w), number average molecular weight (M_n), peak average molecular weight (M_p), and polydispersity for each injection of the sample.

Spider Silk Fragments

Spider silks are natural polymers that consist of three domains: a repetitive middle core domain that dominates the protein chain, and non-repetitive N-terminal and C-terminal domains. The large core domain is organized in a block copolymer-like arrangement, in which two basic sequences, crystalline [poly(A) or poly(GA)] and less crystalline (GGX or GPGXX) polypeptides alternate. Dragline silk is the protein complex composed of major ampullate dragline silk protein 1 (MaSp1) and major ampullate dragline silk protein 2 (*MaSp2*). Both silks are approximately 3500 amino acid long. *MaSp1* can be found in the fibre core and the periphery, whereas *MaSp2* forms clusters in certain core areas. The large central domains of *MaSp1* and *MaSp2* are organized in block copolymer-like arrangements, in which two basic sequences, crystalline [poly(A) or poly(GA)] and less crystalline (GGX or GPGXX) polypeptides alternate in core domain. Specific secondary structures have been assigned to poly(A)/(GA), GGX and GPGXX motifs including β-sheet, α-helix and β-spiral respectively. The primary sequence, composition and secondary structural elements of the repetitive core domain are responsible for mechanical properties of spider silks; whereas, non-repetitive N- and

C-terminal domains are essential for the storage of liquid silk dope in a lumen and fibre formation in a spinning duct.

The main difference between *MaSp1* and *MaSp2* is the presence of proline (P) residues accounting for 15% of the total amino acid content in *MaSp2*, whereas *MaSp1* is proline-free. By calculating the number of proline residues in *N. clavipes* dragline silk, it is possible to estimate the presence of the two proteins in fibers; 81% *MaSp1* and 19% *MaSp2*. Different spiders have different ratios of *MaSp1* and *MaSp2*. For example, a dragline silk fiber from the orb weaver Argiope aurantia contains 41% *MaSp1* and 59% *MaSp2*. Such changes in the ratios of major ampullate silks can dictate the performance of the silk fiber.

At least seven different types of silk proteins are known for one orb-weaver species of spider. Silks differ in primary sequence, physical properties and functions. For example, dragline silks used to build frames, radii and lifelines are known for outstanding mechanical properties including strength, toughness and elasticity. On an equal weight basis, spider silk has a higher toughness than steel and Kevlar. Flageliform silk found in capture spirals has extensibility of up to 500%. Minor ampullate silk, which is found in auxiliary spirals of the orb-web and in prey wrapping, possesses high toughness and strength almost similar to major ampullate silks, but does not supercontract in water.

Spider silks are known for their high tensile strength and toughness. The recombinant silk proteins also confer advantageous properties to cosmetic or dermatological compositions, in particular to be able to improve the hydrating or softening action, good film forming property and low surface density. Diverse and unique biomechanical properties together with biocompatibility and a slow rate of degradation make spider silks excellent candidates as biomaterials for tissue engineering, guided tissue repair and drug delivery, for cosmetic products (e.g. nail and hair strengthener, skin care products), and industrial materials (e.g. nanowires, nanofibers, surface coatings).

In an embodiment, a silk protein may include a polypeptide derived from natural spider silk proteins. The polypeptide is not limited particularly as long as it is derived from natural spider silk proteins, and examples of the polypeptide include natural spider silk proteins and recombinant spider silk proteins such as variants, analogs, derivatives or the like of the natural spider silk proteins. In terms of excellent tenacity, the polypeptide

may be derived from major dragline silk proteins produced in major ampullate glands of spiders. Examples of the major dragline silk proteins include major ampullate spidroin MaSp1 and MaSp2 from *Nephila clavipes*, and ADF3 and ADF4 from *Araneus diadematus*, etc. Examples of the polypeptide derived from major dragline silk proteins include variants, analogs, derivatives or the like of the major dragline silk proteins. Further, the polypeptide may be derived from flagelliform silk proteins produced in flagelliform glands of spiders. Examples of the flagelliform silk proteins include flagelliform silk proteins derived from *Nephila clavipes*, etc.

Examples of the polypeptide derived from major dragline silk proteins include a polypeptide containing two or more units of an amino acid sequence represented by the formula 1: REP1-REP2 (1), preferably a polypeptide containing five or more units thereof, and more preferably a polypeptide containing ten or more units thereof. Alternatively, the polypeptide derived from major dragline silk proteins may be a polypeptide that contains units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) and that has, at a C-terminal, an amino acid sequence represented by any of SEQ ID NOS: 1 to 3 of U.S. Patent No. 9,051,453 or an amino acid sequence having a homology of 90% or more with the amino acid sequence represented by any of SEQ ID NOS: 1 to 3 of U.S. Patent No. 9,051,453. In the polypeptide derived from major dragline silk proteins, units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) may be the same or may be different from each other. In the case of producing a recombinant protein using a microbe such as *Escherichia coli* as a host, the molecular weight of the polypeptide derived from major dragline silk proteins is 500 kDa or less, or 300 kDa or less, or 200 kDa or less, in terms of productivity.

In the formula (1), the REP1 indicates polyalanine. In the REP1, the number of alanine residues arranged in succession is preferably 2 or more, more preferably 3 or more, further preferably 4 or more, and particularly preferably 5 or more. Further, in the REP1, the number of alanine residues arranged in succession is preferably 20 or less, more preferably 16 or less, further preferably 12 or less, and particularly preferably 10 or less. In the formula (1), the REP2 is an amino acid sequence composed of 10 to 200 amino acid residues. The total number of glycine, serine, glutamine and alanine residues contained in the amino acid sequence is 40% or more, preferably 60% or more, and more

preferably 70% or more with respect to the total number of amino acid residues contained therein.

In the major dragline silk, the REP1 corresponds to a crystal region in a fiber where a crystal β sheet is formed, and the REP2 corresponds to an amorphous region in a fiber where most of the parts lack regular configurations and that has more flexibility. Further, the [REP1-REP2] corresponds to a repetitious region (repetitive sequence) composed of the crystal region and the amorphous region, which is a characteristic sequence of dragline silk proteins.

Recombinant Silk Fragments

In some embodiments, the recombinant silk protein refers to recombinant spider silk polypeptides, recombinant insect silk polypeptides, or recombinant mussel silk polypeptides. In some embodiments, the recombinant silk protein fragment disclosed herein include recombinant spider silk polypeptides of *Araneidae* or *Araneoids*, or recombinant insect silk polypeptides of *Bombyx mori*. In some embodiments, the recombinant silk protein fragment disclosed herein include recombinant spider silk polypeptides of *Araneidae* or *Araneoids*. In some embodiments, the recombinant silk protein fragment disclosed herein include block copolymer having repetitive units derived from natural spider silk polypeptides of *Araneidae* or *Araneoids*. In some embodiments, the recombinant silk protein fragment disclosed herein include block copolymer having synthetic repetitive units derived from spider silk polypeptides of *Araneidae* or *Araneoids* and non-repetitive units derived from natural repetitive units of spider silk polypeptides of *Araneidae* or *Araneoids*.

Recent advances in genetic engineering have provided a route to produce various types of recombinant silk proteins. Recombinant DNA technology has been used to provide a more practical source of silk proteins. As used herein "recombinant silk protein" refers to synthetic proteins produced heterologously in prokaryotic or eukaryotic expression systems using genetic engineering methods.

Various methods for synthesizing recombinant silk peptides are known and have been described by Ausubel et al., Current Protocols in Molecular Biology § 8 (John Wiley & Sons 1987, (1990)), incorporated herein by reference. A gram-negative, rod-shaped bacterium *E. coli* is a well-established host for industrial scale production of

proteins. Therefore, the majority of recombinant silks have been produced in *E. coli*. *E. coli* which is easy to manipulate, has a short generation time, is relatively low cost and can be scaled up for larger amounts protein production.

The recombinant silk proteins can be produced by transformed prokaryotic or eukaryotic systems containing the cDNA coding for a silk protein, for a fragment of this protein or for an analog of such a protein. The recombinant DNA approach enables the production of recombinant silks with programmed sequences, secondary structures, architectures and precise molecular weight. There are four main steps in the process: (i) design and assembly of synthetic silk-like genes into genetic 'cassettes', (ii) insertion of this segment into a DNA recombinant vector, (iii) transformation of this recombinant DNA molecule into a host cell and (iv) expression and purification of the selected clones.

The term "recombinant vectors", as used herein, includes any vectors known to the skilled person including plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as adenoviral or baculoviral vectors, or artificial chromosome vectors such as bacterial artificial chromosomes (BAC), yeast artificial chromosomes (YAC), or P1 artificial chromosomes (PAC). Said vectors include expression as well as cloning vectors. Expression vectors comprise plasmids as well as viral vectors and generally contain a desired coding sequence and appropriate DNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism (e.g., bacteria, yeast, or plant) or in in vitro expression systems. Cloning vectors are generally used to engineer and amplify a certain desired DNA fragment and may lack functional sequences needed for expression of the desired DNA fragments.

The prokaryotic systems include Gram-negative bacteria or Gram-positive bacteria. The prokaryotic expression vectors can include an origin of replication which can be recognized by the host organism, a homologous or heterologous promoter which is functional in the said host, the DNA sequence coding for the spider silk protein, for a fragment of this protein or for an analogous protein. Nonlimiting examples of prokaryotic expression organisms are *Escherichia coli, Bacillus subtilis, Bacillus megaterium, Corynebacterium glutamicum, Anabaena, Caulobacter, Gluconobacter, Rhodobacter, Pseudomonas, Para coccus, Bacillus (e.g. Bacillus subtilis) Brevibacterium,*

Corynebacterium, Rhizobium (Sinorhizobium), Flavobacterium, Klebsiella, Enterobacter, Lactobacillus, Lactococcus, Methylobacterium, Propionibacterium, Staphylococcus or Streptomyces cells.

The eukaryotic systems include yeasts and insect, mammalian or plant cells. In this case, the expression vectors can include a yeast plasmid origin of replication or an autonomous replication sequence, a promoter, a DNA sequence coding for a spider silk protein, for a fragment or for an analogous protein, a polyadenylation sequence, a transcription termination site and, lastly, a selection gene. Nonlimiting examples of eukaryotic expression organisms include yeasts, such as *Saccharomyces cerevisiae, Pichia pastoris, basidiosporogenous, ascosporogenous*, filamentous fungi, such as *Aspergillus niger, Aspergillus oryzae, Aspergillus nidulans, Trichoderma reesei, Acremonium chrysogenum, Candida, Hansenula, Kluyveromyces, Saccharomyces (e.g. Saccharomyces cerevisiae), Schizosaccharomyces, Pichia (e.g. Pichia pastoris) or Yarrowia cells* etc., mammalian cells, such as HeLa cells, COS cells, CHO cells etc., insect cells, such as Sf9 cells, MEL cells, etc., "insect host cells" such as *Spodoptera frugiperda* or *Trichoplusia ni* cells. *SF9 cells, SF-21 cells or High-Five cells*, wherein SF-9 and SF-21 are ovarian cells from *Spodoptera frugiperda*, and High-Five cells are egg cells from *Trichoplusia ni*., "plant host cells", such as tobacco, potato or pea cells.

A variety of heterologous host systems have been explored to produce different types of recombinant silks. Recombinant partial spidroins as well as engineered silks have been cloned and expressed in bacteria (*Escherichia coli*), yeast (*Pichia pastoris*), insects (*silkworm larvae*), plants (tobacco, soybean, potato, Arabidopsis), mammalian cell lines (BHT/hamster) and transgenic animals (mice, goats). Most of the silk proteins are produced with an N- or C-terminal His-tags to make purification simple and produce enough amounts of the protein.

In some embodiments, the host suitable for expressing the recombinant spider silk protein using heterogeneous system may include transgenic animals and plants. In some embodiments, the host suitable for expressing the recombinant spider silk protein using heterogeneous system comprises bacteria, yeasts, mammalian cell lines. In some embodiments, the host suitable for expressing the recombinant spider silk protein using heterogeneous system comprises *E. coli*. In some embodiments, the host suitable for

expressing the recombinant spider silk protein using heterogeneous system comprises transgenic *B. mori* silkworm generated using genome editing technologies (e.g. CRISPR).

The recombinant silk protein in this disclosure comprises synthetic proteins which are based on repeat units of natural silk proteins. Besides the synthetic repetitive silk protein sequences, these can additionally comprise one or more natural nonrepetitive silk protein sequences.

In some embodiments, "recombinant silk protein" refers to recombinant silkworm silk protein or fragments thereof. The recombinant production of silk fibroin and silk sericin has been reported. A variety of hosts are used for the production including *E. coli*, *Sacchromyces cerevisiae*, *Pseudomonas sp.*, *Rhodopseudomonas sp.*, *Bacillus sp.*, and *Strepomyces*. See EP 0230702, which is incorporate by reference herein by its entirety.

Provided herein also include design and biological-synthesis of silk fibroin protein-like multiblock polymer comprising GAGAGX hexapeptide (X is A, Y, V or S) derived from the repetitive domain of *B. mori* silk heavy chain (H chain)

In some embodiments, this disclosure provides silk protein-like multiblock polymers derived from the repetitive domain of *B. mori* silk heavy chain (H chain) comprising the GAGAGS hexapeptide repeating units. The GAGAGS hexapeptide is the core unit of H-chain and plays an important role in the formation of crystalline domains. The silk protein-like multiblock polymers containing the GAGAGS hexapeptide repeating units spontaneously aggregate into β-sheet structures, similar to natural silk fibroin protein, where in the silk protein-like multiblock polymers having any weight average molecular weight described herein.

In some embodiments, this disclosure provides silk-peptide like multiblock copolymers composed of the GAGAGS hexapeptide repetitive fragment derived from H chain of *B. mori* silk heavy chain and mammalian elastin VPGVG motif produced by *E. coli*. In some embodiments, this disclosure provides fusion silk fibroin proteins composed of the GAGAGS hexapeptide repetitive fragment derived from H chain of *B. mori* silk heavy chain and GVGVP produced by *E. coli*, where in the silk protein-like multiblock polymers having any weight average molecular weight described herein.

In some embodiments, this disclosure provides *B. mori* silkworm recombinant proteins composed of the $(GAGAGS)_{16}$ repetitive fragment. In some embodiments, this

disclosure provides recombinant proteins composed of the $(GAGAGS)_{16}$ repetitive fragment and the non-repetitive $(GAGAGS)_{16}$ –F-COOH, $(GAGAGS)_{16}$ –F-F-COOH, $(GAGAGS)_{16}$ –F-F-F-COOH, $(GAGAGS)_{16}$ –F-F-F-F-COOH, $(GAGAGS)_{16}$ –F-F-F-F-F-F-F-F-COOH, $(GAGAGS)_{16}$ –F-F-F-F–F-F-F-F-F-F-F-F-COOH produced by *E. coli*, where F has the following amino acid sequence SGFGPVANGGSGEASSESDFGSSGFGPVANASSGEASSESDFAG, and where in the silk protein-like multiblock polymers having any weight average molecular weight described herein.

In some embodiments, "recombinant silk protein" refers to recombinant spider silk protein or fragments thereof. The productions of recombinant spider silk proteins based on a partial cDNA clone have been reported. The recombinant spider silk proteins produced as such comprise a portion of the repetitive sequence derived from a dragline spider silk protein, *Spidroin 1*, from the *spider Nephila clavipes*. see Xu et al. (Proc. Natl. Acad. Sci. U.S.A., 87:7120–7124 (1990). cDNA clone encoding a portion of the repeating sequence of a second fibroin protein, *Spidroin 2*, from dragline silk of *Nephila clavipes* and the recombinant synthesis thereof is described in *J. Biol. Chem.*, 1992, volume 267, pp. 19320–19324. The recombinant synthesis of spider silk proteins including protein fragments and variants of *Nephila clavipes* from transformed *E. coli* is described in U.S. Pat. Nos. 5,728,810 and 5,989,894. cDNA clones encoding minor ampullate spider silk proteins and the expression thereof is described in U.S. Pat. Nos. 5,733,771 and 5,756,677. cDNA clone encoding the flagelliform silk protein from an orb-web spinning spider is described in U.S. Pat. No. 5,994,099. U.S. Pat. No. 6,268,169 describes the recombinant synthesis of spider silk like proteins derived from the repeating peptide sequence found in the natural spider dragline of *Nephila clavipes* by *E. coli, Bacillus subtilis, and Pichia pastoris* recombinant expression systems. WO 03/020916 describes the cDNA clone encoding and recombinant production of spider spider silk proteins having repeative sequences derived from the major ampullate glands of *Nephila madagascariensis*, *Nephila senegalensis, Tetragnatha kauaiensis, Tetragnatha versicolor, Argiope aurantia, Argiope trifasciata, Gasteracantha mammosa, and Latrodectus geometricus*, the flagelliform glands of *Argiope trifasciata*, the ampullate glands of *Dolomedes tenebrosus*, two sets of silk glands from *Plectreurys tristis*, and the

silk glands of the *mygalomorph Euagrus chisoseus*. Each of the above reference is incorporated herein by reference in its entirety.

In some embodiments, the recombinant spider silk protein is a hybrid protein of a spider silk protein and an insect silk protein, a spider silk protein and collagen, a spider silk protein and resilin, or a spider silk protein and keratin. The spider silk repetitive unit comprises or consists of an amino acid sequence of a region that comprises or consists of at least one peptide motif that repetitively occurs within a naturally occurring major ampullate gland polypeptide, such as a dragline spider silk polypeptide, a minor ampullate gland polypeptide, a flagelliform polypeptide, an aggregate spider silk polypeptide, an aciniform spider silk polypeptide or a pyriform spider silk polypeptide.

In some embodiments, the recombinant spider silk protein in this disclosure comprises synthetic spider silk proteins derived from repetitive units of natural spider silk proteins, consensus sequence, and optionally one or more natural non-repetitive spider silk protein sequences. The repeated units of natural spider silk polypeptide may include dragline spider silk polypeptides or flagelliform spider silk polypeptides of *Araneidae* or *Araneoids*.

As used herein, the spider silk "repetitive unit" comprises or consists of at least one peptide motif that repetitively occurs within a naturally occurring major ampullate gland polypeptide, such as a dragline spider silk polypeptide, a minor ampullate gland polypeptide, a flagelliform polypeptide, an aggregate spider silk polypeptide, an aciniform spider silk polypeptide or a pyriform spider silk polypeptide. A "repetitive unit" refers to a region which corresponds in amino acid sequence to a region that comprises or consists of at least one peptide motif (e.g. AAAAAA) or GPGQQ) that repetitively occurs within a naturally occurring silk polypeptide (e.g. MaSpI, ADF-3, ADF-4, or Flag) (i.e. identical amino acid sequence) or to an amino acid sequence substantially similar thereto (i.e. variational amino acid sequence). A "repetitive unit" having an amino acid sequence which is "substantially similar" to a corresponding amino acid sequence within a naturally occurring silk polypeptide (i.e. wild-type repetitive unit) is also similar with respect to its properties, e.g. a silk protein comprising the "substantially similar repetitive unit" is still insoluble and retains its insolubility. A "repetitive unit" having an amino acid sequence which is "identical" to the amino acid

sequence of a naturally occurring silk polypeptide, for example, can be a portion of a silk polypeptide corresponding to one or more peptide motifs of MaSpI, MaSpII, ADF-3 and/or ADF-4. A "repetitive unit" having an amino acid sequence which is "substantially similar" to the amino acid sequence of a naturally occurring silk polypeptide, for example, can be a portion of a silk polypeptide corresponding to one or more peptide motifs of MaSpI, MaSpII, ADF-3 and/or ADF-4, but having one or more amino acid substitution at specific amino acid positions.

As used herein, the term "consensus peptide sequence" refers to an amino acid sequence which contains amino acids which frequently occur in a certain position (e.g. "G") and wherein, other amino acids which are not further determined are replaced by the place holder "X". In some embodiments, the consensus sequence is at least one of (i) GPGXX, wherein X is an amino acid selected from A, S, G, Y, P and Q; (ii) GGX, wherein X is an amino acid selected from Y, P, R, S, A, T, N and Q, preferably Y, P and Q; (iii) A_x, wherein x is an integer from 5 to 10.

The consensus peptide sequences GPGXX and GGX, i.e. glycine rich motifs, provide flexibility to the silk polypeptide and thus, to the thread formed from the silk protein containing said motifs. In detail, the iterated GPGXX motif forms turn spiral structures, which imparts elasticity to the silk polypeptide. Major ampullate and flagelliform silks both have a GPGXX motif. The iterated GGX motif is associated with a helical structure having three amino acids per turn and is found in most spider silks. The GGX motif may provide additional elastic properties to the silk. The iterated polyalanine Ax (peptide) motif forms a crystalline β-sheet structure that provides strength to the silk polypeptide, as described for example in WO 03/057727.

In some embodiments, the recombinant spider silk protein in this disclosure comprises two identical repetitive units each comprising at least one, preferably one, amino acid sequence selected from the group consisting of: GGRPSDTYG and GGRPSSSYG derived from Resilin. Resilin is an elastomeric protein found in most arthropods that provides low stiffness and high strength.

As used herein, "non-repetitive units" refers to an amino acid sequence which is "substantially similar" to a corresponding non-repetitive (carboxy terminal) amino acid sequence within a naturally occurring dragline polypeptide (i.e. wild-type non-repetitive

(carboxy terminal) unit), preferably within ADF-3 (SEQ ID NO:1), ADF-4 (SEQ ID NO:2), NR3 (SEQ ID NO:41), NR4 (SEQ ID NO:42), ADF-4 of the spider *Araneus diadematus* as described in U.S. Pat. No. 8,367,803, C16 peptide (spider silk protein eADF4, molecular weight of 47.7 kDa, AMSilk) comprising the 16 repeats of the sequence GSSAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP, an amino acid sequence adapted from the natural sequence of ADF4 from *A. diadematus*. Non-repetitive ADF-4 and variants thereof display efficient assembly behavior.

Among the synthetic spider silk proteins, the recombinant silk protein in this disclosure comprises in some embodiments the C16-protein having the polypeptide sequence SEQ ID NO: 1 as described in U.S. Patent No. 8288512. Besides the polypeptide sequence shown in SEQ ID NO:1, particularly functional equivalents, functional derivatives and salts of this sequence are also included.

As used herein, "functional equivalents" refers to mutant which, in at least one sequence position of the abovementioned amino acid sequences, have an amino acid other than that specifically mentioned.

In some embodiments, the recombinant spider silk protein in this disclosure comprises, in an effective amount, at least one natural or recombinant silk protein including spider silk protein, corresponding to Spidroin major 1 described by Xu et al., PNAS, USA, 87, 7120, (1990), Spidroin major 2 described by Hinman and Lewis, J. Biol. Chem., 267, 19320, (1922), recombinant spider silk protein as described in U.S. Patent Application No. 2016/0222174 and U.S. Patent Nos. 9,051,453, 9,617,315, 9,689,089, 8,173,772, 8,642,734, 8,367,803 8,097,583, 8,030,024, 7,754,851, 7,148,039, 7,060,260, or alternatively the minor Spidroins described in patent application WO 95/25165. Each of the above-cited references is incorporated herein by reference in its entirety. Additional recombinant spider silk proteins suitable for the recombinant RSPF of this disclosure include ADF3 and ADF4 from the "Major Ampullate" gland of *Araneus diadematus*.

Recombinant silk is also described in other patents and patent applications, incorporated by reference herein: US 2004590196, US 7,754,851, US 2007654470, US 7,951,908, US 2010785960, US 8,034,897, US 20090263430, US 2008226854, US 20090123967, US 2005712095, US 2007991037, US 20090162896, US 200885266, US

8,372,436, US 2007989907, US 2009267596, US 2010319542, US 2009265344, US 2012684607, US 2004583227, US 8,030,024, US 2006643569, US 7,868,146, US 2007991916, US 8,097,583, US 2006643200, US 8,729,238, US 8,877,903, US 20190062557, US 20160280960, US 20110201783, US 2008991916, US 2011986662, US 2012697729, US 20150328363, US 9,034,816, US 20130172478, US 9,217,017, US 20170202995, US 8,721,991, US 2008227498, US 9,233,067, US 8,288,512, US 2008161364, US 7,148,039, US 1999247806, US 2001861597, US 2004887100, US 9,481,719, US 8,765,688, US 200880705, US 2010809102, US 8,367,803, US 2010664902, US 7,569,660, US 1999138833, US 2000591632, US 20120065126, US 20100278882, US 2008161352, US 20100015070, US 2009513709, US 20090194317, US 2004559286, US 200589551, US 2008187824, US 20050266242, US 20050227322, and US 20044418.

Recombinant silk is also described in other patents and patent applications, incorporated by reference herein: US 20190062557, US 20150284565, US 20130225476, US 20130172478, US 20130136779, US 20130109762, US 20120252294, US 20110230911, US 20110201783, US 20100298877, US 10,478,520, US 10,253,213, US 10,072,152, US 9,233,067, US 9,217,017, US 9,034,816, US 8,877,903, US 8,729,238, US 8,721,991, US 8,097,583, US 8,034,897, US 8,030,024, US 7,951,908, US 7,868,146, and US 7,754,851.

In some embodiments, the recombinant spider silk protein in this disclosure comprises or consists of 2 to 80 repetitive units, each independently selected from GPGXX, GGX and A_x as defined herein.

In some embodiments, the recombinant spider silk protein in this disclosure comprises or consists of repetitive units each independently selected from selected from the group consisting of GPGAS, GPGSG, GPGGY, GPGGP, GPGGA, GPGQQ, GPGGG, GPGQG, GPGGS, GGY, GGP, GGA, GGR, GGS, GGT, GGN, GGQ, AAAAA, AAAAAA, AAAAAAA, AAAAAAAA, AAAAAAAAA, AAAAAAAAAA, GGRPSDTYG and GGRPSSSYG, (i) GPYGPGASAAAAAAGGYGPGSGQQ, (ii) GSSAAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP, (iii) GPGQQGPGQQGPGQQGPGQQ: (iv) GPGGAGGPYGPGGAGGPYGPGGAGGPY, (v) GGTTIIEDLDITIDGADGPITISEELTI, (vi)

PGSSAAAAAAAASGPGQGQGQGQGQGGRPSDTYG, (vii) SAAAAAAAAGPGGGNGGRPSDTYGAPGGGNGGRPSSSYG, (viii) GGAGGAGGAGGSGGAGGS (SEQ ID NO: 27), (ix) GPGGAGPGGYGPGGSGPGGYGPGGSGPGGY, (x) GPYGPGASAAAAAAGGYGPGCGQQ, (xi) GPYGPGASAAAAAAGGYGPGKGQQ, (xii) GSSAAAAAAAASGPGGYGPENQGPCGPGGYGPGGP, (xiii) GSSAAAAAAAASGPGGYGPKNQGPSGPGGYGPGGP, (xiv) GSSAAAAAAAASGPGGYGPKNQGPSGPGGYGPGGP, or variants thereof as described in U.S. Pat. No. 8,877,903, for example, a synthetic spider peptide having sequential order of GPGAS, GGY, GPGSG in the peptide chain, or sequential order of AAAAAAAA, GPGGY, GPGGP in the peptide chain, sequential order of AAAAAAAA, GPGQG, GGR in the peptide chain.

In some embodiments, this disclosure provides silk protein-like multiblock peptides that imitate the repeating units of amino acids derived from natural spider silk proteins such as *Spidroin major* 1 domain, *Spidroin major* 2 domain or *Spidroin minor* 1 domain and the profile of variation between the repeating units without modifying their three-dimensional conformation, wherein these silk protein-like multiblock peptides comprise a repeating unit of amino acids corresponding to one of the sequences (I), (II), (III) and/or (IV) below.

$[(XGG)_w(XGA)(GXG)_x(AGA)_y(G)_zAG]_p$ Formula (I) in which: X corresponds to tyrosine or to glutamine, w is an integer equal to 2 or 3, x is an integer from 1 to 3, y is an integer from 5 to 7, z is an integer equal to 1 or 2, and p is an integer and having any weight average molecular weight described herein, and/or

$[(GPG_2YGPGQ_2)_a(X')_2S(A)_b]_p$ Formula (II) in which: X' corresponds to the amino acid sequence GPS or GPG, a is equal to 2 or 3, b is an integer from 7 to 10, and p is an integer and having any weight average molecular weight described herein, and/or

$[(GR)(GA)_l(A)_m(GGX)_n(GA)_l(A)_m]_p$ Formula (III) and/or $[(GGX)_n(GA)_m(A)_l]_p$ Formula (IV) in which: X" corresponds to tyrosine, glutamine or alanine, l is an integer from 1 to 6, m is an integer from 0 to 4, n is an integer from 1 to 4, and p is an integer.

In some embodiments, the recombinant spider silk protein or an analog of a spider silk protein comprising an amino acid repeating unit of sequence (V):

$[(Xaa\ Gly\ Gly)_w(Xaa\ Gly\ Ala)(Gly\ Xaa\ Gly)_x(Ala\ Gly\ Ala)_y(Gly)_zAla\ Gly]_p$ Formula (V), wherein Xaa is tyrosine or glutamine, w is an integer equal to 2 or 3, x is an integer from 1 to 3, y is an integer from 5 to 7, z is an integer equal to 1 or 2, and p is an integer.

In some embodiments, the recombinant spider silk protein in this disclosure is selected from the group consisting of ADF-3 or variants thereof, ADF-4 or variants thereof, MaSpI (SEQ ID NO: 43) or variants thereof, MaSpII (SEQ ID NO: 44) or variants thereof as described in U.S. Pat. No. 8,367,803.

In some embodiments, this disclosure provides water soluble recombinant spider silk proteins produced in mammalian cells. The solubility of the spider silk proteins produced in mammalian cells was attributed to the presence of the COOH-terminus in these proteins, which makes them more hydrophilic. These COOH-terminal amino acids are absent in spider silk proteins expressed in microbial hosts.

In some embodiments, the recombinant spider silk protein in this disclosure comprises water soluble recombinant spider silk protein C16 modified with an amino or carboxyl terminal selected from the amino acid sequences consisting of: GCGGGGGG, GKGGGGGG, GCGGSGGGGSGGGG, GKGGGGGGSGGGG, and GCGGGGGGSGGGG. In some embodiments, the recombinant spider silk protein in this disclosure comprises C_{16}NR4, C_{32}NR4, C16, C32, NR4C_{16}NR4, NR4C_{32}NR4, NR3C_{16}NR3, or NR3C_{32}NR3 such that the molecular weight of the protein ranges as described herein.

In some embodiments, the recombinant spider silk protein in this disclosure comprises recombinant spider silk protein having a synthetic repetitive peptide segments and an amino acid sequence adapted from the natural sequence of ADF4 from *A. diadematus* as described in U.S. Pat. No. 8,877,903. In some embodiments, the RSPF in this disclosure comprises the recombinant spider silk proteins having repeating peptide units derived from natural spider silk proteins such as *Spidroin major* 1 domain, *Spidroin major* 2 domain or *Spidroin minor* 1 domain, wherein the repeating peptide sequence is GSSAAAAAAAASGPGQGQGQGQGQGGRPSDTYG or SAAAAAAAAGPGGGNGGRPSDTYGAPGGGNGGRPSSSYG, as described in U.S. Pat. No. 8,367,803.

In some embodiments, this disclosure provides recombinant spider proteins composed of the GPGGAGPGGYGPGGSGPGGYGPGGSGPGGY repetitive fragment and having a molecular weight as described herein.

As used herein, the term "recombinant silk" refers to recombinant spider and/or silkworm silk protein or fragments thereof. In an embodiment, the spider silk protein is selected from the group consisting of swathing silk (Achniform gland silk), egg sac silk (Cylindriform gland silk), egg case silk (Tubuliform silk), non-sticky dragline silk (Ampullate gland silk), attaching thread silk (Pyriform gland silk), sticky silk core fibers (Flagelliform gland silk), and sticky silk outer fibers (Aggregate gland silk). For example, recombinant spider silk protein, as described herein, includes the proteins described in U.S. Patent Application No. 2016/0222174 and U.S. Patent Nos. 9,051,453, 9,617,315, 9,689,089, 8,173,772, and 8,642,734.

Some organisms make multiple silk fibers with unique sequences, structural elements, and mechanical properties. For example, orb weaving spiders have six unique types of glands that produce different silk polypeptide sequences that are polymerized into fibers tailored to fit an environmental or lifecycle niche. The fibers are named for the gland they originate from and the polypeptides are labeled with the gland abbreviation (e.g. "Ma") and "Sp" for spidroin (short for spider fibroin). In orb weavers, these types include Major Ampullate (MaSp, also called dragline), Minor Ampullate (MiSp), Flagelliform (Flag), Aciniform (AcSp), Tubuliform (TuSp), and Pyriform (PySp). This combination of polypeptide sequences across fiber types, domains, and variation amongst different genus and species of organisms leads to a vast array of potential properties that can be harnessed by commercial production of the recombinant fibers. To date, the vast majority of the work with recombinant silks has focused on the Major Ampullate Spidroins (MaSp).

Aciniform (AcSp) silks tend to have high toughness, a result of moderately high strength coupled with moderately high extensibility. AcSp silks are characterized by large block ("ensemble repeat") sizes that often incorporate motifs of poly serine and GPX. Tubuliform (TuSp or Cylindrical) silks tend to have large diameters, with modest strength and high extensibility. TuSp silks are characterized by their poly serine and poly threonine content, and short tracts of poly alanine. Major Ampullate (MaSp) silks tend to

have high strength and modest extensibility. MaSp silks can be one of two subtypes: MaSp1 and MaSp2. MaSp1 silks are generally less extensible than MaSp2 silks, and are characterized by poly alanine, GX, and GGX motifs. MaSp2 silks are characterized by poly alanine, GGX, and GPX motifs. Minor Ampullate (MiSp) silks tend to have modest strength and modest extensibility. MiSp silks are characterized by GGX, GA, and poly A motifs, and often contain spacer elements of approximately 100 amino acids. Flagelliform (Flag) silks tend to have very high extensibility and modest strength. Flag silks are usually characterized by GPG, GGX, and short spacer motifs.

Silk polypeptides are characteristically composed of a repeat domain (REP) flanked by non-repetitive regions (e.g., C-terminal and N-terminal domains). In an embodiment, both the C-terminal and N-terminal domains are between 75-350 amino acids in length. The repeat domain exhibits a hierarchical architecture. The repeat domain comprises a series of blocks (also called repeat units). The blocks are repeated, sometimes perfectly and sometimes imperfectly (making up a quasi-repeat domain), throughout the silk repeat domain. The length and composition of blocks varies among different silk types and across different species. Table 1 of U.S. Published Application No. 2016/0222174, the entirety of which is incorporated herein, lists examples of block sequences from selected species and silk types, with further examples presented in Rising, A. et al., Spider silk proteins: recent advances in recombinant production, structure-function relationships and biomedical applications, *Cell Mol. Life Sci.,* 68:2, pg 169-184 (2011); and Gatesy, J. et al., Extreme diversity, conservation, and convergence of spider silk fibroin sequences, *Science,* 291:5513, pg. 2603-2605 (2001). In some cases, blocks may be arranged in a regular pattern, forming larger macro-repeats that appear multiple times (usually 2-8) in the repeat domain of the silk sequence. Repeated blocks inside a repeat domain or macro-repeat, and repeated macro-repeats within the repeat domain, may be separated by spacing elements.

The construction of certain spider silk block copolymer polypeptides from the blocks and/or macro-repeat domains, according to certain embodiments of the disclosure, is illustrated in U.S. Published Patent Application No. 2016/0222174.

The recombinant block copolymer polypeptides based on spider silk sequences produced by gene expression in a recombinant prokaryotic or eukaryotic system can be

purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant polypeptide is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant block copolymer polypeptide from cell lysates (remains of cells following disruption of cellular integrity) derived from prokaryotic or eukaryotic cells in which a polypeptide was expressed. Methods for generation of such cell lysates are known to those of skill in the art. In some embodiments, recombinant block copolymer polypeptides are isolated from cell culture supernatant.

Recombinant block copolymer polypeptide may be purified by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant polypeptide or nickel columns for isolation of recombinant polypeptides tagged with 6-8 histidine residues at their N-terminus or C-terminus Alternative tags may comprise the FLAG epitope or the hemagglutinin epitope. Such methods are commonly used by skilled practitioners.

A solution of such polypeptides (i.e., recombinant silk protein) may then be prepared and used as described herein.

In another embodiment, recombinant silk protein may be prepared according to the methods described in U.S. Patent No. 8,642,734, the entirety of which is incorporated herein, and used as described herein.

In an embodiment, a recombinant spider silk protein is provided. The spider silk protein typically consists of from 170 to 760 amino acid residues, such as from 170 to 600 amino acid residues, preferably from 280 to 600 amino acid residues, such as from 300 to 400 amino acid residues, more preferably from 340 to 380 amino acid residues. The small size is advantageous because longer spider silk proteins tend to form amorphous aggregates, which require use of harsh solvents for solubilization and polymerization. The recombinant spider silk protein may contain more than 760 residues, in particular in cases where the spider silk protein contains more than two fragments derived from the N-terminal part of a spider silk protein, The spider silk protein comprises an N-terminal fragment consisting of at least one fragment (NT) derived from

the corresponding part of a spider silk protein, and a repetitive fragment (REP) derived from the corresponding internal fragment of a spider silk protein. Optionally, the spider silk protein comprises a C-terminal fragment (CT) derived from the corresponding fragment of a spider silk protein. The spider silk protein comprises typically a single fragment (NT) derived from the N-terminal part of a spider silk protein, but in preferred embodiments, the N-terminal fragment include at least two, such as two fragments (NT) derived from the N-terminal part of a spider silk protein. Thus, the spidroin can schematically be represented by the formula NT_m-REP, and alternatively NT_m-REP-CT, where m is an integer that is 1 or higher, such as 2 or higher, preferably in the ranges of 1-2, 1-4, 1-6, 2-4 or 2-6. Preferred spidroins can schematically be represented by the formulas NT_2-REP or NT-REP, and alternatively NT_2-REP-CT or NT-REP-CT. The protein fragments are covalently coupled, typically via a peptide bond. In one embodiment, the spider silk protein consists of the NT fragment(s) coupled to the REP fragment, which REP fragment is optionally coupled to the CT fragment.

In one embodiment, the first step of the method of producing polymers of an isolated spider silk protein involves expression of a polynucleic acid molecule which encodes the spider silk protein in a suitable host, such as *Escherichia coli*. The thus obtained protein is isolated using standard procedures. Optionally, lipopolysaccharides and other pyrogens are actively removed at this stage.

In the second step of the method of producing polymers of an isolated spider silk protein, a solution of the spider silk protein in a liquid medium is provided. By the terms "soluble" and "in solution" is meant that the protein is not visibly aggregated and does not precipitate from the solvent at 60,000×g. The liquid medium can be any suitable medium, such as an aqueous medium, preferably a physiological medium, typically a buffered aqueous medium, such as a 10-50 mM Tris-HCl buffer or phosphate buffer. The liquid medium has a pH of 6.4 or higher and/or an ion composition that prevents polymerization of the spider silk protein. That is, the liquid medium has either a pH of 6.4 or higher or an ion composition that prevents polymerization of the spider silk protein, or both.

Ion compositions that prevent polymerization of the spider silk protein can readily be prepared by the skilled person utilizing the methods disclosed herein. A preferred ion

composition that prevents polymerization of the spider silk protein has an ionic strength of more than 300 mM. Specific examples of ion compositions that prevent polymerization of the spider silk protein include above 300 mM NaCl, 100 mM phosphate and combinations of these ions having desired preventive effect on the polymerization of the spider silk protein, e.g. a combination of 10 mM phosphate and 300 mM NaCl.

The presence of an NT fragment improves the stability of the solution and prevents polymer formation under these conditions. This can be advantageous when immediate polymerization may be undesirable, e.g. during protein purification, in preparation of large batches, or when other conditions need to be optimized. It is preferred that the pH of the liquid medium is adjusted to 6.7 or higher, such as 7.0 or higher, or even 8.0 or higher, such as up to 10.5, to achieve high solubility of the spider silk protein. It can also be advantageous that the pH of the liquid medium is adjusted to the range of 6.4-6.8, which provides sufficient solubility of the spider silk protein but facilitates subsequent pH adjustment to 6.3 or lower.

In the third step, the properties of the liquid medium are adjusted to a pH of 6.3 or lower and ion composition that allows polymerization. That is, if the liquid medium wherein the spider silk protein is dissolved has a pH of 6.4 or higher, the pH is decreased to 6.3 or lower. The skilled person is well aware of various ways of achieving this, typically involving addition of a strong or weak acid. If the liquid medium wherein the spider silk protein is dissolved has an ion composition that prevents polymerization, the ion composition is changed so as to allow polymerization. The skilled person is well aware of various ways of achieving this, e.g. dilution, dialysis or gel filtration. If required, this step involves both decreasing the pH of the liquid medium to 6.3 or lower and changing the ion composition so as to allow polymerization. It is preferred that the pH of the liquid medium is adjusted to 6.2 or lower, such as 6.0 or lower. In particular, it may be advantageous from a practical point of view to limit the pH drop from 6.4 or 6.4-6.8 in the preceding step to 6.3 or 6.0-6.3, e.g. 6.2 in this step. In a preferred embodiment, the pH of the liquid medium of this step is 3 or higher, such as 4.2 or higher. The resulting pH range, e.g. 4.2-6.3 promotes rapid polymerization,

In the fourth step, the spider silk protein is allowed to polymerize in the liquid medium having pH of 6.3 or lower and an ion composition that allows polymerization of the spider silk protein. Although the presence of the NT fragment improves solubility of the spider silk protein at a pH of 6.4 or higher and/or an ion composition that prevents polymerization of the spider silk protein, it accelerates polymer formation at a pH of 6.3 or lower when the ion composition allows polymerization of the spider silk protein. The resulting polymers are preferably solid and macroscopic, and they are formed in the liquid medium having a pH of 6.3 or lower and an ion composition that allows polymerization of the spider silk protein. In a preferred embodiment, the pH of the liquid medium of this step is 3 or higher, such as 4.2 or higher. The resulting pH range, e.g. 4.2-6.3 promotes rapid polymerization, Resulting polymer may be provided at the molecular weights described herein and prepared as a solution form that may be used as necessary for article coatings.

Ion compositions that allow polymerization of the spider silk protein can readily be prepared by the skilled person utilizing the methods disclosed herein. A preferred ion composition that allows polymerization of the spider silk protein has an ionic strength of less than 300 mM. Specific examples of ion compositions that allow polymerization of the spider silk protein include 150 mM NaCl, 10 mM phosphate, 20 mM phosphate and combinations of these ions lacking preventive effect on the polymerization of the spider silk protein, e.g. a combination of 10 mM phosphate or 20 mM phosphate and 150 mM NaCl. It is preferred that the ionic strength of this liquid medium is adjusted to the range of 1-250 mM.

Without desiring to be limited to any specific theory, it is envisaged that the NT fragments have oppositely charged poles, and that environmental changes in pH affects the charge balance on the surface of the protein followed by polymerization, whereas salt inhibits the same event.

At neutral pH, the energetic cost of burying the excess negative charge of the acidic pole may be expected to prevent polymerization. However, as the dimer approaches its isoelectric point at lower pH, attractive electrostatic forces will eventually become dominant, explaining the observed salt and pH-dependent polymerization behavior of NT and NT-containing minispidroins. It is proposed that, in some

embodiments, pH-induced NT polymerization, and increased efficiency of fiber assembly of NT-minispidroins, are due to surface electrostatic potential changes, and that clustering of acidic residues at one pole of NT shifts its charge balance such that the polymerization transition occurs at pH values of 6.3 or lower.

In a fifth step, the resulting, preferably solid spider silk protein polymers are isolated from said liquid medium. Optionally, this step involves actively removing lipopolysaccharides and other pyrogens from the spidroin polymers.

Without desiring to be limited to any specific theory, it has been observed that formation of spidroin polymers progresses via formation of water-soluble spidroin dimers. The present disclosure thus also provides a method of producing dimers of an isolated spider silk protein, wherein the first two method steps are as described above. The spider silk proteins are present as dimers in a liquid medium at a pH of 6.4 or higher and/or an ion composition that prevents polymerization of said spider silk protein. The third step involves isolating the dimers obtained in the second step, and optionally removal of lipopolysaccharides and other pyrogens. In a preferred embodiment, the spider silk protein polymer of the disclosure consists of polymerized protein dimers. The present disclosure thus provides a novel use of a spider silk protein, preferably those disclosed herein, for producing dimers of the spider silk protein.

According to another aspect, the disclosure provides a polymer of a spider silk protein as disclosed herein. In an embodiment, the polymer of this protein is obtainable by any one of the methods therefor according to the disclosure. Thus, the disclosure provides various uses of recombinant spider silk protein, preferably those disclosed herein, for producing polymers of the spider silk protein as recombinant silk based coatings. According to one embodiment, the present disclosure provides a novel use of a dimer of a spider silk protein, preferably those disclosed herein, for producing polymers of the isolated spider silk protein as recombinant silk based coatings. In these uses, it is preferred that the polymers are produced in a liquid medium having a pH of 6.3 or lower and an ion composition that allows polymerization of said spider silk protein. In an embodiment, the pH of the liquid medium is 3 or higher, such as 4.2 or higher. The resulting pH range, e.g. 4.2-6.3 promotes rapid polymerization,

Using the method(s) of the present disclosure, it is possible to control the polymerization process, and this allows for optimization of parameters for obtaining silk polymers with desirable properties and shapes.

In an embodiment, the recombinant silk proteins described herein, include those described in U.S. patent No. 8,642,734, the entirety of which is incorporated by reference.

In another embodiment, the recombinant silk proteins described herein may be prepared according to the methods described in U.S. Patent No. 9,051,453, the entirety of which is incorporated herein by reference.

An amino acid sequence represented by SEQ ID NO: 1 of U.S. Patent No. 9,051,453 is identical to an amino acid sequence that is composed of 50 amino acid residues of an amino acid sequence of ADF3 at the C-terminal (NCBI Accession No.: AAC47010, GI: 1263287). An amino acid sequence represented by SEQ ID NO: 2 of U.S. Patent No. 9,051,453 is identical to an amino acid sequence represented by SEQ ID NO: 1 of U.S. Patent No. 9,051,453 from which 20 residues have been removed from the C-terminal. An amino acid sequence represented by SEQ ID NO: 3 of U.S. Patent No. 9,051,453 is identical to an amino acid sequence represented by SEQ ID NO: 1 from which 29 residues have been removed from the C-terminal.

An example of the polypeptide that contains units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) and that has, at a C-terminal, an amino acid sequence represented by any of SEQ ID NOS: 1 to 3 or an amino acid sequence having a homology of 90% or more with the amino acid sequence represented by any of SEQ ID NOS: 1 to 3 of U.S. Patent No. 9,051,453 is a polypeptide having an amino acid sequence represented by SEQ ID NO: 8 of U.S. Patent No. 9,051,453. The polypeptide having the amino acid sequence represented by SEQ ID NO: 8 of U.S. Patent No. 9,051,453 is obtained by the following mutation: in an amino acid sequence of ADF3 (NCBI Accession No.: AAC47010, GI: 1263287) to the N-terminal of which has been added an amino acid sequence (SEQ ID NO: 5 of U.S. Patent No. 9,051,453) composed of a start codon, His 10 tags and an HRV3C Protease (Human rhinovirus 3C Protease) recognition site, 1st to 13th repetitive regions are about doubled and the translation ends at the 1154th amino acid residue. In the polypeptide having the amino acid sequence

represented by SEQ ID NO: 8 of U.S. Patent No. 9,051,453, the C-terminal sequence is identical to the amino acid sequence represented by SEQ ID NO: 3.

Further, the polypeptide that contains units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) and that has, at a C-terminal, an amino acid sequence represented by any of SEQ ID NOS: 1 to 3 of U.S. Patent No. 9,051,453 or an amino acid sequence having a homology of 90% or more with the amino acid sequence represented by any of SEQ ID NOS: 1 to 3 of U.S. Patent No. 9,051,453 may be a protein that has an amino acid sequence represented by SEQ ID NO: 8 of U.S. Patent No. 9,051,453 in which one or a plurality of amino acids have been substituted, deleted, inserted and/or added and that has a repetitious region composed of a crystal region and an amorphous region.

Further, an example of the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) is a recombinant protein derived from ADF4 having an amino acid sequence represented by SEQ ID NO: 15 of U.S. Patent No. 9,051,453. The amino acid sequence represented by SEQ ID NO: 15 of U.S. Patent No. 9,051,453 is an amino acid sequence obtained by adding the amino acid sequence (SEQ ID NO: 5 of U.S. Patent No. 9,051,453) composed of a start codon, His 10 tags and an HRV3C Protease (Human rhinovirus 3C Protease) recognition site, to the N-terminal of a partial amino acid sequence of ADF4 obtained from the NCBI database (NCBI Accession No.: AAC47011, GI: 1263289). Further, the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) may be a polypeptide that has an amino acid sequence represented by SEQ ID NO: 15 of U.S. Patent No. 9,051,453 in which one or a plurality of amino acids have been substituted, deleted, inserted and/or added and that has a repetitious region composed of a crystal region and an amorphous region. Further, an example of the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) is a recombinant protein derived from MaSp2 that has an amino acid sequence represented by SEQ ID NO: 17 of U.S. Patent No. 9,051,453. The amino acid sequence represented by SEQ ID NO: 17 of U.S. Patent No. 9,051,453 is an amino acid sequence obtained by adding the amino acid sequence (SEQ ID NO: 5 of U.S. Patent No. 9,051,453) composed of a start codon, His 10 tags and an HRV3C Protease (Human

rhinovirus 3C Protease) recognition site, to the N-terminal of a partial sequence of MaSp2 obtained from the NCBI web database (NCBI Accession No.: AAT75313, GI: 50363147). Furthermore, the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) may be a polypeptide that has an amino acid sequence represented by SEQ ID NO: 17 of U.S. Patent No. 9,051,453 in which one or a plurality of amino acids have been substituted, deleted, inserted and/or added and that has a repetitious region composed of a crystal region and an amorphous region.

Examples of the polypeptide derived from flagelliform silk proteins include a polypeptide containing 10 or more units of an amino acid sequence represented by the formula 2: REP3 (2), preferably a polypeptide containing 20 or more units thereof, and more preferably a polypeptide containing 30 or more units thereof. In the case of producing a recombinant protein using a microbe such as *Escherichia coli* as a host, the molecular weight of the polypeptide derived from flagelliform silk proteins is preferably 500 kDa or less, more preferably 300 kDa or less, and further preferably 200 kDa or less, in terms of productivity.

In the formula (2), the REP 3 indicates an amino acid sequence composed of Gly-Pro-Gly-Gly-X, where X indicates an amino acid selected from the group consisting of Ala, Ser, Tyr and Val.

A major characteristic of the spider silk is that the flagelliform silk does not have a crystal region, but has a repetitious region composed of an amorphous region. Since the major dragline silk and the like have a repetitious region composed of a crystal region and an amorphous region, they are expected to have both high stress and stretchability. Meanwhile, as to the flagelliform silk, although the stress is inferior to that of the major dragline silk, the stretchability is high. The reason for this is considered to be that most of the flagelliform silk is composed of amorphous regions.

An example of the polypeptide containing 10 or more units of the amino acid sequence represented by the formula 2: REP3 (2) is a recombinant protein derived from flagelliform silk proteins having an amino acid sequence represented by SEQ ID NO: 19 of U.S. Patent No. 9,051,453. The amino acid sequence represented by SEQ ID NO: 19 of U.S. Patent No. 9,051,453 is an amino acid sequence obtained by combining a partial

sequence of flagelliform silk protein of Nephila clavipes obtained from the NCBI database (NCBI Accession No.: AAF36090, GI: 7106224), specifically, an amino acid sequence thereof from the 1220th residue to the 1659th residue from the N-terminal that corresponds to repetitive sections and motifs (referred to as a PR1 sequence), with a partial sequence of flagelliform silk protein of *Nephila clavipes* obtained from the NCBI database (NCBI Accession No.: AAC38847, GI: 2833649), specifically, a C-terminal amino acid sequence thereof from the 816th residue to the 907th residue from the C-terminal, and thereafter adding the amino acid sequence (SEQ ID NO: 5 of U.S. Patent No. 9,051,453) composed of a start codon, His 10 tags and an HRV3C Protease recognition site, to the N-terminal of the combined sequence. Further, the polypeptide containing 10 or more units of the amino acid sequence represented by the formula 2: REP3 (2) may be a polypeptide that has an amino acid sequence represented by SEQ ID NO: 19 of U.S. Patent No. 9,051,453 in which one or a plurality of amino acids have been substituted, deleted, inserted and/or added and that has a repetitious region composed of an amorphous region.

The polypeptide can be produced using a host that has been transformed by an expression vector containing a gene encoding a polypeptide. A method for producing a gene is not limited particularly, and it may be produced by amplifying a gene encoding a natural spider silk protein from a cell derived from spiders by a polymerase chain reaction (PCR), etc., and cloning it, or may be synthesized chemically. Also, a method for chemically synthesizing a gene is not limited particularly, and it can be synthesized as follows, for example: based on information of amino acid sequences of natural spider silk proteins obtained from the NCBI web database, etc., oligonucleotides that have been synthesized automatically with AKTA oligopilot plus 10/100 (GE Healthcare Japan Corporation) are linked by PCR, etc. At this time, in order to facilitate the purification and observation of protein, it is possible to synthesize a gene that encodes a protein having an amino acid sequence of the above-described amino acid sequence to the N-terminal of which has been added an amino acid sequence composed of a start codon and His 10 tags.

Examples of the expression vector include a plasmid, a phage, a virus, and the like that can express protein based on a DNA sequence. The plasmid-type expression

vector is not limited particularly as long as it allows a target gene to be expressed in a host cell and it can amplify itself. For example, in the case of using *Escherichia coli* Rosetta (DE3) as a host, a pET22b(+) plasmid vector, a pCold plasmid vector, and the like can be used. Among these, in terms of productivity of protein, it is preferable to use the pET22b(+) plasmid vector. Examples of the host include animal cells, plant cells, microbes, etc.

The polypeptide used in the present disclosure is preferably a polypeptide derived from ADF3, which is one of two principal dragline silk proteins of Araneus diadematus. This polypeptide has advantages of basically having high strength-elongation and toughness and of being synthesized easily.

Accordingly, the recombinant silk protein (e.g., the recombinant spider silk-based protein) used in accordance with the embodiments, articles, and/or methods described herein, may include one or more recombinant silk proteins described above or recited in U.S. Patent Nos. 8,173,772, 8,278,416, 8,618,255, 8,642,734, 8,691,581, 8,729,235, 9,115,204, 9,157,070, 9,309,299, 9,644,012, 9,708,376, 9,051,453, 9,617,315, 9,968,682, 9,689,089, 9,732,125, 9,856,308, 9,926,348, 10,065,997, 10,316,069, and 10,329,332; and U.S. Patent Publication Nos. 2009/0226969, 2011/0281273, 2012/0041177, 2013/0065278, 2013/0115698, 2013/0316376, 2014/0058066, 2014/0079674, 2014/0245923, 2015/0087046, 2015/0119554, 2015/0141618, 2015/0291673, 2015/0291674, 2015/0239587, 2015/0344542, 2015/0361144, 2015/0374833, 2015/0376247, 2016/0024464, 2017/0066804, 2017/0066805, 2015/0293076, 2016/0222174, 2017/0283474, 2017/0088675, 2019/0135880, 2015/0329587, 2019/0040109, 2019/0135881, 2019/0177363, 2019/0225646, 2019/0233481, 2019/0031842, 2018/0355120, 2019/0186050, 2019/0002644, 2020/0031887, 2018/0273590, 20191/094403, 2019/0031843, 2018/0251501, 2017/0066805, 2018/0127553, 2019/0329526, 2020/0031886, 2018/0080147, 2019/0352349, 2020/0043085, 2019/0144819, 2019/0228449, 2019/0340666, 2020/0000091, 2019/0194710, 2019/0151505, 2018/0265555, 2019/0352330, 2019/0248847, and 2019/0378191, the entirety of which are incorporated herein by reference.

Silk Fibroin-like Protein Fragments

The recombinant silk protein in this disclosure comprises synthetic proteins which are based on repeat units of natural silk proteins. Besides the synthetic repetitive silk protein sequences, these can additionally comprise one or more natural nonrepetitive silk protein sequences. As used herein, "silk fibroin-like protein fragments" refer to protein fragments having a molecular weight and polydispersity as defined herein, and a certain degree of homology to a protein selected from native silk protein, fibroin heavy chain, fibroin light chain, or any protein comprising one or more GAGAGS hexa amino acid repeating units. In some embodiments, a degree of homology is selected from about 99%, about 98%, about 97%, about 96%, about 95%, about 94%, about 93%, about 92%, about 91%, about 90%, about 89%, about 88%, about 87%, about 86%, about 85%, about 84%, about 83%, about 82%, about 81%, about 80%, about 79%, about 78%, about 77%, about 76%, about 75%, or less than 75%.

As described herein, a protein such as native silk protein, fibroin heavy chain, fibroin light chain, or any protein comprising one or more GAGAGS hexa amino acid repeating units includes between about 9% and about 45% glycine, or about 9% glycine, or about 10% glycine, about 43% glycine, about 44% glycine, about 45% glycine, or about 46% glycine. As described herein, a protein such as native silk protein, fibroin heavy chain, fibroin light chain, or any protein comprising one or more GAGAGS hexa amino acid repeating units includes between about 13% and about 30% alanine, or about 13% alanine, or about 28% alanine, or about 29% alanine, or about 30% alanine, or about 31% alanine. As described herein, a protein such as native silk protein, fibroin heavy chain, fibroin light chain, or any protein comprising one or more GAGAGS hexa amino acid repeating units includes between 9% and about 12% serine, or about 9% serine, or about 10% serine, or about 11% serine, or about 12% serine.

In some embodiments, a silk fibroin-like protein described herein includes about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23 %, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about

50%, about 51%, about 52%, about 53%, about 54%, or about 55% glycine. In some embodiments, a silk fibroin-like protein described herein includes about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, or about 39% alanine. In some embodiments, a silk fibroin-like protein described herein includes about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, or about 22% serine. In some embodiments, a silk fibroin-like protein described herein may include independently any amino acid known to be included in natural fibroin. In some embodiments, a silk fibroin-like protein described herein may exclude independently any amino acid known to be included in natural fibroin. In some embodiments, on average 2 out of 6 amino acids, 3 out of 6 amino acids, or 4 out of 6 amino acids in a silk fibroin-like protein described herein is glycine. In some embodiments, on average 1 out of 6 amino acids, 2 out of 6 amino acids, or 3 out of 6 amino acids in a silk fibroin-like protein described herein is alanine. In some embodiments, on average none out of 6 amino acids, 1 out of 6 amino acids, or 2 out of 6 amino acids in a silk fibroin-like protein described herein is serine.

Other Properties of SPF

Compositions of the present disclosure are “biocompatible” or otherwise exhibit “biocompatibility” meaning that the compositions are compatible with living tissue or a living system by not being toxic, injurious, or physiologically reactive and not causing immunological rejection or an inflammatory response. Such biocompatibility can be evidenced by participants topically applying compositions of the present disclosure on their skin for an extended period of time. In an embodiment, the extended period of time is about 3 days. In an embodiment, the extended period of time is about 7 days. In an embodiment, the extended period of time is about 14 days. In an embodiment, the extended period of time is about 21 days. In an embodiment, the extended period of time is about 30 days. In an embodiment, the extended period of time is selected from the group consisting of about 1 month, about 2 months, about 3 months, about 4 months,

about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, and indefinitely. For example, in some embodiments, the coatings described herein are biocompatible coatings.

In some embodiments, compositions described herein, which may be biocompatible compositions (e.g., biocompatible coatings that include silk), may be evaluated and comply with International Standard ISO 10993-1, titled the "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process." In some embodiments, compositions described herein, which may be biocompatible compositions, may be evaluated under ISO 106993-1 for one or more of cytotoxicity, sensitization, hemocompatibility, pyrogenicity, implantation, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and degradation.

Compositions of the present disclosure are "hypoallergenic" meaning that they are relatively unlikely to cause an allergic reaction. Such hypoallergenicity can be evidenced by participants topically applying compositions of the present disclosure on their skin for an extended period of time. In an embodiment, the extended period of time is about 3 days. In an embodiment, the extended period of time is about 7 days. In an embodiment, the extended period of time is about 14 days. In an embodiment, the extended period of time is about 21 days. In an embodiment, the extended period of time is about 30 days. In an embodiment, the extended period of time is selected from the group consisting of about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, and indefinitely.

In an embodiment, the stability of a composition of the present disclosure is about 1 day. In an embodiment, the stability of a composition of the present disclosure is about 2 days. In an embodiment, the stability of a composition of the present disclosure is about 3 days. In an embodiment, the stability of a composition of the present disclosure is about 4 days. In an embodiment, the stability of a composition of the present disclosure is about 5 days. In an embodiment, the stability of a composition of the present disclosure is about 6 days. In an embodiment, the stability of a composition of the present disclosure is about 7 days. In an embodiment, the stability of a composition of the present disclosure is about 8 days. In an embodiment, the stability of a composition of the present disclosure is about

9 days. In an embodiment, the stability of a composition of the present disclosure is about 10 days.

In an embodiment, the stability of a composition of the present disclosure is about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, or about 30 days.

In an embodiment, the stability of a composition of the present disclosure is 10 days to 6 months. In an embodiment, the stability of a composition of the present disclosure is 6 months to 12 months. In an embodiment, the stability of a composition of the present disclosure is 12 months to 18 months. In an embodiment, the stability of a composition of the present disclosure is 18 months to 24 months. In an embodiment, the stability of a composition of the present disclosure is 24 months to 30 months. In an embodiment, the stability of a composition of the present disclosure is 30 months to 36 months. In an embodiment, the stability of a composition of the present disclosure is 36 months to 48 months. In an embodiment, the stability of a composition of the present disclosure is 48 months to 60 months.

In an embodiment, a SPF composition of the present disclosure is not soluble in an aqueous solution due to the crystallinity of the protein. In an embodiment, a SPF composition of the present disclosure is soluble in an aqueous solution. In an embodiment, the SPF of a composition of the present disclosure include a crystalline portion of about two-thirds and an amorphous region of about one-third. In an embodiment, the SPF of a composition of the present disclosure include a crystalline portion of about one-half and an amorphous region of about one-half. In an embodiment, the SPF of a composition of the present disclosure include a 99% crystalline portion and a 1% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 95% crystalline portion and a 5% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 90% crystalline portion and a 10% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 85% crystalline portion and a 15% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 80% crystalline

portion and a 20% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 75% crystalline portion and a 25% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 70% crystalline portion and a 30% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 65% crystalline portion and a 35% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 60% crystalline portion and a 40% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 50% crystalline portion and a 50% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 40% crystalline portion and a 60% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 35% crystalline portion and a 65% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 30% crystalline portion and a 70% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 25% crystalline portion and a 75% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 20% crystalline portion and a 80% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 15% crystalline portion and a 85% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 10% crystalline portion and a 90% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 5% crystalline portion and a 90% amorphous region. In an embodiment, the SPF of a composition of the present disclosure include a 1% crystalline portion and a 99% amorphous region.

As used herein, the term “substantially free of inorganic residuals” means that the composition exhibits residuals of 0.1 % (w/w) or less. In an embodiment, substantially free of inorganic residuals refers to a composition that exhibits residuals of 0.05% (w/w) or less. In an embodiment, substantially free of inorganic residuals refers to a composition that exhibits residuals of 0.01 % (w/w) or less. In an embodiment, the amount of inorganic residuals is between 0 ppm (“non-detectable” or “ND”) and 1000 ppm. In an embodiment, the amount of inorganic residuals is ND to about 500 ppm. In an embodiment, the amount of inorganic residuals is ND to about 400 ppm. In an embodiment, the amount of inorganic residuals is ND to about 300 ppm. In an

embodiment, the amount of inorganic residuals is ND to about 200 ppm. In an embodiment, the amount of inorganic residuals is ND to about 100 ppm. In an embodiment, the amount of inorganic residuals is between 10 ppm and 1000 ppm.

As used herein, the term "substantially free of organic residuals" means that the composition exhibits residuals of 0.1 % (w/w) or less, in an embodiment, substantially free of organic residuals refers to a composition that exhibits residuals of 0.05% (w/w) or less. In an embodiment, substantially free of organic residuals refers to a composition that exhibits residuals of 0.01% (w/w) or less. In an embodiment, the amount of organic residuals is between 0 ppm ("non-detectable" or "ND") and 1000 ppm. In an embodiment, the amount of organic residuals is ND to about 500 ppm. In an embodiment, the amount of organic residuals is ND to about 400 ppm. In an embodiment, the amount of organic residuals is ND to about 300 ppm. In an embodiment, the amount of organic residuals is ND to about 200 ppm. In an embodiment, the amount of organic residuals is ND to about 100 ppm. In an embodiment, the amount of organic residuals is between 10 ppm and 1000 ppm.

Compositions of the present disclosure exhibit "biocompatibility" meaning that the compositions are compatible with living tissue or a living system by not being toxic, injurious, or physiologically reactive and not causing immunological rejection. Such biocompatibility can be evidenced by participants topically applying compositions of the present disclosure on their skin for an extended period of time. In an embodiment, the extended period of time is about 3 days. In an embodiment, the extended period of time is about 7 days, in an embodiment, the extended period of time is about 14 days, in an embodiment, the extended period of time is about 21 days. In an embodiment, the extended period of time is about 30 days. In an embodiment, the extended period of time is selected from the group consisting of about I month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, and indefinitely.

Compositions of the present disclosure are "hypoallergenic" meaning that they are relatively unlikely to cause an allergic reaction. Such hypoallergenicity can be evidenced by participants topically applying compositions of the present disclosure on their skin for an extended period of time. In an embodiment, the extended period of time is about 3

days. In an embodiment, the extended period of time is about 7 days. In an embodiment, the extended period of time is about 14 days. In an embodiment, the extended period of time is about 21 days. In an embodiment, the extended period of time is about 30 days. In an embodiment, the extended period of time is selected from the group consisting of about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, and indefinitely.

Following are non-limiting examples of suitable ranges for various parameters in and for preparation of the silk solutions of the present disclosure. The silk solutions of the present disclosure may include one or more, but not necessarily all, of these parameters and may be prepared using various combinations of ranges of such parameters.

In an embodiment, the percent SPF in the solution is less than 30.0 wt. %. In an embodiment, the percent SPF in the solution is less than 25.0 wt. %. In an embodiment, the percent SPF in the solution is less than 20.0 wt. %. In an embodiment, the percent SPF in the solution is less than 19.0 wt. %. In an embodiment, the percent SPF in the solution is less than 18.0 wt. %. In an embodiment, the percent SPF in the solution is less than 17.0 wt. %. In an embodiment, the percent SPF in the solution is less than 16.0 wt. %. In an embodiment, the percent SPF in the solution is less than 15.0 wt. %. In an embodiment, the percent SPF in the solution is less than 14.0 wt. %. In an embodiment, the percent SPF in the solution is less than 13.0 wt. %. In an embodiment, the percent SPF in the solution is less than 12.0 wt. %. In an embodiment, the percent SPF in the solution is less than 11.0 wt. %. In an embodiment, the percent SPF in the solution is less than 10.0 wt. %. In an embodiment, the percent SPF in the solution is less than 9.0 wt. %. In an embodiment, the percent SPF in the solution is less than 8.0 wt. %. In an embodiment, the percent SPF in the solution is less than 7.0 wt. %. In an embodiment, the percent SPF in the solution is less than 6.0 wt. %. In an embodiment, the percent SPF in the solution is less than 5.0 wt. %. In an embodiment, the percent SPF in the solution is less than 4.0 wt. %. In an embodiment, the percent SPF in the solution is less than 3.0 wt. %. In an embodiment, the percent SPF in the solution is less than 2.0 wt. %. In an embodiment, the percent SPF in the solution is less than 1.0 wt. %. In an embodiment, the percent SPF in the solution is less than 0.9 wt. %. In an embodiment, the percent SPF

in the solution is less than 0.8 wt. %. In an embodiment, the percent SPF in the solution is less than 0.7 wt. %. In an embodiment, the percent SPF in the solution is less than 0.6 wt. %. In an embodiment, the percent SPF in the solution is less than 0.5 wt. %. In an embodiment, the percent SPF in the solution is less than 0.4 wt. %. In an embodiment, the percent SPF in the solution is less than 0.3 wt. %. In an embodiment, the percent SPF in the solution is less than 0.2 wt. %. In an embodiment, the percent SPF in the solution is less than 0.1 wt. %.

In an embodiment, the percent SPF in the solution is greater than 0.1 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.2 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.3 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.4 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.5 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.6 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.7 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.8 wt. %. In an embodiment, the percent SPF in the solution is greater than 0.9 wt. %. In an embodiment, the percent SPF in the solution is greater than 1.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 2.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 3.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 4.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 5.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 6.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 7.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 8.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 9.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 10.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 11.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 12.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 13.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 14.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 15.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 16.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 17.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 18.0 wt. %. In an

embodiment, the percent SPF in the solution is greater than 19.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 20.0 wt. %. In an embodiment, the percent SPF in the solution is greater than 25.0 wt. %.

In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 30.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 25.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 20.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 15.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 10.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 9.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 8.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 7.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 6.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 6.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 5.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 5.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 4.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 4.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 3.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 3.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 2.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 2.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 2.4 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.5 wt. % to about 5.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.5 wt. % to about 4.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.5 wt. % to about 4.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.5 wt. % to about 3.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.5 wt. % to about 3.0 wt. %. In an embodiment, the percent SPF in the solution

ranges from about 0.5 wt. % to about 2.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 4.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 3.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 3.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 2.5 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 2.4 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 2.0 wt. %.

In an embodiment, the percent SPF in the solution ranges from about 20.0 wt. % to about 30.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 10.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 1.0 wt. % to about 10.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 2 wt. % to about 10.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 0.1 wt. % to about 6.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 6.0 wt. % to about 10.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 6.0 wt. % to about 8.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 6.0 wt. % to about 9.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 10.0 wt. % to about 20.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 11.0 wt. % to about 19.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 12.0 wt. % to about 18.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 13.0 wt. % to about 17.0 wt. %. In an embodiment, the percent SPF in the solution ranges from about 14.0 wt. % to about 16.0 wt. %. In an embodiment, the percent SPF in the solution is about 1.0 wt. %. In an embodiment, the percent SPF in the solution is about 1.5 wt. %. In an embodiment, the percent SPF in the solution is about 2.0 wt.%. In an embodiment, the percent SPF in the solution is about 2.4 wt. %. In an embodiment, the percent SPF in the solution is 3.0 wt. %. In an embodiment, the percent SPF in the solution is 3.5 wt. %. In an embodiment, the percent SPF in the solution is about 4.0 wt. %. In an embodiment, the percent SPF in the solution is about 4.5 wt. %. In an embodiment, the percent SPF in the solution is about 5.0 wt. %. In an embodiment, the percent SPF in the solution is about 5.5 wt. %. In an

embodiment the percent SPF in the solution is about 6.0 wt. %. In an embodiment, the percent SPF in the solution is about 6.5 wt. %. In an embodiment, the percent SPF in the solution is about 7.0 wt. %. In an embodiment, the percent SPF in the solution is about 7.5 wt. %. In an embodiment, the percent SPF in the solution is about 8.0 wt. %. In an embodiment, the percent SPF in the solution is about 8.5 wt. %. In an embodiment, the percent SPF in the solution is about 9.0 wt. %. In an embodiment, the percent SPF in the solution is about 9.5 wt. %. In an embodiment, the percent SPF in the solution is about 10.0 wt. %.

In an embodiment, the percent sericin in the solution is non-detectable to 25.0 wt. %. In an embodiment, the percent sericin in the solution is non-detectable to 5.0 wt. %. In an embodiment, the percent sericin in the solution is 1.0 wt. %. In an embodiment, the percent sericin in the solution is 2.0 wt. %. In an embodiment, the percent sericin in the solution is 3.0 wt. %. In an embodiment, the percent sericin in the solution is 4.0 wt. %. In an embodiment, the percent sericin in the solution is 5.0 wt. %. In an embodiment, the percent sericin in the solution is 10.0 wt. %. In an embodiment, the percent sericin in the solution is 25.0 wt. %.

In some embodiments, the silk fibroin protein fragments of the present disclosure are shelf stable (they will not slowly or spontaneously gel when stored in an aqueous solution and there is no aggregation of fragments and therefore no increase in molecular weight over time), from 10 days to 3 years depending on storage conditions, percent SPF, and number of shipments and shipment conditions. Additionally, pH may be altered to extend shelf life and/or support shipping conditions by preventing premature folding and aggregation of the silk. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 1 year. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 2 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 2 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 5 years. In an

embodiment, the stability of the LiBr-silk fragment solution is 2 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 2 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 2 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 3 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 3 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 4 to 5 years.

In an embodiment, the stability of a composition of the present disclosure is 10 days to 6 months. In an embodiment, the stability of a composition of the present disclosure is 6 months to 12 months. In an embodiment, the stability of a composition of the present disclosure is 12 months to 18 months. In an embodiment, the stability of a composition of the present disclosure is 18 months to 24 months. In an embodiment, the stability of a composition of the present disclosure is 24 months to 30 months. In an embodiment, the stability of a composition of the present disclosure is 30 months to 36 months. In an embodiment, the stability of a composition of the present disclosure is 36 months to 48 months. In an embodiment, the stability of a composition of the present disclosure is 48 months to 60 months.

In an embodiment, a composition of the present disclosure having SPF has non-detectable levels of LiBr residuals. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is between 10 ppm and 1000 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is between 10 ppm and 300 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 25 ppm. In an embodiment, the amount of the Li Br residuals in a composition of the present disclosure is less than 50 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 75 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 100 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 200 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 300 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 400 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 500

ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 600 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 700 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 800 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 900 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is less than 1000 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 500 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 450 ppm. In an embodiment, the amount of the LiBr residue in a composition of the present disclosure is non-detectable to 400 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 350 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 300 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 250 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 200 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 150 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is non-detectable to 100 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is 100 ppm to 200 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is 200 ppm to 300 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is 300 ppm to 400 ppm. In an embodiment, the amount of the LiBr residuals in a composition of the present disclosure is 400 ppm to 500 ppm.

In an embodiment, a composition of the present disclosure having SPF, has non-detectable levels of Na_2CO_3 residuals. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 100 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals

in a composition of the present disclosure is less than 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 500 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 600 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 700 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 800 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 900 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is less than 1000 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 500 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 450 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 350 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 250 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 150 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is non-detectable to 100 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is 100 ppm to 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is 200 ppm to 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is 300 ppm to 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a composition of the present disclosure is 400 ppm to 500 ppm.

A unique feature of the SPF compositions of the present disclosure are shelf stability (they will not slowly or spontaneously gel when stored in an aqueous solution and there is no aggregation of fragments and therefore no increase in molecular weight over time), from 10 days to 3 years depending on storage conditions, percent silk, and number of shipments and shipment conditions. Additionally pH may be altered to extend shelf-life and/or support shipping conditions by preventing premature folding and aggregation of the silk. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 2 weeks at room temperature (RT). In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 4 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 6 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 8 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 10 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 12 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability ranging from about 4 weeks to about 52 weeks at RT.

Table 18 below shows shelf stability test results for embodiments of SPF compositions of the present disclosure.

Table 18. Shelf Stability of SPF Compositions of the Present Disclosure

% Silk	Temperature	Time to Gelation
2	RT	4 weeks
2	4 °C	>9 weeks
4	RT	4 weeks
4	4 °C	>9 weeks
6	RT	2 weeks
6	4 °C	>9 weeks

In some embodiments, the water solubility of the silk film derived from silk fibroin protein fragments as described herein can be modified by solvent annealing (water annealing or methanol annealing), chemical crosslinking, enzyme crosslinking and heat treatment.

In some embodiments, the process of annealing may involve inducing beta-sheet formation in the silk fibroin protein fragment solutions used as a coating material. Techniques of annealing (e.g., increase crystallinity) or otherwise promoting "molecular packing" of silk fibroin-protein based fragments have been described. In some embodiments, the amorphous silk film is annealed to introduce beta-sheet in the presence of a solvent selected from the group of water or organic solvent. In some embodiments, the amorphous silk film is annealed to introduce beta-sheet in the presence of water (water annealing process). In some embodiments, the amorphous silk fibroin protein fragment film is annealed to introduce beta-sheet in the presence of methanol. In some embodiments, annealing (e.g., the beta sheet formation) is induced by addition of an organic solvent. Suitable organic solvents include, but are not limited to methanol, ethanol, acetone, isopropanol, or combination thereof.

In some embodiments, annealing is carried out by so-called "water-annealing" or "water vapor annealing" in which water vapor is used as an intermediate plasticizing agent or catalyst to promote the packing of beta-sheets. In some embodiments, the process of water annealing may be performed under vacuum. Suitable such methods have been described in Jin H-J et al. (2005), Water-stable Silk Films with Reduced Beta-Sheet Content, Advanced Functional Materials, 15: 1241-1247; Xiao H. et al. (2011), Regulation of Silk Material Structure by Temperature-Controlled Water Vapor Annealing, Biomacromolecules, 12(5): 1686-1696.

The important feature of the water annealing process is to drive the formation of crystalline beta-sheet in the silk fibroin protein fragment peptide chain to allow the silk fibroin self-assembling into a continuous film. In some embodiments, the crystallinity of the silk fibroin protein fragment film is controlled by controlling the temperature of water vapor and duration of the annealing. In some embodiments, the annealing is performed at a temperature ranging from about 65 °C to about 110 °C. In some embodiments, the temperature of the water is maintained at about 80 °C. In some embodiments, annealing is performed at a temperature selected from the group of about 65 °C, about 70 °C, about 75 °C, about 80 °C, about 85 °C, about 90 °C, about 95 °C, about 100 °C, about 105 °C, and about 110 °C.

In some embodiments, the annealing process lasts a period of time selected from the group of about 1 minute to about 40 minutes, about 1 minute to about 50 minutes, about 1 minute to about 60 minutes, about 1 minute to about 70 minutes, about 1 minute to about 80 minutes, about 1 minute to about 90 minutes, about 1 minute to about 100 minutes, about 1 minute to about 110 minutes, about 1 minute to about 120 minutes, about 1 minute to about 130 minutes, about 5 minutes to about 40 minutes, about 5 minutes to about 50 minutes, about 5 minutes to about 60 minutes, about 5 minutes to about 70 minutes, about 5 minutes to about 80 minutes, about 5 minutes to about 90 minutes, about 5 minutes to about 100 minutes, about 5 minutes to about 110 minutes, about 5 minutes to about 120 minutes, about 5 minutes to about 130 minutes, about 10 minutes to about 40 minutes, about 10 minutes to about 50 minutes, about 10 minutes to about 60 minutes, about 10 minutes to about 70 minutes, about 10 minutes to about 80 minutes, about 10 minutes to about 90 minutes, about 10 minutes to about 100 minutes, about 10 minutes to about 110 minutes, about 10 minutes to about 120 minutes, about 10 minutes to about 130 minutes, about 15 minutes to about 40 minutes, about 15 minutes to about 50 minutes, about 15 minutes to about 60 minutes, about 15 minutes to about 70 minutes, about 15 minutes to about 80 minutes, about 15 minutes to about 90 minutes, about 15 minutes to about 100 minutes, about 15 minutes to about 110 minutes, about 15 minutes to about 120 minutes, about 15 minutes to about 130 minutes, about 20 minutes to about 40 minutes, about 20 minutes to about 50 minutes, about 20 minutes to about 60 minutes, about 20 minutes to about 70 minutes, about 20 minutes to about 80 minutes, about 20 minutes to about 90 minutes, about 20 minutes to about 100 minutes, about 20 minutes to about 110 minutes, about 20 minutes to about 120 minutes, about 20 minutes to about 130 minutes, about 25 minutes to about 40 minutes, about 25 minutes to about 50 minutes, about 25 minutes to about 60 minutes, about 25 minutes to about 70 minutes, about 25 minutes to about 80 minutes, about 25 minutes to about 90 minutes, about 25 minutes to about 100 minutes, about 25 minutes to about 110 minutes, about 25 minutes to about 120 minutes, about 25 minutes to about 130 minutes, about 30 minutes to about 40 minutes, about 30 minutes to about 50 minutes, about 30 minutes to about 60 minutes, about 30 minutes to about 70 minutes, about 30 minutes to about 80 minutes, about 30 minutes to about 90 minutes, about 30 minutes to about 100 minutes,

about 30 minutes to about 110 minutes, about 30 minutes to about 120 minutes, about 30 minutes to about 130 minutes, about 35 minutes to about 40 minutes, about 35 minutes to about 50 minutes, about 35 minutes to about 60 minutes, about 35 minutes to about 70 minutes, about 35 minutes to about 80 minutes, about 35 minutes to about 90 minutes, about 35 minutes to about 100 minutes, about 35 minutes to about 110 minutes, about 35 minutes to about 120 minutes, about 35 minutes to about 130 minutes, about 40 minutes to about 50 minutes, about 40 minutes to about 60 minutes, about 40 minutes to about 70 minutes, about 40 minutes to about 80 minutes, about 40 minutes to about 90 minutes, about 40 minutes to about 100 minutes, about 40 minutes to about 110 minutes, about 40 minutes to about 120 minutes, about 40 minutes to about 130 minutes, about 45 minutes to about 50 minutes, about 45 minutes to about 60 minutes, about 45 minutes to about 70 minutes, about 45 minutes to about 80 minutes, about 45 minutes to about 90 minutes, about 45 minutes to about 100 minutes, about 45 minutes to about 110 minutes, about 45 minutes to about 120 minutes, and about 45 minutes to about 130 minutes. In some embodiments, the annealing process lasts a period of time ranging from about 1 minute to about 60 minutes. In some embodiments, the annealing process lasts a period of time ranging from about 45 minutes to about 60 minutes. The longer water annealing post-processing corresponded an increased crystallinity of silk fibroin protein fragments.

In some embodiments, the annealed silk fibroin protein fragment film is immersing the wet silk fibroin protein fragment film in 100 % methanol for 60 minutes at room temperature. The methanol annealing changed the composition of silk fibroin protein fragment film from predominantly amorphous random coil to crystalline antiparallel beta-sheet structure.

In some embodiments, the SPF described herein can be used to produce SPF powders, nanoparticles, and/or microparticles. Silk microparticles have been described for example in WO 2016/110873, which is incorporated by reference herein in its entirety. This can be accomplished by placing the silk solution in a lyophilizer at an appropriate temperature (e.g., room temperature), at a pressure of less than about 100 millitorr (mtorr) until the water and other volatiles have been evaporated (about 1.0 wt. % to about 10 wt. % moisture content), and a fine SPF powder remains. The solid silk

powder resulted from lyophilization is then pulverized to form fine powders of desired particle size.

In some embodiments, an SPF solution can be casted on a substrate to form a silk film containing silk fibroin protein fragments after drying. The silk film is then pulverized to form fine powders.

In some embodiments, an SPF solution can be dried by subjecting to thin film evaporation process (also known as Rototherm) followed by milling. The silk solution is placed in a thin film evaporator under reduced pressure, gentle heating and water is continuously removed from the aqueous solution to result in a solid of variable particle size. The particle size can be varied by controlling the evaporation process parameters including pressure, temperature, rotational speed of the cylinder, thickness of the liquid film in the evaporator. The dry protein powder resulted from the rototherm evaporation contains less than 10.0 wt. % moisture content.

In some embodiments, an SPF solution can be used to prepare SPF microparticles by precipitation with methanol.

Alternative flash drying, fluid-bed drying, spray drying or vacuum drying can be applied to remove water from an SPF solution.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles can be stored and handled without refrigeration or other special handling procedures.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles comprise low molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders, nanoparticles, and/or microparticles comprise mid-molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders, nanoparticles, and/or microparticles comprise a mixture of low molecular weight silk fibroin protein fragments and mid-molecular weight silk fibroin protein fragment.

In some embodiments, the SPF powder are solid particles having median particle size ranging from 1.0 μm to 1000 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 500 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 300 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 250 μm. In some embodiments, the

SPF powder are microparticles having median particle size ranging from 1.0 μm to 200 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 100 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 50.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 25.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 10.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 30.0 μm to 50.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 35.0 μm to 45.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 35.0 μm to 55.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 25.0 μm to 45.0 μm. In some embodiments, the SPF powder are microparticles having median particle size selected from the group consisting of 1.0 μm, 2.0 μm, 3.0 μm, 4.0 μm, 5.0 μm, 6.0 μm, 7.0 μm, 8.0 μm, 9.0 μm, 10.0 μm, 11.0 μm, 12.0 μm, 13.0 μm, 14.0 μm, 15.0 μm, 16.0 μm, 17.0 μm, 18.0 μm, 19.0 μm, 20.0 μm, 21.0 μm, 22.0 μm, 23.0 μm, 24.0 μm, 25.0 μm, 26.0 μm, 27.0 μm, 28.0 μm, 29.0 μm, 30.0 μm, 31.0 μm, 32.0 μm, 33.0 μm, 34.0 μm, 35.0 μm, 36.0 μm, 37.0 μm, 38.0 μm, 39.0 μm, 40.0 μm, 41.0 μm, 42.0 μm, 43.0 μm, 44.0 μm, 45.0 μm, 46.0 μm, 47.0 μm, 48.0 μm, 49.0 μm, 50.0 μm, 51.0 μm, 52.0 μm, 53.0 μm, 54.0 μm, 55.0 μm, 56.0 μm, 57.0 μm, 58.0 μm, 59.0 μm, 60.0 μm, 61.0 μm, 62.0 μm, 63.0 μm, 64.0 μm, 65.0 μm, 66.0 μm, 67.0 μm, 68.0 μm, 69.0 μm, 70.0 μm, 71.0 μm, 72.0 μm, 73.0 μm, 74.0 μm, 75.0 μm, 76.0 μm, 77.0 μm, 78.0 μm, 79.0 μm, 80.0 μm, 81.0 μm, 82.0 μm, 83.0 μm, 84.0 μm, 85.0 μm, 86.0 μm, 87.0 μm, 88.0 μm, 89.0 μm, 90.0 μm, 91.0 μm, 92.0 μm, 93.0 μm, 94.0 μm, 95.0 μm, 96.0 μm, 97.0 μm, 98.0 μm, 99.0 μm, 100.0 μm, 110 μm, 120 μm, 130 μm, 140 μm, 150 μm, 160 μm, 170 μm, 180 μm, 190 μm, 200 μm, 210 μm, 220 μm, 230 μm, 240 μm, 250 μm, 260 μm, 270 μm, 280 μm, 290 μm, 300 μm, 310 μm, 320 μm, 330 μm, 340 μm, 350 μm, 360 μm, 370 μm, 380 μm, 390 μm, 400 μm, 410 μm, 420 μm, 430 μm, 440 μm, 450 μm, 460 μm, 470 μm, 480 μm, 490 μm, 500 μm, 510 μm, 520 μm, 530 μm, 540 μm, 550 μm, 560 μm, 570 μm, 580 μm, 590 μm, 600 μm, 610 μm, 620 μm, 630 μm, 640 μm, 650 μm, 660 μm, 670 μm, 680 μm, 690 μm, 700 μm, 710 μm, 720 μm, 730

μm, 740 μm, 750 μm, 760 μm, 770 μm, 780 μm, 790 μm, 800 μm, 810 μm, 820 μm, 830 μm, 840 μm, 850 μm, 860 μm, 870 μm, 880 μm, 890 μm, 900 μm, 910 μm, 920 μm, 930 μm, 940 μm, 950 μm, 960 μm, 970 μm, 980 μm, 990 μm, and 1000 μm.

In some embodiments, the SPF powder are microparticles having median particle size less than 500 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 325 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 250 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 100 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 50 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 10 μm.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles described herein may find applications as delivery systems for therapeutically active agent, e.g., delivery systems for sustained release of drugs.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles are present in a composition described herein in an amount selected from the group consisting of about 0.001 wt. %, 0.01 wt. %, about 0.1 wt. %, about 0.2 wt. %, about 0.3 wt. %, about 0.4 wt. %, about 0.5 wt. %, about 0.6 wt. %, about 0.7 wt. %, about 0.8 wt. %, about 0.9 wt. %, about 1.0 wt. %, about 1.1 wt. %, about 1.2 wt. %, about 1.3 wt. %, about 1.4 wt. %, about 1.5 wt. %, about 1.6 wt. %, about 1.7 wt. %, about 1.8 wt. %, about 1.9 wt. %, about 2.0 wt. %, about 2.1 wt. %, about 2.2 wt. %, about 2.3 wt. %, about 2.4 wt. %, about 2.5 wt. %, about 2.6 wt. %, about 2.7 wt. %, about 2.8 wt. %, about 2.9 wt. %, about 3.0 wt. %, about 3.1 wt. %, about 3.2 wt. %, about 3.3 wt. %, about 3.4 wt. %, about 3.5 wt. %, about 3.6 wt. %, about 3.7 wt. %, about 3.8 wt. %, about 3.9 wt. %, about 4.0 wt. %, about 4.1 wt. %, about 4.2 wt. %, about 4.3 wt. %, about 4.4 wt. %, about 4.5 wt. %, about 4.6 wt. %, about 4.7 wt. %, about 4.8 wt. %, about 4.9 wt. %, about 5.0 wt. %, about 5.1 wt. %, about 5.2 wt. %, about 5.3 wt. %, about 5.4 wt. %, about 5.5 wt. %, about 5.6 wt. %, about 5.7 wt. %, about 5.8 wt. %, about 5.9 wt. %, about 6.0 wt. %, about 6.1 wt. %, about 6.2 wt. %, about 6.3 wt. %, about 6.4 wt. %, about 6.5 wt. %, about 6.6 wt. %, about 6.7 wt. %, about 6.8 wt. %, about 6.9 wt. %, about 7.0 wt. %, about 7.1 wt. %, about 7.2 wt. %, about 7.3 wt. %,

about 7.4 wt. %, about 7.5 wt. %, about 7.6 wt. %, about 7.7 wt. %, about 7.8 wt. %, about 7.9 wt. %, about 8.0 wt. %, about 8.1 wt. %, about 8.2 wt. %, about 8.3 wt. %, about 8.4 wt. %, about 8.5 wt. %, about 8.6 wt. %, about 8.7 wt. %, about 8.8 wt. %, about 8.9 wt. %, about 9.0 wt. %, about 9.1 wt. %, about 9.2 wt. %, about 9.3 wt. %, about 9.4 wt. %, about 9.5 wt. %, about 9.6 wt. %, about 9.7 wt. %, about 9.8 wt. %, about 9.9 wt. %, about 10.0 wt. % by the total weight of the composition.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles are present in a composition described herein in an amount selected from the group consisting of about 0.1 mg/mL, about 0.2 mg/mL, about 0.3 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, about 0.6 mg/mL, about 0.7 mg/mL, about 0.8 mg/mL, about 0.9 mg/mL, about 1.0 mg/mL, about 1.1 mg/mL, about 1.2 mg/mL, about 1.3 mg/mL, about 1.4 mg/mL, about 1.5 mg/mL, about 1.6 mg/mL, about 1.7 mg/mL, about 1.8 mg/mL, about 1.9 mg/mL, about 2.0 mg/mL, about 2.1 mg/mL, about 2.2 mg/mL, about 2.3 mg/mL, about 2.4 mg/mL, about 2.5 mg/mL, about 2.6 mg/mL, about 2.7 mg/mL, about 2.8 mg/mL, about 2.9 mg/mL, and about 3.0 mg/mL.

In some embodiments, the SPF as described herein can be used to prepare SPF microparticles by precipitation with methanol. Alternative flash drying, fluid-bed drying, spray drying or vacuum drying can be applied to remove water from the silk solution. The SPF powder can then be stored and handled without refrigeration or other special handling procedures. In some embodiments, the SPF powders comprise low molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders comprise mid-molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders comprise a mixture of low molecular weight silk fibroin protein fragments and mid-molecular weight silk fibroin protein fragment.

In some embodiments, the disclosure provides a composition or tissue filler SPF described herein, including without limitation a soft tissues filler can be used to produce SPF powders, nanoparticles, and including without limitation a gel, and all methods of use described herein, comprising SPF nano- or microparticles. This can be accomplished by placing the silk solution in a lyophilizer at an appropriate temperature (e.g., room temperature), at a pressure of less than about 100 millitorr (mtorr) until the water and other volatiles have been evaporated (about 1.0 wt. % to about 10 wt. % moisture

content), and a fine SPF powder remains. The solid silk powder resulted from lyophilization is then pulverized to form fine powders of desired particle size.

In some embodiments, the particles are integrated into the gel. an SPF solution can be casted on a substrate to form a silk film containing silk fibroin protein fragments after drying. The silk film is then pulverized to form fine powders.

In some embodiments, the particles are covalently integrated into the gel. An SPF solution can be dried by subjecting to thin film evaporation process (also known as Rototherm) followed by milling. The silk solution is placed in a thin film evaporator under reduced pressure, gentle heating and water is continuously removed from the aqueous solution to result in a solid of variable particle size. The particle size can be varied by controlling the evaporation process parameters including pressure, temperature, rotational speed of the cylinder, thickness of the liquid film in the evaporator. The dry protein powder resulted from the rototherm evaporation contains less than 10.0 wt. % moisture content.

In some embodiments, the particles are non-covalently integrated into the gel. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein. An SPF solution can be used to prepare SPF microparticles by precipitation with methanol.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising any nano- and/or microparticles particles known in the art. In some embodiments, the nano- and/or microparticles comprise caprolactone. In some embodiments, the nano- and/or microparticles comprise cellulose. In some embodiments, the nano- and/or microparticles are integrated into the gel. In some embodiments, the nano- and/or microparticles are covalently attached. In some embodiments, the nano- and/or microparticles are non-covalently attached. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising nanofibers or microfibers integrated into the gel. In some embodiments, the nanofibers or microfibers are covalently attached. In some embodiments, the nanofibers or microfibers are non-covalently attached. In some embodiments, the composition or tissue filler includes lidocaine or any other anesthetic as described herein. In some embodiments, the composition or tissue filler does not include an anesthetic as described herein. In some embodiments, the nanofibers or microfibers comprise SPF described herein. In some embodiments, the nanofibers or microfibers comprise caprolactone. In some embodiments, the nanofibers or microfibers comprise cellulose.

In some embodiments, the disclosure provides a gel, for example and without limitation a hydrogel, and without limitation for use in any methods of use described herein, the gel and/or hydrogel comprising SPF nano- or microparticles. In some embodiments, the gel and/or hydrogel may or may not include HA as described herein. In some embodiments, the gel and/or hydrogel matrix does not include SPF as described herein, except for the SPF nano- or microparticles embedded in the matrix. In some embodiments, the gel and/or hydrogel is any gel or hydrogel known in the art. In some embodiments, the particles are integrated into the gel. In some embodiments, the particles are covalently integrated into the gel. In some embodiments, the particles are non-covalently integrated into the gel. In some embodiments, the gel or hydrogel include lidocaine or any other anesthetic as described herein. In some embodiments, the gel or hydrogel do not include an anesthetic as described herein.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, configured to deliver another molecule, compound, drug, and the like. In some embodiments, the molecule, compound, drug, or the like, comprises free silk and/or free SPF as described herein. In some embodiments, free silk and/or free SPF boosts collagen expression. In some embodiments, the molecule, compound, drug, or the like, comprises retinol. In some embodiments, the molecule, compound, drug, or the like, comprises a vitamin, including

without limitation vitamin C. In some embodiments, the molecule, compound, drug, or the like, comprises and inflammatory agent. In some embodiments, the molecule, compound, drug, or the like, comprises an anti-inflammatory agent. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate epithelial cell regeneration. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate wound healing. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents to stimulate pain management. In some embodiments, the molecule, compound, drug, or the like, comprises one or more agents able to provide sustained release. In some embodiments, the molecule, compound, drug, or the like, comprises one or more lubricant agents.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising an imaging agent. In some embodiments, the imaging agent is selected from iodine, DOPA, and imaging nanoparticles. In some embodiments, the imaging agent is selected from a paramagnetic imaging agent and a superparamagnetic imaging agent. In some embodiments, the imaging agent is selected from NP-based magnetic resonance imaging (MRI) contrast agents, positron emission tomography (PET)/single photon emission computed tomography (SPECT) imaging agents, ultrasonically active particles, and optically active (e.g., luminescent, fluorescent, infrared) particles. In some embodiments, the imaging agent is a SPECT imaging agent, a PET imaging agent, an optical imaging agent, an MRI or MRS imaging agent, an ultrasound imaging agent, a multimodal imaging agent, an X-ray imaging agent, or a CT imaging agent.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, for use to deliver drugs relevant to a specific area, including without limitation an area of injection.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising micro

particles or micro capsules. In some embodiments, microparticles or micro capsules further comprise a drug.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, wherein the composition or tissue filler is radio opaque.

In some embodiments, the disclosure provides a composition or tissue filler described herein, including without limitation a soft tissues filler, and including without limitation a gel, and all methods of use described herein, further comprising a substantially solid silk composition comprising SPF described herein, having an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight, and a polydispersity between 1 and about 5. In some embodiments, the SPF have a polydispersity between 1 and about 1.5. In some embodiments, the SPF have a polydispersity between about 1.5 and about 2.0. In some embodiments, the SPF have a polydispersity between about 1.5 and about 3.0. In some embodiments, the SPF have a polydispersity between about 2.0 and about 2.5. In some embodiments, the SPF have a polydispersity between about 2.5 and about 3.0. In some embodiments, the composition further comprises about 0.01% (w/w) to about 10% (w/w) sericin relative to the SPF. In some embodiments, the SPF are formulated into particles. In some embodiments, the particles have a size of between about 1 μm and about 1000 μm. In some embodiments, the SPF in the substantially solid silk composition are obtained from a precursor solution comprising SPF fragments having an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight, and a polydispersity between 1 and about 5. In some embodiments, the SPF in the precursor solution have a polydispersity between 1 and about 1.5. In some embodiments, the SPF in the precursor solution have a polydispersity between about 1.5 and about 2.0. In some embodiments, the SPF in the precursor solution have a polydispersity between about 1.5 and about 3.0. In some embodiments, the SPF in the precursor solution have a polydispersity between about 2.0 and about 2.5. In some embodiments, the SPF in the precursor solution have a polydispersity between about 2.5 and about 3.0. In some embodiments, the precursor solution further comprises about

0.01% (w/w) to about 10% (w/w) sericin relative to the SPF in the precursor solution. In some embodiments, the SPF in the precursor solution do not spontaneously or gradually gelate and do not visibly change in color or turbidity when in the precursor solution for at least 10 days prior to obtaining the silk fibroin fragments in the substantially solid silk composition. In some embodiments, the SPF in the substantially solid silk composition are obtained from the precursor solution by a process selected from a lyophilization process, a thin film evaporation process, a salting-out process, and a PVA-assisted method. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 0.01 wt. % to about 10.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 0.01 wt. % to about 1.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 1.0 wt. % to about 2.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 2.0 wt. % to about 3.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 3.0 wt. % to about 4.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 4.0 wt. % to about 5.0 wt. % relative to the total weight. In some embodiments, the substantially solid silk composition is present in the composition or tissue filler at about 5.0 wt. % to about 6.0 wt. % relative to the total weight.

Alternative flash drying, fluid-bed drying, spray drying or vacuum drying can be applied to remove water from an SPF solution.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles can be stored and handled without refrigeration or other special handling procedures.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles comprise low molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders, nanoparticles, and/or microparticles comprise mid-molecular weight silk fibroin protein fragments. In some embodiments, the SPF powders, nanoparticles, and/or

microparticles comprise a mixture of low molecular weight silk fibroin protein fragments and mid-molecular weight silk fibroin protein fragment.

In some embodiments, the SPF powder are solid particles having median particle size ranging from 1.0 μm to 1000 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 500 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 300 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 250 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 200 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 100 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 50.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 25.0 μm. In some embodiments, the SPF powder are microparticles having median particle size ranging from 1.0 μm to 10.0 μm. In some embodiments, the SPF powder are microparticles having median particle size selected from the group consisting of 1.0 μm, 2.0 μm, 3.0 μm, 4.0 μm, 5.0 μm, 6.0 μm, 7.0 μm, 8.0 μm, 9.0 μm, 10.0 μm, 11.0 μm, 12.0 μm, 13.0 μm, 14.0 μm, 15.0 μm, 16.0 μm, 17.0 μm, 18.0 μm, 19.0 μm, 20.0 μm, 21.0 μm, 22.0 μm, 23.0 μm, 24.0 μm, 25.0 μm, 26.0 μm, 27.0 μm, 28.0 μm, 29.0 μm, 30.0 μm, 31.0 μm, 32.0 μm, 33.0 μm, 34.0 μm, 35.0 μm, 36.0 μm, 37.0 μm, 38.0 μm, 39.0 μm, 40.0 μm, 41.0 μm, 42.0 μm, 43.0 μm, 44.0 μm, 45.0 μm, 46.0 μm, 47.0 μm, 48.0 μm, 49.0 μm, 50.0 μm, 51.0 μm, 52.0 μm, 53.0 μm, 54.0 μm, 55.0 μm, 56.0 μm, 57.0 μm, 58.0 μm, 59.0 μm, 60.0 μm, 61.0 μm, 62.0 μm, 63.0 μm, 64.0 μm, 65.0 μm, 66.0 μm, 67.0 μm, 68.0 μm, 69.0 μm, 70.0 μm, 71.0 μm, 72.0 μm, 73.0 μm, 74.0 μm, 75.0 μm, 76.0 μm, 77.0 μm, 78.0 μm, 79.0 μm, 80.0 μm, 81.0 μm, 82.0 μm, 83.0 μm, 84.0 μm, 85.0 μm, 86.0 μm, 87.0 μm, 88.0 μm, 89.0 μm, 90.0 μm, 91.0 μm, 92.0 μm, 93.0 μm, 94.0 μm, 95.0 μm, 96.0 μm, 97.0 μm, 98.0 μm, 99.0 μm, 100.0 μm, 110 μm, 120 μm, 130 μm, 140 μm, 150 μm, 160 μm, 170 μm, 180 μm, 190 μm, 200 μm, 210 μm, 220 μm, 230 μm, 240 μm, 250 μm, 260 μm, 270 μm, 280 μm, 290 μm, 300 μm, 310 μm, 320 μm, 330 μm, 340 μm, 350 μm, 360 μm, 370 μm, 380 μm, 390 μm, 400 μm, 410 μm, 420 μm, 430 μm, 440 μm, 450 μm, 460 μm, 470 μm, 480 μm, 490

μm, 500 μm, 510 μm, 520 μm, 530 μm, 540 μm, 550 μm, 560 μm, 570 μm, 580 μm, 590 μm, 600 μm, 610 μm, 620 μm, 630 μm, 640 μm, 650 μm, 660 μm, 670 μm, 680 μm, 690 μm, 700 μm, 710 μm, 720 μm, 730 μm, 740 μm, 750 μm, 760 μm, 770 μm, 780 μm, 790 μm, 800 μm, 810 μm, 820 μm, 830 μm, 840 μm, 850 μm, 860 μm, 870 μm, 880 μm, 890 μm, 900 μm, 910 μm, 920 μm, 930 μm, 940 μm, 950 μm, 960 μm, 970 μm, 980 μm, 990 μm, and 1000 μm.

In some embodiments, the SPF powder are microparticles having median particle size less than 500 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 325 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 250 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 100 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 50 μm. In some embodiments, the SPF powder are microparticles having median particle size less than 10 μm.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles described herein may find applications as delivery systems for therapeutically active agent, e.g., delivery systems for sustained release of drugs.

In some embodiments, the SPF powders, nanoparticles, and/or microparticles are present in a composition described herein in an amount selected from the group consisting of about 0.001 wt. %, 0.01 wt. %, about 0.1 wt. %, about 0.2 wt. %, about 0.3 wt. %, about 0.4 wt. %, about 0.5 wt. %, about 0.6 wt. %, about 0.7 wt. %, about 0.8 wt. %, about 0.9 wt. %, about 1.0 wt. %, about 1.1 wt. %, about 1.2 wt. %, about 1.3 wt. %, about 1.4 wt. %, about 1.5 wt. %, about 1.6 wt. %, about 1.7 wt. %, about 1.8 wt. %, about 1.9 wt. %, about 2.0 wt. %, about 2.1 wt. %, about 2.2 wt. %, about 2.3 wt. %, about 2.4 wt. %, about 2.5 wt. %, about 2.6 wt. %, about 2.7 wt. %, about 2.8 wt. %, about 2.9 wt. %, about 3.0 wt. %, about 3.1 wt. %, about 3.2 wt. %, about 3.3 wt. %, about 3.4 wt. %, about 3.5 wt. %, about 3.6 wt. %, about 3.7 wt. %, about 3.8 wt. %, about 3.9 wt. %, about 4.0 wt. %, about 4.1 wt. %, about 4.2 wt. %, about 4.3 wt. %, about 4.4 wt. %, about 4.5 wt. %, about 4.6 wt. %, about 4.7 wt. %, about 4.8 wt. %, about 4.9 wt. %, about 5.0 wt. %, about 5.1 wt. %, about 5.2 wt. %, about 5.3 wt. %, about 5.4 wt. %, about 5.5 wt. %, about 5.6 wt. %, about 5.7 wt. %, about 5.8 wt. %,

about 5.9 wt. %, about 6.0 wt. %, about 6.1 wt. %, about 6.2 wt. %, about 6.3 wt. %, about 6.4 wt. %, about 6.5 wt. %, about 6.6 wt. %, about 6.7 wt. %, about 6.8 wt. %, about 6.9 wt. %, about 7.0 wt. %, about 7.1 wt. %, about 7.2 wt. %, about 7.3 wt. %, about 7.4 wt. %, about 7.5 wt. %, about 7.6 wt. %, about 7.7 wt. %, about 7.8 wt. %, about 7.9 wt. %, about 8.0 wt. %, about 8.1 wt. %, about 8.2 wt. %, about 8.3 wt. %, about 8.4 wt. %, about 8.5 wt. %, about 8.6 wt. %, about 8.7 wt. %, about 8.8 wt. %, about 8.9 wt. %, about 9.0 wt. %, about 9.1 wt. %, about 9.2 wt. %, about 9.3 wt. %, about 9.4 wt. %, about 9.5 wt. %, about 9.6 wt. %, about 9.7 wt. %, about 9.8 wt. %, about 9.9 wt. %, about 10.0 wt. % by the total weight of the composition.

Disclosed herein are tissue fillers that include silk protein fragments (SPF). In some embodiments, this disclosure describes dermal fillers that give longer-lasting results while avoiding complications have focused on the modification of hyaluronic acid-based hydrogels. In some embodiments, this disclosure describes an activated silk hydrogel platform in which silk fibroin is successfully integrated into hyaluronic acid-based hydrogels, enabling the efficient optimization of mechanical, optical, and longevity properties of the hydrogel. In some embodiments, this disclosure describes the method of making silk-HA hydrogels using the activated silk hydrogel platform using mixtures of hyaluronic acid, silk fibroin, and polyethylene glycol.

In some embodiments, this disclosure describes a silk fibroin/hyaluronic acid/polyethylene glycol hydrogel system. In some embodiments, this disclosure describes silk-HA hydrogels exhibiting physicochemical properties (e.g., mechanical strength, elasticity, water content of the hydrogel is similar to soft tissue) suitable for application as dermal filler to a wide variety of cosmetic and medical indications.

In some embodiments, the tissue fillers are prepared from compositions described herein that may include SPF and hyaluronic acid (HA). In some embodiments, the tissue fillers described herein may be dermal fillers.

In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using HA having a MW of between about 5 kDa and about 5 MDa, between about 100 kDa and about 4 MDa, or between about 500 kDa and about 3 MDa. In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using HA having a MW of about 50 kDa, about 100 kDa, about 150 kDa, about

200 kDa, about 250 kDa, about 300 kDa, about 350 kDa, about 400 kDa, about 450 kDa, about 500 kDa, about 550 kDa, about 600 kDa, about 650 kDa, about 700 kDa, about 750 kDa, about 800 kDa, about 850 kDa, about 900 kDa, about 950 kDa, about 1000 kDa, about 1050 kDa, about 1100 kDa, about 1150 kDa, about 1200 kDa, about 1250 kDa, about 1300 kDa, about 1350 kDa, about 1400 kDa, about 1450 kDa, about 1500 kDa, about 1550 kDa, about 1600 kDa, about 1650 kDa, about 1700 kDa, about 1750 kDa, about 1800 kDa, about 1850 kDa, about 1900 kDa, about 1950 kDa, about 2000 kDa, about 2050 kDa, about 2100 kDa, about 2150 kDa, about 2200 kDa, about 2250 kDa, about 2300 kDa, about 2350 kDa, about 2400 kDa, about 2450 kDa, about 2500 kDa, about 2550 kDa, about 2600 kDa, about 2650 kDa, about 2700 kDa, about 2750 kDa, about 2800 kDa, about 2850 kDa, about 2900 kDa, about 2950 kDa, about 3000 kDa, about 3050 kDa, about 3100 kDa, about 3150 kDa, about 3200 kDa, about 3250 kDa, about 3300 kDa, about 3350 kDa, about 3400 kDa, about 3450 kDa, about 3500 kDa, about 3550 kDa, about 3600 kDa, about 3650 kDa, about 3700 kDa, about 3750 kDa, about 3800 kDa, about 3850 kDa, about 3900 kDa, about 3950 kDa, or about 4000 kDa. Any of the above MW of HA can be mixed with any other of the above MW of HA, in any possible proportion. In some embodiments, a tissue and/or dermal filler is made by mixing a high MW HA can be mixed with a low MW HA, where the high MW HA is in a proportion of about 0.01%, or about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1%, or about 2%, or about 3%, or about 4%, or about 5%, or about 6%, or about 7%, or about 8%, or about 9%, or about 10%, or about 11%, or about 12%, or about 13%, or about 14%, or about 15%, or about 16%, or about 17%, or about 18%, or about 19%, or about 20%, or about 21%, or about 22%, or about 23%, or about 24%, or about 25%, or about 26%, or about 27%, or about 28%, or about 29%, or about 30%, or about 31%, or about 32%, or about 33%, or about 34%, or about 35%, or about 36%, or about 37%, or about 38%, or about 39%, or about 40%, or about 41%, or about 42%, or about 43%, or about 44%, or about 45%, or about 46%, or about 47%, or about 48%, or about 49%, or about 50%, or about 51%, or about 52%, or about 53%, or about 54%, or about 55%, or about 56%, or about 57%, or about 58%, or about 59%, or about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%,

or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or about 99.5%, or about 99.9%.

In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using silk SPF having a MW between about 5 kDa and about 35 kDa. In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using silk SPF having a MW of about 5 kDa, or about 6 kDa, or about 7 kDa, or about 8 kDa, or about 9 kDa, or about 10 kDa, or about 11 kDa, or about 12 kDa, or about 13 kDa, or about 14 kDa, or about 15 kDa, or about 16 kDa, or about 17 kDa, or about 19 kDa, or about 19 kDa, or about 20 kDa, or about 21 kDa, or about 22 kDa, or about 23 kDa, or about 24 kDa, or about 25 kDa, or about 26 kDa, or about 27 kDa, or about 28 kDa, or about 29 kDa, or about 30 kDa.

In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using an initial concentration of HA of about 80 mg/ml, or about 81 mg/ml, or about 82 mg, ml, or about 83 mg/ml, or about 84 mg/ml, or about 85 mg/ml, or about 86 mg/ml, or about 87 mg/ml, or about 88 mg/ml, or about 89 mg/ml, or about 90 mg/ml, or about 91 mg/ml, or about 92 mg/ml, or about 93 mg/ml, or about 94 mg/ml, or about 95 mg/ml, or about 96 mg/ml, or about 97 mg/ml, or about 98 mg/ml, or about 99 mg/ml, or about 100 mg/ml, or about 101 mg/ml, or about 102 mg/ml, or about 103 mg/ml, or about 104 mg/ml, or about 105 mg/ml, or about 106 mg/ml, or about 107 mg/ml, or about 108 mg/ml, or about 109 mg/ml, or about 110 mg/ml, or about 111 mg/ml, or about 112 mg/ml, or about 113 mg/ml, or about 114 mg/ml, or about 115 mg/ml, or about 116 mg/ml, or about 117 mg/ml, or about 118 mg/ml, or about 119 mg/ml, or about 120 mg/ml, or higher.

In some embodiments, the tissue and/or dermal fillers described herein have a silk SPF concentration of about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1%, or about 1.1%, or about 1.2%, or about 1.3%, or about 1.4%, or about 1.5%, or about 1.6%, or

about 1.7%, or about 1.8%, or about 1.9%, or about 2%, or about 2.1%, or about 2.2%, or about 2.3%, or about 2.4%, or about 2.5%, or about 2.6%, or about 2.7%, or about 2.8%, or about 2.9%, or about 3%, or about 3.1%, or about 3.2%, or about 3.3%, or about 3.4%, or about 3.5%, or about 3.6%, or about 3.7%, or about 3.8%, or about 3.9%, or about 4%, or about 4.1%, or about 4.2%, or about 4.3%, or about 4.4%, or about 4.5%, or about 4.6%, or about 4.7%, or about 4.8%, or about 4.9%, or about 5% of total HA and silk SPF.

In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using a PEGDE cross-linker having a Mn of about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, about 1100, or about 1200.

In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using reaction conditions including a cross-linking step at about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, about 40 °C, about 41 °C, about 42 °C, about 43 °C, about 44 °C, about 45 °C, about 46 °C, about 47 °C, about 48 °C, about 49 °C, about 50 °C, about 51 °C, about 52 °C, about 53 °C, about 54 °C, or about 55 °C. In some embodiments, the tissue and/or dermal fillers are made by a process described herein by using reaction conditions including a cross-linking step of about 15 minutes, about 16 minutes, about 17 minutes, about 18 minutes, about 19 minutes, about 20 minutes, about 21 minutes, about 22 minutes, about 23 minutes, about 24 minutes, about 25 minutes, about 26 minutes, about 27 minutes, about 28 minutes, about 29 minutes, about 30 minutes, about 31 minutes, about 32 minutes, about 33 minutes, about 34 minutes, about 35 minutes, about 36 minutes, about 37 minutes, about 38 minutes, about 39 minutes, about 40 minutes, about 41 minutes, about 42 minutes, about 43 minutes, about 44 minutes, about 45 minutes, about 46 minutes, about 47 minutes, about 48 minutes, about 49 minutes, about 50 minutes, about 51 minutes, about 52 minutes, about 53 minutes, about 54 minutes, about 55 minutes, about 56 minutes, about 57 minutes, about 58 minutes, about 59 minutes, about 60 minutes, about 61 minutes, about 62 minutes, about 63 minutes, about 64 minutes, or about 65 minutes.

In some embodiments, the tissue and/or dermal fillers include free HA, for example un-crosslinked HA. In some embodiments, the tissue and/or dermal fillers

include about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1%, or about 1.1%, or about 1.2%, or about 1.3%, or about 1.4%, or about 1.5%, or about 1.6%, or about 1.7%, or about 1.8%, or about 1.9%, or about 2%, or about 2.1%, or about 2.2%, or about 2.3%, or about 2.4%, or about 2.5%, or about 2.6%, or about 2.7%, or about 2.8%, or about 2.9%, or about 3%, or about 3.1%, or about 3.2%, or about 3.3%, or about 3.4%, or about 3.5%, or about 3.6%, or about 3.7%, or about 3.8%, or about 3.9%, or about 4%, or about 4.1%, or about 4.2%, or about 4.3%, or about 4.4%, or about 4.5%, or about 4.6%, or about 4.7%, or about 4.8%, or about 4.9%, or about 5%, about 5.1%, or about 5.2%, or about 5.3%, or about 5.4%, or about 5.5%, or about 5.6%, or about 5.7%, or about 5.8%, or about 5.9%, or about 6%, or about 6.1%, or about 6.2%, or about 6.3%, or about 6.4%, or about 6.5%, or about 6.6%, or about 6.7%, or about 6.8%, or about 6.9%, or about 7%, or about 7.1%, or about 7.2%, or about 7.3%, or about 7.4%, or about 7.5%, or about 7.6%, or about 7.7%, or about 7.8%, or about 7.9%, or about 8%, or about 8.1%, or about 8.2%, or about 8.3%, or about 8.4%, or about 8.5%, or about 8.6%, or about 8.7%, or about 8.8%, or about 8.9%, or about 9%, or about 9.1%, or about 9.2%, or about 9.3%, or about 9.4%, or about 9.5%, or about 9.6%, or about 9.7%, or about 9.8%, or about 9.9%, or about 10% of total HA (crosslinked HA and un-crosslinked HA). In some embodiments, the tissue and/or dermal fillers do not include free HA.

In some embodiments, the tissue and/or dermal fillers include HA at about 10 mg/ml, about 11 mg/ml, about 12 mg/ml, about 13 mg/ml, about 14 mg/ml, about 15 mg/ml, about 16 mg/ml, about 17 mg/ml, about 18 mg/ml, about 19 mg/ml, about 20 mg/ml, about 21 mg/ml, about 22 mg/ml, about 23 mg/ml, about 24 mg/ml, about 25 mg/ml, about 26/mg/ml, about 27 mg/ml, about 28 mg/ml, about 29 mg/ml, or about 30 mg/ml.

In some embodiments, the tissue and/or dermal fillers have a MoD of about 10.0%, about 10.1%, about 10.2%, about 10.3%, about 10.4%, about 10.5%, about 10.6%, about 10.7%, about 10.8%, about 10.9%, about 11.0%, about 11.1%, about 11.2%, about 11.3%, about 11.4%, about 11.5%, about 11.6%, about 11.7%, about 11.8%, about 11.9%, about 12.0%, about 12.1%, about 12.2%, about 12.3%, about 12.4%, about 12.5%, about 12.6%, about 12.7%, about 12.8%, about 12.9%, about

13.0%, about 13.1%, about 13.2%, about 13.3%, about 13.4%, about 13.5%, about 13.6%, about 13.7%, about 13.8%, about 13.9%, about 14.0%, about 14.1%, about 14.2%, about 14.3%, about 14.4%, about 14.5%, about 14.6%, about 14.7%, about 14.8%, about 14.9%, about 15.0%, about 15.1%, about 15.2%, about 15.3%, about 15.4%, about 15.5%, about 15.6%, about 15.7%, about 15.8%, about 15.9%, about 16.0%, about 16.1%, about 16.2%, about 16.3%, about 16.4%, about 16.5%, about 16.6%, about 16.7%, about 16.8%, about 16.9%, about 17.0%, about 17.1%, about 17.2%, about 17.3%, about 17.4%, about 17.5%, about 17.6%, about 17.7%, about 17.8%, about 17.9%, about 18.0%, about 18.1%, about 18.2%, about 18.3%, about 18.4%, about 18.5%, about 18.6%, about 18.7%, about 18.8%, about 18.9%, about 19.0%, about 19.1%, about 19.2%, about 19.3%, about 19.4%, about 19.5%, about 19.6%, about 19.7%, about 19.8%, about 19.9%, or about 20.0%.

In some embodiments, the tissue and/or dermal fillers have an injection force of about 5 N, about 6 N, about 7 N, about 8 N, about 9 N, about 10 N, about 11 N, about 12 N, about 13 N, about 14 N, about 15 N, about 16 N, about 17 N, about 18 N, about 19 N, about 20 N, about 21 N, about 22 N, about 23 N, about 24 N, or about 25 N. In some embodiments, the tissue and/or dermal fillers have an injection force of about 26 N, about 27 N, about 28 N, about 29 N, about 30 N, about 31 N, about 32 N, about 33 N, about 34 N, about 35 N, about 36 N, about 37 N, about 38 N, about 39 N, about 40 N, about 41 N, about 42 N, about 43 N, about 44 N, about 45 N, about 46 N, about 47 N, about 48 N, about 49 N, or about 50 N. In some embodiments, the injection force relate to injection through a 30 G needle.

The tissue fillers provided herein include compositions further including one or more components such as SPF, for example crosslinked SPF and/or non-crosslinked SPF (e.g., free SPF), hyaluronic acid, for example crosslinked HA and/or non-crosslinked HA. As used herein, crosslinked SPF refers to SPF which is crosslinked with an identical or non-identical SPF. Crosslinked SPF can also be referred to as homo-crosslinked SPF. As used herein, crosslinked HA refers to HA which is crosslinked with an identical or non-identical HA. Crosslinked HA can also be referred to as homo-crosslinked HA. The tissue fillers provided herein can also include SPF crosslinked to HA, and/or HA

crosslinked to SPF. SPF crosslinked to HA, and/or HA crosslinked to SPF, can also be referred to as crosslinked SPF-HA, or hetero-crosslinked SPF-HA.

In some embodiments, the compositions of the invention are monophasic. In some embodiments, the compositions of the invention are biphasic, or multiphasic. In some embodiments, the compositions of the invention include a non-crosslinked polymeric phase, for example non-crosslinked SPF, and/or non-crosslinked HA. In some embodiments, the compositions of the invention include a crosslinked phase, for example crosslinked SPF, and/or crosslinked HA. In some embodiments, the compositions of the invention include a liquid phase, for example water, and/or an aqueous solution. In some embodiments, the aqueous solution can include SPF. In some embodiments, the aqueous phase can include HA. In some embodiments, the liquid phase may include a non-crosslinked polymer such as non-crosslinked HA and/or non-crosslinked SPF.

In some embodiments, a composition of the invention comprises a carrier phase. As such, the disclosed compositions can be monophasic or multiphasic compositions. As used herein, the term “carrier phase” is synonymous with “carrier” and refers to a material used to increase fluidity of a hydrogel. A carrier is advantageously a physiologically-acceptable carrier and may include one or more conventional excipients useful in pharmaceutical compositions. As used herein, the term “a physiologically-acceptable carrier” refers to a carrier in accord with, or characteristic of, the normal functioning of a living organism. As such, administration of a composition comprising a hydrogel and a carrier has substantially no long term or permanent detrimental effect when administered to a mammal. The present tissue fillers include a carrier where a major of the volume is water or saline. However, other useful carriers include any physiologically tolerable material which improves upon extrudability or intrudability of the hydrogel through a needle or into a target host environment. Potential carriers could include but are not limited to physiological buffer solutions, serum, other protein solutions, gels composed of polymers including proteins, glycoproteins, proteoglycans, or polysaccharides. Any of the indicated potential carriers may be either naturally derived, wholly synthetic, or combinations of thereof.

In one embodiment, a composition provided herein includes one or more of modified SPF, crosslinked SPF, non-crosslinked SPF, modified HA, crosslinked HA,

non-crosslinked HA, homo-crosslinked SPF, homo-crosslinked HA, and hetero-crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF and non-crosslinked SPF. In some embodiments, the compositions provided herein include crosslinked SPF and non-crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF and crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include non-crosslinked SPF and non-crosslinked HA. In some embodiments, the compositions provided herein include non-crosslinked SPF and crosslinked HA. In some embodiments, the compositions provided herein include non-crosslinked SPF and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, and non-crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, and crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF, crosslinked HA, and non-crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF, crosslinked HA, and crosslinked SPF-HA. In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked HA, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include non-crosslinked SPF, crosslinked HA, and non-crosslinked HA. In some embodiments, the compositions provided herein include non-crosslinked SPF, crosslinked HA, and crosslinked SPF-HA. In some embodiments, the compositions provided herein include non-crosslinked SPF, non-crosslinked HA, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, crosslinked HA, and non-crosslinked HA. In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, crosslinked HA, and crosslinked SPF-HA. In some embodiments, the compositions

provided herein include crosslinked SPF, non-crosslinked SPF, non-crosslinked HA, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF, crosslinked HA, non-crosslinked HA, and crosslinked SPF-HA. In some embodiments, the compositions provided herein include non-crosslinked SPF, crosslinked HA, non-crosslinked HA, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF, non-crosslinked SPF, crosslinked HA, non-crosslinked HA, and crosslinked SPF-HA.

In some embodiments, the compositions provided herein include crosslinked SPF. In some embodiments, the compositions provided herein include SPF and hyaluronic acids (HA). In one aspect, the SPF/HA based compositions described herein include HA crosslinked moieties. In some embodiments, the compositions include SPF-HA cross linked moieties. In some embodiments, the compositions include non-cross linked HA. In some embodiments, the compositions may include non-cross linked SPF. In some embodiments, the compositions may include at least one additional agent. In some embodiments, the compositions include crosslinked SPF-SPF, SPF-HA, and or HA-HA, with variable stability, resulting in compositions of various degrees of bioabsorbability, and /or bioresorbability.

In some embodiments, the HA is crosslinked into a matrix. In some embodiments, the HA matrix encapsulates or semi-encapsulates one or more SPF. In some embodiments, the HA is crosslinked with one or more SPF.

In some embodiments, the tissue fillers, or portions thereof, are biocompatible, biodegradable, bioabsorbable, bioresorbable, or a combination thereof. In some embodiments, the tissue fillers provided herein include a fluid component, for example a single fluid or a solution including substantially one or more fluids. In some embodiments, the tissue fillers include water or an aqueous solution. In some embodiments, the tissue fillers are injectable, implantable, or deliverable under the skin by any means known in the art such as, for example, following surgical resection of the tissue. In some embodiments, the compositions are tissue and/or dermal fillers. In some embodiments, the compositions are sterile.

In some embodiments, the tissue fillers described herein may include about 1% (w/w) SPF and about 0.3% (w/w) lidocaine.

Provided herein are methods of manufacturing compositions including silk protein fragments (SPFs) and hyaluronic acid (HA), methods of delivery of compositions including SPF and HA, and methods of treatment using compositions including SPF and HA.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one skilled in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference in their entireties.

The percentage symbol "%" used herein includes "wt. %" or % w/w, % v/v, or % w/v.

As used herein, the term "a", "an", or "the" generally is construed to cover both the singular and the plural forms.

As used herein, the term "about" generally refers to a particular numeric value that is within an acceptable error range as determined by one of ordinary skill in the art, which will depend in part on how the numeric value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean a range of ±20 %, ±10 %, or ±5 % of a given numeric value.

As used herein, the term "fibroin" or "silk protein" refers to a type of structural protein produced by certain spider and insect species that produce silk (See defition provided in WIPO Pearl-WIPO's Multilingual Terminology Portal database, https://wipopearl.wipo.int/en/linguistic). Fibroin may include silkworm fibroin, insect or spider silk protein (e.g., spidroin), recombinant spider protein, silk proteins present in other spider silk types, e.g., tubuliform slik protein (TuSP), flagelliform silk protein, minor ampullate silk proteins, aciniform silk protein, pyriform silk protein, aggregate silk glue), silkworm fibroin produced by genetically modified silkworm, or recombinant silkworm fibroin.

As used herein, the term "silk fibroin" refers to silkworm fibroin, silk fibroin produced by genetically modified silkworm, or recombinant silkworm fibroin (See (1) Narayan Ed., Encyclopedia of Biomedical Engineering, Vol. 2, Elsevier, 2019; (2) Kobayashi et al. Eds, Encyclopedia of Polymeric Nanomaterials, Springer, 2014, https://link.springer.com/referenceworkentry/10.1007%2F978-3-642-36199-9_323-1). In an embodiment, silk fibroin is obtained from *Bombyx mori*.

As used herein, the terms "silk fibroin peptide," "silk fibroin protein-based fragment," and "silk fibroin fragment" are used interchangeably. Molecular weight or number of amino acids units are defined when molecular size becomes an important parameter.

As used herein, the term polymer "polydispersity (PD)" is generally used as a measure of the broadness of a molecular weight distribution of a polymer, and is defined by the formula polydispersity PD = Mw/Mn.

As used herein, the term "low molecular weight silk fibroin protein based fragment" (Low-MW silk) refers to silk fibroin fragments having a weight average molecular weight (Mw) of about 200 Da to about 25 kDa, or lower than about 28 kDa, or between about 15 kDa and about 28 kDa.

As used herein, the term "medium molecular weight silk fibroin fragment" (Med-MW silk) refers to silk fibroin fragments having a weight average molecular weight ranging from about 25 kDa to about 60 kDa, or about 39 kDa to about 54 kDa.

The term "gelation" as used herein refers to a process involving continuous increase in viscosity accompanied by gradual enhancement of elastic properties. The main cause of gelation in polymer systems is the enhancement of interactions between the dissolved polymer or their aggregates. In contrast to micellization, gelation occurs from the semi-dilute to the high concentration of block copolymer solution and results from an arrangement of ordered micelles.

The term "hydrogel" as used herein refers to three dimensional networks made of cross-linked hydrophilic or amphiphilic polymers that are swollen in liquid without dissolving in them. Hydrogel has the capability to absorb a large amount of water.

Hydrogels are low-volume-fraction 3D networks of molecules, fibers or particles with intermediate voids, filled with water or aqueous media. Hydrogels can be classified into two classes: one class is physical gel resulted from physical association of polymer chains, and the other class is chemical gels (or irreversible gel) of which the network linked by covalent bonds. The inclusion of functional groups as pendant groups or on the backbone of the 3D network allows the synthesis of hydrogels that swell in response to a variety of stimuli including temperature, electromagnetic fields, chemicals and biomolecules. In an embodiment, the physical forms of the silk-HA hydrogel described herein may include microgels (hydrogel microparticles) and bulk hydrogels.

As used herein, the terms "substantially sericin free" or "substantially devoid of sericin" refer to silk fibers in which a majority of the sericin protein has been removed, and/or SPF made from silk fibers in which a majority of the sericin protein has been removed. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 10.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 9.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 8.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 7.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 6.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.01% (w/w) and about 5.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.05% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 0.1% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin

refers to silk fibroin and SPF having between about 0.5% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 1.0% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 1.5% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 2.0% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having between about 2.5% (w/w) and about 4.0% (w/w) sericin. In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having a sericin content between about 0.01% (w/w) and about 0.1 % (w/w). In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having a sericin content below about 0.1 % (w/w). In an embodiment, silk fibroin and SPF that are substantially devoid of sericin refers to silk fibroin and SPF having a sericin content below about 0.05% (w/w). In an embodiment, when a silk source is added to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes, a degumming loss of about 26 wt.% to about 31 wt.% is obtained.

As used herein, the term “substantially homogeneous” may refer to pure silk fibroin-based protein fragments that are distributed in a normal distribution about an identified molecular weight. As used herein, the term “substantially homogeneous” may refer to an even distribution of an additive, for example lidocaine, throughout a composition of the present disclosure.

As used herein, the term “substantially free of inorganic residuals” means that the composition exhibits residuals of 0.1 % (w/w) or less. In an embodiment, substantially free of inorganic residuals refers to a composition that exhibits residuals of 0.05% (w/w) or less. In an embodiment, substantially free of inorganic residuals refers to a composition that exhibits residuals of 0.01% (w/w) or less. In an embodiment, the amount of inorganic residuals is between 0 ppm (“non-detectable” or “ND”) and 1000 ppm. In an embodiment, the amount of inorganic residuals is ND to about 500 ppm. In an embodiment, the amount of inorganic residuals is ND to about 400 ppm. In an

embodiment, the amount of inorganic residuals is ND to about 300 ppm. In an embodiment, the amount of inorganic residuals is ND to about 200 ppm. In an embodiment, the amount of inorganic residuals is ND to about 100 ppm. In an embodiment, the amount of inorganic residuals is between 10 ppm and 1000 ppm.

As used herein, the term "substantially free of organic residuals" means that the composition exhibits residuals of 0.1% (w/w) or less. In an embodiment, substantially free of organic residuals refers to a composition that exhibits residuals of 0.05% (w/w) or less. In an embodiment, substantially free of organic residuals refers to a composition that exhibits residuals of 0.01% (w/w) or less. In an embodiment, the amount of organic residuals is between 0 ppm ("non-detectable" or "ND") and 1000 ppm. In an embodiment, the amount of organic residuals is ND to about 500 ppm. In an embodiment, the amount of organic residuals is ND to about 400 ppm. In an embodiment, the amount of organic residuals is ND to about 300 ppm. In an embodiment, the amount of organic residuals is ND to about 200 ppm. In an embodiment, the amount of organic residuals is ND to about 100 ppm. In an embodiment, the amount of organic residuals is between 10 ppm and 1000 ppm.

As used herein, the term "non-crosslinked" refers to a lack of intermolecular bonds joining individual matrix polymer molecules, macromolecules, and/or monomer chains. As such, a non-crosslinked matrix polymer is not linked to any other matrix polymer by an intermolecular bond.

Tissue fillers, compositions, or portions thereof, of the present disclosure exhibit "biocompatibility" or are "biocompatible" meaning that the compositions are compatible with living tissue or a living system by not being substantially toxic, injurious, or physiologically reactive and not causing immunological rejection. The term "biocompatible" encompasses the terms "bioabsorbable," "bioresorbable," and "biodegradable," which are defined herein.

Tissue fillers, compositions, or portions thereof, of the present disclosure may be "bioabsorbable," "bioresorbable," and/or "biodegradable". As used herein, the terms "bioabsorbable" refers to materials or substances that dissipate upon implantation within a body, independent of which mechanisms by which dissipation can occur, such as dissolution, degradation, absorption and excretion. As used herein, the term

"bioresorbable" means capable of being absorbed by the body. As used herein, the term "biodegradable" refers to materials which can decompose under physiological conditions into byproducts. Such physiological conditions include, for example, hydrolysis (decomposition via hydrolytic cleavage), enzymatic catalysis (enzymatic degradation), mechanical interactions, and the like. As used herein, the term "biodegradable" also encompasses the term "bioresorbable", which describes a material or substance that decomposes under physiological conditions to break down to products that undergo bioresorption into the host-organism, namely, become metabolites of the biochemical systems of the host organism. As used herein, the terms "bioresorbable" and "bioresorption" encompass processes such as cell-mediated degradation, enzymatic degradation and/or hydrolytic degradation of the bioresorbable polymer, and/or elimination of the bioresorbable polymer from living tissue as will be appreciated by the person skilled in the art. In some embodiments, the SPF-HA compositions and materials described herein may be biocompatible, bioresorbable, bioabsorbable, and/or biodegradable.

Where the tissue fillers described herein are biodegradable or bioresorbable, they may resist biodegradation or bioresorption for at least about 1 day, or at least about 2 days, or at least about 3 days, or at least about 4 days, at least about 5 days, or at least about 10 days, or at least about 15 days, or at least about 20 days, or at least about 25 days, or at least about 30 days, or at least about 35 days, or at least about 40 days, or at least about 45 days, or at least about 50 days, or at least about 60 days, or at least about 70 days, or at least about 80 days, or at least about 90 days, or at least about 100 days, or at least about 110 days, or at least about 120 days, or at least about 130 days, or at least about 140 days, or at least about 140 days, or at least about 150 days, or at least about 160 days, or at least about 170 days, or at least about 180 days, or at least about 190 days, or at least about 200 days, or at least about 250 days, or at least about 300 days, or at least about 1 year, or at least about 2 years or they may resist biodegradation for less than about 5 days, or at most about 10 days, or at most about 15 days, or at most about 20 days, or at most about 25 days, or at most about 30 days, or at most about 35 days, or at most about 40 days, or at most about 45 days, or at most about 50 days, or at most about 60 days, or at most about 70 days, or at most about 80 days, or at most about 90 days, or

at most about 100 days, or at most about 110 days, or at most about 120 days, or at most about 130 days, or at most about 140 days, or at most about 140 days, or at most about 150 days, or at most about 160 days, or at most about 170 days, or at most about 180 days, or at most about 190 days, or at most about 200 days, or at most about 250 days, or at most about 300 days, or at most about 1 year, or at most about 2 years.

Where the tissue fillers described herein are bioabsorbable they may resist bioabsorption for at least about 1 day, or at least about 2 days, or at least about 3 days, or at least about 4 days, at least about 5 days, or at least about 10 days, or at least about 15 days, or at least about 20 days, or at least about 25 days, or at least about 30 days, or at least about 35 days, or at least about 40 days, or at least about 45 days, or at least about 50 days, or at least about 60 days, or at least about 70 days, or at least about 80 days, or at least about 90 days, or at least about 100 days, or at least about 110 days, or at least about 120 days, or at least about 130 days, or at least about 140 days, or at least about 140 days, or at least about 150 days, or at least about 160 days, or at least about 170 days, or at least about 180 days, or at least about 190 days, or at least about 200 days, or at least about 250 days, or at least about 300 days, or at least about 1 year, or at least about 2 years or they may resist bioabsorption for less than about 5 days, or at most about 10 days, or at most about 15 days, or at most about 20 days, or at most about 25 days, or at most about 30 days, or at most about 35 days, or at most about 40 days, or at most about 45 days, or at most about 50 days, or at most about 60 days, or at most about 70 days, or at most about 80 days, or at most about 90 days, or at most about 100 days, or at most about 110 days, or at most about 120 days, or at most about 130 days, or at most about 140 days, or at most about 140 days, or at most about 150 days, or at most about 160 days, or at most about 170 days, or at most about 180 days, or at most about 190 days, or at most about 200 days, or at most about 250 days, or at most about 300 days, or at most about 1 year, or at most about 2 years.

As described herein, the degree of biodegradation, bioabsorption, and bioresorption may be modified and/or controlled by, for example, adding one or more agents to compositions described herein that retard biodegradation, bioabsorption, and/or bioresorption. In addition, the degree of biodegradation, bioabsorption, and bioresorption may be modified and/or controlled by increasing or decreasing the degree of polymeric

cross-linking present in the polymeric materials described herein. For example, the rate of biodegradation, bioabsorption, and/or bioresorption of the compositions described here may be increased by reducing the amount of crosslinking in the polymeric materials described herein. Alternatively, the rate of biodegradation, bioabsorption, and/or bioresorption of the tissue fillers and compositions described here may be decreased by increasing the amount of crosslinking in the polymeric materials described herein.

Tissue fillers and compositions of the present disclosure are "hypoallergenic" meaning that they are relatively unlikely to cause an allergic reaction. Such hypoallergenicity can be evidenced by participants topically applying compositions of the present disclosure on their skin for an extended period of time. In an embodiment, the extended period of time is about 3 days. In an embodiment, the extended period of time is about 7 days. In an embodiment, the extended period of time is about 14 days. In an embodiment, the extended period of time is about 21 days. In an embodiment, the extended period of time is about 30 days. In an embodiment, the extended period of time is selected from the group consisting of about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, and indefinitely.

As used herein, "low molecular weight" silk refers to silk protein fragments having a molecular weight in a range of about 5 kDa to about 20 kDa, or about 200 Da to about 25 kDa, or lower than about 28 kDa, or between about 15 kDa and about 28 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 11 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 12 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 13 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 14 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 15 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 16 kDa.

As used herein, "medium molecular weight" silk refers to silk protein fragments having a molecular weight in a range of about 20 kDa to about 55 kDa, or about 25 kDa to about 60 kDa, or about 39 kDa to about 54 kDa. In some embodiments, a target low

molecular weight for certain silk protein fragments may be about 40 kDa. In some embodiments, a target medium molecular weight for certain silk protein fragments may be about 48 kDa.

As used herein, "high molecular weight" silk refers to silk protein fragments having a molecular weight in a range of about 55 kDa to about 150 kDa. In some embodiments, a target low molecular weight for certain silk protein fragments may be about 100 kDa to about 145 kDa. In some embodiments, a target high molecular weight for certain silk protein fragments may be about 100 kDa.

In some embodiments, the molecular weights described herein, e.g., low molecular weight SPF, medium molecular weight SPF, high molecular weight SPF, may be converted to the approximate number of amino acids contained within the respective natural or recombinant proteins, such as natural or recombinant silk proteins, as would be understood by a person having ordinary skill in the art. For example, the average weight of an amino acid may be about 110 daltons, i.e., 110 g/mol. Therefore, in some embodiments, dividing the molecular weight of a linear protein by 110 daltons may be used to approximate the number of amino acid residues contained therein.

As used herein, the term "polydispersity" refers to a measure of the distribution of molecular mass in a given polymer sample. Polydispersity may be calculated by dividing the weight average molecular weight (Mw) by the number average molecular weight (Mn). As used herein, the term "weight average molecular weight" (Mw) generally refers to a molecular weight measurement that depends on the contributions of polymer molecules according to their sizes. The weight average molecular weight may be defined by the formula: $Mw = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i}$, where M_i is the molecular weight of a chain and N_i is the number of chains of that molecular weight. As used herein, the term "number average molecular weight" (Mn) generally refers to a molecular weight measurement that is calculated by dividing the total weight of all the polymer molecules in a sample with the total number of polymer molecules in the sample. The number average molecular weight may be defined by the formula: $Mn = \frac{\Sigma N_i M_i}{\Sigma N_i}$, where M_i is the molecular weight of a chain and N_i is the number of chains of that molecular weight. For example, a monodisperse polymer, where all polymer chains are equal has a polydispersity (Mw/Mn) of 1. In

general, molecular weight averages may be determined by gel permeation chromatography (GPC) and size exclusion chromatography (SEC). The larger the polydispersity index, the broader the molecular weight.

As used herein, the term "tissue filler" refers broadly a material that may be provided in and about soft tissue to add volume, add support, or otherwise treat a soft tissue deficiency. The term "tissue filler" also encompasses tissue and/or dermal fillers; however, the term "dermal filler" should not be construed as imposing any limitations as to the location and type of delivery of such filler. Nevertheless, dermal fillers described herein may generally encompass the use and delivery of such dermal fillers at multiple levels beneath the dermis. As used herein, the term "soft tissue" may refer to those tissues that connect, support, or surround other structures and organs of the body. For example, soft tissues described herein may include, without limitation, skin, dermal tissues, subdermal tissues, cutaneous tissues, subcutaneous tissues, intradural tissue, muscles, tendons, ligaments, fibrous tissues, fat, blood vessels and arteries, and nerves, and synovial (intradermal) tissues.

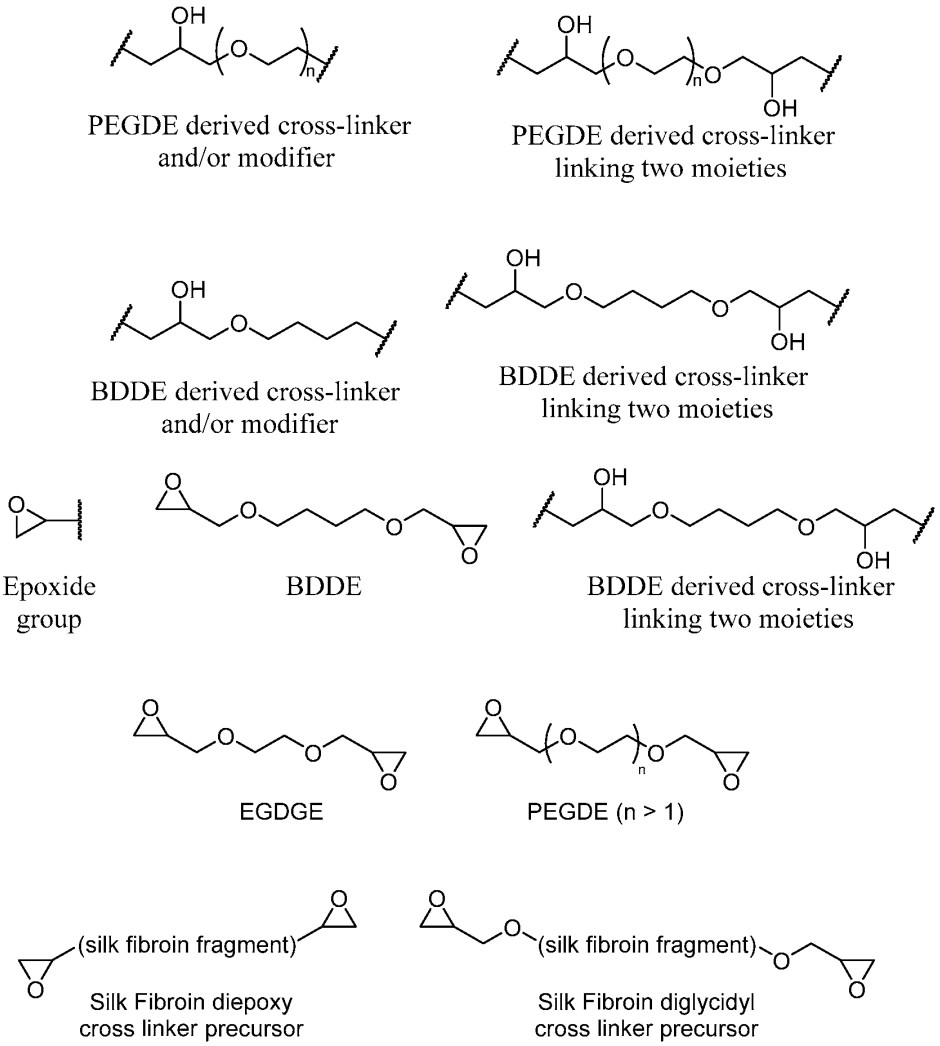
As used herein, "auto cross-linking" refers to either a) cross-linking between two strands of polymers of similar chemical nature, for example cross-linking between two strands of hyaluronic acid, or cross-linking between two strands of SPFs, or b) cross-linking between cross-linking groups on the same polymers strand to create a cyclic ester (lactone), a cyclic amide, a cyclic construct including a cross-linking moiety, or the like, for example cross-linking between two groups on the same strand of hyaluronic acid, or cross-linking between two groups on the same SPF strand.

As used herein, "zero-length cross linking," and/or "cross-linking including a bond," and/or "cross-linking using an activating agent," refers to cross-linking between two groups on either separate polymer strands, or the same polymer strand, where the groups react directly with each other, and no additional cross-linking moiety is inserted between them. Cross-linking between a carboxylic acid group and an amine or alcohol, where one of the groups is activated by an activating agent, for example a carbodiimide, is an example of zero-length cross-linking.

As used herein, the term "epoxy derived cross-linker" refers to a molecular bridge between two moieties in the same or separate polymer chains, which is obtained by

employing a cross-linking precursor including an epoxide group, for example 1,4-butanediol diglycidyl ether (BDDE), polyethylene glycol diglycidyl ether (PEGDE, or PEGDGE), or a silk fibroin or silk fibroin fragment polyepoxy linker. Without wishing to be bound by any particular theory, by reacting with a reactive center in a polymer chain, including in the side chain of the polymer, the epoxide ring opens to form a secondary alcohol and a new bond (Scheme 1). Reactive groups include, but are not limited to, nucleophilic groups such as carboxylic groups, amino groups, or hydroxyl groups.

Scheme 1



As used herein, the "Tyndall effect," and/or "tyndalling," is an adverse event occurring in some patients administered with tissue fillers. Tyndall effect is characterized by the appearance of a blue discoloration at the skin site where a tissue filler had been injected, which represents visible tissue and/or dermal filler composition seen through the translucent epidermis. The Tyndall effect can be seen when light-scattering particulate-matter is dispersed in an otherwise-light-transmitting medium, when the cross-section of particles is in a specific range, usually somewhat below or near the wavelength of visible light. Under the Tyndall effect, longer-wavelength light (e.g., red) is transmitted to a greater degree through the medium, while shorter-wavelength light (e.g., blue) is reflected to a greater degree via scattering, giving the overall impression that the medium is colored blue.

Silk Protein Fragments

In some embodiments, the silk protein-based compositions and silk protein fragments, or methods of producing the same, may include those described in U.S. Patent Application Publication Nos. 2015/00933340, 2015/0094269, 2016/0193130, 2016/0022560, 2016/0022561, 2016/0022562, 2016/0022563, and 2016/0222579, 2016/0281294, and U.S. Patent Nos. 9,187,538, 9,522,107, 9,517,191, 9,522,108, 9,511,012, and 9,545,369, the entirety of which are incorporated herein by reference.

As used herein, silk protein fragments (SPFs) refer generally to a mixture, composition, or population of peptides and/or proteins originating from silk. In some embodiments, SPFs are produced as substantially pure and highly scalable SPF mixture solutions that may be used across multiple industries for a variety of applications. The solutions are generated from raw pure intact silk protein material and processed in order to remove any sericin and achieve the desired weight average molecular weight (MW) and polydispersity of the fragment mixture. Select method parameters may be altered to achieve distinct final silk protein fragment characteristics depending upon the intended use. The resulting final fragment solution is pure silk protein fragments and water with PPM to non-detectable levels of process contaminants, levels acceptable in the pharmaceutical, medical and consumer cosmetic markets. The concentration, size and polydispersity of silk protein fragments in the solution may further be altered depending

upon the desired use and performance requirements. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, and have a polydispersity ranging from about 1.5 and about 3.0. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 5 kDa to about 150 kDa, and have a polydispersity ranging from about 1.5 and about 3.0. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 6 kDa to about 17 kDa, and have a polydispersity ranging from about 1.5 and about 3.0. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 17 kDa to about 39 kDa, and have a polydispersity ranging from about 1.5 and about 3.0. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 39 kDa to about 80 kDa, and have a polydispersity ranging from about 1.5 and about 3.0. In an embodiment, the pure silk fibroin-based protein fragments in the solution are substantially devoid of sericin, have an average weight average molecular weight ranging from about 80 kDa to about 150 kDa, and have a polydispersity ranging from about 1.5 and about 3.0.

In an embodiment, the silk protein fragments described herein may be prepared in a solution or as a solid, whereby the solid is suspended in a physiological solution (e.g., water, saline, and the like) or a gel of HA, as described herein. In some embodiments, the silk protein fragments described herein may be prepared in liposomes or microspheres before depositing the same in a gel of HA.

In an embodiment, the silk solutions of the present disclosure may be used to generate the tissue filler compositions described herein. In an embodiment, the solutions may be used to generate gels that may be homogenized with HA and additional agents to prepare the tissue fillers described herein. Depending on the silk solution utilized and the methods for casting the films or gels, various properties are achieved.

In some embodiments, the percent SPF content, by weight, in the tissue fillers described herein is at least 0.01%, or at least 0.1%, or at least 0.2%, or at least 0.3%, or at least 0.4%, or at least 0.5%, or at least 0.6%, or at least 0.7%, or at least 0.8%, or at least 0.9%, or at least 1%, or at least 2%, or at least 3%, or at least 4%, or at least 5%, or at least 6%, or at least 7%, or at least 8%, or at least 9%, or at least 10%, or at least 11%, or at least 12%, or at least 13%, or at least 14%, or at least 15%, or at least 16%, or at least 17%, or at least 18%, or at least 19%, or at least 20%, or at least 21%, or at least 22%, or at least 23%, or at least 24%, or at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least 35%, or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45%, or at least 46%, or at least 47%, or at least 48%, or at least 49%, or at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55%, or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 61%, or at least 62%, or at least 63%, or at least 64%, or at least 65%, or at least 66%, or at least 67%, or at least 68%, or at least 69%, or at least 70%, or at least 71%, or at least 72%, or at least 73%, or at least 74%, or at least 75%, or at least 76%, or at least 77%, or at least 78%, or at least 79%, or at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or at least 99.5%, or at least 99.9%.

In some embodiments, the percent SPF content, by weight, in the tissue fillers described herein is at most 0.01%, or at most 0.1%, or at most 0.2%, or at most 0.3%, or at most 0.4%, or at most 0.5%, or at most 0.6%, or at most 0.7%, or at most 0.8%, or at most 0.9%, or at most 1%, or at most 2%, or at most 3%, or at most 4%, or at most 5%, or at most 6%, or at most 7%, or at most 8%, or at most 9%, or at most 10%, or at most 11%, or at most 12%, or at most 13%, or at most 14%, or at most 15%, or at most 16%, or at most 17%, or at most 18%, or at most 19%, or at most 20%, or at most 21%, or at most 22%, or at most 23%, or at most 24%, or at most 25%, or at most 26%, or at most 27%, or at most 28%, or at most 29%, or at most 30%, or at most 31%, or at most 32%,

or at most 33%, or at most 34%, or at most 35%, or at most 36%, or at most 37%, or at most 38%, or at most 39%, or at most 40%, or at most 41%, or at most 42%, or at most 43%, or at most 44%, or at most 45%, or at most 46%, or at most 47%, or at most 48%, or at most 49%, or at most 50%, or at most 51%, or at most 52%, or at most 53%, or at most 54%, or at most 55%, or at most 56%, or at most 57%, or at most 58%, or at most 59%, or at most 60%, or at most 61%, or at most 62%, or at most 63%, or at most 64%, or at most 65%, or at most 66%, or at most 67%, or at most 68%, or at most 69%, or at most 70%, or at most 71%, or at most 72%, or at most 73%, or at most 74%, or at most 75%, or at most 76%, or at most 77%, or at most 78%, or at most 79%, or at most 80%, or at most 81%, or at most 82%, or at most 83%, or at most 84%, or at most 85%, or at most 86%, or at most 87%, or at most 88%, or at most 89%, or at most 90%, or at most 91%, or at most 92%, or at most 93%, or at most 94%, or at most 95%, or at most 96%, or at most 97%, or at most 98%, or at most 99%, or at most 99.5%, or at most 99.9%.

In some embodiments, the percent SPF content, by weight, in the tissue fillers described herein is about 0.01%, or about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1%, or about 2%, or about 3%, or about 4%, or about 5%, or about 6%, or about 7%, or about 8%, or about 9%, or about 10%, or about 11%, or about 12%, or about 13%, or about 14%, or about 15%, or about 16%, or about 17%, or about 18%, or about 19%, or about 20%, or about 21%, or about 22%, or about 23%, or about 24%, or about 25%, or about 26%, or about 27%, or about 28%, or about 29%, or about 30%, or about 31%, or about 32%, or about 33%, or about 34%, or about 35%, or about 36%, or about 37%, or about 38%, or about 39%, or about 40%, or about 41%, or about 42%, or about 43%, or about 44%, or about 45%, or about 46%, or about 47%, or about 48%, or about 49%, or about 50%, or about 51%, or about 52%, or about 53%, or about 54%, or about 55%, or about 56%, or about 57%, or about 58%, or about 59%, or about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%,

or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%, or about 99.5%, or about 99.9%.

In some embodiments, the percent SPF content, by weight, in the tissue fillers described herein is between about 0.01% to about 100%, or about 0.01% to about 99.9%, or about 0.01% to about 75%; or between about 0.1% to about 95%, or about 1% to about 95%, or about 10% to about 95%; or between about 0.1% to about 1%, or about 0.1% to about 2%, or about 0.1% to about 3%, or about 0.1% to about 4%, or about 0.1% to about 5%, or about 0.1% to about 6%, or about 0.1% to about 7%, or about 0.1% to about 8%, or about 0.1% to about 9%, or about 0.1% to about 10%, or about 0.1% to about 11%, or about 0.1% to about 12%, or about 0.1% to about 13%, or about 0.1% to about 14%, or about 0.1% to about 15%, or about 0.1% to about 16%, or about 0.1% to about 17%, or about 0.1% to about 18%, or about 0.1% to about 19%, or about 0.1% to about 20%, or about 0.1% to about 21%, or about 0.1% to about 22%, or about 0.1% to about 23%, or about 0.1% to about 24%, or about 0.1% to about 25%; or between about 1% to about 2%, or about 1% to about 3%, or about 1% to about 4%, or about 1% to about 5%, or about 1% to about 6%, or about 1% to about 7%, or about 1% to about 8%, or about 1% to about 9%, or about 1% to about 10%, or about 1% to about 11%, or about 1% to about 12%, or about 1% to about 13%, or about 1% to about 14%, or about 1% to about 15%, or about 1% to about 16%, or about 1% to about 17%, or about 1% to about 18%, or about 1% to about 19%, or about 1% to about 20%, or about 1% to about 21%, or about 1% to about 22%, or about 1% to about 23%, or about 1% to about 24%, or about 1% to about 25%; or between about 10% to about 20%, or about 10% to about 25%, or about 10% to about 30%, or about 10% to about 35%, or about 10% to about 40%, or about 10% to about 45%, or about 10% to about 50%, or about 10% to about 55%, or about 10% to about 60%, or about 10% to about 65%, or about 10% to about 70%, or about 10% to about 75%, or about 10% to about 80%, or about 10% to about 85%, or about 10% to about 90%, or about 10% to about 100%.

The SPF described herein can have a variety of mechanical and physical properties depending on the degree of crystallinity of the SPF peptides and/or proteins. In an embodiment, an SPF composition of the present disclosure is not soluble in an aqueous solution due to the crystallinity of the protein. In an embodiment, an SPF

composition of the present disclosure is soluble in an aqueous solution. In an embodiment, the SPFs of a composition of the present disclosure include a crystalline portion of about two-thirds and an amorphous region of about one-third. In an embodiment, the SPFs of a composition of the present disclosure include a crystalline portion of about one-half and an amorphous region of about one-half. In an embodiment, the SPFs of a composition of the present disclosure include a 99% crystalline portion and a 1% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 95% crystalline portion and a 5% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 90% crystalline portion and a 10% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 85% crystalline portion and a 15% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 80% crystalline portion and a 20% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 75% crystalline portion and a 25% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 70% crystalline portion and a 30% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 65% crystalline portion and a 35% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 60% crystalline portion and a 40% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 50% crystalline portion and a 50% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 40% crystalline portion and a 60% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 35% crystalline portion and a 65% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 30% crystalline portion and a 70% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 25% crystalline portion and a 75% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 20% crystalline portion and a 80% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 15% crystalline portion and a 85%

amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 10% crystalline portion and a 90% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 5% crystalline portion and a 90% amorphous region. In an embodiment, the SPFs of a composition of the present disclosure include a 1% crystalline portion and a 99% amorphous region.

In some embodiments, the physical and mechanical properties of the SPF vary with the degree of presence in the SPF composition of α-helix and/or random coil regions. In some embodiments, an SPF hydrogel disclosed herein has a protein structure that is substantially-free of α-helix and random coil regions. In aspects of these embodiments, a hydrogel has a protein structure including, e.g., about 5% α-helix and random coil regions, about 10% α-helix and random coil regions, about 15% α-helix and random coil regions, about 20% α-helix and random coil regions, about 25% α-helix and random coil regions, about 30% α-helix and random coil regions, about 35% α-helix and random coil regions, about 40% α-helix and random coil regions, about 45% α-helix and random coil regions, or about 50% α-helix and random coil regions. In other aspects of these embodiments, a hydrogel has a protein structure including, e.g., at most 5% α-helix and random coil regions, at most 10% α-helix and random coil regions, at most 15% α-helix and random coil regions, at most 20% α-helix and random coil regions, at most 25% α-helix and random coil regions, at most 30% α-helix and random coil regions, at most 35% α-helix and random coil regions, at most 40% α-helix and random coil regions, at most 45% α-helix and random coil regions, or at most 50% α-helix and random coil regions. In yet other aspects of these embodiments, a hydrogel has a protein structure including, e.g., about 5% to about 10% α-helix and random coil regions, about 5% to about 15% α-helix and random coil regions, about 5% to about 20% α-helix and random coil regions, about 5% to about 25% α-helix and random coil regions, about 5% to about 30% α-helix and random coil regions, about 5% to about 40% α-helix and random coil regions, about 5% to about 50% α-helix and random coil regions, about 10% to about 20% α-helix and random coil regions, about 10% to about 30% α-helix and random coil regions, about 15% to about 25% α-helix and random coil regions, about 15% to about 30% α-helix and random coil regions, or about 15% to about 35% α-helix and random coil regions.

In some embodiments, SPF solution compositions of the present disclosure have shelf stability, i.e., they will not slowly or spontaneously gel when stored in an aqueous solution and there, without apparent aggregation of fragments and/or increase in molecular weight over time, from 10 days to 3 years depending on storage conditions, percent silk, and number of shipments and shipment conditions. Additionally, pH may be altered to extend shelf-life and/or support shipping conditions by preventing premature folding and aggregation of the silk. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 2 weeks at room temperature (RT). In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 4 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 6 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 8 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 10 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability for up to 12 weeks at RT. In an embodiment, a SPF solution composition of the present disclosure has a shelf stability ranging from about 4 weeks to about 52 weeks at RT. **Table 1** below shows shelf stability test results for embodiments of SPF compositions of the present disclosure.

Table 1. Shelf Stability of SPF Compositions of the Present Disclosure

% Silk	Temperature	Time to Gelation
2	RT	4 weeks
2	4 °C	>9 weeks
4	RT	4 weeks
4	4 °C	>9 weeks
6	RT	2 weeks
6	4 °C	>9 weeks

A known additive such as a vitamin (e.g., vitamin C) can be added to a SPF solution composition of the present disclosure to create a gel that is stable from 10 days to 3 years at room temperature (RT). Both examples, a SPF composition and the same with an additive, can be lyophilized for enhanced storage control ranging from 10 days to

10 years depending on storage and shipment conditions. The lyophilized silk powder can also be used as a raw ingredient in the medical, consumer, and electronic markets. Additionally, lyophilized silk powder can be resuspended in water, HFIP, or organic solution following storage to create silk solutions of varying concentrations, including higher concentration solutions than those produced initially. In another embodiment, the silk fibroin-based protein fragments are dried using a rototherm evaporator or other methods known in the art for creating a dry protein form containing less than 10% water by mass.

The SPFs used in the tissue fillers and methods disclosed herein can be manipulated and incorporated in various ways, for example in the form of a solution, which may be combined with other materials (e.g., HA) to prepare the tissue filler compositions described herein. Following are non-limiting examples of suitable ranges for various parameters in and for preparation of the silk solutions of the present disclosure. The silk solutions of the present disclosure may include one or more, but not necessarily all, of these parameters and may be prepared using various combinations of ranges of such parameters.

In an embodiment, the percent silk in the solution is less than 30%. In an embodiment, the percent silk in the solution is less than 25%. In an embodiment, the percent silk in the solution is less than 20%. In an embodiment, the percent silk in the solution is less than 19%. In an embodiment, the percent silk in the solution is less than 18%. In an embodiment, the percent silk in the solution is less than 17%. In an embodiment, the percent silk in the solution is less than 16%. In an embodiment, the percent silk in the solution is less than 15%. In an embodiment, the percent silk in the solution is less than 14%. In an embodiment, the percent silk in the solution is less than 13%. In an embodiment, the percent silk in the solution is less than 12%. In an embodiment, the percent silk in the solution is less than 11%. In an embodiment, the percent silk in the solution is less than 10%. In an embodiment, the percent silk in the solution is less than 9%. In an embodiment, the percent silk in the solution is less than 8%. In an embodiment, the percent silk in the solution is less than 7%. In an embodiment, the percent silk in the solution is less than 6%. In an embodiment, the percent silk in the solution is less than 5%. In an embodiment, the percent silk in the solution is less than

4%. In an embodiment, the percent silk in the solution is less than 3%. In an embodiment, the percent silk in the solution is less than 2%. In an embodiment, the percent silk in the solution is less than 1%. In an embodiment, the percent silk in the solution is less than 0.9%. In an embodiment, the percent silk in the solution is less than 0.8%. In an embodiment, the percent silk in the solution is less than 0.7%. In an embodiment, the percent silk in the solution is less than 0.6%. In an embodiment, the percent silk in the solution is less than 0.5%. In an embodiment, the percent silk in the solution is less than 0.4%. In an embodiment, the percent silk in the solution is less than 0.3%. In an embodiment, the percent silk in the solution is less than 0.2%. In an embodiment, the percent silk in the solution is less than 0.1%. In an embodiment, the percent silk in the solution is greater than 0.1%. In an embodiment, the percent silk in the solution is greater than 0.2%. In an embodiment, the percent silk in the solution is greater than 0.3%. In an embodiment, the percent silk in the solution is greater than 0.4%. In an embodiment, the percent silk in the solution is greater than 0.5%. In an embodiment, the percent silk in the solution is greater than 0.6%. In an embodiment, the percent silk in the solution is greater than 0.7%. In an embodiment, the percent silk in the solution is greater than 0.8%. In an embodiment, the percent silk in the solution is greater than 0.9%. In an embodiment, the percent silk in the solution is greater than 1%. In an embodiment, the percent silk in the solution is greater than 2%. In an embodiment, the percent silk in the solution is greater than 3%. In an embodiment, the percent silk in the solution is greater than 4%. In an embodiment, the percent silk in the solution is greater than 5%. In an embodiment, the percent silk in the solution is greater than 6%. In an embodiment, the percent silk in the solution is greater than 7%. In an embodiment, the percent silk in the solution is greater than 8%. In an embodiment, the percent silk in the solution is greater than 9%. In an embodiment, the percent silk in the solution is greater than 10%. In an embodiment, the percent silk in the solution is greater than 11%. In an embodiment, the percent silk in the solution is greater than 12%. In an embodiment, the percent silk in the solution is greater than 13%. In an embodiment, the percent silk in the solution is greater than 14%. In an embodiment, the percent silk in the solution is greater than 15%. In an embodiment, the percent silk in the solution is greater than 16%. In an embodiment, the percent silk in the solution is greater than 17%. In an embodiment, the percent silk in the solution is greater

than 18%. In an embodiment, the percent silk in the solution is greater than 19%. In an embodiment, the percent silk in the solution is greater than 20%. In an embodiment, the percent silk in the solution is greater than 25%. In an embodiment, the percent silk in the solution is between 0.1% and 30%. In an embodiment, the percent silk in the solution is between 0.1% and 25%. In an embodiment, the percent silk in the solution is between 0.1% and 20%. In an embodiment, the percent silk in the solution is between 0.1% and 15%. In an embodiment, the percent silk in the solution is between 0.1% and 10%. In an embodiment, the percent silk in the solution is between 0.1% and 9%. In an embodiment, the percent silk in the solution is between 0.1% and 8%. In an embodiment, the percent silk in the solution is between 0.1% and 7%. In an embodiment, the percent silk in the solution is between 0.1% and 6.5%. In an embodiment, the percent silk in the solution is between 0.1% and 6%. In an embodiment, the percent silk in the solution is between 0.1% and 5.5%. In an embodiment, the percent silk in the solution is between 0.1% and 5%. In an embodiment, the percent silk in the solution is between 0.1% and 4.5%. In an embodiment, the percent silk in the solution is between 0.1% and 4%. In an embodiment, the percent silk in the solution is between 0.1% and 3.5%. In an embodiment, the percent silk in the solution is between 0.1% and 3%. In an embodiment, the percent silk in the solution is between 0.1% and 2.5%. In an embodiment, the percent silk in the solution is between 0.1% and 2.0%. In an embodiment, the percent silk in the solution is between 0.1% and 2.4%. In an embodiment, the percent silk in the solution is between 0.5% and 5%. In an embodiment, the percent silk in the solution is between 0.5% and 4.5%. In an embodiment, the percent silk in the solution is between 0.5% and 4%. In an embodiment, the percent silk in the solution is between 0.5% and 3.5%. In an embodiment, the percent silk in the solution is between 0.5% and 3%. In an embodiment, the percent silk in the solution is between 0.5% and 2.5%. In an embodiment, the percent silk in the solution is between 1 and 4%. In an embodiment, the percent silk in the solution is between 1 and 3.5%. In an embodiment, the percent silk in the solution is between 1 and 3%. In an embodiment, the percent silk in the solution is between 1 and 2.5%. In an embodiment, the percent silk in the solution is between 1 and 2.4%. In an embodiment, the percent silk in the solution is between 1 and 2%. In an embodiment, the percent silk in the solution is between 20% and 30%. In an embodiment, the percent silk in the solution is between

0.1% and 6%. In an embodiment, the percent silk in the solution is between 6% and 10%. In an embodiment, the percent silk in the solution is between 6% and 8%. In an embodiment, the percent silk in the solution is between 6% and 9%. In an embodiment, the percent silk in the solution is between 10% and 20%. In an embodiment, the percent silk in the solution is between 11% and 19%. In an embodiment, the percent silk in the solution is between 12% and 18%. In an embodiment, the percent silk in the solution is between 13% and 17%. In an embodiment, the percent silk in the solution is between 14% and 16%.

In an embodiment, the silk compositions described herein may be combined with HA to form a tissue filler composition. In an embodiment, the percent silk in the tissue filler composition by weight is less than 30%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 25%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 20%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 19%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 18%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 17%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 16%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 15%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 14%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 13%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 12%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 11%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 10%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 9%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 8%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 7%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 6%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 5%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 4%. In an embodiment, the percent silk in the tissue filler composition by weight is less than

3%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 2%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 1%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.9%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.8%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.7%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.6%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.5%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.4%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.3%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.2%. In an embodiment, the percent silk in the tissue filler composition by weight is less than 0.1%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.1%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.2%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.3%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.4%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.5%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.6%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.7%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.8%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 0.9%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 1%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 2%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 3%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 4%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 5%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 6%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 7%. In an embodiment, the percent silk in the tissue filler composition by

weight is greater than 8%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 9%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 10%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 11%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 12%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 13%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 14%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 15%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 16%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 17%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 18%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 19%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 20%. In an embodiment, the percent silk in the tissue filler composition by weight is greater than 25%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 30%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 25%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 20%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 15%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 10%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 9%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 8%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 7%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 6.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 6%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 5.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 4.5%. In an embodiment, the percent silk in the tissue

filler composition by weight is between 0.1% and 4%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 3.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 3%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 2.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 2.0%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 2.4%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 4.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 4%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 3.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 3%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.5% and 2.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 4%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 3.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 3%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 2.5%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 2.4%. In an embodiment, the percent silk in the tissue filler composition by weight is between 1 and 2%. In an embodiment, the percent silk in the tissue filler composition by weight is between 20% and 30%. In an embodiment, the percent silk in the tissue filler composition by weight is between 0.1% and 6%. In an embodiment, the percent silk in the tissue filler composition by weight is between 6% and 10%. In an embodiment, the percent silk in the tissue filler composition by weight is between 6% and 8%. In an embodiment, the percent silk in the tissue filler composition by weight is between 6% and 9%. In an embodiment, the percent silk in the tissue filler composition by weight is between 10% and 20%. In an embodiment, the percent silk in the tissue filler composition by weight is between 11% and 19%. In an embodiment, the percent silk in the tissue filler composition by weight is between 12% and 18%. In an embodiment, the percent silk in the tissue filler composition by weight is

between 13% and 17%. In an embodiment, the percent silk in the tissue filler composition by weight is between 14% and 16%.

In an embodiment, the percent sericin in the solution or tissue filler composition is non-detectable to 30%. In an embodiment, the percent sericin in the solution or tissue filler composition is non-detectable to 5%. In an embodiment, the percent sericin in the solution or tissue filler composition is 1%. In an embodiment, the percent sericin in the solution or tissue filler composition is 2%. In an embodiment, the percent sericin in the solution or tissue filler composition is 3%. In an embodiment, the percent sericin in the solution or tissue filler composition is 4%. In an embodiment, the percent sericin in the solution or tissue filler composition is 5%. In an embodiment, the percent sericin in the solution or tissue filler composition is 10%. In an embodiment, the percent sericin in the solution or tissue filler composition is 30%.

In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 1 year. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 2 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 0 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 2 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 1 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 2 to 3 years. In an embodiment, the stability of the LiBr-silk fragment solution is 2 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 2 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 3 to 4 years. In an embodiment, the stability of the LiBr-silk fragment solution is 3 to 5 years. In an embodiment, the stability of the LiBr-silk fragment solution is 4 to 5 years.

In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 10 days to 6 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 6

months to 12 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 12 months to 18 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 18 months to 24 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 24 months to 30 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 30 months to 36 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 36 months to 48 months. In an embodiment, the stability of a silk-fibroin based protein fragment compositions that may be included in the tissue fillers of the present disclosure is 48 months to 60 months.

In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have having an average weight average molecular weight ranging from 1 kDa to 250 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have having an average weight average molecular weight ranging from 5 kDa to 150 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have having an average weight average molecular weight ranging from 1 kDa to 6 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 6 kDa to 17 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 17 kDa to 39 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 39 kDa to 80 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 80 kDa to 150 kDa.

In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging

from 1 kDa to 250 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 240 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 230 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 220 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 210 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 200 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 190 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 180 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 170 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 160 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 150 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 140 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 130 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 120 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa

to 110 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 100 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 90 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 80 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 70 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 60 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 50 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 40 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 30 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 20 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 kDa to 10 kDa.

In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 1 to 5 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 5 to 10 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 10 to 15 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average

weight average molecular weight ranging from 15 to 20 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 20 to 25 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 25 to 30 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 30 to 35 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 35 to 40 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 40 to 45 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 45 to 50 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 50 to 55 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 55 to 60 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 60 to 65 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 65 to 70 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 70 to 75 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 75 to 80 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 80 to 85 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 85 to 90

kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 90 to 95 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 95 to 100 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 100 to 105 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 105 to 110 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 110 to 115 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 115 to 120 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 120 to 125 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 125 to 130 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 130 to 135 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 135 to 140 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 140 to 145 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 145 to 150 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 150 to 155 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 155 to 160 kDa. In an embodiment, silk

fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 160 to 165 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 165 to 170 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 170 to 175 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 175 to 180 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 180 to 185 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 185 to 190 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 190 to 195 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 195 to 200 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 200 to 205 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have having an average weight average molecular weight ranging from 205 to 210 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 210 to 215 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 215 to 220 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 220 to 225 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 225 to 230 kDa. In an embodiment, silk

fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 230 to 235 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 235 to 240 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 240 to 245 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 245 to 250 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 250 to 255 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 255 to 260 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 260 to 265 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 265 to 270 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 270 to 275 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 275 to 280 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 280 to 285 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 285 to 290 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 290 to 295 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 295 to 300 kDa. In an embodiment, silk fibroin-based

protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 300 to 305 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 305 to 310 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 310 to 315 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 315 to 320 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 320 to 325 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 325 to 330 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 330 to 335 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 35 to 340 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 340 to 345 kDa. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have an average weight average molecular weight ranging from 345 to 350 kDa.

In an embodiment, the tissue fillers described herein may include silk protein comprising one or more of low molecular weight silk, medium molecular weight silk, and high molecular weight silk.

In an embodiment, the tissue fillers described herein may include silk protein comprising one or more of low molecular weight silk, medium molecular weight silk, and high molecular weight silk. In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk and medium molecular weight silk. In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk and high molecular weight silk. In an embodiment,

the tissue fillers described herein may include silk protein comprising medium molecular weight silk and high molecular weight silk. In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk, medium molecular weight silk, and high molecular weight silk.

In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk and medium molecular weight silk. In some embodiments, the w/w ratio between low molecular weight silk and medium molecular weight silk is between about 99:1 to about 1:99, between about 95:5 to about 5:95, between about 90:10 to about 10:90, between about 75:25 to about 25:75, between about 65:35 to about 35:65, or between about 55:45 to about 45:55. In some embodiments, the w/w ratio between low molecular weight silk and medium molecular weight silk is between about 99:1 to about 55:45, between about 95:5 to about 45:55, between about 90:10 to about 35:65, between about 75:25 to about 15:85, between about 65:35 to about 10:90, or between about 55:45 to about 1:99. In an embodiment, the w/w ratio between low molecular weight silk and medium molecular weight silk is about 99:1, about 98:2, about 97:3, about 96:4, about 95:5, about 94:6, about 93:7, about 92:8, about 91:9, about 90:10, about 89:11, about 88:12, about 87:13, about 86:14, about 85:15, about 84:16, about 83:17, about 82:18, about 81:19, about 80:20, about 79:21, about 78:22, about 77:23, about 76:24, about 75:25, about 74:26, about 73:27, about 72:28, about 71:29, about 70:30, about 69:31, about 68:32, about 67:33, about 66:34, about 65:35, about 64:36, about 63:37, about 62:38, about 61:39, about 60:40, about 59:41, about 58:42, about 57:43, about 56:44, about 55:45, about 54:46, about 53:47, about 52:48, about 51:49, about 50:50, about 49:51, about 48:52, about 47:53, about 46:54, about 45:55, about 44:56, about 43:57, about 42:58, about 41:59, about 40:60, about 39:61, about 38:62, about 37:63, about 36:64, about 35:65, about 34:66, about 33:67, about 32:68, about 31:69, about 30:70, about 29:71, about 28:72, about 27:73, about 26:74, about 25:75, about 24:76, about 23:77, about 22:78, about 21:79, about 20:80, about 19:81, about 18:82, about 17:83, about 16:84, about 15:85, about 14:86, about 13:87, about 12:88, about 11:89, about 10:90, about 9:91, about 8:92, about 7:93, about 6:94, about 5:95, about 4:96, about 3:97, about 2:98, or about 1:99. In an embodiment, the w/w ratio between low molecular weight silk and medium molecular weight silk is about 9:1, about

8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1. In an embodiment, the w/w ratio between low molecular weight silk and medium molecular weight silk is about 1:9, about 1:8, about 1:7, about 1:6, about 1:5, about 1:4, about 1:3, about 1:2, or about 1:1.

In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk and high molecular weight silk. In some embodiments, the w/w ratio between low molecular weight silk and high molecular weight silk is between about 99:1 to about 1:99, between about 95:5 to about 5:95, between about 90:10 to about 10:90, between about 75:25 to about 25:75, between about 65:35 to about 35:65, or between about 55:45 to about 45:55. In some embodiments, the w/w ratio between low molecular weight silk and high molecular weight silk is between about 99:1 to about 55:45, between about 95:5 to about 45:55, between about 90:10 to about 35:65, between about 75:25 to about 15:85, between about 65:35 to about 10:90, or between about 55:45 to about 1:99. In an embodiment, the w/w ratio between low molecular weight silk and high molecular weight silk is about 99:1, about 98:2, about 97:3, about 96:4, about 95:5, about 94:6, about 93:7, about 92:8, about 91:9, about 90:10, about 89:11, about 88:12, about 87:13, about 86:14, about 85:15, about 84:16, about 83:17, about 82:18, about 81:19, about 80:20, about 79:21, about 78:22, about 77:23, about 76:24, about 75:25, about 74:26, about 73:27, about 72:28, about 71:29, about 70:30, about 69:31, about 68:32, about 67:33, about 66:34, about 65:35, about 64:36, about 63:37, about 62:38, about 61:39, about 60:40, about 59:41, about 58:42, about 57:43, about 56:44, about 55:45, about 54:46, about 53:47, about 52:48, about 51:49, about 50:50, about 49:51, about 48:52, about 47:53, about 46:54, about 45:55, about 44:56, about 43:57, about 42:58, about 41:59, about 40:60, about 39:61, about 38:62, about 37:63, about 36:64, about 35:65, about 34:66, about 33:67, about 32:68, about 31:69, about 30:70, about 29:71, about 28:72, about 27:73, about 26:74, about 25:75, about 24:76, about 23:77, about 22:78, about 21:79, about 20:80, about 19:81, about 18:82, about 17:83, about 16:84, about 15:85, about 14:86, about 13:87, about 12:88, about 11:89, about 10:90, about 9:91, about 8:92, about 7:93, about 6:94, about 5:95, about 4:96, about 3:97, about 2:98, or about 1:99.

In an embodiment, the tissue fillers described herein may include silk protein comprising medium molecular weight silk and high molecular weight silk. In some embodiments, the w/w ratio between medium molecular weight silk and high molecular weight silk is between about 99:1 to about 1:99, between about 95:5 to about 5:95, between about 90:10 to about 10:90, between about 75:25 to about 25:75, between about 65:35 to about 35:65, or between about 55:45 to about 45:55. In some embodiments, the w/w ratio between medium molecular weight silk and high molecular weight silk is between about 99:1 to about 55:45, between about 95:5 to about 45:55, between about 90:10 to about 35:65, between about 75:25 to about 15:85, between about 65:35 to about 10:90, or between about 55:45 to about 1:99. In an embodiment, the w/w ratio between medium molecular weight silk and high molecular weight silk is about 99:1, about 98:2, about 97:3, about 96:4, about 95:5, about 94:6, about 93:7, about 92:8, about 91:9, about 90:10, about 89:11, about 88:12, about 87:13, about 86:14, about 85:15, about 84:16, about 83:17, about 82:18, about 81:19, about 80:20, about 79:21, about 78:22, about 77:23, about 76:24, about 75:25, about 74:26, about 73:27, about 72:28, about 71:29, about 70:30, about 69:31, about 68:32, about 67:33, about 66:34, about 65:35, about 64:36, about 63:37, about 62:38, about 61:39, about 60:40, about 59:41, about 58:42, about 57:43, about 56:44, about 55:45, about 54:46, about 53:47, about 52:48, about 51:49, about 50:50, about 49:51, about 48:52, about 47:53, about 46:54, about 45:55, about 44:56, about 43:57, about 42:58, about 41:59, about 40:60, about 39:61, about 38:62, about 37:63, about 36:64, about 35:65, about 34:66, about 33:67, about 32:68, about 31:69, about 30:70, about 29:71, about 28:72, about 27:73, about 26:74, about 25:75, about 24:76, about 23:77, about 22:78, about 21:79, about 20:80, about 19:81, about 18:82, about 17:83, about 16:84, about 15:85, about 14:86, about 13:87, about 12:88, about 11:89, about 10:90, about 9:91, about 8:92, about 7:93, about 6:94, about 5:95, about 4:96, about 3:97, about 2:98, or about 1:99.

In an embodiment, the tissue fillers described herein may include silk protein comprising low molecular weight silk, medium molecular weight silk, and high molecular weight silk. In an embodiment, the w/w ratio between low molecular weight silk, medium molecular weight silk, and high molecular weight silk is about 1:1:8, 1:2:7, 1:3:6, 1:4:5, 1:5:4, 1:6:3, 1:7:2, 1:8:1, 2:1:7, 2:2:6, 2:3:5, 2:4:4, 2:5:3, 2:6:2, 2:7:1, 3:1:6,

3:2:5, 3:3:4, 3:4:3, 3:5:2, 3:6:1, 4:1:5, 4:2:4, 4:3:3, 4:4:2, 4:5:1, 5:1:4, 5:2:3, 5:3:2, 5:4:1, 6:1:3, 6:2:2, 6:3:1, 7:1:2, 7:2:1, or 8:1:1. In an embodiment, the w/w ratio between low molecular weight silk, medium molecular weight silk, and high molecular weight silk is about 3:0.1:0.9, 3:0.2:0.8, 3:0.3:0.7, 3:0.4:0.6, 3:0.5:0.5, 3:0.6:0.4, 3:0.7:0.3, 3:0.8:0.2, or 3:0.9:0.1.

In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have a polydispersity ranging from about 1 to about 5.0. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have a polydispersity ranging from about 1.5 to about 3.0. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have a polydispersity ranging from about 1 to about 1.5. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have a polydispersity ranging from about 1.5 to about 2.0. In an embodiment, silk fibroin-based protein fragments incorporated into the tissue fillers described herein have a polydispersity ranging from about 2.0 to about 2.5. In an embodiment, a composition of the present disclosure having pure silk fibroin-based protein fragments, has a polydispersity ranging from about is 2.0 to about 3.0. In an embodiment, a composition of the present disclosure having pure silk fibroin-based protein fragments, has a polydispersity ranging from about is 2.5 to about 3.0.

In an embodiment, a tissue filler described herein that includes SPF has non-detectable levels of LiBr residuals. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is between 10 ppm and 1000 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is between 10 ppm and 300 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 25 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 50 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 75 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 100 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 200 ppm. In an embodiment, the amount

of the LiBr residuals in a tissue filler described herein that includes SPF is less than 300 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 400 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 500 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 600 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 700 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 800 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 900 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is less than 1000 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 500 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 450 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 400 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 350 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 300 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 250 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 200 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 150 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is non-detectable to 100 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is 100 ppm to 200 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is 200 ppm to 300 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is 300 ppm to 400 ppm. In an embodiment, the amount of the LiBr residuals in a tissue filler described herein that includes SPF is 400 ppm to 500 ppm.

In an embodiment, a tissue filler described herein that includes SPF having pure silk fibroin-based protein fragments, has non-detectable levels of Na_2CO_3 residuals. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 100 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 500 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 600 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 700 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 800 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 900 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is less than 1000 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 500 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 450 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 350 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 250 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 150 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is non-detectable to 100

ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is 100 ppm to 200 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is 200 ppm to 300 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is 300 ppm to 400 ppm. In an embodiment, the amount of the Na_2CO_3 residuals in a tissue filler described herein that includes SPF is 400 ppm to 500 ppm.

In an embodiment, the water solubility of pure silk fibroin-based protein fragments of the present disclosure is 50 to 100%. In an embodiment, the water solubility of pure silk fibroin-based protein fragments of the present disclosure is 60 to 100%. In an embodiment, the water solubility of pure silk fibroin-based protein fragments of the present disclosure is 70 to 100%. In an embodiment, the water solubility of pure silk fibroin-based protein fragments of the present disclosure is 80 to 100%. In an embodiment, the water solubility is 90 to 100%. In an embodiment, the silk fibroin-based fragments of the present disclosure are non-soluble in aqueous solutions.

In an embodiment, the solubility of pure silk fibroin-based protein fragments of the present disclosure in organic solutions is 50 to 100%. In an embodiment, the solubility of pure silk fibroin-based protein fragments of the present disclosure in organic solutions is 60 to 100%. In an embodiment, the solubility of pure silk fibroin-based protein fragments of the present disclosure in organic solutions is 70 to 100%. In an embodiment, the solubility of pure silk fibroin-based protein fragments of the present disclosure in organic solutions is 80 to 100%. In an embodiment, the solubility of pure silk fibroin-based protein fragments of the present disclosure in organic solutions is 90 to 100%. In an embodiment, the silk fibroin-based fragments of the present disclosure are non-soluble in organic solutions.

Methods of making silk protein fragments used in the compositions of the present disclosure are demonstrated in U.S. Patent Application Publication Nos. 2015/00933340, 2015/0094269, 2016/0193130, 2016/0022560, 2016/0022561, 2016/0022562, 2016/0022563, and 2016/0222579, 2016/0281294, and U.S. Patent Nos. 9,187,538, 9,522,107, 9,517,191, 9,522,108, 9,511,012, and 9,545,369, the entirety of which are incorporated herein by reference. However, an exemplary method is demonstrated in Fig.

1, which is a flow chart showing various embodiments for producing pure silk fibroin-based protein fragments (SPFs) of the present disclosure. It should be understood that not all of the steps illustrated are necessarily required to fabricate all silk solutions of the present disclosure. As illustrated in Fig. 1, step A, cocoons (heat-treated or non-heat-treated), silk fibers, silk powder or spider silk can be used as the silk source. If starting from raw silk cocoons from *Bombyx mori*, the cocoons can be cut into small pieces, for example pieces of approximately equal size, step B1. The raw silk is then extracted and rinsed to remove any sericin, step C1a. This results in substantially sericin free raw silk. In an embodiment, water is heated to a temperature between 84 °C and 100 °C (ideally boiling) and then Na_2CO_3 (sodium carbonate) is added to the boiling water until the Na_2CO_3 is completely dissolved. The raw silk is added to the boiling water/Na_2CO_3 (100 °C) and submerged for approximately 15 - 90 minutes, where boiling for a longer time results in smaller silk protein fragments. In an embodiment, the water volume equals about 0.4 x raw silk weight and the Na_2CO_3 volume equals about 0.848 x raw silk weight. In an embodiment, the water volume equals 0.1 x raw silk weight and the Na_2CO_3 volume is maintained at 2.12 g/L. This is demonstrated in Fig. 6 and Fig. 7: silk mass (x-axis) was varied in the same volume of extraction solution (i.e., the same volume of water and concentration of Na_2CO_3) achieving sericin removal (substantially sericin free) as demonstrated by an overall silk mass loss of 26 to 31 percent (y-axis). Subsequently, the water dissolved Na_2CO_3 solution is drained and excess water/Na_2CO_3 is removed from the silk fibroin fibers (e.g., ring out the fibroin extract by hand, spin cycle using a machine, etc.). The resulting silk fibroin extract is rinsed with warm to hot water to remove any remaining adsorbed sericin or contaminate, typically at a temperature range of about 40 °C to about 80 °C, changing the volume of water at least once (repeated for as many times as required). The resulting silk fibroin extract is a substantially sericin-depleted silk fibroin. In an embodiment, the resulting silk fibroin extract is rinsed with water at a temperature of about 60 °C. In an embodiment, the volume of rinse water for each cycle equals 0.1 L to 0.2 L x raw silk weight. It may be advantageous to agitate, turn or circulate the rinse water to maximize the rinse effect. After rinsing, excess water is removed from the extracted silk fibroin fibers (e.g., ring out fibroin extract by hand or using a machine). Alternatively, methods known to one skilled in the art such as pressure,

temperature, or other reagents or combinations thereof may be used for the purpose of sericin extraction. Alternatively, the silk gland (100% sericin free silk protein) can be removed directly from a worm. This would result in liquid silk protein, without any alteration of the protein structure, free of sericin.

The extracted fibroin fibers are then allowed to dry completely. Once dry, the extracted silk fibroin is dissolved using a solvent added to the silk fibroin at a temperature between ambient and boiling, step C1b. In an embodiment, the solvent is a solution of Lithium bromide (LiBr) (boiling for LiBr is 140 °C). Alternatively, the extracted fibroin fibers are not dried but wet and placed in the solvent; solvent concentration can then be varied to achieve similar concentrations as to when adding dried silk to the solvent. The final concentration of LiBr solvent can range from 0.1 M to 9.3 M. Fig. 8 is a table summarizing the Molecular Weights of silk dissolved from different concentrations of Lithium Bromide (LiBr) and from different extraction and dissolution sizes. Complete dissolution of the extracted fibroin fibers can be achieved by varying the treatment time and temperature along with the concentration of dissolving solvent. Other solvents may be used including, but not limited to, phosphate phosphoric acid, calcium nitrate, calcium chloride solution or other concentrated aqueous solutions of inorganic salts. To ensure complete dissolution, the silk fibers should be fully immersed within the already heated solvent solution and then maintained at a temperature ranging from about 60 °C to about 140 °C for 1-168 hrs. In an embodiment, the silk fibers should be fully immersed within the solvent solution and then placed into a dry oven at a temperature of about 100 °C for about 1 hour.

The temperature at which the silk fibroin extract is added to the LiBr solution (or vice versa) has an effect on the time required to completely dissolve the fibroin and on the resulting molecular weight and polydispersity of the final SPF mixture solution. In an embodiment, silk solvent solution concentration is less than or equal to 20% w/v. In addition, agitation during introduction or dissolution may be used to facilitate dissolution at varying temperatures and concentrations. The temperature of the LiBr solution will provide control over the silk protein fragment mixture molecular weight and polydispersity created. In an embodiment, a higher temperature will more quickly dissolve the silk offering enhanced process scalability and mass production of silk

solution. In an embodiment, using a LiBr solution heated to a temperature between 80 °C - 140 °C reduces the time required in an oven in order to achieve full dissolution. Varying time and temperature at or above 60 °C of the dissolution solvent will alter and control the MW and polydispersity of the SPF mixture solutions formed from the original molecular weight of the native silk fibroin protein.

Alternatively, whole cocoons may be placed directly into a solvent, such as LiBr, bypassing extraction, step B2. This requires subsequent filtration of silk worm particles from the silk and solvent solution and sericin removal using methods know in the art for separating hydrophobic and hydrophilic proteins such as a column separation and/or chromatography, ion exchange, chemical precipitation with salt and/or pH, and or enzymatic digestion and filtration or extraction, all methods are common examples and without limitation for standard protein separation methods, step C2. Non-heat treated cocoons with the silkworm removed, may alternatively be placed into a solvent such as LiBr, bypassing extraction. The methods described above may be used for sericin separation, with the advantage that non-heat treated cocoons will contain significantly less worm debris.

Dialysis may be used to remove the dissolution solvent from the resulting dissolved fibroin protein fragment solution by dialyzing the solution against a volume of water, step E1. Pre-filtration prior to dialysis is helpful to remove any debris (i.e., silk worm remnants) from the silk and LiBr solution, step D. In one example, a 3 μm or 5 μm filter is used with a flow-rate of 200-300 mL/min to filter a 0.1% to 1.0% silk-LiBr solution prior to dialysis and potential concentration if desired. A method disclosed herein, as described above, is to use time and/or temperature to decrease the concentration from 9.3 M LiBr to a range from 0.1 M to 9.3 M to facilitate filtration and downstream dialysis, particularly when considering creating a scalable process method. Alternatively, without the use of additional time or temperate, a 9.3 M LiBr-silk protein fragment solution may be diluted with water to facilitate debris filtration and dialysis. The result of dissolution at the desired time and temperate filtration is a translucent particle-free room temperature shelf-stable silk protein fragment-LiBr solution of a known MW and polydispersity. It is advantageous to change the dialysis water regularly until the solvent has been removed (e.g., change water after 1 hour, 4 hours, and then

every 12 hours for a total of 6 water changes). The total number of water volume changes may be varied based on the resulting concentration of solvent used for silk protein dissolution and fragmentation. After dialysis, the final silk solution maybe further filtered to remove any remaining debris (i.e., silk worm remnants).

Alternatively, Tangential Flow Filtration (TFF), which is a rapid and efficient method for the separation and purification of biomolecules, may be used to remove the solvent from the resulting dissolved fibroin solution, step E2. TFF offers a highly pure aqueous silk protein fragment solution and enables scalability of the process in order to produce large volumes of the solution in a controlled and repeatable manner. The silk and LiBr solution may be diluted prior to TFF (20% down to 0.1% silk in either water or LiBr). Pre-filtration as described above prior to TFF processing may maintain filter efficiency and potentially avoids the creation of silk gel boundary layers on the filter's surface as the result of the presence of debris particles. Pre-filtration prior to TFF is also helpful to remove any remaining debris (i.e., silk worm remnants) from the silk and LiBr solution that may cause spontaneous or long-term gelation of the resulting water only solution, step D. TFF, recirculating or single pass, may be used for the creation of water-silk protein fragment solutions ranging from 0.1% silk to 30.0% silk (more preferably, 0.1% - 6.0% silk). Different cutoff size TFF membranes may be required based upon the desired concentration, molecular weight and polydispersity of the silk protein fragment mixture in solution. Membranes ranging from 1-100 kDa may be necessary for varying molecular weight silk solutions created for example by varying the length of extraction boil time or the time and temperate in dissolution solvent (e.g., LiBr). In an embodiment, a TFF 5 or 10 kDa membrane is used to purify the silk protein fragment mixture solution and to create the final desired silk-to-water ratio. As well, TFF single pass, TFF, and other methods known in the art, such as a falling film evaporator, may be used to concentrate the solution following removal of the dissolution solvent (e.g., LiBr) (with resulting desired concentration ranging from 0.1% to 30% silk). This can be used as an alternative to standard HFIP concentration methods known in the art to create a water-based solution. A larger pore membrane could also be utilized to filter out small silk protein fragments and to create a solution of higher molecular weight silk with and/or without tighter polydispersity values. Fig. 5 is a table summarizing Molecular Weights

for some embodiments of silk protein solutions of the present disclosure. Silk protein solution processing conditions were as follows: 100 °C extraction for 20 min, room temperature rinse, LiBr in 60 °C oven for 4-6 hours. TFF processing conditions for water-soluble films were as follows: 100 °C extraction for 60 min, 60 °C rinse, 100 °C LiBr in 100 °C oven for 60 min. Figs. 12-23 further demonstrate manipulation of extraction time, LiBr dissolution conditions, and TFF processing and resultant example molecular weights and polydispersities. These examples are not intended to be limiting, but rather to demonstrate the potential of specifying parameters for specific molecular weight silk fragment solutions.

An assay for LiBr and Na_2CO_3 detection was performed using an HPLC system equipped with evaporative light scattering detector (ELSD). The calculation was performed by linear regression of the resulting peak areas for the analyte plotted against concentration. More than one sample of a number of formulations of the present disclosure was used for sample preparation and analysis. Generally, four samples of different formulations were weighed directly in a 10 mL volumetric flask. The samples were suspended in 5 mL of 20 mM ammonium formate (pH 3.0) and kept at 2-8 °C for 2 hours with occasional shaking to extract analytes from the film. After 2 hours the solution was diluted with 20 mM ammonium formate (pH 3.0). The sample solution from the volumetric flask was transferred into HPLC vials and injected into the HPLC-ELSD system for the estimation of sodium carbonate and lithium bromide.

The analytical method developed for the quantitation of Na_2CO_3 and LiBr in silk protein formulations was found to be linear in the range 10 - 165 μg/mL, with RSD for injection precision as 2% and 1% for area and 0.38% and 0.19% for retention time for sodium carbonate and lithium bromide respectively. The analytical method can be applied for the quantitative determination of sodium carbonate and lithium bromide in silk protein formulations.

The final silk protein fragment solution is pure silk protein fragments and water with PPM to undetectable levels of particulate debris and/or process contaminants, including LiBr and Na_2CO_3. Fig. 3 and Fig. 4 are tables summarizing LiBr and Na_2CO_3 concentrations in solutions of the present disclosure. In Fig. 3, the processing conditions included 100 °C extraction for 60 min, 60 °C rinse, 100 °C LiBr in 100 °C oven for 60

min. TFF conditions including pressure differential and number of dia-filtration volumes were varied. In Fig. 4, the processing conditions included 100 °C boil for 60 min, 60 °C rinse, LiBr in 60 °C oven for 4-6 hours.

Either the silk fragment-water solutions, the lyophilized silk protein fragment mixture, or any other compositions including SPFs, can be sterilized following standard methods in the art not limited to filtration, heat, radiation or e-beam. It is anticipated that the silk protein fragment mixture, because of its shorter protein polymer length, will withstand sterilization better than intact silk protein solutions described in the art. Additionally, silk articles created from the SPF mixtures described herein may be sterilized as appropriate to application. For example, an SPF tissue and/or dermal filler loaded with a molecule to be used in medical applications with an open wound/incision, may be sterilized standard methods such as by radiation or e-beam.

Fig. 2 is a flow chart showing various parameters that can be modified during the process of producing a silk protein fragment solution of the present disclosure during the extraction and the dissolution steps. Select method parameters may be altered to achieve distinct final solution characteristics depending upon the intended use, e.g., molecular weight and polydispersity. It should be understood that not all of the steps illustrated are necessarily required to fabricate all silk solutions of the present disclosure.

In an embodiment, a process for producing a silk protein fragment solution of the present disclosure includes forming pieces of silk cocoons from the *Bombyx mori* silk worm; extracting the pieces at about 100 °C in a solution of water and Na_2CO_3 for about 60 minutes, wherein a volume of the water equals about 0.4 x raw silk weight and the amount of Na_2CO_3 is about 0.848 x the weight of the pieces to form a silk fibroin extract; triple rinsing the silk fibroin extract at about 60 °C for about 20 minutes per rinse in a volume of rinse water, wherein the rinse water for each cycle equals about 0.2 L x the weight of the pieces; removing excess water from the silk fibroin extract; drying the silk fibroin extract; dissolving the dry silk fibroin extract in a LiBr solution, wherein the LiBr solution is first heated to about 100 °C to create a silk and LiBr solution and maintained; placing the silk and LiBr solution in a dry oven at about 100 °C for about 60 minutes to achieve complete dissolution and further fragmentation of the native silk protein structure into mixture with desired molecular weight and polydispersity; filtering the solution to

remove any remaining debris from the silkworm; diluting the solution with water to result in a 1% silk solution; and removing solvent from the solution using Tangential Flow Filtration (TFF). In an embodiment, a 10 kDa membrane is utilized to purify the silk solution and create the final desired silk-to-water ratio. TFF can then be used to further concentrate the pure silk solution to a concentration of 2% silk to water.

Each process step from raw cocoons to dialysis is scalable to increase efficiency in manufacturing. Whole cocoons are currently purchased as the raw material, but pre-cleaned cocoons or non-heat treated cocoons, where worm removal leaves minimal debris, have also been used. Cutting and cleaning the cocoons is a manual process, however for scalability this process could be made less labor intensive by, for example, using an automated machine in combination with compressed air to remove the worm and any particulates, or using a cutting mill to cut the cocoons into smaller pieces. The extraction step, currently performed in small batches, could be completed in a larger vessel, for example an industrial washing machine where temperatures at or in between 60 °C to 100 °C can be maintained. The rinsing step could also be completed in the industrial washing machine, eliminating the manual rinse cycles. Dissolution of the silk in LiBr solution could occur in a vessel other than a convection oven, for example a stirred tank reactor. Dialyzing the silk through a series of water changes is a manual and time intensive process, which could be accelerated by changing certain parameters, for example diluting the silk solution prior to dialysis. The dialysis process could be scaled for manufacturing by using semi-automated equipment, for example a tangential flow filtration system.

Varying extraction (i.e., time and temperature), LiBr (i.e., temperature of LiBr solution when added to silk fibroin extract or vice versa) and dissolution (i.e., time and temperature) parameters results in solvent and silk solutions with different viscosities, homogeneities, and colors. Increasing the temperature for extraction, lengthening the extraction time, using a higher temperature LiBr solution at emersion and over time when dissolving the silk and increasing the time at temperature (e.g., in an oven as shown here, or an alternative heat source) all resulted in less viscous and more homogeneous solvent and silk solutions. While almost all parameters resulted in a viable silk solution, methods

that allow complete dissolution to be achieved in fewer than 4 to 6 hours are preferred for process scalability.

Molecular weight of the silk protein fragments may be controlled based upon the specific parameters utilized during the extraction step, including extraction time and temperature; specific parameters utilized during the dissolution step, including the LiBr temperature at the time of submersion of the silk in to the lithium bromide and time that the solution is maintained at specific temperatures; and specific parameters utilized during the filtration step. By controlling process parameters using the disclosed methods, it is possible to create SPF mixture solutions with polydispersity equal to or lower than 2.5 at a variety of different molecular weight ranging from 1 kDa to 250 kDa, 5 kDa to 200 kDa, 5 kDa to 150 kDa, 10 kDa to 150 kDa, or 10 kDa to 80 kDa. By altering process parameters to achieve silk solutions with different molecular weights, a range of fragment mixture end products, with desired polydispersity of equal to or less than 2.5 may be targeted based upon the desired performance requirements. For example, a lower molecular weight silk film containing a drug may have a faster release rate compared to a higher molecular weight SPF preparation. Additionally, SPF mixture solutions with a polydispersity of greater than 2.5 can be achieved. Further, two solutions with different average molecular weights and polydispersities can be mixed to create combination solutions. Alternatively, a liquid silk gland (100% sericin free silk protein) that has been removed directly from a worm could be used in combination with any of the SPF mixture solutions of the present disclosure. Molecular weight of the pure silk fibroin-based protein fragment composition was determined using High Pressure Liquid Chromatography (HPLC) with a Refractive Index Detector (RID). Polydispersity was calculated using Cirrus GPC Online GPC/SEC Software Version 3.3 (Agilent).

Parameters were varied during the processing of raw silk cocoons into silk solution. Varying these parameters affected the MW of the resulting silk solution. Parameters manipulated included (i) time and temperature of extraction, (ii) temperature of LiBr, (iii) temperature of dissolution oven, and (iv) dissolution time. Molecular weight was determined with mass spec as shown in Figs. 9-25.

Experiments were carried out to determine the effect of varying the extraction time. Figs. 9-15 are graphs showing these results, and Tables 2-8 summarize the results. Below is a summary:

- A sericin extraction time of 30 minutes resulted in larger MW than a sericin extraction time of 60 minutes
- MW decreases with time in the oven
- 140 °C LiBr and oven resulted in the low end of the confidence interval to be below a MW of 9500 Da
- 30 min extraction at the 1 hour and 4 hour time points have undigested silk
- 30 min extraction at the 1 hour time point resulted in a significantly high molecular weight with the low end of the confidence interval being 35,000 Da
- The range of MW reached for the high end of the confidence interval was 18000 to 216000 Da (important for offering solutions with specified upper limit)

Table 2. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 100 °C Lithium Bromide (LiBr) and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30	1	57247	12780	35093	93387	1.63
60	1	31520	1387	11633	85407	2.71
30	4	40973	2632	14268	117658	2.87
60	4	25082	1248	10520	59803	2.38
30	6	25604	1405	10252	63943	2.50
60	6	20980	1262	10073	43695	2.08

Table 3. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, boiling Lithium Bromide (LiBr) and 60 °C Oven Dissolution for 4 hr.

Sample	Boil Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 4 hr	30	49656	4580	17306	142478	2.87
60 min, 4 hr	60	30042	1536	11183	80705	2.69

Table 4. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 °C Lithium Bromide (LiBr) and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 1 hr	30	1	58436		22201	153809	2.63
60 min, 1 hr	60	1	31700		11931	84224	2.66
30 min, 4 hr	30	4	61956.5	13337	21463	178847	2.89
60 min, 4 hr	60	4	25578.5	2446	9979	65564	2.56

Table 5. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C Lithium Bromide (LiBr) and 80 °C Oven Dissolution for 6 hr.

Sample	Boil Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 6 hr	30	63510		18693	215775	3.40
60 min, 6 hr	60	25164	238	9637	65706	2.61

Table 6. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 80 °C Lithium Bromide (LiBr) and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 4 hr	30	4	59202	14028	19073	183760	3.10
60 min, 4 hr	60	4	26312.5	637	10266	67442	2.56
30 min, 6 hr	30	6	46824		18076	121293	2.59
60 min, 6 hr	60	6	26353		10168	68302	2.59

Table 7. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 100 °C Lithium Bromide (LiBr) and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 4 hr	30	4	47853		19758	115900	2.42
60 min, 4 hr	60	4	25082	1248	10520	59804	2.38
30 min, 6 hr	30	6	55421	8992	19153	160366	2.89
60 min, 6 hr	60	6	20980	1262	10073	43694	2.08

Table 8. The effect of extraction time (30 min vs 60 min) on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 140 °C Lithium Bromide (LiBr) and 140 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30 min, 4 hr	30	4	9024.5	1102	4493	18127	2.00865
60 min, 4 hr	60	4	15548		6954	34762	2.2358
30 min, 6 hr	30	6	13021		5987	28319	2.1749
60 min, 6 hr	60	6	10888		5364	22100	2.0298

Experiments were carried out to determine the effect of varying the extraction temperature. **Fig. 16** is a graph showing these results, and **Table 9** summarizes the results. Below is a summary:

- Sericin extraction at 90 °C resulted in higher MW than sericin extraction at 100 °C extraction
- Both 90 °C and 100 °C show decreasing MW over time in the oven

Table 9. The effect of extraction temperature (90 °C vs. 100 °C) on molecular weight of silk processed under the conditions of 60 min. Extraction Temperature, 100 °C Lithium Bromide (LiBr) and 100 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	Boil Time	Oven Time	Average Mw	Standard deviation	Confidence Interval	PD

90 °C, 4 hr	60	4	37308	4204	13368	104119	2.79
100 °C, 4 hr	60	4	25082	1248	10520	59804	2.38
90 °C, 6 hr	60	6	34224	1135	12717	92100	2.69
100 °C, 6 hr	60	6	20980	1262	10073	43694	2.08

Experiments were carried out to determine the effect of varying the Lithium Bromide (LiBr) temperature when added to silk. **Figs. 17-18** are graphs showing these results, and **Tables 10-11** summarize the results. Below is a summary:

- No impact on MW or confidence interval (all CI ~10500-6500 Da)
- Studies illustrated that the temperature of LiBr-silk dissolution, as LiBr is added and begins dissolving, rapidly drops below the original LiBr temperature due to the majority of the mass being silk at room temp

Table 10. The effect of Lithium Bromide (LiBr) temperature on molecular weight of silk processed under the conditions of 60 min. Extraction Time., 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	LiBr Temp (°C)	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
60 °C LiBr, 1 hr	60	1	31700		11931	84223	2.66
100 °C LiBr, 1 hr	100	1	27907	200	10735	72552	2.60
RT LiBr, 4 hr	RT	4	29217	1082	10789	79119	2.71
60 °C LiBr, 4 hr	60	4	25578	2445	9978	65564	2.56
80 °C LiBr, 4 hr	80	4	26312	637	10265	67441	2.56
100 °C LiBr, 4 hr	100	4	27681	1729	11279	67931	2.45
Boil LiBr, 4 hr	Boil	4	30042	1535	11183	80704	2.69
RT LiBr, 6 hr	RT	6	26543	1893	10783	65332	2.46
80 °C LiBr, 6 hr	80	6	26353		10167	68301	2.59
100 °C LiBr, 6 hr	100	6	27150	916	11020	66889	2.46

Table 11. The effect of Lithium Bromide (LiBr) temperature on molecular weight of silk processed under the conditions of 30 min. Extraction Time, 100 °C Extraction Temperature and 60 °C Oven Dissolution (Oven/Dissolution Time was varied).

Sample	LiBr Temp (°C)	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
60 °C LiBr, 4 hr	60	4	61956	13336	21463	178847	2.89
80 °C LiBr, 4 hr	80	4	59202	14027	19073	183760	3.10
100 °C LiBr, 4 hr	100	4	47853		19757	115899	2.42
80 °C LiBr, 6 hr	80	6	46824		18075	121292	2.59
100 °C LiBr, 6 hr	100	6	55421	8991	19152	160366	2.89

Experiments were carried out to determine the effect of oven/dissolution temperature. **Figs. 19-23** are graphs showing these results, and **Tables 12-16** summarize the results. Below is a summary:

- Oven temperature has less of an effect on 60 min extracted silk than 30 min extracted silk. Without wishing to be bound by theory, it is believed that the 30 min silk is less degraded during extraction and therefore the oven temperature has more of an effect on the larger MW, less degraded portion of the silk.
- For 60 °C vs. 140 °C oven the 30 min extracted silk showed a very significant effect of lower MW at higher oven temp, while 60 min extracted silk had an effect but much less
- The 140 °C oven resulted in a low end in the confidence interval at ~6000 Da

Table 12. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 30 min. Extraction Time, and 100 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time	Oven Temp (°C)	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30	60	4	47853		19758	115900	2.42

30	100	4	40973	2632	14268	117658	2.87
30	60	6	55421	8992	19153	160366	2.89
30	100	6	25604	1405	10252	63943	2.50

Table 13. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 100 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

oil Time	Oven Temp	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
60	60	1	27908	200	10735	72552	2.60
60	100	1	31520	1387	11633	85407	2.71
60	60	4	27681	1730	11279	72552	2.62
60	100	4	25082	1248	10520	59803	2.38
60	60	6	27150	916	11020	66889	2.46
60	100	6	20980	1262	10073	43695	2.08

Table 14. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 140 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time	Oven Temp(°C)	Oven Time	Average	Standard deviation	Confidence Interval		PD
60	60	4	30042	1536	11183	80705	2.69
60	40	4	15548		7255	33322	2.14

Table 15. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 30 min. Extraction Time, and 140 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time	Oven Temp (°C)	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
30	60	4	49656	4580	17306	142478	2.87

30	140	4	9025	1102	4493	18127	2.01
30	60	6	59383	11640	17641	199889	3.37
30	140	6	13021		5987	28319	2.17

Table 16. The effect of oven/dissolution temperature on molecular weight of silk processed under the conditions of 100 °C Extraction Temperature, 60 min. Extraction Time, and 80 °C Lithium Bromide (LiBr) (Oven/Dissolution Time was varied).

Boil Time	Oven Temp (°C)	Oven Time	Average Mw	Standard deviation	Confidence Interval		PD
60	60	4	26313	637	10266	67442	2.56
60	80	4	30308	4293	12279	74806	2.47
60	60	6	26353		10168	68302	2.59
60	80	6	25164	238	9637	65706	2.61

In an embodiment, the methods disclosed herein result in a solution with characteristics that can be controlled during manufacturing, including, but not limited to: MW – may be varied by changing extraction and/or dissolution time and temp (e.g., LiBr temperature), pressure, and filtration (e.g., size exclusion chromatography); Structure – removal or cleavage of heavy or light chain of the fibroin protein polymer; Purity – hot water rinse temperature for improved sericin removal or filter capability for improved particulate removal that adversely affects shelf stability of the silk fragment protein mixture solution; Color – the color of the solution can be controlled with, for example, LiBr temp and time; Viscosity; Clarity; and Stability of solution. The resultant pH of the solution is typically about 7 and can be altered using an acid or base as appropriate to storage requirements.

The above-described SPF mixture solutions may be utilized to produce SPF containing tissue fillers, as described herein.

A method for preparing an aqueous solution of pure silk fibroin-based protein fragments having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa includes the steps of: degumming a silk source by adding the silk

source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 60 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in an oven having a temperature of about 140 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk protein fragments, the aqueous solution comprising: fragments having an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, and wherein the aqueous solution of pure silk fibroin-based protein fragments comprises a polydispersity of between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin-based protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin-based protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin-based protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin-based protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin-based protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide. A film may be

fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The film may comprise from about 1.0 wt. % to about 50.0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin-based protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

A method for preparing an aqueous solution of pure silk fibroin-based protein fragments having an average weight average molecular weight ranging from about 5 kDa to about 150 kDa includes the steps of: degumming a silk source by adding the silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 60 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in an oven having a temperature of about 140 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk protein fragments, the aqueous solution comprising: fragments having an average weight average molecular weight ranging from about 5 kDa to about 150 kDa, and wherein the aqueous solution of pure silk fibroin-based protein fragments comprises a polydispersity of between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin-based protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin-based protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the

aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin-based protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin-based protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin-based protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide. A film may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The film may comprise from about 1.0 wt. % to about 50.0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin-based protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

A method for preparing an aqueous solution of pure silk fibroin-based protein fragments having an average weight average molecular weight ranging from about 6 kDa to about 17 kDa includes the steps of: degumming a silk source by adding the silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 60 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in an oven having a temperature

of about 140 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of silk protein fragments, the aqueous solution comprising: fragments having an average weight average molecular weight ranging from about 6 kDa to about 17 kDa, and wherein the aqueous solution of pure silk fibroin-based protein fragments comprises a polydispersity of between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin-based protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin-based protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin-based protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin-based protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin-based protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide. A film may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The film may comprise from about 1.0 wt. % to about 50.0 wt. % of vitamin C or a derivative thereof. The film may have a water content ranging from about 2.0 wt. % to about 20.0 wt. %. The film may comprise from about 30.0 wt. % to about 99.5 wt. % of pure silk fibroin-based protein fragments. A gel may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or

a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

A method for preparing an aqueous solution of pure silk fibroin-based protein fragments having an average weight average molecular weight ranging from about 17 kDa to about 39 kDa includes the steps of: adding a silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of between about 30 minutes to about 60 minutes so as to result in degumming; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 80 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in a dry oven having a temperature in the range between about 60 °C to about 100 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of pure silk fibroin-based protein fragments, wherein the aqueous solution of pure silk fibroin-based protein fragments comprises lithium bromide residuals of between about 10 ppm and about 300 ppm, wherein the aqueous solution of silk protein fragments comprises sodium carbonate residuals of between about 10 ppm and about 100 ppm, wherein the aqueous solution of pure silk fibroin-based protein fragments comprises fragments having an average weight average molecular weight ranging from about 17 kDa to about 39 kDa, and wherein the aqueous solution of pure silk fibroin-based protein fragments comprises a polydispersity of between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin-based protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin-based protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of

pure silk fibroin-based protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin-based protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin-based protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin-based protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide.

A gel may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

According to aspects illustrated herein, there is disclosed a method for preparing an aqueous solution of pure silk fibroin-based protein fragments having an average weight average molecular weight ranging from about 39 kDa to about 80 kDa, the method including the steps of: adding a silk source to a boiling (100 °C) aqueous solution of sodium carbonate for a treatment time of about 30 minutes so as to result in degumming; removing sericin from the solution to produce a silk fibroin extract comprising non-detectable levels of sericin; draining the solution from the silk fibroin extract; dissolving the silk fibroin extract in a solution of lithium bromide having a starting temperature upon placement of the silk fibroin extract in the lithium bromide solution that ranges from about 80 °C to about 140 °C; maintaining the solution of silk fibroin-lithium bromide in a dry oven having a temperature in the range between about 60 °C to about 100 °C for a period of at least 1 hour; removing the lithium bromide from the silk fibroin extract; and producing an aqueous solution of pure silk fibroin-based protein fragments, wherein the aqueous solution of pure silk fibroin-based protein fragments comprises lithium bromide residuals of between about 10 ppm and about 300 ppm, sodium carbonate residuals of between about 10 ppm and about 100 ppm, fragments having an average weight average molecular weight ranging from about 40 kDa to about

65 kDa, and wherein the aqueous solution of pure silk fibroin-based protein fragments comprises a polydispersity of between about 1.5 and about 3.0. The method may further comprise drying the silk fibroin extract prior to the dissolving step. The aqueous solution of pure silk fibroin-based protein fragments may comprise lithium bromide residuals of less than 300 ppm as measured using a high-performance liquid chromatography lithium bromide assay. The aqueous solution of pure silk fibroin-based protein fragments may comprise sodium carbonate residuals of less than 100 ppm as measured using a high-performance liquid chromatography sodium carbonate assay. The method may further comprise adding a therapeutic agent to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a molecule selected from one of an antioxidant or an enzyme to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding a vitamin to the aqueous solution of pure silk fibroin-based protein fragments. The vitamin may be vitamin C or a derivative thereof. The aqueous solution of pure silk fibroin-based protein fragments may be lyophilized. The method may further comprise adding an alpha hydroxy acid to the aqueous solution of pure silk fibroin-based protein fragments. The alpha hydroxy acid may be selected from the group consisting of glycolic acid, lactic acid, tartaric acid and citric acid. The method may further comprise adding hyaluronic acid or its salt form at a concentration of about 0.5% to about 10.0% to the aqueous solution of pure silk fibroin-based protein fragments. The method may further comprise adding at least one of zinc oxide or titanium dioxide.

A gel may be fabricated from the aqueous solution of pure silk fibroin-based protein fragments produced by this method. The gel may comprise from about 0.5 wt. % to about 20.0 wt. % of vitamin C or a derivative thereof. The gel may have a silk content of at least 2% and a vitamin content of at least 20%.

Hyaluronic Acid and Hyaluronic Acid Gels

A biodegradable polymer component of the present invention is hyaluronate, also known as hyaluronic acid (HA). HA consists of alternating residues of D-glucuronic acid and N-acetyl-D-glucosamine. This water soluble polymer is naturally found in nearly all tissue, especially in the extracellular matrix, the eyes and synovial fluid of joints. HA is commercially available in pure form. Small gel particle HA fillers may be used stimulate

natural collagen production that is presumed to be induced by mechanical stretching of the dermis and activation of dermal fibroblasts.

HA concentration in the resulting tissue and/or dermal fillers of the invention contributes to tissue and/or dermal filler stiffness and longevity. In some embodiments, an increased concentration of HA in the resulting tissue and/or dermal fillers described herein may increase the stiffness and/or longevity of the resulting tissue and/or dermal filler as compared to a tissue and/or dermal filler having a comparatively lesser concentration of HA.

In some embodiments, HA incorporated in the tissue fillers described herein has a molecular weight of 100,000 daltons or greater, 150,000 daltons or greater, 1 million daltons or greater, or 2 million daltons or greater. In some embodiments, HA incorporated in the tissue fillers described herein has a molecular weight of 100,000 daltons or less, 150,000 daltons or less, 1 million daltons or less, or 2 million daltons or less. In some embodiments, the HA incorporated in the tissue fillers described herein has a high molecular weight (e.g., an HA molecular weight of about 1 MDa to about 4 MDa). In some embodiments, the HA incorporated in the tissue fillers described herein has a low molecular weight (e.g., an HA molecular weight of less than about 1 MDa).

In some embodiments, the HA source may be a hyaluronate salt such as, for example, sodium hyaluronate. In some embodiments, the HA is crosslinked. Crosslinked HA can be formulated into a variety of shapes, such as membranes, gels, semi-gels, sponges, or microspheres. In some embodiments, the crosslinked HA is in fluid gel form, i.e., it takes the shape of its container. The viscosity of an HA gel or semi-gel can be altered by the addition of unconjugated HA and/or hyaluronate. Viscosity can also be tuned by varying the degree of SPF-SPF, SPF-HA, and/or HA-HA cross-linking as described herein. In some embodiment, about 4% to about 12% of the HA may be crosslinked as HA-HA or HA-SPF.

In an embodiment, the SPF compositions described herein may be combined with HA to form a tissue filler composition. In an embodiment, the percent HA in the tissue filler composition by weight is less than 99%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 98%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 97%. In an embodiment, the percent

HA in the tissue filler composition by weight is less than 96%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 95%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 94%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 93%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 92%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 91%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 90%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 85%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 80%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 75%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 70%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 65%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 60%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 55%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 50%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 45%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 40%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 35%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 30%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 25%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 20%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 19%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 18%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 17%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 16%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 15%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 14%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 13%. In an embodiment, the percent HA in the tissue filler composition by

weight is less than 12%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 11%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 10%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 9%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 8%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 7%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 6%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 5%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 4%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 3%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 2%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 1%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.9%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.8%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.7%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.6%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.5%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.4%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.3%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.2%. In an embodiment, the percent HA in the tissue filler composition by weight is less than 0.1%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.1%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.2%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.3%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.4%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.5%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.6%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.7%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than

0.8%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 0.9%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 1%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 2%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 3%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 4%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 5%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 6%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 7%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 8%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 9%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 10%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 11%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 12%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 13%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 14%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 15%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 16%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 17%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 18%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 19%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 20%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 25%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 30%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 35%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 40%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 45%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 50%. In an embodiment, the

percent HA in the tissue filler composition by weight is greater than 55%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 60%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 65%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 70%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 75%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 80%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 85%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 90%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 91%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 92%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 93%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 94%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 95%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 96%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 97%. In an embodiment, the percent HA in the tissue filler composition by weight is greater than 98%.

In an embodiment, the percent HA in the tissue filler composition by weight is about 0.1%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.2%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.3%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.4%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.5%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.6%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.7%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.8%. In an embodiment, the percent HA in the tissue filler composition by weight is about 0.9%. In an embodiment, the percent HA in the tissue filler composition by weight is about 1%. In an embodiment, the percent HA in the tissue filler composition by weight is about 2%. In an embodiment, the percent

HA in the tissue filler composition by weight is about 3%. In an embodiment, the percent HA in the tissue filler composition by weight is about 4%. In an embodiment, the percent HA in the tissue filler composition by weight is about 5%. In an embodiment, the percent HA in the tissue filler composition by weight is about 6%. In an embodiment, the percent HA in the tissue filler composition by weight is about 7%. In an embodiment, the percent HA in the tissue filler composition by weight is about 8%. In an embodiment, the percent HA in the tissue filler composition by weight is about 9%. In an embodiment, the percent HA in the tissue filler composition by weight is about 10%. In an embodiment, the percent HA in the tissue filler composition by weight is about 11%. In an embodiment, the percent HA in the tissue filler composition by weight is about 12%. In an embodiment, the percent HA in the tissue filler composition by weight is about 13%. In an embodiment, the percent HA in the tissue filler composition by weight is about 14%. In an embodiment, the percent HA in the tissue filler composition by weight is about 15%. In an embodiment, the percent HA in the tissue filler composition by weight is about 16%. In an embodiment, the percent HA in the tissue filler composition by weight is about 17%. In an embodiment, the percent HA in the tissue filler composition by weight is about 18%. In an embodiment, the percent HA in the tissue filler composition by weight is about 19%. In an embodiment, the percent HA in the tissue filler composition by weight is about 20%. In an embodiment, the percent HA in the tissue filler composition by weight is about 25%. In an embodiment, the percent HA in the tissue filler composition by weight is about 30%. In an embodiment, the percent HA in the tissue filler composition by weight is about 35%. In an embodiment, the percent HA in the tissue filler composition by weight is about 40%. In an embodiment, the percent HA in the tissue filler composition by weight is about 45%. In an embodiment, the percent HA in the tissue filler composition by weight is about 50%. In an embodiment, the percent HA in the tissue filler composition by weight is about 55%. In an embodiment, the percent HA in the tissue filler composition by weight is about 60%. In an embodiment, the percent HA in the tissue filler composition by weight is about 65%. In an embodiment, the percent HA in the tissue filler composition by weight is about 70%. In an embodiment, the percent HA in the tissue filler composition by weight is about 75%. In an embodiment, the percent HA in the tissue filler composition by weight

is about 80%. In an embodiment, the percent HA in the tissue filler composition by weight is about 85%. In an embodiment, the percent HA in the tissue filler composition by weight is about 90%. In an embodiment, the percent HA in the tissue filler composition by weight is about 91%. In an embodiment, the percent HA in the tissue filler composition by weight is about 92%. In an embodiment, the percent HA in the tissue filler composition by weight is about 93%. In an embodiment, the percent HA in the tissue filler composition by weight is about 94%. In an embodiment, the percent HA in the tissue filler composition by weight is about 95%. In an embodiment, the percent HA in the tissue filler composition by weight is about 96%. In an embodiment, the percent HA in the tissue filler composition by weight is about 97%. In an embodiment, the percent HA in the tissue filler composition by weight is about 98%.

In an embodiment, the percent HA in the tissue filler composition by weight is between about 0.1% to about 1%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 0.5% to about 1.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 1% to about 5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 1.5% to about 5.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 2% to about 6%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 2.5% to about 6.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 3% to about 7%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 3.5% to about 7.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 4% to about 8%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 4.5% to about 8.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 5% to about 9%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 5.5% to about 9.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 6% to about 10%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 6.5% to about 10.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 7% to about 11%. In an embodiment, the percent

HA in the tissue filler composition by weight is between about 7.5% to about 11.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 8% to about 12%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 8.5% to about 12.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 9% to about 13%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 9.5% to about 13.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 10% to about 14%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 10.5% to about 14.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 11% to about 15%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 11.5% to about 15.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 12% to about 16%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 12.5% to about 16.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 13% to about 17%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 13.5% to about 17.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 14% to about 18%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 14.5% to about 18.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 15% to about 19%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 15.5% to about 19.5%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 16% to about 20%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 20% to about 30%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 30% to about 40%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 40% to about 50%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 50% to about 60%. In an embodiment, the percent HA in the tissue filler composition by weight is

between about 60% to about 70%. In an embodiment, the percent HA in the tissue filler composition by weight is between about 80% to about 90%

In some embodiments, the percent HA, by weight, in the tissue filler compositions described herein is about 1% to about 2%, or about 1% to about 3%, or about 1% to about 4%, or about 1% to about 5%, or about 1% to about 6%, or about 1% to about 7%, or about 1% to about 8%, or about 1% to about 9%, or about 1% to about 10%, or about 1% to about 11%, or about 1% to about 12%, or about 1% to about 13%, or about 1% to about 14%, or about 1% to about 15%, or about 1% to about 16%, or about 1% to about 17%, or about 1% to about 18%, or about 1% to about 19%, or about 1% to about 20%, or about 1% to about 21%, or about 1% to about 22%, or about 1% to about 23%, or about 1% to about 24%, or about 1% to about 25%, or about 1% to about 30%, or about 1% to about 40%, or about 1% to about 50%, or about 1% to about 60%, or about 1% to about 70%, or about 1% to about 80%, or about 1% to about 95%; or about 10% to about 20%, or about 10% to about 25%, or about 10% to about 30%, or about 10% to about 35%, or about 10% to about 40%, or about 10% to about 45%, or about 10% to about 50%, or about 10% to about 55%, or about 10% to about 60%, or about 10% to about 65%, or about 10% to about 70%, or about 10% to about 75%, or about 10% to about 80%, or about 10% to about 85%, or about 10% to about 90%, or about 10% to about 95%.

In some embodiments, the HA described herein may be acquired from commercial sources or may be produced by *Streptococcus equi* bacteria.

Tissue fillers described herein that include HA may be characterized for their *in vitro* biological activities and *in vivo* biological activities. For example, *in vitro* assays may be performed on a portion of the tissue fillers described herein for cell toxicity, resistance to enzymatic degradation, syringeability (e.g., solution viscosity, injection flow rate, syringe/needle diameter), and/or particle morphology analysis. See, e.g., Park, et al., J. Eur. Acad. Dermatol. Venerol. (2014) 28:565-568. *In vivo* assays may be performed to determine initial morphological patterns, total remaining filler present, histological evaluations, and may include the examination of granuloma formation or cutaneous adverse reactions. See, e.g., Park, et al., J. Eur. Acad. Dermatol. Venerol. (2014) 28:565-568; and Ramot, et al., Toxicology Pathology (2015) 43: 267-271.

Gelation

In an embodiment, silk gels may be provided with a gelation aid. In some embodiments, the gelation aid may be an acid, electricity, mixing, and/or sonication.

In an embodiment, when producing a silk gel, an acid can be added to a silk solution described herein to help facilitate gelation. In an embodiment, when producing a silk gel that includes a neutral or a basic molecule and/or therapeutic agent, an acid can be added to facilitate gelation. In an embodiment, when producing a silk gel, increasing the pH (making the gel more basic) increases the shelf stability of the gel. In an embodiment, when producing a silk gel, increasing the pH (making the gel more basic) allows for a greater quantity of an acidic molecule to be loaded into the gel.

In an embodiment, when producing a silk gel, electricity can be passed through a silk solution described herein to help facilitate gelation.

In an embodiment, when producing a silk gel, mixing of a silk solution described herein can be used to help facilitate gelation.

In an embodiment, when producing a silk gel, sonication of a silk solution described herein can be used to help facilitate gelation.

In an embodiment, natural additives may be added to the silk gel to further stabilize additives. For example, trace elements such as selenium or magnesium or L-methionine can be used. Further, light-block containers can be added to further increase stability.

In some embodiments, gelation enhancers can be used to accelerate SPF gelation. In some embodiments, an SPF solution can be mixed with pure alcohol or aqueous alcohol solution at varied volume ratios accompanied by mixing, either through stirring, shaking or any other form of agitation. In some embodiments, this alcohol solution enhancer may then have a quantity of an amphiphilic peptide added as a further enhancer of the final gel outcome. The extent of acceleration may be heightened or lessened as appropriate by adding a larger or smaller enhancer component to the system.

In some embodiments, gelation rate may be enhanced by increasing the concentration of SPF in a solution used for making a gel. Various methods can be used to that end, including but not limited to: dialysis of intermediate SPF solution against a buffer incorporating a hygroscopic species such as polyethylene glycol, a lyophilization

step, and/or an evaporation step. Increased temperature may also be used as an enhancer of the gelation process. In addition, manipulation of intermediate silk solution pH by methods including but not limited to direct titration and gas exchange can be used to enhance the gelation process. Introduction of select ionic species including calcium and potassium in particular may also be used to accelerate gelation rate.

In some embodiments, gelation can be helped by the use of nucleating agents, including organic and inorganic species, both soluble and insoluble in an SPF intermediate. Nucleating agents can include but are not limited to peptide sequences which bind silk molecules, previously gelled silk, and poorly soluble β-sheet rich structures. In some embodiments, a further means of accelerating the gelation process is through the introduction of mechanical excitation, which can be imparted through a shearing device, ultrasound device, or mechanical mixer.

The time necessary for complete silk solution gelation may vary from seconds to hours or days, depending on the values of the above mentioned parameters as well as the initial state of aggregation and organization found in the SPF solution. The volume fraction of added enhancer may vary from about 0% to about 99% of the total system volume (i.e., either component may be added to a large excess of the other or in any relative concentration within the interval). The concentration of SPF solution used can range from about 1% (w/v), to about 20% (w/v), and any other appropriate range. The enhancer can be added to SPF solution or the SPF solution can be added to enhancer. The formed SPF hydrogel may be further chemically or physically crosslinked to gain altered mechanical properties.

In some embodiments, an enhancer solution is added to an SPF solution, or vice-versa, the SPF solution having a concentration of SPF of about 1% (w/v), about 2% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 6% (w/v), about 7% (w/v), about 8% (w/v), about 9% (w/v), about 10% (w/v), about 12% (w/v), about 15% (w/v), about 18% (w/v), about 20% (w/v), about 25% (w/v), or about 30% (w/v). In some embodiments, an enhancer solution is added to an SPF solution, or vice-versa, the SPF solution having a concentration of SPF of at least 1% (w/v), at least 2% (w/v), at least 3% (w/v), at least 4% (w/v), at least 5% (w/v), at least 6% (w/v), at least 7% (w/v), at least 8% (w/v), at least 9% (w/v), at least 10% (w/v), at least 12% (w/v), at least 15% (w/v), at

least 18% (w/v), at least 20% (w/v), at least 25% (w/v), or at least 30% (w/v). In some embodiments, an enhancer solution is added to an SPF solution, or vice-versa, the SPF solution having a concentration of SPF of about 1% (w/v) to about 5% (w/v), about 1% (w/v) to about 10% (w/v), about 1% (w/v) to about 15% (w/v), about 1% (w/v) to about 20% (w/v), about 1% (w/v) to about 25% (w/v), about 1% (w/v) to about 30% (w/v), about 5% (w/v) to about 10% (w/v), about 5% (w/v) to about 15% (w/v), about 5% (w/v) to about 20% (w/v), about 5% (w/v) to about 25% (w/v), about 5% (w/v) to about 30% (w/v), about 10% (w/v) to about 15% (w/v), about 10% (w/v) to about 20% (w/v), about 10% (w/v) to about 25% (w/v), or about 10% (w/v) to about 30% (w/v).

Gels and Hydrogels – Modifying and Cross-Linking

In some embodiments, the invention provides compositions comprising one or more hydrogels comprising one or more crosslinked matrix polymers. As used herein, the term "crosslinked" refers to the intermolecular bonds joining the individual polymer molecules, macromolecules, and/or monomer chains, into a more stable structure like a gel. As such, a crosslinked matrix polymer has at least one intermolecular bond joining at least one individual polymer molecule to another one, where the first individual polymer molecule can be of similar, or different, chemical nature to the other. Matrix polymers disclosed herein may be crosslinked using dialdehydes and disulfides cross-linking agents including, without limitation, multifunctional PEG-based crosslinking agents, divinyl sulfones, diglycidyl ethers, and bis-epoxides. Non-limiting examples of SPF, and/or HA, cross-linking agents include divinyl sulfone (DVS), 1,4-butanediol diglycidyl ether (BDDE), UV light, glutaraldehyde, 1,2-bis(2,3-epoxypropoxy)ethylene (EGDGE), 1,2,7,8-diepoxyoctane (DEO), biscarbodiimide (BCD), pentaerythritol tetraglycidyl ether (PETGE), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, or combinations thereof. In some embodiments, the HA cross-linking agent may include BDDE or DVS. In some embodiments, the HA and/or SPF cross-linking agent may be BDDE, DVS, UV light, glutaraldehyde, or a carbodiimide, as described herein.

In some embodiments, the tissue fillers described herein may contain residual cross-linking agent. In some embodiments, the tissue fillers described herein may contain only trace amounts of the cross-linking agent such as, for example, no greater than about

2 ppm, or no greater than about 1.9 ppm, or no greater than about 1.8 ppm, or no greater than about 1.7 ppm, or no greater than about 1.6 ppm, or no greater than about 1.5 ppm, or no greater than about 1.4 ppm, or no greater than about 1.3 ppm, or no greater than about 1.2 ppm, or no greater than about 1.1 ppm, or no greater than about 1.0 ppm, or no greater than about 0.9 ppm, or no greater than about 0.8 ppm, or no greater than about 0.7 ppm, or no greater than about 0.6 ppm, or no greater than about 0.5 ppm, or no greater than about 0.4 ppm, or no greater than about 0.3 ppm, or no greater than about 0.2 ppm, or no greater than about 0.1 ppm. In some embodiments, the tissue fillers described herein may contain trace amounts BDDE, but at a concentration no greater than about 2 ppm, or no greater than about 1.9 ppm, or no greater than about 1.8 ppm, or no greater than about 1.7 ppm, or no greater than about 1.6 ppm, or no greater than about 1.5 ppm, or no greater than about 1.4 ppm, or no greater than about 1.3 ppm, or no greater than about 1.2 ppm, or no greater than about 1.1 ppm, or no greater than about 1.0 ppm, or no greater than about 0.9 ppm, or no greater than about 0.8 ppm, or no greater than about 0.7 ppm, or no greater than about 0.6 ppm, or no greater than about 0.5 ppm, or no greater than about 0.4 ppm, or no greater than about 0.3 ppm, or no greater than about 0.2 ppm, or no greater than about 0.1 ppm. As understood by a person having ordinary skill in the art, the amount of residual cross-linking agent present in a particular tissue filler sample may be determined by gas chromatography-mass spectrometry.

In some embodiments, the tissue fillers described herein may include a matrix that may include an SPF matrix portion and an HA matrix portion, where the SPF matrix portion includes a mixture of crosslinked and non-crosslinked SPF and the HA matrix portion includes a mixture of crosslinked and non-crosslinked HA.

In some embodiments, the tissue fillers of the invention include linker modified HA. In some embodiments, the tissue fillers of the invention include linker modified SPF. Bifunctional cross-linkers can react at both ends to connect two different HA molecules, two different SPF molecules, or an HA molecule with an SPF molecule. In some embodiments, the cross-linker bonds with an HA molecule only at one end, leaving the other end pendant. In some embodiments, the cross-linker bonds with an SPF molecule only at one end, leaving the other end pendant.

As used herein, the degree of modification (MoD) can be defined as (see for example J. Kablik et al., Dermatol Surg, 2009 (35): 302–312):

Total % Degree of Modification = % Crosslink + % Pendant

In order to determine the MoD, it can also be defined as (see for example L. Kenne et al., Carbohydrate Polymers, 2013 (91): 410– 418):

$$MoD = \frac{n_{linked\ crosslinkers}}{n_{HA\ disaccharides} + n_{SPF\ repeating\ units}}$$

where $n_{\text{linked crosslinkers}}$ is the number of linked cross-linker molecules, $n_{\text{HA disaccharides}}$ is the number or disaccharides in HA, and $n_{\text{SPF repeating units}}$ is the number of repeating units in SPF. These numbers can be determined by NMR using characteristic chemical shifts of crosslinker, HA, and SPF (See "Chemical Characterization of Hydrogels Crosslinked with Polyethylene Glycol for Soft Tissue Augmentation," Monticelli et al., Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1077-1081).

In some embodiments, the MoD is between about 1% and 25%, between about 2% and about 20%, or between about 3.5% and about 17.5%. In some embodiments, the MoD is about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, about 5.6%, about 5.7%, about 5.8%, about 5.9%, about 6.0%, about 6.1%, about 6.2%, about 6.3%, about 6.4%, about 6.5%, about 6.6%, about 6.7%, about 6.8%, about 6.9%, about 7.0%, about 7.1%, about 7.2%, about 7.3%, about 7.4%, about 7.5%, about 7.6%, about 7.7%, about 7.8%, about 7.9%, about 8.0%, about 8.1%, about 8.2%, about 8.3%, about 8.4%, about 8.5%, about 8.6%, about 8.7%, about 8.8%, about 8.9%, about 9.0%, about 9.1%, about 9.2%, about 9.3%, about 9.4%, about 9.5%, about 9.6%, about 9.7%, about 9.8%, about 9.9%, about 10.0%, about 10.1%, about 10.2%, about 10.3%, about 10.4%, about 10.5%, about 10.6%, about 10.7%, about 10.8%, about 10.9%, about 11.0%, about 11.1%, about 11.2%, about 11.3%, about 11.4%, about 11.5%, about 11.6%, about 11.7%, about 11.8%, about

11.9%, about 12.0%, about 12.1%, about 12.2%, about 12.3%, about 12.4%, about 12.5%, about 12.6%, about 12.7%, about 12.8%, about 12.9%, about 13.0%, about 13.1%, about 13.2%, about 13.3%, about 13.4%, about 13.5%, about 13.6%, about 13.7%, about 13.8%, about 13.9%, about 14.0%, about 14.1%, about 14.2%, about 14.3%, about 14.4%, about 14.5%, about 14.6%, about 14.7%, about 14.8%, about 14.9%, about 15.0%, about 15.1%, about 15.2%, about 15.3%, about 15.4%, about 15.5%, about 15.6%, about 15.7%, about 15.8%, about 15.9%, about 16.0%, about 16.1%, about 16.2%, about 16.3%, about 16.4%, about 16.5%, about 16.6%, about 16.7%, about 16.8%, about 16.9%, about 17.0%, about 17.1%, about 17.2%, about 17.3%, about 17.4%, about 17.5%, about 17.6%, about 17.7%, about 17.8%, about 17.9%, about 18.0%, about 18.1%, about 18.2%, about 18.3%, about 18.4%, about 18.5%, about 18.6%, about 18.7%, about 18.8%, about 18.9%, about 19.0%, about 19.1%, about 19.2%, about 19.3%, about 19.4%, about 19.5%, about 19.6%, about 19.7%, about 19.8%, about 19.9%, or about 20.0%.

In some embodiments, the tissue fillers of the invention include crosslinked SPF. In some embodiments, the tissue fillers of the invention include crosslinked HA. An SPF fragment can be crosslinked to another SPF fragment, or with HA. SPF-SPF, SPF-HA, and HA-HA crosslinked species can be obtained by using cross-linking agents of various lengths, including zero length.

In some embodiments, the tissue fillers described herein may be provided in the form of a hydrogel having crosslinked HA and/or crosslinked SPF. The crosslinked HA and/or crosslinked SPF (or SPF-HA crosslinked species) may have a measurable degree of cross-linking. As used herein, the term “degree of crosslinking” refers to the number of cross-linking units (or molecules or residues) relative to the number of monomeric units in the polymer macromolecule, which was crosslinked. In some embodiments, the monomeric units are the amino acids in SPF. In some embodiments, the monomeric units are the disaccharide monomer units of HA. Thus, a composition that that has a crosslinked matrix polymer with a 4% degree of crosslinking means that on average there are four crosslinking molecules for every 100 monomeric units. Every other parameter being equal, the greater the degree of crosslinking, the harder the gel becomes. Without being limited to any one theory of the invention, the degree of cross-linking in HA and/or

SPF may result in stiffer resulting materials or compositions prepared therefrom. For example, the higher the degree of cross-linking, the longer such materials are likely to persist in the body. Indeed, without being limited to any one theory, biocompatible materials that include crosslinked materials will have varied rates of bioresorption, bioabsorption, and/or biodegradation depending on the degree of crosslinking where degree of cross-linking is inversely proportional to the rate of bioresorption, bioabsorption, and/or biodegradation. Furthermore, greater crosslinking in the tissue fillers described herein may reduce hydrophilicity and the lifting capacity of such tissue fillers.

In a non-limiting example, a crosslinked SPF that has a degree of crosslinking of about 5%, has about 5 cross-linking moieties for every 100 monomeric units, e.g., amino acids, in the crosslinked SPF.

Non-limiting examples of a degree of crosslinking include about 1% to about 15%, or about 2% to about 14%, or about 1% to about 2%, about 1.5% to about 2.5%, or about 2% to about 3%, or about 2.5% to about 3.5%, or about 3% to about 4%, or about 3.5% to about 4.5%, or about 4% to about 5%, or about 4.5% to about 5.5%, or about 5% to about 6%, or about 5.5% to about 6.5%, or about 6% to about 7%, or about 6.5% or about 7.5%, or about 7% to about 8%, or about 7.5% or about 8.5%, or about 8% to about 9%, or about 8.5% to about 9.5%, or about 9% to about 10%, or about 9.5% to about 10.5%, or about 10% to about 11%, or about 10.5% to about 11.5%, or about 11% to about 12%, or about 11.5% to about 12.5%, or about 12% to about 13%, or about 12.5% to about 13.5%, or about 13% to about 14%, or about 13.5% to about 14.5%, or about 14% to about 15%.

In some embodiments, the degree of crosslinking is at least 1%. In some embodiments, the degree of crosslinking is at least 2%. In some embodiments, the degree of crosslinking is at least 3%. In some embodiments, the degree of crosslinking is at least 4%. In some embodiments, the degree of crosslinking is at least 5%. In some embodiments, the degree of crosslinking is at least 6%. In some embodiments, the degree of crosslinking is at least 7%. In some embodiments, the degree of crosslinking is at least 8%. In some embodiments, the degree of crosslinking is at least 9%. In some embodiments, the degree of crosslinking is at least 10%. In some embodiments, the

degree of crosslinking is at least 11%. In some embodiments, the degree of crosslinking is at least 12%. In some embodiments, the degree of crosslinking is at least 13%. In some embodiments, the degree of crosslinking is at least 14%. In some embodiments, the degree of crosslinking is at least 15%.

In some embodiments, a composition of the invention comprises crosslinked SPF where the degree of crosslinking is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, or at least 15%. In some embodiments, a composition comprises crosslinked SPF where the degree of crosslinking is at most 1%, at most 2%, at most 3%, at most 4%, at most 5%, at most 6%, at most 7%, at most 8%, at most 9%, at most 10%, at most 11%, at most 12%, at most 13%, at most 14%, or at most 15%. In some embodiments, a composition comprises crosslinked SPF where the degree of crosslinking is about 1% to about 15%, about 2% to about 11%, about 3% to about 10%, about 1% to about 5%, about 10% to about 15%, about 11% to about 15%, about 6% to about 10%, or about 6% to about 8%, or about 1% to about 2%, about 1.5% to about 2.5%, or about 2% to about 3%, or about 2.5% to about 3.5%, or about 3% to about 4%, or about 3.5% to about 4.5%, or about 4% to about 5%, or about 4.5% to about 5.5%, or about 5% to about 6%, or about 5.5% to about 6.5%, or about 6% to about 7%, or about 6.5% or about 7.5%, or about 7% to about 8%, or about 7.5% or about 8.5%, or about 8% to about 9%, or about 8.5% to about 9.5%, or about 9% to about 10%, or about 9.5% to about 10.5%, or about 10% to about 11%, or about 10.5% to about 11.5%, or about 11% to about 12%, or about 11.5% to about 12.5%, or about 12% to about 13%, or about 12.5% to about 13.5%, or about 13% to about 14%, or about 13.5% to about 14.5%, or about 14% to about 15%.

In some embodiments, a composition of the invention comprises crosslinked HA where the degree of crosslinking is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, or at least 15%. In some embodiments, a composition comprises crosslinked HA where the degree of crosslinking is at most 1%, at most 2%, at most 3%, at most 4%, at most 5%, at most 6%, at most 7%, at most 8%, at most 9%, at most 10%, at most 11%, at most 12%, at most 13%, at most 14%, or at most

15%. In some embodiments, a composition comprises crosslinked HA where the degree of crosslinking is about 1% to about 15%, about 2% to about 11%, about 3% to about 10%, about 1% to about 5%, about 10% to about 15%, about 11% to about 15%, about 6% to about 10%, or about 6% to about 8%, or about 1% to about 2%, about 1.5% to about 2.5%, or about 2% to about 3%, or about 2.5% to about 3.5%, or about 3% to about 4%, or about 3.5% to about 4.5%, or about 4% to about 5%, or about 4.5% to about 5.5%, or about 5% to about 6%, or about 5.5% to about 6.5%, or about 6% to about 7%, or about 6.5% or about 7.5%, or about 7% to about 8%, or about 7.5% or about 8.5%, or about 8% to about 9%, or about 8.5% to about 9.5%, or about 9% to about 10%, or about 9.5% to about 10.5%, or about 10% to about 11%, or about 10.5% to about 11.5%, or about 11% to about 12%, or about 11.5% to about 12.5%, or about 12% to about 13%, or about 12.5% to about 13.5%, or about 13% to about 14%, or about 13.5% to about 14.5%, or about 14% to about 15%.

In some embodiments, a composition of the invention comprises crosslinked SPF-HA where the degree of crosslinking is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, or at least 15%. In some embodiments, a composition comprises crosslinked SPF-HA where the degree of crosslinking is at most 1%, at most 2%, at most 3%, at most 4%, at most 5%, at most 6%, at most 7%, at most 8%, at most 9%, at most 10%, at most 11%, at most 12%, at most 13%, at most 14%, or at most 15%. In some embodiments, a composition comprises crosslinked SPF-HA where the degree of crosslinking is about 1% to about 15%, about 2% to about 11%, about 3% to about 10%, about 1% to about 5%, about 10% to about 15%, about 11% to about 15%, about 6% to about 10%, or about 6% to about 8%, or about 1% to about 2%, about 1.5% to about 2.5%, or about 2% to about 3%, or about 2.5% to about 3.5%, or about 3% to about 4%, or about 3.5% to about 4.5%, or about 4% to about 5%, or about 4.5% to about 5.5%, or about 5% to about 6%, or about 5.5% to about 6.5%, or about 6% to about 7%, or about 6.5% or about 7.5%, or about 7% to about 8%, or about 7.5% or about 8.5%, or about 8% to about 9%, or about 8.5% to about 9.5%, or about 9% to about 10%, or about 9.5% to about 10.5%, or about 10% to about 11%, or about 10.5% to about 11.5%, or about 11% to about 12%, or about 11.5% to about 12.5%, or about 12% to about 13%, or

about 12.5% to about 13.5%, or about 13% to about 14%, or about 13.5% to about 14.5%, or about 14% to about 15%.

For example, 1 mole of SPF to 1 mole of HA may be cross linked wherein the mole of HA could have a molecular weight of about 1 kDa to about 2 M kDa. In some embodiments, 1 mole of SPF to 1 million moles of HA, or vis versa, where SPF can be 100 Da to 350 kDa, whereby any percentage of each mole can be crosslinked or free. A method of cross-linking SPF to other SPF can include one or more steps. In a first step, the epoxide, such as BDDE, is added to an SPF solution in excess and the reaction is allowed to proceed. Epoxides can react with various groups on the SPF macromolecule, such as carboxyl, amine, alcohol, thiol, and the like, resulting in linkages such as esters, secondary or tertiary amines, ethers, thioethers, and the like. Where both epoxides of BDDE have reacted with the functional groups in one or more SPF macromolecules, the SPF becomes crosslinked. In an embodiment, cross-linking of HA may be performed via a reaction with BDDE under alkaline conditions to yield a covalent linkage between HA and the cross-linker as described in Schanté et al., Carbohydrate Polymers (2011) 85:469-489. The degree of modification or crosslinking may be determined by NMR in accordance with methods known in the art (e.g., Edsman et al., Dermatol. Surg. (2012) 38: 1170-1179).

Methods of linking peptides are known in the art. The linking of the individual isolated SPF into oligomeric and/or crosslinked SPF peptides as set forth herein, can be effected by chemical conjugation procedures well known in the art, such as by creating peptide linkages, use of condensation agents, and by employing well known bifunctional cross-linking reagents. The conjugation may be direct, which includes linkages not involving any intervening group, e.g., direct peptide linkages, or indirect, wherein the linkage contains an intervening moiety, such as a protein or peptide, e.g., plasma albumin, or other spacer molecule. For example, the linkage may be via a heterobifunctional or homobifunctional cross-linker, e.g., carbodiimide, glutaraldehyde, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and derivatives, bis-maleimide, 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, and the like.

Cross-linking can also be accomplished without exogenous cross-linkers by utilizing reactive groups on the molecules being conjugated. Methods for chemically

cross-linking peptide molecules are generally known in the art, and a number of hetero- and homobifunctional agents are described in, e.g., U.S. Pat. Nos. 4,355,023, 4,657,853, 4,676,980, 4,925,921, and 4,970,156, and Immuno Technology Catalogue and Handbook, Pierce Chemical Co. (1989), each of which is incorporated herein by reference. Such conjugation, including cross-linking, should be performed so as not to substantially affect the desired function of the peptide oligomer or entity conjugated thereto, including therapeutic agents, and moieties capable of binding substances of interest.

It will be understood to one skilled in the art that alternative linkers can be used to link SPF peptides, for example the use of chemical protein cross-linkers. For example a homobifunctional cross-linker such as disuccinimidyl-suberimidate-dihydrochloride; dimethyl-adipimidate-dihydrochloride; 1,5,-2,4 dinitrobenzene or heterobifunctional cross-linkers such as N-hydroxysuccinimidyl 2,3-dibromopropionate; 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride; and succinimidyl-4-[n-maleimidomethyl]-cyclohexane-1-carboxylate.

The present invention also provides compositions including crosslinked SPF to HA. SPF to HA cross-linking can be achieved by various methods, for example by epoxide methods, periodate methods, and/or tresyl chloride methods. In some embodiments, SPF are crosslinked to HA using an epoxide, for example a multifunctional epoxide. For example, a bifunctional epoxide such as 1,4 butanediol diglycidyl ether (BDDE) can be used. Other multifunctional epoxides include, but are not limited to, polyglycerolpolyglycidyl ether (PGPGE), pentaerythriolpolyglycidyl ether (PEPGE) and diglycerolpolyglycidyl ether (DGPGE). Zero-length cross-linking between SPF and HA is also provided using an activating agent.

A method of cross-linking SPF to other macromolecules, for example HA, can include one or more steps. In a first step, the epoxide, such as BDDE, is added to an SPF solution in excess and the reaction is allowed to proceed. Epoxides can react with various groups on the SPF macromolecule, such as carboxyl, amine, alcohol, thiol, and the like, resulting in linkages such as esters, secondary or tertiary amines, ethers, thioethers, and the like. Where only one epoxide has reacted with SPF, there remains a free epoxide attached to the SPF available for cross-linking with another SPF, or a different macromolecule, for example HA, or the like. The order of adding the reagents can be

varied. For example BDDE can be added to HA first, and then SPF is added to form crosslinked SPF-HA. In some embodiments, SPF and HA can be mixed first, and then BDDE is added to the mixture. In some embodiments, adding BDDE to a mixture of SPF and HA results in a composition including crosslinked SPF to SPF, crosslinked HA to HA, and crosslinked SPF to HA.

In some embodiments, the crosslinked SPF-HA can be prepared using the tresyl chloride method, including one or more steps. In one step, crosslinked HA and/or non-crosslinked HA can be activated with tresyl chloride, i.e., 2,2,2-trifluoroethanesulfonyl chloride, or any other suitable acid chloride. Tresyl chloride is added for example drop-wise to a base/solvent solution, for example, pyridine/acetone solution, containing crosslinked and/or non-crosslinked HA. In some embodiments, the tresyl chloride is reactive with all four of the hydroxyl groups on the sugar rings of crosslinked and/or non-crosslinked HA. In an optional step, the resulting HA-tresylate is washed. In a step, SPF fragments are added which will react with the HA-tresylate.

In some embodiments, the tresyl chloride method can be used to attach an SPF directly to crosslinked and/or non-crosslinked HA. In other embodiments, the tresyl chloride method can be used to attach an SPF to crosslinked and/or non-crosslinked HA via a spacer, for example 6-amino-1-hexanol. In some embodiments, the spacer can first be coupled to crosslinked or non-crosslinked HA via tresyl activation and coupling. For coupling an SPF to the spacer, the tresyl activation and coupling are thereafter repeated. Any suitable spacer can be used, i.e., spacers having at least some characteristics similar to 6-amino-1-hexanol, i.e., a primary amine for coupling to the HA-tresylate, and a reactive group, for example a hydroxyl group, for activation and coupling of the SPF.

In some embodiments, tresyl chloride does not cross-link HA. The HA matrix used in the tresyl chloride method may, however, be crosslinked for additional stability. The cross-linking can be effected, for example, by using a multifunctional epoxide, such as BDDE, as described above. Cross-linking can be done either before or after peptide coupling.

The tresyl chloride method has advantages over other immobilization methods, including efficient coupling under very mild conditions, no side reactions during

activation and coupling, and the RGD peptides can be bound directly to the carbon atoms of the HA support.

In various embodiments, tissue fillers described herein may include gels and hydrogels that are HA-based. HA-based as used herein refers to compositions or materials including crosslinked HA and compositions including crosslinked HA plus one or more other crosslinked polymers. In addition, HA can refer to hyaluronic acid and any of its hyaluronate salts, including, but not limited to, sodium hyaluronate (NaHA), potassium hyaluronate, magnesium hyaluronate, calcium hyaluronate, and combinations thereof. The use of more than one biocompatible polymer is specifically not excluded from the present description. Tissue fillers described herein, which may be in the form gels and hydrogels, can include more than one biocompatible polymer, such as, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more biocompatible polymers in addition to HA and/or SPF. Suitable biocompatible polymers include polysaccharides (e.g., HA, chitosan, chondroitin sulfate, alginate, carboxymethylcellulose), poly(ethyleneglycol), poly(lactic acid), poly(hydroxyethylmethacrylate), poly(methylmethacrylate), proteins other than SPF (e.g., elastin and collagen).

HA described herein may be intermolecularly crosslinked. In some embodiments, the cross-linking stabilizes HA physical properties. In some embodiments, the present invention provides formation of stable crosslinked HA using multifunctional epoxides. As used herein, the term “multifunctional” epoxide means a chemical reagent having two or more epoxides present, such as lower aliphatic epoxides or their corresponding epihalohydrins. Examples of multifunctional epoxides include, but are not limited to, the diepoxide 1,4 butanediol diglycidyl ether (BDDE), polyglycerolpolyglycidyl ether (PGPGE), pentaerythriolpolyglycidyl ether (PEPGE) and diglycerolpolyglycidyl ether (DGPGE). In a preferred embodiment, the diepoxide BDDE is used as the cross-linking agent. The sugar moieties of HA cross-link via the two epoxides of BDDE. In other embodiments, cross-linking agents include alkyldiepoxy bodies such as 1,3-butadiene diepoxide, 1,2,7,8-diepoxyoctane, 1,5-hexadiene diepoxide and the like, diglycidyl ether bodies such as ethylene glycol diglycidyl ether, 1,4-butanediol diglycidyl ether, bisphenol A diglycidyl ether and the like, divinylsulfone, and epichlorohydrin. Among them, particularly, divinylsulfone, 1,4-butanediol diglycidyl ether, and ethylene glycol

diglycidyl ether can be suitably used. In the present invention, two or more kinds of crosslinking agents may be used by appropriately combining them.

In some embodiments, HA is crosslinked to HA. A method of cross-linking HA to HA can include one or more steps. In a first step, an epoxide, such as BDDE, is added to an HA solution in excess and the reaction is allowed to proceed. Epoxides can react with from one to four of the hydroxyl groups on the sugar rings of HA to form one to four ether linkages. Alternatively, or in addition to reacting with the hydroxyl groups, the epoxide can react with the carboxylic acid of the polysaccharide to form an ester bond. Where both epoxides of BDDE have reacted with the functional groups in the sugar rings of one or more HA macromolecules, the HA becomes crosslinked.

In some embodiments, the cross cross-linking agent can be a zero length cross-linking agent such as a chemical bond obtained by employing an activating agent such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), or BCDI. In some embodiments, the zero-length cross-linking activating agent is reacted with the HA in the presence of N-hydroxysuccinimide (NHS), sulfo-NHS (or sulfonyl-NHS) or 4-dimethylaminopyridine (DMAP). In some embodiments, gels and hydrogels described herein are formed by reacting at least one cross-linkable biocompatible polymer, such as HA and/or a protein, e.g. an SPF protein, or any other additional protein, with at least one cross-linking activating agent.

In some embodiments, crosslinked SPF-SPF, crosslinked SPF-HA, and/or crosslinked HA-HA, can have variable residence times after application, for example after being injected as tissue filler, an intra-dermal, subdermal, or generally, as a dermal filler.fillers. In some embodiments, residence times can be affected in the sodium periodate method depending on the number of reactive groups in the SPF which are available for attachment to another SPF macromolecule, or to HA. An example of a reactive group in SPF which can attach to HA is a primary amine. An SPF containing two reactive groups, such as two primary amines, can itself cross-link the HA in the periodate method, thereby creating a more stable conjugate. In other embodiments, where only one reactive group is present in the SPF, such as only one primary amine, for example at the amino terminus, SPF-HA cross-linking is reduced resulting in a more biodegradable matrix.

In some embodiments, BDDE crosslinked HA can have a variable residence time after application, for example after being injected as a tissue filler, an intra-dermal, subdermal, or generally dermal filler. In some embodiments, BDDE crosslinked HA can persist in tissue and/or dermal tissue anywhere from one to at least thirty days, depending on the amount of cross-linking. The variable residence time of the cross linked HA can be tuned by introducing hydrolyzable bonds during the epoxide cross-linking. In some embodiments, the materials crosslinked with epoxide at a lower pH have a greater amount of ester bond formation and therefore are more rapidly hydrolyzable.

In one embodiment, the cross-linking agent is a zero-length cross-linking activating agent. Generally, zero-length cross-linking activating agents couple polymers without adding any additional spacer arm atoms, and therefore zero-length cross-linking activating agents are not incorporated into the crosslinked polymer matrix. Suitable zero-length cross-linking agents include carbodiimides, such as, for example, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and BCDI. Non-water soluble carbodiimides include dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC), which may also be suitable.

Carbodiimide-mediated coupling between carboxylates and alcohol or amine functional groups proceeds readily at ambient temperature, neutral pH and under aqueous conditions. Neutral pH can be, for example, between about 6.0 and about 8.0, such as between about 6.5 and about 7.5, such as about 7.0. Typically in water, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) can be used to mediate esterification between carboxylates and alcohols or amidation between carboxylates and amines. Thus, crosslinked HA is formed by exploiting reactive groups present on HA (e.g., carboxylate and alcohol). In addition, by taking advantage of the high reactivity of amine groups on proteins, for example SPF proteins, amidation between lysine side-chains of proteins with carboxylate groups of HA is achieved to form HA-protein crosslinked hydrogels. Cross-linking agents and unreacted polymers can be removed by dialysis.

In some embodiments, EDC is used in conjunction with N-hydroxysuccinimide (NHS) or sulfonyl-NHS (sulfo-NHS), collectively referred to as “NHS” herein. NHS stabilizes reactive intermediates formed by EDC; thus, the addition of NHS can increase

the coupling efficiency of EDC. Alternatively, 4-dimethylaminopyridine (DMAP) can be used to catalyze the coupling reaction.

In some embodiments, the HA-based tissue fillers of the invention include crosslinked HA-based compositions and at least partially crosslinked HA-based compositions. Uncrosslinked HA as used herein refers to both truly uncrosslinked (e.g., "free") HA chains as well as lightly crosslinked chains and fragments thereof that are generally in soluble liquid form.

In some embodiments, the hydrogel compositions of the invention includes at least some cross-linking between HA and SPF.

Non-limiting Exemplary Embodiments

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 5 kDa to about 150 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 6

kDa to about 17 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 17 kDa to about 39 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 39 kDa to about 80 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including low molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF

to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including medium molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including low molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, medium molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%; wherein the w/w ratio between low molecular weight SPF and medium molecular weight SPF is about 3:1.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including high molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking occurring as a result of using an epoxy derived cross-linker, e.g., BDDE, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5

and about 3.0 and an average weight average molecular weight ranging from about 1 kDa to about 250 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 5 kDa to about 150 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 6 kDa to about 17 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 17 kDa to about 39 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking

occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0 and an average weight average molecular weight ranging from about 39 kDa to about 80 kDa, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including low molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including medium molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including low molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, medium molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%; wherein the w/w ratio between low molecular weight SPF and medium molecular weight SPF is about 3:1.

In one embodiment, the invention relates to a biocompatible tissue and/or dermal filler including high molecular weight silk protein fragments (SPF) having a polydispersity of between about 1.5 and about 3.0, hyaluronic acid (HA), water, and between about 0.05% to about 0.5% lidocaine, e.g., about 0.3% lidocaine; wherein a portion of up to 100% w/w of SPF are crosslinked, and a portion of up to 100% w/w of HA is cross linked, the cross-linking occurring between one or more of SPF to SPF, SPF to HA, and HA to HA; the cross-linking including zero-length cross-linking occurring as a result of using an activating agent, e.g., BCDI, and with a degree of cross-linking of up to 15%.

In one embodiment, the invention relates to biocompatible tissue and/or dermal filler formulations described in Table 16-B.

Table 16-B

Silk average weight average Mw	HA MW PEGDE MW (and/or PPGDE MW)	HA/silk ratio	Total Silk + HA (mg/mL)	MoD
about 12 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 14 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 15 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 16 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 100 kDa	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

LMW	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
MMW	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 700 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 12 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 13 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 14 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 16 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 48 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 100 kDa	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
MMW	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

HMW	HA about 750 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 12 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 14 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 15 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 16 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 100 kDa	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

LMW	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
MMW	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 800 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 12 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 13 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 14 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 16 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 48 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 100 kDa	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
MMW	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

HMW	HA about 850 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 12 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 14 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 15 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 16 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 100 kDa	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

LMW	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
MMW	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 950 kDa PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE about 500 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 700 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 750 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 800 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 850 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 13 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 15 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
about 48 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
LMW	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%
HMW	HA about 950 kDa PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 12 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 13 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 14 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 15 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 16 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 48 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

about 100 kDa	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

LMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

MMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

HMW	HA one or more of about 550 kDa; about 1.0 MDa; about 1.2 MDa; about 1.5 MDa; about 2.2 MDa; about 2.8 MDa; about 2.9 MDa; about 3.0 MDa; and about 3.1 MDA PEGDE one or more of about 200 Da, about 1000 Da, about 2,000 Da, and about 6000 Da; and/or PPGDE one or more of about 380 Da, and about 640 Da	about 92/8; about 93/7; about 94/6; about 95/5; about 96/4; about 97/3; about 18/12; about 27/3; about 29.4/0.6; about 99/1; about 92.5/7.5; about 90/10	about 18; about 19; about 20; about 21; about 22; about 23; about 24; about 25; about 26; about 27; about 28; about 29; about 30	about 5%; about 6%; about 7%; about 8%; about 9%; about 10%; about 11%; about 12%; about 13%; about 14%; about 15%

Additional Agents

In some embodiments, the tissue fillers described herein include an active agent, such as a drug. In some embodiments, the active agent can be one or more of enzyme inhibitors, anesthetic agents, medicinal neurotoxins, antioxidants, anti-infective agents, anti-inflammatory agents, vasodilators, ultraviolet (UV) light blocking agents, dyes (e.g., tattoo dye, ink or pigment), a reflective agent, hormones, immunosuppressants, and combinations thereof. The tissue fillers described herein can include an active agent selected from the group consisting of enzyme inhibitors, anesthetic agents, medicinal neurotoxins (e.g., botulinum toxin and clostridium toxin), antioxidants, anti-infective

agents (e.g., antibiotics), vasodilators, dyes (e.g., tattoo ink or pigment, reflective agents, anti-inflammatory agents, ultraviolet (UV) light blocking agents, dyes, hormones, immunosuppressants, and combinations thereof.

In some embodiments, the immunosuppressant is rapamycin, or rapamycin-like compound.

In some embodiments, the active agent may be an antibiotic selected from the group consisting of a penicillin (e.g., penicillin V, amoxicillin), an erythromycin (e.g., erythromycin stearate), a lincosamide (e.g., clindamycin), and a cephalosporin (e.g. cephalexin), and a combination thereof.

In some embodiments, the active agent may be a vasodilator selected from the group consisting of nitroglycerin, labetalol, thrazide, isosorbide dinitrate, pentaerythritol tetranitrate, digitalis, hydralazine, diazoxide, amrinone, L-arginine, bamethan sulphate, bencyclane fumarate, benfurodil hemisuccinate, benzyl nicotinate, buflomedil hydrochloride, buphenine hydrochloride, butalamine hydrochloride, cetiedil citrate, ciclonicate, cinepazide maleate, cyclandelate, di-isopropylammonium dichloroacetate, ethyl nicotinate, hepronicate, hexyl nicotinate, ifenprodil tartrate, inositol nicotinate, isoxsuprine hydrochloride, kallidinogenase, methyl nicotinate, naftidrofuryl oxalate, nicametate citrate, niceritrol, nicoboxil, nicofuranose, nicotinyl alcohol, nicotinyl alcohol tartrate, nitric oxide, nonivamide, oxpentifylline, papaverine, papaveroline, pentifylline, peroxynitrite, pinacidil, pipratecol, propentofyltine, raubasine, suloctidil, teasuprine, thymoxamine hydrochloride, tocopherol nicotinate, tolazoline, xanthinol nicotinate, diazoxide, hydralazine, minoxidil, and sodium nitroprusside, and a combination thereof.

In some embodiments, the tissue fillers described herein may include an active agent at a concentration, by weight, of at least 0.01%, or at least 0.02%, or at least 0.03%, or at least 0.04%, or at least 0.05%, or at least 0.06%, or at least 0.07%, or at least 0.08%, or at least 0.09%, or at least 0.1%, or at least 0.2%, or at least 0.3%, or at least 0.4%, or at least 0.5%, or at least 0.6%, or at least 0.7%, or at least 0.8%, or at least 0.9%, or at least 1.0%, or at least 1.5%, or at least 2.0%, or at least 2.5%, or at least 3.0%, or at least 3.5%, or at least 4.0%, or at least 4.5%, or at least 5.0%, or at least 5.5%, or at least 6.0%, or at least 6.5%, or at least 7.0%, or at least 7.5%, or at least 8.0%, or at least 8.5%, or at least

9.0%, or at least 9.5%, or at least 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 35%, or at least 40%, or at least 45%, or at least 50%.

In some embodiments, the tissue fillers described herein may include an active agent at a concentration, by weight, of at most 0.01%, or at most 0.02%, or at most 0.03%, or at most 0.04%, or at most 0.05%, or at most 0.06%, or at most 0.07%, or at most 0.08%, or at most 0.09%, or at most 0.1%, or at most 0.2%, or at most 0.3%, or at most 0.4%, or at most 0.5%, or at most 0.6%, or at most 0.7%, or at most 0.8%, or at most 0.9%, or at most 1.0%, or at most 1.5%, or at most 2.0%, or at most 2.5%, or at most 3.0%, or at most 3.5%, or at most 4.0%, or at most 4.5%, or at most 5.0%, or at most 5.5%, or at most 6.0%, or at most 6.5%, or at most 7.0%, or at most 7.5%, or at most 8.0%, or at most 8.5%, or at most 9.0%, or at most 9.5%, or at most 10%, or at most 15%, or at most 20%, or at most 25%, or at most 30%, or at most 35%, or at most 40%, or at most 45%, or at most 50%.

In some embodiments, the tissue fillers described herein may include an active agent at a concentration, by weight, of about 0.01% to about 0.1%, or about 0.05% to about 0.15%, or about 0.1% to about 0.2%, or about 0.15% to about 0.25%, or about 0.2% to about 0.3%, or about 0.25% to about 0.35%, or about 0.3% to about 0.4%, or about 0.35% to about 0.45%, or about 0.4% to about 0.5%, or about 0.45% to about 0.55%, or about 0.5% to about 0.6%, or about 0.55% to about 0.65%, or about 0.6% to about 0.7%, or about 0.65% to about 0.75%, or about 0.7% to about 0.8%, or about 0.75% to about 0.85%, or about 0.8% to about 0.9%, or about 0.85% to about 0.95%, or about 1% to about 2%, or about 1.5% to about 2.5%, or about 2% to about 3%, or about 2.5% to about 3.5%, or about 3% to about 4%, or about 3.5% to about 4.5%, or about 4% to about 5%, or about 4.5% to about 5.5%, or about 5% to about 6%, or about 5.5% to about 6.5%, or about 6% to about 7%, or about 6.5% to about 7.5%, or about 7% to about 8%, or about 7.5% to about 8.5%, or about 8% to about 9%, or about 8.5% to about 9.5%, or about 9% to about 10%, or about 10% to about 15%, or about 15% to about 20%, or about 20% to about 25%, or about 25% to about 30%, or about 30% to about 35%, or about 35% to about 40%, or about 40% to about 45%, or about 45% to about 50%.

In some embodiments, the tissue fillers described herein may include an active agent at a concentration, by weight, of about 0.01%, or about 0.02%, or about 0.03%, or

about 0.04%, or about 0.05%, or about 0.06%, or about 0.07%, or about 0.08%, or about 0.09%, or about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1.0%, or about 1.5%, or about 2.0%, or about 2.5%, or about 3.0%, or about 3.5%, or about 4.0%, or about 4.5%, or about 5.0%, or about 5.5%, or about 6.0%, or about 6.5%, or about 7.0%, or about 7.5%, or about 8.0%, or about 8.5%, or about 9.0%, or about 9.5%, or about 10%, or about 11%, or about 12%, or about 13%, or about 14%, or about 15%, or about 16%, or about 17%, or about 18%, or about 19%, or about 20%, or about 21%, or about 22%, or about 23%, or about 24%, or about 25%, or about 26%, or about 27%, or about 28%, or about 29%, or about 30%, or about 31%, or about 32%, or about 33%, or about 34%, or about 35%, or about 36%, or about 37%, or about 38%, or about 39%, or about 40%, or about 41%, or about 42%, or about 43%, or about 44%, or about 45%, or about 46%, or about 47%, or about 48%, or about 49%, or about 50%.

In some embodiments, the tissue fillers described herein include a fibrosis-inhibiting agent. In some embodiments, tissue fillers described herein may further include a compound that acts to have an inhibitory effect on pathological processes in or around the treatment site. In certain aspects, the active agent may be selected from one of the following classes of compounds: anti-inflammatory agents (e.g., dexamethasone, cortisone, fludrocortisone, prednisone, prednisolone, 6α-methylprednisolone, triamcinolone, betamethasone, and aspirin).

In some embodiments, with the active agent may, but is not limited to, antioxidants and enzymes. In an embodiment, the active agent may include, but is not limited to, selenium, ubiquinone derivatives, thiol-based antioxidants, saccharide-containing antioxidants, polyphenols, botanical extracts, caffeic acid, apigenin, pycnogenol, resveratrol, folic acid, vitamin B12, vitamin B6, vitamin B3, vitamin E, vitamin C and derivatives thereof, vitamin D, vitamin A, astaxathin, lutein, lycopene, essential fatty acids (omegas 3 and 6), iron, zinc, magnesium, flavonoids (soy, curcumin, silymarin, pycnongeol), growth factors, aloe, hyaluronic acid, extracellular matrix proteins, cells, nucleic acids, biomarkers, biological reagents, zinc oxide, benzoyl peroxide, retinoids, titanium, allergens in a known dose (for sensitization treatment), essential oils including, but not limited to, lemongrass or rosemary oil, and fragrances.

Considering the active agents more broadly, the active agents may include therapeutic agents such as small molecules, drugs, proteins, peptides and nucleic acids.

In certain embodiments, the tissue fillers described herein can include one or more anesthetic agents in an amount effective to ameliorate or mitigate pain or discomfort at the tissue filler injection site. The local anesthetic can be selected from the group of ambucaine, amolanone, amylocalne, benoxinate, benzocaine, betoxycaine, biphenamine, bupivacaine, butacaine, butamben, butanilicaine, butethamine, butoxycaine, carticaine, chloroprocaine, cocaethylene, cocaine, cyclomethycaine, dibucaine, dimethisoquin, dimethocaine, diperodon, dicyclomine, ecgonidine, ecgonine, ethyl chloride, etidocaine, beta-eucaine, euprocin, fenalcomine, formocaine, hexylcaine, hydroxytetracaine, isobutyl p-aminobenzoate, leucinocaine mesylate, levoxadrol, lidocaine, mepivacaine, meprylcaine, metabutoxycaine, methyl chloride, myrtecaine, naepaine, octacaine, orthocaine, oxethazaine, parethoxycaine, phenacaine, phenol, piperocaine, piridocaine, polidocanol, pramoxine, prilocalne, procaine, propanocaine, proparacaine, propipocaine, propoxycaine, pseudococaine, pyrrocaine, ropivacaine, salicyl alcohol, tetracaine, tolycaine, trimecaine, zolamine, and salts thereof.

In some embodiments, the tissue fillers described herein may include lidocaine or other anesthetic recited above at a concentration, by weight, of at least 0.01%, or at least 0.02%, or at least 0.03%, or at least 0.04%, or at least 0.05%, or at least 0.06%, or at least 0.07%, or at least 0.08%, or at least 0.09%, or at least 0.1%, or at least 0.2%, or at least 0.3%, or at least 0.4%, or at least 0.5%, or at least 0.6%, or at least 0.7%, or at least 0.8%, or at least 0.9%, or at least 1.0%, or at least 1.5%, or at least 2.0%, or at least 2.5%, or at least 3.0%, or at least 3.5%, or at least 4.0%, or at least 4.5%, or at least 5.0%, or at least 5.5%, or at least 6.0%, or at least 6.5%, or at least 7.0%, or at least 7.5%, or at least 8.0%, or at least 8.5%, or at least 9.0%, or at least 9.5%, or at least 10%.

In some embodiments, the tissue fillers described herein may include lidocaine or other anesthetic recited above at a concentration, by weight, of at most 0.01%, or at most 0.02%, or at most 0.03%, or at most 0.04%, or at most 0.05%, or at most 0.06%, or at most 0.07%, or at most 0.08%, or at most 0.09%, or at most 0.1%, or at most 0.2%, or at most 0.3%, or at most 0.4%, or at most 0.5%, or at most 0.6%, or at most 0.7%, or at most 0.8%, or at most 0.9%, or at most 1.0%, or at most 1.5%, or at most 2.0%, or at

most 2.5%, or at most 3.0%, or at most 3.5%, or at most 4.0%, or at most 4.5%, or at most 5.0%, or at most 5.5%, or at most 6.0%, or at most 6.5%, or at most 7.0%, or at most 7.5%, or at most 8.0%, or at most 8.5%, or at most 9.0%, or at most 9.5%, or at most 10%.

In some embodiments, the tissue fillers described herein may include lidocaine or other anesthetic recited above at a concentration, by weight, of about 0.01%, or about 0.02%, or about 0.03%, or about 0.04%, or about 0.05%, or about 0.06%, or about 0.07%, or about 0.08%, or about 0.09%, or about 0.1%, or about 0.2%, or about 0.3%, or about 0.4%, or about 0.5%, or about 0.6%, or about 0.7%, or about 0.8%, or about 0.9%, or about 1.0%, or about 1.5%, or about 2.0%, or about 2.5%, or about 3.0%, or about 3.5%, or about 4.0%, or about 4.5%, or about 5.0%, or about 5.5%, or about 6.0%, or about 6.5%, or about 7.0%, or about 7.5%, or about 8.0%, or about 8.5%, or about 9.0%, or about 9.5%, or about 10%.

In some embodiments, the tissue fillers described herein may include lidocaine or other anesthetic recited above at a concentration, by weight, of about 0.01% to about 0.02%, or about 0.03% to about 0.04%, or about 0.05% to about 0.06% to about 0.07%, or about 0.08% to about 0.09%, or about 0.1% to about 0.2%, or about 0.3% to about 0.4%, or about 0.5% to about 0.6%, or about 0.7% to about 0.8%, or about 0.9% to about 1.0%, or about 1% to about 1.5%, or about 1.5% to about 2.0%, or about 2.0% to about 2.5%, or about 2.5% to about 3.0%, or about 3.0% to about 3.5%, or about 3.5% to about 4.0%, or about 4.0% to about 4.5%, or about 4.5% to about 5.0%, or about 5.0% to about 5.5%, or about 5.5% to about 6.0%, or about 6.0% to about 6.5%, or about 6.5% to about 7.0%, or about 7.5% to about 8.0%, or about 8.0% to about 8.5%, or about 8.5% to about 9.0%, or about 9.5% to about 10%.

In one embodiment, the anesthetic agent is lidocaine, such as in the form of lidocaine HCl. The tissue fillers described herein may have a lidocaine or other anesthetic in a concentration of between about 0.1% and about 5% by weight of the composition, for example, about 0.2% to about 1.0% by weight of the tissue filler. In one embodiment, the tissue filler has a lidocaine concentration of about 0.3% by weight (w/w %) of the tissue filler. The concentration of lidocaine in the tissue fillers described herein can be therapeutically effective meaning the concentration is adequate to provide a therapeutic

benefit such as, for example, ameliorating or mitigating pain or discomfort at the tissue filler injection site.

Optical Properties

When light encounters a material, it can interact with it in several ways. These interactions depend on the nature of the light, i.e., its wavelength, frequency, energy, etc., and the nature of the material. Light interacts with an object by some combination of reflection, and transmittance with refraction. An optically transparent material allows much of the light that falls on it to be transmitted, with little light being reflected. Materials which do not allow the transmission of light are called optically opaque, or simply opaque.

In some embodiments, the invention provides a tissue filler described herein having transparency and/or translucency. Transparency (also called pellucidity or diaphaneity) is the physical property of allowing light to pass through a material, whereas translucency (also called translucence or translucidity) only allows light to pass through diffusely. The opposite property is opacity. Transparent materials are clear, while translucent ones cannot be seen through clearly. The tissue fillers disclosed herein may, or may not, exhibit optical properties such as transparency and/or translucency. In some embodiments, including methods for superficial line filling, it would be an advantage to have an opaque hydrogel. Factors used to control a tissue filler's optical properties include, without limitation, SPF concentration, degree of crystallinity, and/or hydrogel homogeneity.

In some embodiments, the tissue fillers described herein are opaque.

In an embodiment, a tissue filler described herein is optically transparent. In aspects of this embodiment, a tissue filler described herein transmits, e.g., about 75% of the light, about 80% of the light, about 85% of the light, about 90% of the light, about 95% of the light, or about 100% of the light. In other aspects of this embodiment, a tissue filler described herein, e.g., at least 75% of the light, at least 80% of the light, at least 85% of the light, at least 90% of the light, or at least 95% of the light. In yet other aspects of this embodiment, an a tissue filler described herein transmits, e.g., about 75% to about 100% of the light, about 80% to about 100% of the light, about 85% to about 100% of the light, about 90% to about 100% of the light, or about 95% to about 100% of the light.

In another embodiment, a tissue filler described herein is optically opaque. In aspects of this embodiment, a tissue filler described herein transmits, e.g., about 0.1% of the light, about 1% of the light, about 10% of the light, about 15% of the light, about 20% of the light, about 25% of the light, about 30% of the light, about 35% of the light, about 40% of the light, about 45% of the light, about 50% of the light, about 55% of the light, about 60% of the light, about 65% of the light, about 70% of the light, about 75% of the light, about 80% of the light, about 85% of the light, about 90% of the light, about 95% of the light, or about 100% of the light. In other aspects of this embodiment, a tissue filler described herein transmits, e.g., at most 0.1% of the light, at most 1% of the light, at most 10% of the light, at most 15% of the light, at most 20% of the light, at most 25% of the light, at most 30% of the light, at most 35% of the light, at most 40% of the light, at most 45% of the light, at most 50% of the light, at most 55% of the light, at most 60% of the light, at most 65% of the light, at most 70% of the light, or at most 75% of the light. In other aspects of this embodiment, a tissue filler described herein transmits, e.g., at least 0.1% of the light, at least 1% of the light, at least 10% of the light, at least 15% of the light, at least 20% of the light, at least 25% of the light, at least 30% of the light, at least 35% of the light, at least 40% of the light, at least 45% of the light, at least 50% of the light, at least 55% of the light, at least 60% of the light, at least 65% of the light, at least 70% of the light, or at least 75% of the light. In other aspects of this embodiment, a tissue filler described herein transmits, e.g., about 0.1% to about 15%, about 0.1% to about 20%, about 0.1% to about 25%, about 0.1% to about 30%, about 0.1% to about 35%, about 0.1% to about 40%, about 0.1% to about 45%, about 0.1% to about 50%, about 0.1% to about 55%, about 0.1% to about 60%, about 0.1% to about 65%, about 0.1% to about 70%, about 0.1% to about 75%, about 1% to about 15%, about 1% to about 20%, about 1% to about 25%, about 1% to about 30%, about 1% to about 35%, about 1% to about 40%, about 1% to about 45%, about 1% to about 50%, about 1% to about 55%, about 1% to about 60%, about 1% to about 65%, about 1% to about 70%, about 1% to about 75%, about 10% to about 20%, about 10% to about 25%, about 10% to about 30%, about 10% to about 35%, about 10% to about 40%, about 10% to about 45%, about 10% to about 50%, about 10% to about 55%, about 10% to about 60%, about 10% to about 65%, about 10% to about 70%, about 10% to about 75%, about 25% to about 35%, about

25% to about 40%, about 25% to about 45%, about 25% to about 50%, about 25% to about 55%, about 25% to about 60%, about 25% to about 65%, about 25% to about 70%, or about 25% to about 75%, of the light.

In some embodiments, a tissue filler described herein is optically translucent. In aspects of this embodiments, a tissue filler described herein diffusely transmits, e.g., about 75% of the light, about 80% of the light, about 85% of the light, about 90% of the light, about 95% of the light, or about 100% of the light. In other aspects of these embodiments, a tissue filler diffusely transmits, e.g., at least 0.1% of the light, at least 1% of the light, at least 5% of the light, at least 10% of the light, at least 15% of the light, at least 20% of the light, at least 25% of the light, at least 30% of the light, at least 35% of the light, at least 40% of the light, at least 45% of the light, at least 50% of the light, at least 55% of the light, at least 60% of the light, at least 65% of the light, at least 70% of the light, 75% of the light, at least 80% of the light, at least 85% of the light, at least 90% of the light, or at least 95% of the light. In other aspects of these embodiments, a tissue filler diffusely transmits, e.g., at most 0.1% of the light, at most 1% of the light, at most 5% of the light, at most 10% of the light, at most 15% of the light, at most 20% of the light, at most 25% of the light, at most 30% of the light, at most 35% of the light, at most 40% of the light, at most 45% of the light, at most 50% of the light, at most 55% of the light, at most 60% of the light, at most 65% of the light, at most 70% of the light, 75% of the light, at most 80% of the light, at most 85% of the light, at most 90% of the light, at most 95% of the light, or at most 100% of the light. In yet other aspects of these embodiments, a tissue filler diffusely transmits, e.g., about 0.1% to about 100% of the light, about 1% to about 100% of the light, about 5% to about 100% of the light, about 10% to about 100% of the light, about 15% to about 100% of the light, about 20% to about 100% of the light, about 25% to about 100% of the light, about 30% to about 100% of the light, about 35% to about 100% of the light, about 45% to about 100% of the light, about 50% to about 100% of the light, about 55% to about 100% of the light, about 60% to about 100% of the light, about 65% to about 100% of the light, about 70% to about 100% of the light, about 75% to about 100% of the light, about 80% to about 100% of the light, about 85% to about 100% of the light, about 90% to about 100% of the light, or about 95% to about 100% of the light.

In some embodiments, a tissue filler described herein may be described by its attenuation coefficient, which is defined as a description of material's ability to scatter or absorb light.

Tissue filler and skin properties can influence the manifestation of the adverse Tyndall effect event in skin following delivery of certain tissue fillers known in the art. Fillers with high stiffness and elasticity can be used to correct areas on the face like nasolabial folds, cheeks, and chin without any fear of facial discoloration, as the materials are injected in the mid and deep dermis regions. However, when fillers are used for more superficial applications, for example to correct fine line wrinkles, or mistakenly applied too superficially in the upper regions of the dermis, a bluish discoloration of the skin is often observed. This phenomenon, which is thought to be the result of Tyndall effect, leaves a semi-permanent discoloration of the application sites. In some embodiments, the effect disappears after the administration of enzymes, for example hyaluronidase, in order to degrade the filler material. Consequently, Tyndall effect is more common in patients treated for superficial fine line wrinkles. Prolonged manifestation of Tyndall effect, typically for as long as the filler lasts in the skin, is an undesired side effect and a cause of concern for patients.

In some embodiments, the tissue fillers described herein mitigate the Tyndall effect due to their homogeneity and resulting opacity.

In some embodiments, the tissue fillers described herein do not result in Tyndall effect, or do not result in any visually perceptible blue discoloration resulting from Tyndall effect. In some embodiments, the tissue fillers described herein do not result in Tyndall effect, or do not result in any visually perceptible blue discoloration resulting from Tyndall effect. In some embodiments, the invention relates to tissue fillers and methods for improving aesthetic appearance, comprising administering, to a dermal region of a patient, a substantially optically transparent dermal filler composition that exhibits no or insignificant Tyndall effect. The appearance of a blue discoloration at the skin site where a tissue filler had been injected, (Tyndall effect) is a significant adverse event experienced by some dermal filler patients. Tyndall effect is more common in patients treated for superficial fine line wrinkles. Embodiments of the present invention have been developed which provide long lasting, translucent fillers which can be injected

superficially to treat fine lines and wrinkles, even in regions of relatively thin skin, without any resulting blue discoloration from Tyndall effect. Fine lines or superficial wrinkles are generally understood to be those wrinkles or creases in skin that are typically found in regions of the face (forehead, lateral canthus, vermillion border/perioral lines) where the skin is thinnest, that is, the skin has a dermis thickness of less than 1 mm. On the forehead the average dermal thickness is about 0.95 mm for normal skin and about 0.81 mm for wrinkled skin. Dermis around the lateral canthus is even thinner (e.g., about 0.61 mm for normal skin and about 0.41 mm for wrinkled skin). The average outer diameter of a 30 or 32 gauge needle (needles that are typically used for fine line gel application) is about 0.30 and about 0.24 mm.In some embodiments, the tissue fillers described herein do not result in Tyndall effect, or do not result in any visually perceptible blue discoloration resulting from Tyndall effect.

In an embodiment, a tissue filler disclosed herein is optically opaque. In aspects of this embodiment, a tissue filler disclosed herein transmits, e.g., about 5% of the light, about 10% of the light, about 15% of the light, about 20% of the light, about 25% of the light, about 30% of the light, about 35% of the light, about 40% of the light, about 45% of the light, about 50% of the light, about 55% of the light, about 60% of the light, about 65% of the light, or about 70% of the light. In other aspects of this embodiment, a tissue filler disclosed herein transmits, e.g., at most 5% of the light, at most 10% of the light, at most 15% of the light, at most 20% of the light, at most 25% of the light, at most 30% of the light, at most 35% of the light, at most 40% of the light, at most 45% of the light, at most 50% of the light, at most 55% of the light, at most 60% of the light, at most 65% of the light, at most 70% of the light, or at most 75% of the light. In other aspects of this embodiment, a tissue filler disclosed herein transmits, e.g., about 5% to about 15%, about 5% to about 20%, about 5% to about 25%, about 5% to about 30%, about 5% to about 35%, about 5% to about 40%, about 5% to about 45%, about 5% to about 50%, about 5% to about 55%, about 5% to about 60%, about 5% to about 65%, about 5% to about 70%, about 5% to about 75%, about 15% to about 20%, about 15% to about 25%, about 15% to about 30%, about 15% to about 35%, about 15% to about 40%, about 15% to about 45%, about 15% to about 50%, about 15% to about 55%, about 15% to about 60%, about 15% to about 65%, about 15% to about 70%, about 15% to about 75%, about 25% to about

35%, about 25% to about 40%, about 25% to about 45%, about 25% to about 50%, about 25% to about 55%, about 25% to about 60%, about 25% to about 65%, about 25% to about 70%, or about 25% to about 75%, of the light.

In some embodiments, a tissue filler disclosed herein exhibits, e.g., about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100% reduction in tyndalling. In other aspects of these embodiments, a tissue filler disclosed herein exhibits, e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, reduction in tyndalling. In other aspects of these embodiments, a tissue filler disclosed herein exhibits, e.g., about 20% to about 100%, about 50% to about 100%, about 70% to about 100%, about 15% to about 35%, about 20% to about 40%, about 25% to about 45%, about 30% to about 50%, about 35% to about 55%, about 40% to about 60%, about 45% to about 65%, about 50% to about 70%, about 55% to about 75%, about 60% to about 80%, about 65% to about 85%, about 70% to about 90%, about 75% to about 95%, or about 80% to about 100%, reduction in tyndalling.

Water Content

In an embodiment, the tissue fillers described herein may include water. For example, some tissue fillers described herein may be gels, such as hydrogels, and may include water absorbed, entrapped, or otherwise disposed therein.

In some embodiments, the crosslinked silk-HA hydrogel is a low swelling hydrogel. In some embodiments, the crosslinked silk-HA hydrogel is a high swelling hydrogel. In some embodiments, the degree of swelling for the hydrogel formulations of the present disclosure may be modulated by controlling the degree of crosslinking or by varying HA contents. The higher the degree of crosslinking is present in the hydrogel, the lower the degree of swelling of the hydrogel will be due to tighter hydrogel structure. The more the HA content is present in the hydrogel, the higher the degree of swelling will be due to the presence of more hydroxyl groups (-OH) in the HA structure.

In an embodiment, the percent water content, by weight, in the tissue fillers of the present disclosure is 1% to 95%. In an embodiment, the percent water content, by weight, in the tissue fillers described herein is at least 1%, or at least 2%, or at least 3%, or at least 4%, or at least 5%, or at least 6%, or at least 7%, or at least 8%, or at least 9%, or at least 10%, or at least 11%, or at least 12%, or at least 13%, or at least 14%, or at least 15%, or at least 16%, or at least 17%, or at least 18%, or at least 19%, or at least 20%, or at least 21%, or at least 22%, or at least 23%, or at least 24%, or at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least 35%, or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45%, or at least 46%, or at least 47%, or at least 48%, or at least 49%, or at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55%, or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 61%, or at least 62%, or at least 63%, or at least 64%, or at least 65%, or at least 66%, or at least 67%, or at least 68%, or at least 69%, or at least 70%, or at least 71%, or at least 72%, or at least 73%, or at least 74%, or at least 75%, or at least 76%, or at least 77%, or at least 78%, or at least 79%, or at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%.

In an embodiment, the percent water content, by weight, in the tissue fillers described herein is at most 1%, or at most 2%, or at most 3%, or at most 4%, or at most 5%, or at most 6%, or at most 7%, or at most 8%, or at most 9%, or at most 10%, or at most 11%, or at most 12%, or at most 13%, or at most 14%, or at most 15%, or at most 16%, or at most 17%, or at most 18%, or at most 19%, or at most 20%, or at most 21%, or at most 22%, or at most 23%, or at most 24%, or at most 25%, or at most 26%, or at most 27%, or at most 28%, or at most 29%, or at most 30%, or at most 31%, or at most 32%, or at most 33%, or at most 34%, or at most 35%, or at most 36%, or at most 37%, or at most 38%, or at most 39%, or at most 40%, or at most 41%, or at most 42%, or at most 43%, or at most 44%, or at most 45%, or at most 46%, or at most 47%, or at most 48%, or at most 49%, or at most 50%, or at most 51%, or at most 52%, or at most 53%,

or at most 54%, or at most 55%, or at most 56%, or at most 57%, or at most 58%, or at most 59%, or at most 60%, or at most 61%, or at most 62%, or at most 63%, or at most 64%, or at most 65%, or at most 66%, or at most 67%, or at most 68%, or at most 69%, or at most 70%, or at most 71%, or at most 72%, or at most 73%, or at most 74%, or at most 75%, or at most 76%, or at most 77%, or at most 78%, or at most 79%, or at most 80%, or at most 81%, or at most 82%, or at most 83%, or at most 84%, or at most 85%, or at most 86%, or at most 87%, or at most 88%, or at most 89%, or at most 90%, or at most 91%, or at most 92%, or at most 93%, or at most 94%, or at most 95%.

In an embodiment, the percent water content, by weight, in the tissue fillers described herein is 1% to 2%, or 2% to 3%, or 3% to 4%, or 4% to 5%, or 5% to 6%, or 6% to 7%, or 7% to 8%, or 8% to 9%, or 9% to 10%, or 10% to 11%, or 11% to 12%, or 12% to 13%, or 13% to 14%, or 14% to 15%, or 15% to 16%, or 16% or 17%, or 17% to 18%, or 18% to 19%, or 19% to 20%, or 20% to 21%, or 21% to 22%, or 22% to 23%, or 23% to 24%, or 24% to 25%, or 25% to 26%, or 26% to 27%, or 27% to 28%, or 28% to 29%, or 30% to 31%, or 31% to 32%, or 32% to 33%, or 33% to 34%, or 34% to 35%, or 35% to 36%, or 36% to 37%, or 37% to 38%, or 38% to 39%, or 39% to 40%, or 40% to 41%, or 41% to 42%, or 42% to 43%, or 43% to 44%, or 44% to 45%, or 45% to 46%, or 46% to 47%, or 47% to 48%, or 48% to 49%, or 49% to 50%, or 50% to 51%, or 51% to 52%, or 52% to 53%, or 53% to 54%, or 54% to 55%, or 55% to 56%, or 56% to 57%, or 57% to 58%, or 58% to 59%, or 59% to 60%, or 60% to 61%, or 61% to 62%, or 62% to 63%, or 63% to 64%, or 64% to 65%, or 65% to 66%, or 66% to 67%, or 67% to 68%, or 68% to 69%, or 69% to 70%, or 70% to 71%, or 71% to 72%, or 72% to 73%, or 73% to 74%, or 74% to 75%, or 75% to 76%, or 76% to 77%, or 77% to 78%, or 78% to 79%, or 79% to 80%, or 80% to 81%, or 81% to 82%, or 82% to 83%, or 83% to 84%, or 84% to 85%, or 85% to 86%, or 86% to 87%, or 87% to 88%, or 88% to 89%, or 89% to 90%, or 90% to 91%, or 91% to 92%, or 92% to 93%, or 93% to 94%, or 94% to 95%, or 95% to 96%, or 96% to 97%, or 97% to 98%.

In an embodiment, the percent water content, by weight, in the tissue fillers described herein is about 1%, or about 2%, or about 3%, or about 4%, or about 5%, or about 6%, or about 7%, or about 8%, or about 9%, or about 10%, or about 11%, or about 12%, or about 13%, or about 14%, or about 15%, or about 16%, or about 17%, or about

18%, or about 19%, or about 20%, or about 21%, or about 22%, or about 23%, or about 24%, or about 25%, or about 26%, or about 27%, or about 28%, or about 29%, or about 30%, or about 31%, or about 32%, or about 33%, or about 34%, or about 35%, or about 36%, or about 37%, or about 38%, or about 39%, or about 40%, or about 41%, or about 42%, or about 43%, or about 44%, or about 45%, or about 46%, or about 47%, or about 48%, or about 49%, or about 50%, or about 51%, or about 52%, or about 53%, or about 54%, or about 55%, or about 56%, or about 57%, or about 58%, or about 59%, or about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%.

Mechanical Properties

The tissue fillers described herein, or components thereof, may be provided in a number of physical states depending upon the selected therapy and mode of delivery. In some embodiments, the tissue fillers of the invention are fluids, for example liquids. In some embodiments, the tissue fillers of the invention are viscous fluids. In some embodiments, the tissue fillers of the invention are solids. In some embodiments, the tissue fillers of the invention are elastic solids.

A number of rheological properties may be evaluated when examining the tissue fillers described herein, as shown in Table 17:

Table 17

Rheology Terms Used to Describe Tissue Fillers	
Elasticity	Ability of tissue filler to spring back to its original shape after deformation
Elastic Modulus	Measure of stored energy in viscoelastic material represented by symbol G’
Viscosity	Flow characteristics of tissue filler (gel thickness)
Viscous Modulus	Measure of dissipated energy in viscoelastic material represented by G”

Complex Modulus	Total resistance to deformation of tissue filler determined by vector sum of G' and G" (G*)
Complex Viscosity	Viscosity calculated from frequency sweep represented by n*
Viscoelastic	Describes tissue fillers which possess elastic and viscous properties
Shear force	External force which is applied parallel to tissue filler by placing between two plates that twist in opposite directions
Shear thinning	Decreasing tissue filler viscosity with increasing rate of deformation

In some embodiments, the tissue fillers of the invention are viscoelastic materials, which exhibit mechanical properties of both elastic, and viscous materials. In some embodiments, the tissue fillers of the invention may be described as gels. Methods for assessing the mechanical or rheological properties (e.g., viscoelastic properties) of a material are known in the art, such as for example described in U.S. Patent Application Publication No. 2006/0105022 and Stocks, et al., J. Drugs. Dermatol. (2011) 10:974-980, the entirety of which are incorporated herein by reference. Viscoelasticity of a material can be characterized by using dynamic mechanical analysis, for example by applying an oscillatory stress to a sample and measuring the resulting strain. Elastic materials typically exhibit in-phase stress and strain, i.e., application of stress results in immediate strain. In viscous materials, strain is de-phased from the application of stress by 90 degrees. In viscoelastic materials, the phase difference between strain and stress is more than 0, but less than 90 degrees. In some embodiments, the viscoelasticity of SPF materials of the invention can be characterized by means of the complex dynamic modulus G, which includes the storage modulus G' (also referred to as the elastic modulus), and the loss modulus G'' (also referred to as the viscous modulus):

$$G = G' + iG''$$

where $i^2 = -1$, $G' = \frac{\sigma_0}{\varepsilon_0}\cos\delta$, and $G'' = \frac{\sigma_0}{\varepsilon_0}\sin\delta$, σ_0 is the amplitude of stress, ε_0 is the amplitude of strain, and δ is the phase shift.

The elastic modulus G' and the loss modulus G'' are measured by subjecting an SPF gel sample to an oscillatory stress in a rotational, or shear rheometer. The sample is

placed between two plates, one fixed and one being able to rotate, or oscillate with a given frequency. The values of the elastic modulus G' and the loss modulus G'' are frequency dependent. Ranges of frequency used in measuring the elastic modulus G' and the loss modulus G'' are typically between, but not limited to, 0.1 to 10 Hz. In some embodiments, the elastic modulus G' and the loss modulus G'' are measured at an oscillatory frequency of 1 Hz.

In some embodiments, rheological properties of the tissue fillers described herein, e.g., G' and G", can be measured with an oscillatory parallel plate rheometer. A plate of various diameters, for example 25 mm can be used at a gap height between plates of various distances, for example 1 mm. Measurements can be performed at various temperatures. In some embodiments, measurements are performed at a constant temperature of 25 °C. In some embodiments, a measurement includes a frequency sweep between two frequency values, for example from 1 to 10 Hz, at a specific strain value, for example at a constant strain of 2%. In some embodiments, measurements include a logarithmic increase of frequency, followed by a strain sweep which can be for example between 1 to 300% at a constant frequency, for example 5 Hz with a logarithmic increase in strain. In some embodiments, the storage modulus G' and the loss modulus G" can be obtained from a strain sweep at a specific percentage strain value, for example at 1% strain.

In some embodiments, the complex modulus (i.e., the sum of G' and *i*G") provides a comprehensive measure of total resistance to deformation of a particular tissue filler described herein. Complex modulus may be tested using a rheometer where a particular tissue filler (e.g., a gel) may be squeezed between two parallel circular plates and variable rotational strain is provided by rotating one plate at varying frequencies.

In some embodiments, the characteristics of a particular tissue filler may be examined via that tissue filler's percent elasticity, where percent elasticity is equal to 100 × G'/(G' + G").

In some embodiments, the characteristics of a particular tissue filler may be examined via that tissue filler's recovery coefficient:

$$Recovery\ Coefficient = \frac{Viscosity\ value\ obtained\ during\ increasing\ sweep\ frequency}{Viscosity\ value\ obtained\ during\ decreasing\ sweep\ frequency}$$

where: a recovery coefficient of about 1 means that the particular tissue filler (e.g., a gel) retained its structure despite applied forces; a recovery coefficient of greater than 1 means that the particular tissue filler (e.g., a gel) experienced structural breakdown; and a recovery coefficient of less than 1 gel experienced increased structural performance.

Without being limited to any one theory of the invention, increasing G' results in a relative increase in a material's ability to better resist alterations in shape and the material may be described as being firmer, harder, or more elastic than a material (e.g., gel tissue filler) with a lower G'. Accordingly, increasing G' may result in a corresponding increase in a material's ability to provide structural support and/or volumization.

Without being limited to any one theory of the invention, increasing G" results in a more viscous material (e.g., gel) as compared to a material having a lower G". Moreover, there is a greater energy loss as dissipated heat for materials with higher G". In some embodiments, G' increases with an increasing degree of cross-linking. In some embodiments, G'' increases with an increasing degree of cross-linking. In some embodiments, both G' and G'' increase with an increasing degree of cross-linking. In some embodiments, the tissue fillers of the invention have a G' from about less than 50 Pa, to about more than 15000 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 50 Pa to about 500,000 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 100 Pa to about 500,000 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 75 Pa to about 150 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 100 Pa to about 250 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 150 Pa to about 275 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 150 Pa to about 500 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 250 Pa to about 750 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 375 Pa to about 675 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 425 Pa to about 850 Pa. In some

embodiments, the tissue fillers of the invention have a G' from about 500 Pa to about 1000 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 650 Pa to about 1050 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 750 Pa to about 1250 Pa. In some embodiments, the tissue fillers of the invention have a G' from about 950 Pa to about 1500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 50 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 100 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 150 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 200 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 225 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 275 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 300 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 325 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 350 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 375 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 400 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 425 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 450 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 475 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 525 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 550 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 575 Pa. In some embodiments, the tissue fillers of the invention have a G' of about at least Pa. In some embodiments, the tissue fillers of the invention have a G' of about 625 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 650 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 675 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 700 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 725 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 750 Pa. In some

embodiments, the tissue fillers of the invention have a G' of at least 775 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 800 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 825 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 850 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 875 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 900 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 925 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 950 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 975 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 1050 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1100 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1150 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1200 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1300 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1350 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1400 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1450 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 1500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 50 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 100 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 150 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 200 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 225 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 275 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 300 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 325 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 350 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 375 Pa. In

some embodiments, the tissue fillers of the invention have a G' of at most 400 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 425 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 450 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 475 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 525 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 550 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 575 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most Pa. In some embodiments, the tissue fillers of the invention have a G' of about 625 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 650 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 675 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 700 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 725 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 775 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 800 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 825 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 850 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 875 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 900 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 925 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 950 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 975 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 1050 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1100 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1150 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1200 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1300 Pa. In

some embodiments, the tissue fillers of the invention have a G' of at most 1350 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1400 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1450 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 1500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 50 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 100 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 150 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 200 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 225 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 275 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 300 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 325 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 350 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 375 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 400 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 425 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 450 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 475 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 525 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 550 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 575 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 600 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 625 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 650 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 675 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 700 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 725 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 775 Pa. In some

embodiments, the tissue fillers of the invention have a G' of about 800 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 825 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 850 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 875 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 900 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 925 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 950 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 975 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 1050 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1100 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1150 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1200 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1300 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1350 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1400 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1450 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1500 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 2000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 2250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 2500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 2750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 3000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 3250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 3500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 3750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 4000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 4250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 4500 Pa. In

some embodiments, the tissue fillers of the invention have a G' of at least 4750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 5000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 5250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 5500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 5750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about at least 6000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 6250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 6500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 6750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 7000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 7250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 7500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 7750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 8000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 8250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 8500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 8750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 9000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 9250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 9500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 9750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 10000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at least 10500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 11000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 11500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 12000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 12500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 13000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 13500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 14000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least

14500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at least 15000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 200 0Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 2250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 2500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 2750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 3000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 3250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 3500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 3750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 4000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 4250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 4500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 4750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 5000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 5250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 5500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 5750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 6000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 6250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 6500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 6750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 7000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 7250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 7500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 7750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 8000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 8250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 8500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 8750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 9000 Pa. In

some embodiments, the tissue fillers of the invention have a G' of at most 9250 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 9500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 9750 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 10000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of at most 10500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 11000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 11500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 12000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 12500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 13000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 13500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 14000 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 14500 Pa. In some embodiments, the tissue fillers of the invention have a G' of at most 15000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 2000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 2250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 2500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 2750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 3000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 3250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 3500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 3750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 4000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 4250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 4500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 4750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 5000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 5250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 5500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 5750 Pa. In

some embodiments, the tissue fillers of the invention have a G' of about 6000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 6250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 6500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 6750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 7000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 7250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 7500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 7750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 8000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 8250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 8500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 8750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 9000 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 9250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 9500 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 9750 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 10000 Pa.

In some embodiments, the tissue fillers of the invention have a G' of about 1050 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1100 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1150 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1200 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1250 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1300 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1350 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1400 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1450 Pa. In some embodiments, the tissue fillers of the invention have a G' of about 1500 Pa.

In some embodiments, the tissue fillers of the invention have a G'' from about less than 5 Pa, to about more than 200 Pa. In some embodiments, the tissue fillers of the invention have a G'' from about 5 Pa to about 200 Pa. In some embodiments, the tissue fillers of the invention have a G'' from about 5 Pa to about 25 Pa. In some embodiments,

the tissue fillers of the invention have a $G"$ from about 15 Pa to about 35 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 10 Pa to about 50 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 15 Pa to about 75 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 20 Pa to about 85 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 25 Pa to about 100 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 35 Pa to about 125 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 45 Pa to about 115 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 75 Pa to about 150 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 100 Pa to about 175 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ from about 115 Pa to about 200 Pa.

In some embodiments, the tissue fillers of the invention have a $G"$ of at least 5 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 10 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 15 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 20 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 25 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 30 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 35 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 40 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 45 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 50 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 55 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 60 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 65 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 70 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 75 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 80 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 85 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 90 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of at least 95 Pa. In some

embodiments, the tissue fillers of the invention have a G'' of at least 100 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 105 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 110 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 115 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 120 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 125 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 130 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 135 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 140 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 145 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 150 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 155 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 160 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 165 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 170 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 175 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 180 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 185 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 190 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 195 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at least 200 Pa.

In some embodiments, the tissue fillers of the invention have a G'' of at most 5 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 10 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 15 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 20 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 25 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 30 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 35 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 40 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 45 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 50 Pa. In some

embodiments, the tissue fillers of the invention have a G'' of at most 55 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 60 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 65 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 70 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 75 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 80 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 85 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 90 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 95 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 100 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 105 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 110 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 115 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 120 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 125 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 130 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 135 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 140 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 145 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 150 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 155 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 160 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 165 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 170 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 175 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 180 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 185 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 190 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 195 Pa. In some embodiments, the tissue fillers of the invention have a G'' of at most 200 Pa.

In some embodiments, the tissue fillers of the invention have a $G"$ of about 5 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 10 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 15 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 20 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 25 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 30 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 35 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 40 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 45 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 50 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 55 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 60 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 65 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 70 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 75 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 80 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 85 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 90 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 95 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 100 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 105 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 110 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 115 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 120 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 125 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 130 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 135 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 140 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 145 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 150 Pa. In some embodiments, the tissue fillers of the invention have a $G"$ of about 155 Pa. In some

embodiments, the tissue fillers of the invention have a G'' of about 160 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 165 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 170 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 175 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 180 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 185 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 190 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 195 Pa. In some embodiments, the tissue fillers of the invention have a G'' of about 200 Pa.

In some embodiments, a tissue filler disclosed herein exhibits dynamic viscosity. Viscosity is resistance of a fluid to shear or flow caused by either shear stress or tensile stress. Viscosity describes a fluid's internal resistance to flow caused by intermolecular friction exerted when layers of fluids attempt to slide by one another and may be thought of as a measure of fluid friction. The less viscous the fluid, the greater its ease of movement (fluidity).

Viscosity can be defined in two ways; dynamic viscosity (μ; η is sometimes used) or kinematic viscosity (v). Dynamic viscosity, also known as absolute or complex viscosity, is the tangential force per unit area required to move one horizontal plane with respect to the other at unit velocity when maintained a unit distance apart by the fluid. The SI physical unit of dynamic viscosity is the Pascal-second (Pas), which is identical to $Nm^{-2}s$. Dynamic viscosity can be expressed as $\tau = \mu\, dvx/dz$, where τ = shearing stress, μ = dynamic viscosity, and dvx/dz is the velocity gradient over time. For example, if a fluid with a viscosity of one Pa·s is placed between two plates, and one plate is pushed sideways with a shear stress of one Pascal, it moves a distance equal to the thickness of the layer between the plates in one second. Kinematic viscosity (v) is the ratio of dynamic viscosity to density, a quantity in which no force is involved and is defined as follows: $v = \mu/\rho$, where μ is the dynamic viscosity, and ρ is density (kg/m^3). Kinematic viscosity is usually measured by a glass capillary viscometer as has an SI unit of m^2/s. The viscosity of a fluid is temperature dependent, and thus dynamic and kinematic viscosity are reported in reference to temperature.

In some embodiments, a tissue filler disclosed herein exhibits a dynamic viscosity of, for example, at least 10 Pa·s, at least 20 Pa·s, at least 30 Pa·s, at least 40 Pa·s, at least 50 Pa·s, at least 60 Pa·s, at least 70 Pa·s, at least 80 Pa·s, at least 90 Pa·s, at least 100 Pa·s, at least 125 Pa·s, at least 150 Pa·s, at least 175 Pa·s, at least 200 Pas, at least 225 Pa·s, at least 250 Pa·s, at least 275 Pa·s, at least 300 Pa·s, at least 400 Pa·s, at least 500 Pa·s, at least 600 Pa·s, at least 700 Pa·s, at least 750 Pa·s, at least 800 Pa·s, at least 900 Pa·s, at least 1,000 Pa·s, at least 1,100 Pa·s, or at least 1,200 Pa·s. In some embodiments, a tissue filler disclosed herein exhibits a dynamic viscosity of, for example, at most 10 Pa·s, at most 20 Pa·s, at most 30 Pa·s, at most 40 Pa·s, at most 50 Pa·s, at most 60 Pa·s, at most 70 Pa·s, at most 80 Pa·s, at most 90 Pa·s, at most 100 Pa·s, at most 125 Pa·s, at most 150 Pa·s, at most 175 Pa·s, at most 200 Pa·s, at most 225 Pa·s, at most 250 Pa·s, at most 275 Pa·s, at most 300 Pa·s, at most 400 Pa·s, at most 500 Pa·s, at most 600 Pa·s, at most 700 Pa·s, at most 750 Pa·s, at most 800 Pa·s, at most 900 Pa·s, or at most 1000 Pa·s. In some embodiments, a tissue filler disclosed herein exhibits a dynamic viscosity of, for example, about 10 Pa·s to about 100 Pa·s, about 10 Pa·s to about 150 Pa·s, about 10 Pa·s to about 250 Pa·s, about 50 Pa·s to about 100 Pa·s, about 50 Pa·s to about 150 Pa·s, about 50 Pa·s to about 250 Pa·s, about 100 Pa·s to about 500 Pa·s, about 100 Pa·s to about 750 Pa·s, about 100 Pa·s to about 1,000 Pa·s, about 100 Pa·s to about 1,200 Pa·s, about 300 Pa·s to about 500 Pa·s, about 300 Pa·s to about 750 Pa·s, about 300 Pa·s to about 1,000 Pa·s, or about 300 Pa·s to about 1,200 Pa·s.

In an embodiment, the tissue fillers described herein may substantially maintain their G' and/or G" *in vivo* for at least 1 day, or at least 2 days, or at least 3 days, or at least 4 days, or at least 5 days, or at least 6 days, or at least 1 week, or at least 2 weeks, or at least 3 weeks, or at least 1 month, or at least 2 months, or at least 3 months, or at least 4 months, or at least 5 months, or at least 6 months, or at least 7 months, or at least 8 months, or at least 9 months, or at least 10 months, or at least 11 months, or at least 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their G' and/or G" *in vivo* for at most 1 day, or at most 2 days, or at most 3 days, or at most 4 days, or at most 5 days, or at most 6 days, or at most 1 week, or at most 2 weeks, or at most 3 weeks, or at most 1 month, or at most 2 months, or at most 3 months, or at

most 4 months, or at most 5 months, or at most 6 months, or at most 7 months, or at most 8 months, or at most 9 months, or at most 10 months, or at most 11 months, or at most 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their G' and/or G" *in vivo* for about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 1 week, or about 2 weeks, or about 3 weeks, or about 1 month, or about 2 months, or about 3 months, or about 4 months, or about 5 months, or about 6 months, or about 7 months, or about 8 months, or about 9 months, or about 10 months, or about 11 months, or about 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their elasticity *in vivo* for at least 1 day, or at least 2 days, or at least 3 days, or at least 4 days, or at least 5 days, or at least 6 days, or at least 1 week, or at least 2 weeks, or at least 3 weeks, or at least 1 month, or at least 2 months, or at least 3 months, or at least 4 months, or at least 5 months, or at least 6 months, or at least 7 months, or at least 8 months, or at least 9 months, or at least 10 months, or at least 11 months, or at least 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their elasticity *in vivo* for at most 1 day, or at most 2 days, or at most 3 days, or at most 4 days, or at most 5 days, or at most 6 days, or at most 1 week, or at most 2 weeks, or at most 3 weeks, or at most 1 month, or at most 2 months, or at most 3 months, or at most 4 months, or at most 5 months, or at most 6 months, or at most 7 months, or at most 8 months, or at most 9 months, or at most 10 months, or at most 11 months, or at most 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their elasticity *in vivo* for about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 1 week, or about 2 weeks, or about 3 weeks, or about 1 month, or about 2 months, or about 3 months, or about 4 months, or about 5 months, or about 6 months, or about 7 months, or about 8 months, or about 9 months, or about 10 months, or about 11 months, or about 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their viscosity *in vivo* for at least 1 day, or at least 2 days, or at least 3 days, or at least 4

days, or at least 5 days, or at least 6 days, or at least 1 week, or at least 2 weeks, or at least 3 weeks, or at least 1 month, or at least 2 months, or at least 3 months, or at least 4 months, or at least 5 months, or at least 6 months, or at least 7 months, or at least 8 months, or at least 9 months, or at least 10 months, or at least 11 months, or at least 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their viscosity *in vivo* for at most 1 day, or at most 2 days, or at most 3 days, or at most 4 days, or at most 5 days, or at most 6 days, or at most 1 week, or at most 2 weeks, or at most 3 weeks, or at most 1 month, or at most 2 months, or at most 3 months, or at most 4 months, or at most 5 months, or at most 6 months, or at most 7 months, or at most 8 months, or at most 9 months, or at most 10 months, or at most 11 months, or at most 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their viscosity *in vivo* for about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 1 week, or about 2 weeks, or about 3 weeks, or about 1 month, or about 2 months, or about 3 months, or about 4 months, or about 5 months, or about 6 months, or about 7 months, or about 8 months, or about 9 months, or about 10 months, or about 11 months, or about 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their volume *in vivo* for at least 1 day, or at least 2 days, or at least 3 days, or at least 4 days, or at least 5 days, or at least 6 days, or at least 1 week, or at least 2 weeks, or at least 3 weeks, or at least 1 month, or at least 2 months, or at least 3 months, or at least 4 months, or at least 5 months, or at least 6 months, or at least 7 months, or at least 8 months, or at least 9 months, or at least 10 months, or at least 11 months, or at least 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their volume *in vivo* for at most 1 day, or at most 2 days, or at most 3 days, or at most 4 days, or at most 5 days, or at most 6 days, or at most 1 week, or at most 2 weeks, or at most 3 weeks, or at most 1 month, or at most 2 months, or at most 3 months, or at most 4 months, or at most 5 months, or at most 6 months, or at most 7 months, or at most 8

months, or at most 9 months, or at most 10 months, or at most 11 months, or at most 1 year.

In an embodiment, the tissue fillers described herein may substantially maintain their volume *in vivo* for about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 1 week, or about 2 weeks, or about 3 weeks, or about 1 month, or about 2 months, or about 3 months, or about 4 months, or about 5 months, or about 6 months, or about 7 months, or about 8 months, or about 9 months, or about 10 months, or about 11 months, or about 1 year.

Methods of Manufacture

The tissue fillers provided herein may be prepared by combining an SPF based component with an HA based component with or without any additional agents. In certain embodiments, one or both of the SPF and HA may be crosslinked prior to combination. In some embodiments, the SPF and HA may be combined and then crosslinked with a cross-linking agent as described herein. In some embodiments, the SPF may be crosslinked with a cross linking agent and then added to a HA, which may or may not be cross linked, and then the combination thereof may be subjected to additional cross linking. In some embodiments, the HA may be crosslinked with a cross linking agent and then added to a SPF, which may or may not be cross linked, and then the combination thereof may be subjected to additional cross linking.

In some embodiments, the tissue fillers described herein may be prepared by combining an SPF based component, and HA based component, and an additional agent, as described hereinabove. In such embodiments, one or both of the SPF and HA may be crosslinked prior to combination. In some embodiments, the SPF and HA may be combined with the additional agent and then crosslinked with a cross-linking agent as described herein. In some embodiments, the additional agent may be added after combining the SPF and HA.

In some embodiments, the tissue filler described herein may include SPF and HA in a weight ratio (SPF:HA) of 0.1:1 to 0.1:10, or 0.1:1 to 0.1:100, or 0.1:1000; 1:1 to 1:10, or 1:1 to 1:100, or 1:1 to 1:1000.

In some embodiments, the tissue filler described herein may include SPF and HA in a weight ratio (HA:SPF) of 0.1:1 to 0.1:10, or 0.1:1 to 0.1:100, or 0.1:1000; 1:1 to 1:10, or 1:1 to 1:100, or 1:1 to 1:1000.

In some embodiments, a resulting HA/SPF combination (whether crosslinked or non-crosslinked) may be homogenized such as through mechanical blending of initially crosslinked HA and/or SPF.

In some embodiments, a solution of SPF may be provided and crosslinked with a cross linking agent to yield a crosslinked SPF, to which HA may be added in either its crosslinked form, non-crosslinked form, or a mixture thereof. The resulting mixture may then be homogenized and any additional agents (e.g., lidocaine may be added).

In some embodiments, a solution of SPF may be provided and crosslinked with a cross linking agent in the presence of HA to yield a crosslinked SPF-HA composition, to which HA may, or may not, be added in its non-crosslinked form. The resulting mixture may then be homogenized and any additional agents (e.g., lidocaine may be added).

In some embodiments the specific SPF formulations provided herein may be combined with HA, or may utilize the cross-linking procedures, using the preparations set forth in U.S. Patent Nos. 8,288,347 or 8,450,475, or U.S. Patent Application Publication Nos. 2006/0105022, 2016/0376382, or 2017/0315828, the entirety of which are incorporated herein by reference.

In some embodiments, the methods described herein may include a sterilization step where the tissue filler or a portion thereof is exposed, for example, to temperatures of 120 °C to about 130 °C and pressures of about 12 to about 20 pounds per square inch for a time of about 1 to about 15 minutes.

In some embodiments, the methods described herein may include a de-gassing step wherein the SPF, HA, or SPF/HA solutions described herein that are used in preparing the resulting tissue fillers are de-gassed.

In some embodiments, the tissue fillers described herein may be prepared according to the general methods described in Examples 5 to 20. In the methods described therein, silk may be prepared in an aqueous solution, an aqueous/alcohol solution, wherein the alcohol may be ethanol or methanol, for example. In the methods described therein, any of the crosslinking agents described herein may be used as applicable to cross

link SPF to SPF, SPF to HA, or HA to HA, as would be understood by a person having ordinary skill in art.

Methods of Treatment

In an embodiment, the tissue fillers described herein may be provided in methods of treating one or more conditions in a patient in need thereof. In some embodiments, a therapeutically effective amount of a tissue filler may be delivered into a tissue of a patient in need thereof to treat a condition or other tissue deficiency.

As used herein, the term "treating,", "treat", or "treatment" refers to reducing or eliminating in a patient a cosmetic or clinical symptom of a condition, such as a soft tissue condition, or delaying or preventing in an individual the onset of a cosmetic or clinical symptom of a condition.

In some embodiments, the condition treated by the tissue fillers described herein may include a soft tissue condition. Soft tissue conditions include, without limitation, augmentations, reconstructions, diseases, disorders, defects, or imperfections of a body part, region or area. In one aspect, a soft tissue condition treated by the disclosed tissue fillers include, without limitation, a facial augmentation, a facial reconstruction, a facial disease, a facial disorder, a facial defect, or a facial imperfection. In some embodiments, a soft tissue condition treated by the tissue fillers described herein include, without limitation, skin dehydration, a lack of skin elasticity, skin roughness, a lack of skin tautness, a skin stretch line or mark, skin paleness, a dermal divot, a sunken check, a sunken temple, a thin lip, a urethra defect, a skin defect, a breast defect, a retro-orbital defect, a facial fold, or a wrinkle. In some embodiments, a soft tissue condition treated by the tissue fillers described herein include, without limitation, breast imperfection, defect, disease and/or disorder, such as, e.g., a breast augmentation, a breast reconstruction, mastopexy, micromastia, thoracic hypoplasia, Poland's syndrome, defects due to implant complications like capsular contraction and/or rupture; a facial imperfection, defect, disease or disorder, such as, e.g., a facial augmentation, a facial reconstruction, Parry-Romberg syndrome, lupus erythematosus profundus, dermal divots, sunken cheeks, sunken temples, thin lips, nasal imperfections or defects, retro-orbital imperfections or defects, a facial fold, line and/or wrinkle like a glabellar line, a nasolabial line, a perioral line, and/or a marionette line, and/or other contour deformities or imperfections of the

face; a neck imperfection, defect, disease or disorder; a skin imperfection, defect, disease and/or disorder; other soft tissue imperfections, defects, diseases and/or disorders, such as, e.g., an augmentation or a reconstruction of the upper arm, lower arm, hand, shoulder, back, torso including abdomen, buttocks, upper leg, lower leg including calves, foot including plantar fat pad, eye, genitals, or other body part, region or area, or a disease or disorder affecting these body parts, regions or areas; urinary incontinence, fecal incontinence, other forms of incontinence; and gastroesophageal reflux disease (GERD).

In some embodiments, the tissue fillers described herein may be delivered to soft tissues including, without limitation skin, dermal tissues, subdermal tissues, cutaneous tissues, subcutaneous tissues, intradural tissue, muscles, tendons, ligaments, fibrous tissues, fat, blood vessels and arteries, nerves, and synovial (intradermal) tissues.

In some embodiments, the tissue fillers described herein can be placed directly in a wound to aid in healing by providing an artificial biodegradable matrix along with cell attachment, migration, and proliferation signals. In some embodiments, the tissue fillers described herein can be coated on a biodegradable mesh or other implanted material, or it can itself be formed into sheets or other structures, or can be maintained in a hydrated form.

In some embodiments, the amount of a composition used with any of the methods as disclosed herein will be determined based on the alteration and/or improvement desired, the reduction and/or elimination of a condition symptom desired, the clinical and/or cosmetic effect desired by the individual and/or physician, and the body part or region being treated. The effectiveness of composition administration may be manifested by one or more of the following clinical and/or cosmetic measures: altered and/or improved soft tissue shape, altered and/or improved soft tissue size, altered and/or improved soft tissue contour, altered and/or improved tissue function, tissue ingrowth support and/or new collagen deposition, sustained engraftment of the tissue filler, improved patient satisfaction and/or quality of life, and decreased use of implantable foreign material. For example, for breast augmentation procedures, effectiveness of the compositions and methods may be manifested by one or more of the following clinical and/or cosmetic measures: increased breast size, altered breast shape, altered breast contour, sustained engraftment, reduction in the risk of capsular contraction, decreased

rate of liponecrotic cyst formation, improved patient satisfaction and/or quality of life, and decreased use of breast implant.

In some embodiments, effectiveness of the tissue fillers and methods in treating a facial soft tissue may be manifested by one or more of the following clinical and/or cosmetic measures: increased size, shape, and/or contour of facial feature like increased size, shape, and/or contour of lip, cheek, temple, or eye region; altered size, shape, and/or contour of facial feature like altered size, shape, and/or contour of lip, cheek, temple, or eye region shape; reduction or elimination of a wrinkle, fold or line in the skin; resistance to a wrinkle, fold or line in the skin; rehydration of the skin; increased elasticity to the skin; reduction or elimination of skin roughness; increased and/or improved skin tautness; reduction or elimination of stretch lines or marks; increased and/or improved skin tone, shine, brightness and/or radiance; increased and/or improved skin color, reduction or elimination of skin paleness; sustained engraftment of composition; decreased side effects; improved patient satisfaction and/or quality of life.

In some embodiments, the invention provides for tissue fillers and methods of treatment involving a dermal region. As used herein, the term “dermal region” refers to the region of skin comprising the epidermal-dermal junction and the dermis including the superficial dermis (papillary region) and the deep dermis (reticular region). The skin is composed of three primary layers: the epidermis, which provides waterproofing and serves as a barrier to infection; the dermis, which serves as a location for the appendages of skin; and the hypodermis (subcutaneous adipose layer). The epidermis contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells which make up the epidermis are keratinocytes, melanocytes, Langerhans cells, and Merkels cells.

The dermis is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many mechanoreceptor/nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal from its own cells as well as from the stratum basal of the epidermis. The dermis is structurally divided into two

areas: a superficial area adjacent to the epidermis, called the papillary region, and a deep thicker area known as the reticular region.

The papillary region is composed of loose areolar connective tissue. It is named for its fingerlike projections called papillae that extend toward the epidermis. The papillae provide the dermis with a "bumpy" surface that interdigitates with the epidermis, strengthening the connection between the two layers of skin. The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenous, elastic, and reticular fibers that weave throughout it. These protein fibers give the dermis its properties of strength, extensibility, and elasticity. Also located within the reticular region are the roots of the hair, sebaceous glands, sweat glands, receptors, nails, and blood vessels. Stretch marks from pregnancy are for example located in the dermis.

The hypodermis lies below the dermis. Its purpose is to attach the dermal region of the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the hypodermis contains 50% of body fat). Fat serves as padding and insulation for the body.

In some embodiments, a tissue filler disclosed herein is administered to a skin region of an individual by injection into a dermal region or a hypodermal region. In some embodiments, a tissue filler disclosed herein is administered to a dermal region of an individual by injection into, e.g., an epidermal-dermal junction region, a papillary region, a reticular region, or any combination thereof.

In some embodiments, the invention provides methods of treating a soft tissue condition of an individual, including administering one or more tissue fillers disclosed herein to a site of the soft tissue condition of the individual, wherein the administration of the composition improves the soft tissue condition, thereby treating the soft tissue condition. In some embodiments, a soft tissue condition is a breast tissue condition, a facial tissue condition, a neck condition, a skin condition, an upper arm condition, a lower arm condition, a hand condition, a shoulder condition, a back condition, a torso including abdominal condition, a buttock condition, an upper leg condition, a lower leg condition

including calf condition, a foot condition including plantar fat pad condition, an eye condition, a genital condition, or a condition effecting another body part, region or area.

In some embodiments, the invention provides methods of treating a skin condition including administering to an individual suffering from a skin condition one or more tissue fillers disclosed herein, wherein the administration of the tissue filler improves the skin condition, thereby treating the skin condition. In some embodiments, a skin condition includes skin dehydration, and the method of treatment includes administering to an individual suffering from skin dehydration one or more tissue fillers disclosed herein, wherein the administration of the tissue filler rehydrates the skin, thereby treating skin dehydration. In another aspect of these embodiments, a method of treating a lack of skin elasticity includes administering to an individual suffering from a lack of skin elasticity a tissue filler disclosed herein, wherein the administration of the tissue filler increases the elasticity of the skin, thereby treating a lack of skin elasticity. In yet another aspect of these embodiments, a method of treating skin roughness includes administering to an individual suffering from skin roughness a composition disclosed herein, wherein the administration of the composition decreases skin roughness, thereby treating skin roughness. In some embodiments, a method of treating a lack of skin tautness includes administering to an individual suffering from a lack of skin tautness a tissue filler disclosed herein, wherein the administration of the tissue filler makes the skin tauter, thereby treating a lack of skin tautness.

In some embodiments, the invention provides methods of treating a skin stretch line or mark, including administering to an individual suffering from a skin stretch line or mark one or more tissue fillers disclosed herein, wherein the administration of the one or more tissue fillers reduces or eliminates the skin stretch line or mark, thereby treating a skin stretch line or mark. In some embodiments, a method of treating skin paleness includes administering to an individual suffering from skin paleness a tissue filler disclosed herein, wherein the administration of the tissue filler increases skin tone or radiance, thereby treating skin paleness. In some embodiments, a method of treating skin wrinkles includes administering to an individual suffering from skin wrinkles a tissue filler disclosed herein, wherein the administration of the tissue filler reduces or eliminates skin wrinkles, thereby treating skin wrinkles. In yet another aspect of these embodiments,

a method of treating skin wrinkles includes administering to an individual a tissue filler disclosed herein, wherein the administration of the tissue filler makes the skin resistant to skin wrinkles, thereby treating skin wrinkles.

In some embodiments, the invention provides administration of a composition disclosed herein wherein such administration promotes new collagen deposition or formation. The tissue fillers described herein may support tissue ingrowth and new deposition or formation of collagen.

Without being limited to any one theory of the invention, the molecular weight of SPFs used in the preparation tissue fillers described herein may be adjusted to provide a mild inflammatory response at a selected tissue in order trigger the deposition or formation of collagen through the resulting tissue proliferation and maturation responses that follow the initial inflammatory response. Indeed, higher molecular weight SPFs may result in an increased inflammatory response while lower molecular weight SPFs may result in little or no inflammatory response.

Without being limited to any one theory of the invention, the tissue fillers described herein provide the unexpected attribute that a resulting inflammatory response, and thereby collagen formation through the proliferation and maturation tissue response, may be tuned because the SPF solutions used herein have narrow rather than broad polydispersities. In an embodiment, administration of a tissue filler disclosed herein increases new collagen deposition.

In some embodiments, administration of a tissue disclosed herein increases new collagen deposition or formation by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about

73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%, relative to the same or similar tissue filler comprising HA, but lacking SPF.

In some embodiments, administration of a tissue filler disclosed herein increases new collagen deposition or formation by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100%, at least 125%, at least 150%, at least 175%, at least 200%, at least 225%, at least 250%, at least 275%, or at least 300%, relative to the same or similar tissue filler comprising HA, but lacking SPF.

In some embodiments, administration of a tissue filler disclosed herein increases new collagen deposition or formation by at most 1%, at most 2%, at most 3%, at most 4%, at most 5%, at most 6%, at most 7%, at most 8%, at most 9%, at most 10%, at most 11%, at most 12%, at most 13%, at most 14%, at most 15%, at most 16%, at most 17%, at most 18%, at most 19%, at most 20%, at most 21%, at most 22%, at most 23%, at most 24%, at most 25%, at most 26%, at most 27%, at most 28%, at most 29%, at most 30%, at most 31%, at most 32%, at most 33%, at most 34%, at most 35%, at most 36%, at most

37%, at most 38%, at most 39%, at most 40%, at most 41%, at most 42%, at most 43%, at most 44%, at most 45%, at most 46%, at most 47%, at most 48%, at most 49%, at most 50%, at most 51%, at most 52%, at most 53%, at most 54%, at most 55%, at most 56%, at most 57%, at most 58%, at most 59%, at most 60%, at most 61%, at most 62%, at most 63%, at most 64%, at most 65%, at most 66%, at most 67%, at most 68%, at most 69%, at most 70%, at most 71%, at most 72%, at most 73%, at most 74%, at most 75%, at most 76%, at most 77%, at most 78%, at most 79%, at most 80%, at most 81%, at most 82%, at most 83%, at most 84%, at most 85%, at most 86%, at most 87%, at most 88%, at most 89%, at most 90%, at most 91%, at most 92%, at most 93%, at most 94%, at most 95%, at most 96%, at most 97%, at most 98%, at most 99%, at most 100%, at most 125%, at most 150%, at most 175%, at most 200%, at most 225%, at most 250%, at most 275%, or at most 300%, relative to the same or similar tissue filler comprising HA, but lacking SPF.

In some embodiments, administration of a tissue filler disclosed herein increases new collagen deposition or formation by about 1% to about 10%, about 10% to about 50%, about 10% to about 100%, about 50% to about 150%, about 100% to about 200%, about 150% to about 250%, about 200% to about 300%, about 350% to about 450%, about 400% to about 500%, about 550% to about 650%, about 600% to about 700%, relative to the same or similar tissue filler comprising HA, but lacking SPF.

In some embodiments, the amount of a tissue filler used with any of the methods disclosed herein will typically be a therapeutically effective amount. As used herein, the term “therapeutically effective amount” is synonymous with “effective amount”, “therapeutically effective dose”, and/or “effective dose,” and refers to the amount of tissue filler that will elicit the expected biological, cosmetic, or clinical response in a patient in need thereof. As a non-limiting example, an effective amount is an amount sufficient to achieve one or more of the clinical and/or cosmetic measures disclosed herein. The appropriate effective amount to be administered for a particular application of the disclosed methods can be determined by those skilled in the art, using the guidance provided herein. For example, an effective amount can be extrapolated from any and all *in vitro* and *in vivo* assays as described herein. One skilled in the art will recognize that the condition of the individual can be monitored throughout the course of therapy and that the

effective amount of a composition disclosed herein that is administered can be adjusted accordingly.

In some embodiments, the amount of a tissue filler administered is at least 0.001 g, or at least 0.002 g, or at least 0.003 g, or at least 0.004 g, or at least 0.005 g, or at least 0.006 g, or at least 0.007 g, or at least 0.008 g, or at least 0.009 g, or at least 0.01 g, or at least 0.02 g, or at least 0.03 g, or at least 0.04 g, or at least 0.05 g, or at least 0.06 g, or at least 0.07 g, or at least 0.08 g, or at least 0.09 g, or at least 0.1 g, or at least 0.2 g, or at least 0.3 g, or at least 0.4 g, or at least 0.5 g, or at least 0.6 g, or at least 0.7 g, or at least 0.8 g, or at least 0.9 g, or at least 1 g, or at least 2 g, or at least 3 g, or at least 4 g, or at least 5 g, or at least 6 g, or at least 7 g, or at least 8 g, or at least 9 g, or at least 10 g, or at least 11 g, or at least 12 g, or at least 13 g, or at least 14 g, or at least 15 g, or at least 20 g, or at least 25 g, or at least 30 g, or at least 35 g, or at least 40 g, or at least 45 g, or at least 50 g, or at least 55 g, or at least 60 g, or at least 65 g, or at least 70 g, or at least 75 g, or at least 80 g, or at least 85 g, or at least 90 g, or at least 95 g, or at least 100 g.

In some embodiments, the amount of a tissue filler administered is at most 0.001 g, or at most 0.002 g, or at most 0.003 g, or at most 0.004 g, or at most 0.005 g, or at most 0.006 g, or at most 0.007 g, or at most 0.008 g, or at most 0.009 g, or at most 0.01 g, or at most 0.02 g, or at most 0.03 g, or at most 0.04 g, or at most 0.05 g, or at most 0.06 g, or at most 0.07 g, or at most 0.08 g, or at most 0.09 g, or at most 0.1 g, or at most 0.2 g, or at most 0.3 g, or at most 0.4 g, or at most 0.5 g, or at most 0.6 g, or at most 0.7 g, or at most 0.8 g, or at most 0.9 g, or at most 1 g, or at most 2 g, or at most 3 g, or at most 4 g, or at most 5 g, or at most 6 g, or at most 7 g, or at most 8 g, or at most 9 g, or at most 10 g, or at most 11 g, or at most 12 g, or at most 13 g, or at most 14 g, or at most 15 g, or at most 20 g, or at most 25 g, or at most 30 g, or at most 35 g, or at most 40 g, or at most 45 g, or at most 50 g, or at most 55 g, or at most 60 g, or at most 65 g, or at most 70 g, or at most 75 g, or at most 80 g, or at most 85 g, or at most 90 g, or at most 95 g, or at most 100 g.

In some embodiments, the amount of a tissue filler administered is about 0.001 g, or about 0.002 g, or about 0.003 g, or about 0.004 g, or about 0.005 g, or about 0.006 g, or about 0.007 g, or about 0.008 g, or about 0.009 g, or about 0.01 g, or about 0.02 g, or about 0.03 g, or about 0.04 g, or about 0.05 g, or about 0.06 g, or about 0.07 g, or about 0.08 g, or about 0.09 g, or about 0.1 g, or about 0.2 g, or about 0.3 g, or about 0.4 g, or

about 0.5 g, or about 0.6 g, or about 0.7 g, or about 0.8 g, or about 0.9 g, or about 1 g, or about 2 g, or about 3 g, or about 4 g, or about 5 g, or about 6 g, or about 7 g, or about 8 g, or about 9 g, or about 10 g, or about 11 g, or about 12 g, or about 13 g, or about 14 g, or about 15 g, or about 20 g, or about 25 g, or about 30 g, or about 35 g, or about 40 g, or about 45 g, or about 50 g, or about 55 g, or about 60 g, or about 65 g, or about 70 g, or about 75 g, or about 80 g, or about 85 g, or about 90 g, or about 95 g, or about 100 g.

In some embodiments, the amount of a tissue filler administered is 0.001 g to 0.01 g, or 0.01 g to 0.1 g, or 0.1 g to 1 g, or 1 g to 10 g, or 10 g to 20 g, or 20 g to 30 g, or 30 g to 40 g, or 40 g to 50 g, or 50 g to 60 g, or 60 g to 70 g, or 70 g to 80 g, or 80 g to 90 g, or 90 g to 100 g.

In some embodiments, the volume of a tissue filler administered is at least 0.01 mL, or at least 0.02 mL, or at least 0.03 mL, or at least 0.04 mL, or at least 0.05 mL, or at least 0.06 mL, or at least 0.07 mL, or at least 0.08 mL, or at least 0.09 mL, or at least 0.10 mL, or at least 0.15 mL, or at least 0.20 mL, or at least 0.25 mL, or at least 0.30 mL, or at least 0.35 mL, or at least 0.40 mL, or at least 0.45 mL, or at least 0.50 mL, or at least 0.55 mL, or at least 0.60 mL, or at least 0.65 mL, or at least 0.70 mL, or at least 0.75 mL, or at least 0.80 mL, or at least 0.85 mL, or at least 0.90 mL, or at least 0.95 mL, or at least 1 mL, or at least 2 mL, or at least 3 mL, or at least 4 mL, or at least 5 mL, or at least 6 mL, or at least 7 mL, or at least, 8 mL, or at least 9 mL, or at least 10 mL, or at least 15 mL, or at least 20 mL, or at least 25 mL, or at least 30 mL, or at least 35 mL, or at least 40 mL, or at least 45 mL, or at least 50 mL, or at least 55 mL, or at least 60 mL, or at least 65 mL, or at least 70 mL, or at least 75 mL, or at least 80 mL, or at least 85 mL, or at least 90 mL, or at least 95 mL, or at least 100 mL, or at least 110 mL, or at least 120 mL, or at least 130 mL, or at least 140 mL, or at least 150 mL, or at least 160 mL, or at least 170 mL, or at least 180 mL, or at least 190 mL, or at least 200 mL, or at least 210 mL, or at least 220 mL, or at least 230 mL, or at least 240 mL, or at least 250 mL, or at least 260 mL, or at least 270 mL, or at least 280 mL, or at least 290 mL, or at least 300 mL, or at least 325, 350 mL, or at least 375 mL, or at least 400 mL, or at least 425 mL, or at least 450 mL, or at least 475 mL, or at least 500 mL, or at least 525 mL, or at least 550 mL, or at least 575 mL, or at least 600 mL, or at least 625 mL, or at least 650 mL, or at least 675 mL, or at least 700 mL, or at least 725 mL, or at least 750 mL, or at least 775 mL, or at least 800

mL, or at least 825 mL, or at least 850 mL, or at least 875 mL, or at least 900 mL, or at least 925 mL, or at least 950 mL, or at least 975 mL, or at least 1000 mL.

In some embodiments, the volume of a tissue filler administered is at most 0.01 mL, or at most 0.02 mL, or at most 0.03 mL, or at most 0.04 mL, or at most 0.05 mL, or at most 0.06 mL, or at most 0.07 mL, or at most 0.08 mL, or at most 0.09 mL, or at most 0.10 mL, or at most 0.15 mL, or at most 0.20 mL, or at most 0.25 mL, or at most 0.30 mL, or at most 0.35 mL, or at most 0.40 mL, or at most 0.45 mL, or at most 0.50 mL, or at most 0.55 mL, or at most 0.60 mL, or at most 0.65 mL, or at most 0.70 mL, or at most 0.75 mL, or at most 0.80 mL, or at most 0.85 mL, or at most 0.90 mL, or at most 0.95 mL, or at most 1 mL, or at most 2 mL, or at most 3 mL, or at most 4 mL, or at most 5 mL, or at most 6 mL, or at most 7 mL, or at most, 8 mL, or at most 9 mL, or at most 10 mL, or at most 15 mL, or at most 20 mL, or at most 25 mL, or at most 30 mL, or at most 35 mL, or at most 40 mL, or at most 45 mL, or at most 50 mL, or at most 55 mL, or at most 60 mL, or at most 65 mL, or at most 70 mL, or at most 75 mL, or at most 80 mL, or at most 85 mL, or at most 90 mL, or at most 95 mL, or at most 100 mL, or at most 110 mL, or at most 120 mL, or at most 130 mL, or at most 140 mL, or at most 150 mL, or at most 160 mL, or at most 170 mL, or at most 180 mL, or at most 190 mL, or at most 200 mL, or at most 210 mL, or at most 220 mL, or at most 230 mL, or at most 240 mL, or at most 250 mL, or at most 260 mL, or at most 270 mL, or at most 280 mL, or at most 290 mL, or at most 300 mL, or at most 325, 350 mL, or at most 375 mL, or at most 400 mL, or at most 425 mL, or at most 450 mL, or at most 475 mL, or at most 500 mL, or at most 525 mL, or at most 550 mL, or at most 575 mL, or at most 600 mL, or at most 625 mL, or at most 650 mL, or at most 675 mL, or at most 700 mL, or at most 725 mL, or at most 750 mL, or at most 775 mL, or at most 800 mL, or at most 825 mL, or at most 850 mL, or at most 875 mL, or at most 900 mL, or at most 925 mL, or at most 950 mL, or at most 975 mL, or at most 1000 mL.

In some embodiments, the volume of a tissue filler administered is about 0.01 mL, or about 0.02 mL, or about 0.03 mL, or about 0.04 mL, or about 0.05 mL, or about 0.06 mL, or about 0.07 mL, or about 0.08 mL, or about 0.09 mL, or about 0.10 mL, or about 0.15 mL, or about 0.20 mL, or about 0.25 mL, or about 0.30 mL, or about 0.35 mL, or about 0.40 mL, or about 0.45 mL, or about 0.50 mL, or about 0.55 mL, or about 0.60 mL,

or about 0.65 mL, or about 0.70 mL, or about 0.75 mL, or about 0.80 mL, or about 0.85 mL, or about 0.90 mL, or about 0.95 mL, or about 1 mL, or about 2 mL, or about 3 mL, or about 4 mL, or about 5 mL, or about 6 mL, or about 7 mL, or about, 8 mL, or about 9 mL, or about 10 mL, or about 11 mL, or about 12 mL, or about 13 mL, or about 14 mL, or about 15 mL, or about 16 mL, or about 17 mL, or about 18 mL, or about 19 mL, or about 20 mL, or about 21 mL, or about 22 mL, or about 23 mL, or about 24 mL, or about 25 mL, or about 26 mL, or about 27 mL, or about 28 mL, or about 30 mL, or about 35 mL, or about 36 mL, or about 37 mL, or about 38 mL, or about 39 mL, or about 40 mL, or about 41 mL, or about 42 mL, or about 43 mL, or about 44 mL, or about 45 mL, or about 46 mL, or about 47 mL, or about 48 mL, or about 49 mL, or about 50 mL, or about 51 mL, or about 52 mL, or about 53 mL, or about 54 mL, or about 55 mL, or about 56 mL, or about 57 mL, or about 58 mL, or about 59 mL, or about 60 mL, or about 61 mL, or about 62 mL, or about 63 mL, or about 64 mL, or about 65 mL, or about 66 mL, or about 67 mL, or about 68 mL, or about 69 mL, or about 70 mL, or about 71 mL, or about 72 mL, or about 73 mL, or about 74 mL, or about 75 mL, or about 76 mL, or about 77 mL, or about 78 mL, or about 79 mL, or about 80 mL, or about 81 mL, or about 82 mL, or about 83 mL, or about 84 mL, or about 85 mL, or about 86 mL, or about 87 mL, or about 88 mL, or about 89 mL, or about 90 mL, or about 91 mL, or about 92 mL, or about 93 mL, or about 94 mL, or about 95 mL, or about 96 mL, or about 97 mL, or about 98 mL, or about 99 mL, or about 100 mL, or about 110 mL, or about 120 mL, or about 130 mL, or about 140 mL, or about 150 mL, or about 160 mL, or about 170 mL, or about 180 mL, or about 190 mL, or about 200 mL, or about 210 mL, or about 220 mL, or about 230 mL, or about 240 mL, or about 250 mL, or about 260 mL, or about 270 mL, or about 280 mL, or about 290 mL, or about 300 mL, or about 310 mL, or about 320 mL, or about 330 mL, or about 340 mL, or about 350 mL, or about 360 mL, or about 370 mL, or about 380 mL, or about 390 mL, or about 400 mL, or about 410 mL, or about 420 mL, or about 430 mL, or about 440 mL, or about 450 mL, or about 460 mL, or about 470 mL, or about 480 mL, or about 490 mL, or about 500 mL, or about 510 mL, or about 520 mL, or about 530 mL, or about 540 mL, or about 550 mL, or about 560 mL, or about 570 mL, or about 580 mL, or about 590 mL, or about 600 mL, or about 610 mL, or about 620 mL, or about 630 mL, or about 640 mL, or about 650 mL, or about 660 mL, or about 670 mL, or about 680

mL, or about 690 mL, or about 700 mL, or about 710 mL, or about 720 mL, or about 730 mL, or about 740 mL, or about 750 mL, or about 760 mL, or about 770 mL, or about 780 mL, or about 790 mL, or about 800 mL, or about 810 mL, or about 820 mL, or about 830 mL, or about 840 mL, or about 850 mL, or about 860 mL, or about 870 mL, or about 880 mL, or about 890 mL, or about 900 mL, or about 910 mL, or about 920 mL, or about 930 mL, or about 940 mL, or about 950 mL, or about 960 mL, or about 970 mL, or about 980 mL, or about 990 mL, or about 1000 mL.

In some embodiments, the volume of a tissue filler administered is 0.01 mL to 0.10 mL, or 0.10 mL to 1 mL, or 1 mL to 10 mL, or 10 mL to 100 mL, or 50 mL to 100 mL, or 100 mL to 150 mL, or 150 mL to 200 mL, or 200 mL to 250 mL, or 250 mL to 300 mL, or 300 mL to 350 mL, or 350 mL to 400 mL, or 400 mL to 450 mL, or 450 mL to 500 mL, or 500 mL to 550 mL, or 550 mL to 600 mL, or 600 mL to 650 mL, or 650 mL to 700 mL, or 700 mL to 750 mL, or 750 mL to 800 mL, or 800 mL to 850 mL, or 850 mL to 900 mL, or 900 mL to 950 mL, or 950 mL to 1000 mL, or 1 mL to 25 mL, or 1 mL to 50 mL, or 1 mL to 75 mL, or 1 mL to 100 mL, or 10 mL to 25 mL, or 10 mL 50 mL, or 10 mL to 75 mL, or 100 mL to 250 mL, or 100 mL to 500 mL, or 100 mL to 750 mL, or 100 mL to 1000 mL.

In some embodiments, the invention provides for administering a tissue filler disclosed herein. As used herein, the term “administering” means any delivery mechanism that provides a tissue filler disclosed herein to an individual that potentially results in a clinically, therapeutically, or experimentally beneficial result. The actual delivery mechanism used to administer a tissue filler to an individual can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of condition, the location of the condition, the cause of the condition, the severity of the condition, the degree of relief desired, the duration of relief desired, the particular tissue filler used, the rate of biodegradability, bioabsorbability, bioresorbability, and the like, of the particular tissue filler used, the nature of the components included in the particular tissue filler used, the particular route of administration, the particular characteristics, history and risk factors of the patient, such as, e.g., age, weight, general health and the like, or any combination thereof. In an aspect of this embodiment, a tissue filler disclosed herein is administered to a region of a patient by injection, wherein the

region may be in the skin, dermal tissues, subdermal tissues, cutaneous tissues, subcutaneous tissues, intradural tissue, muscles, tendons, ligaments, fibrous tissues, fat, blood vessels and arteries, nerves, or synovial (intradermal) tissues.

In some embodiments, the route of administration of a tissue filler administered to a patient will be determined based on the cosmetic and/or clinical effect desired by the patient and/or physician and the body part or region being treated. A tissue filler disclosed herein may be administered by any means known to persons of ordinary skill in the art including, without limitation, syringe with needle, catheter, topically, or by direct surgical implantation. The tissue filler disclosed herein can be administered into a skin region such as, e.g., a dermal region or a hypodermal region. In addition, a tissue filler disclosed herein may be administered once, twice, thrice, or a plurality of times as required by the specific therapy.

In some embodiments, a tissue filler disclosed herein is injectable. As used herein, the term "injectable" refers to a tissue material having the properties necessary to administer the tissue filler into a skin region of an individual using an injection device with a needle such as, for example, a fine needle. As used herein, the term "fine needle" refers to a needle that is 27 gauge or smaller. In some embodiments, a fine needle can be a 27 gauge to 30 gauge needle. Injectability of a tissue filler disclosed herein can be accomplished by varying certain parameters of the tissue filers disclosed herein by, for example, adjusting the degree of cross-linking, otherwise varying G' and/or G" parameters, adding non-cross linked polymers (e.g., SPF or HA), and the like.

In some embodiments, a tissue filler disclosed herein is injectable through a fine needle. In some embodiments, a tissue filler disclosed herein is injectable through a needle of, for example, 20 gauge, or 21 gauge, or 22 gauge, or 23 gauge, or 24 gauge, or 25 gauge, or 26 gauge, or 27 gauge, or 28 gauge, or 29 gauge, or 30 gauge, or 31 gauge, or 32 gauge, or 33 gauge, or 34 gauge. In some embodiments, the tissue filler described herein are injectable through a needle of 20 gauge, or 21 gauge, or 22 gauge, or 23 gauge, or 24 gauge, or 25 gauge, or 26 gauge, or 27 gauge, or 28 gauge, or 29 gauge, or 30 gauge.

In some embodiments, a tissue filler disclosed herein is injectable with a syringe having a volume of about 0.8 to about 1.0 mL.

In some embodiments, the tissue fillers described herein may be delivered to void spaces in or about soft tissues for the purpose of, for example, tissue augmentation (e.g., breast or buttock augmentation). When delivering the tissue fillers described herein to such void spaces, larger syringes and needles may be used (e.g., needles that are 27 gauge or larger).

In some embodiments, the tissue fillers described herein may be applied to a wound without the use of a needle in order to coat the wound or a medical device proximate to the wound.

In some embodiments, the tissue fillers described herein may be applied to a surface of a medical device.

In one aspect, the disclosure includes a method of treatment or prevention of a disorder, disease, or condition alleviated by administering a treatment to a subject in need thereof. In some embodiments, the method comprises administering to the subject a composition of the disclosure. In some embodiments, the composition is injected into a tissue. In some embodiments, the composition comprises a tissue filler of the disclosure. In some embodiments, the composition is administered by injection as described herein.

In some embodiments, the tissue is associated with the disorder, disease, or condition that can be alleviated by administering , as would be understood by one of ordinary skill in the art. For example, a treatment, such as radiation, cryotherapy, or drug treatment,tissue can be associated with a disorder, disease, or condition when administering a composition of the disclosure into the tissue results in the alleviation, treatment, prevention, or amelioration of the disorder, disease, or condition.

Any type of tissue is contemplated by the disclosure. Tissue is a broad term that encompasses a portion of a body: for example, a tumor tissue, a group of cells, a group of cells, , interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body, e.g., a rectum, ovary, prostate, and the like. Non-limiting examples of diseases, disorders, conditions include cervical cancer, rectal cancer, pulmonary tumors, mediastinum lymphoma, breast cancer, uterine cancer, pancreatic cancer, head and neck cancers, lung cancer, liver cancer, vaginal cancers, benign prostatic hyperplasia (BPH), menorrhagia, uterine fibroids adenocarcinomas. heat/thermal ablation (radiofrequency or microwave); and drug treatment (local) such as alcohol tissue ablation or hyperosmolar

ablation using NaCl crystals or hyperosmolar solution, nerve, cartilage, bone, brain, or portion thereof. See, for example, US 8,257,723, which is incorporated by reference herein in its entirety.

In some embodiments, the tissue is an organ. In some embodiments, the tissue is a portion of an organ. Non-limiting examples of a tissue include the urethra, the urethral sphincter, the lower esophageal sphincter, the diaphragm, the rectum, a vocal cord, and the larynx.

In some embodiments, the composition is administered into a region of a rectal wall. In some embodiments, the region of the rectal wall is in the vicinity of the anal sphincter. In some embodiments, the composition is administered into the wall of the internal sphincter. In some embodiments, the composition is administered into the internal sphincter.

Any disorder, disease, or condition that can be alleviated, treated, prevented, or ameliorated using the compositions of the disclosure is contemplated by the present disclosure. In some embodiments, the disorder, disease, or condition is a gynecological related, urological related, gastroenterological related, or cancer related. Non-limiting examples of disorders, diseases, or conditions include urinary incontinence, gastroesophageal reflux disease (GERD), vesicoureteral reflux, fecal incontinence, dental tissue defects, vocal cord tissue defects, larynx defects, and other non-dermal soft tissue defects.

In some embodiments, the composition may remain in place for between one day and twelve months after introduction of the composition into the body. In some embodiments, the composition may remain in place for other periods, including from one week to three months and two to eight weeks. In some embodiments, the composition described herein can be biodegraded in less than about two months after implantation. In some embodiments, the composition is removed by biodegradation in the subject.

In one aspect, the present disclosure describes methods of tissue debulking. In a non-limiting example, a tissue that is bulked with a biodegradable composition of the disclosure can be debulked by causing the composition to degrade. In one aspect, the methods described herein further comprise a tissue debulking step. In some embodiments, the debulking step comprises administering to the subject a composition

that causes biodegradation. In some embodiments, the composition causes hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof. In some embodiments, the debulking step comprises administering to the subject a composition comprising an enzyme. In some embodiments, the enzyme is a hyaluronidase.

In one aspect of the disclosure, the composition described herein is radiopaque. As used herein, the term “radiopaque” is used to describe a material that is not transparent to X-rays or other forms of radiation. In some embodiments, the composition protects a tissue by blocking radiation being administered to another tissue. In some embodiments, the composition blocks about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% about 80%, about 90%, or about 100% of the radiation. In some embodiments, the tissue receives about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% about 80%, about 90%, or about 100% less radiation than it would have in the absence of the composition described herein.

As would be understood by one of ordinary skill in the art, composition volumes for administering within the methods described herein are dependent on the configuration of the tissues to be treated and the tissues to be separated from each other. In many cases, a volume of about 20 cubic centimeters (cc's or mls) is suitable.

In some embodiment is a kit for introducing a compositions described herein into a body. The kit may include a compositions and a device for delivering the filler to the body. Embodiments include kits wherein the delivery device is a syringe, and other embodiments include a needle for the syringe, and may include a needle for administering the compositions and/or the anesthetic.

The following clauses describe certain embodiments.

Clause 1a. A biocompatible composition comprising silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG), wherein a portion of the HA is modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol. Clause 1b. A biocompatible composition comprising silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG), wherein a portion of the HA is

modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol, and wherein a portion of the silk fibroin or silk fibroin fragments are free and/or uncrosslinked.

Clause 2. The composition of clause 1, wherein a portion of the silk fibroin or silk fibroin fragments are modified or crosslinked.

Clause 3. The composition of any one of clauses 1 or 2, wherein a portion of the silk fibroin or silk fibroin fragments are crosslinked to HA.

Clause 4. The composition of any one of clauses 1 to 3, wherein a portion of the silk fibroin or silk fibroin fragments are crosslinked to silk fibroin or silk fibroin fragments.

Clause 5. The tissue filler of any one of clauses 1 to 4, wherein the silk fibroin or silk fibroin fragments are substantially devoid of sericin.

Clause 6a. The composition of any one of clauses 1 to 5, wherein a portion of silk fibroin or silk fibroin fragments have an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight. Clause 6b. The composition of any one of clauses 1 to 5, wherein a portion of silk fibroin or silk fibroin fragments have an average weight average molecular weight selected from between about 1 kDa and about 5 kDa, from between about 5 kDa and about 10 kDa, from between about 6 kDa and about 17 kDa, from between about 10 kDa and about 15 kDa, from between about 14 kDa and about 30 kDa, from between about 15 kDa and about 20 kDa, from between about 17 kDa and about 39 kDa, from between about 20 kDa and about 25 kDa, from between about 25 kDa and about 30 kDa, from between about 30 kDa and about 35 kDa, from between about 35 kDa and about 40 kDa, from between about 39 kDa and about 54 kDa, from between about 39 kDa and about 80 kDa, from between about 40 kDa and about 45 kDa, from between about 45 kDa and about 50 kDa, from between about 50 kDa and about 55 kDa, from between about 55 kDa and about 60 kDa, from between about 60 kDa and about 100 kDa, or from between about 80 kDa and about 144 kDa.

Clause 7a. The composition of any one of clauses 1 to 6, wherein the silk fibroin or silk fibroin fragments have a polydispersity of between 1 and about 5.0. Clause 7b.

The composition of any one of clauses 1 to 6, wherein the silk fibroin or silk fibroin fragments have a polydispersity from 1 to about 1.5, from about 1.5 to about 2.0, from about 2.0 to about 2.5, from about 2.5 to about 3.0, from about 3.0 to about 3.5, from about 3.5 to about 4.0, from about 4.0 to about 4.5, or from about 4.5 to about 5.0.

Clause 8. The composition of any one of clauses 1 to 6, wherein the silk fibroin or silk fibroin fragments have a polydispersity of between about 1.5 and about 3.0.

Clause 9a. The composition of any one of clauses 1 to 8, wherein the composition has a degree of modification (MoD) of about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15%. Clause 9b. The composition of any one of clauses 1 to 8, wherein the composition has a degree of modification (MoD) of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, or about 25%.

Clause 10. The composition of any one of clauses 1 to 9, wherein modification or cross-linking is obtained using as cross-linker a monoepoxy- or diepoxy-PEG, a monoglycidyl-, diglycidyl-, or polyglycidyl-PEG, a monoglycidyl- or diglycidyl-PEG, a monoepoxy- or diepoxy-PPG, a monoglycidyl-, diglycidyl-, or polyglycidyl-PPG, a monoglycidyl- or diglycidyl-PPG, or any combinations thereof.

Clause 11a. The composition of any one of clauses 1 to 10, further comprising lidocaine. Clause 11b. The composition of any one of clauses 1 to 10, further comprising lidocaine at a concentration of about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, or about 1%.

Clause 12. The composition of any one of clauses 1 to 11, wherein the composition is a gel or a hydrogel.

Clause 13. The composition of any one of clauses 1 to 12, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27

mg/mL, about 28 mg/mL, about 29 mg/mL, about 30 mg/mL, about 31 mg/mL, about 32 mg/mL, about 33 mg/mL, about 34 mg/mL, about 35 mg/mL, about 36 mg/mL, about 37 mg/mL, about 38 mg/mL, about 39 mg/mL, or about 40 mg/mL.

Clause 14. The composition of any one of clauses 1 to 13, wherein the ratio of HA to silk fibroin or silk fibroin fragments in the composition is about 91/9, about 92/8, about 93/7, about 94/6, about 95/5, about 96/4, about 97/3, about 18/12, about 27/3, about 29.4/0.6, about 99/1, about 92.5/7.5, about 90/10, about 80/20, about 70/30, about 60/40, or about 50/50.

Clause 15. The composition of any one of clauses 1 to 13, wherein the ratio of HA to silk fibroin or silk fibroin fragments in the composition is about 50/50, about 51/49, about 52/48, about 53/47, about 54/46, about 55/45, about 56/44, about 57/43, about 58/42, about 59/41, about 60/40, about 61/39, about 62/38, about 63/37, about 64/36, about 65/35, about 66/34, about 67/33, about 68/32, about 69/31, about 70/30, about 71/29, about 72/28, about 73/27, about 74/26, about 75/25, about 76/24, about 77/23, about 78/22, about 79/21, about 80/20, about 81/19, about 82/18, about 83/17, about 84/16, about 85/15, about 86/14, about 87/13, about 88/12, about 89/11, about 90/10, about 91/9, about 92/8, about 93/7, about 94/6, about 95/5, about 96/4, about 97/3, about 98/2, or about 99/1.

Clause 16. The composition of any one of clauses 1 to 17, wherein the total concentration of free and/or uncrosslinked silk fibroin or silk fibroin fragments in the composition is about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, or about 8 mg/mL.

Clause 17. The composition of any one of clauses 1 to 16, wherein a portion of the free and/or uncrosslinked silk fibroin or silk fibroin fragments comprises silk microparticles having a median particle size ranging from 1.0 μm to 50.0 μm, from 1.0 μm to 25.0 μm, from 1.0 μm to 10.0 μm, from 30.0 μm to 50.0 μm, from 35.0 μm to 45.0 μm, from 35.0 μm to 55.0 μm, or from 25.0 μm to 45.0 μm.

Clause 18. The composition of any one of clauses 1 to 17, wherein the composition is injectable through 30G or 27G needles, and having an injection force through a 30G needle between about 10 N and about 80 N.

Clause 19. The composition of any one of clauses 1 to 17, wherein the composition is injectable through a 30G needle with an injection force of about 1 N, about 2 N, about 3 N, about 4 N, about 5 N, about 6 N, about 7 N, about 8 N, about 9 N, about 10 N, about 11 N, about 12 N, about 13 N, about 14 N, about 15 N, about 16 N, about 17 N, about 18 N, about 19 N, about 20 N, about 21 N, about 22 N, about 23 N, about 24 N, about 25 N, about 26 N, about 27 N, about 28 N, about 29 N, about 30 N, about 31 N, about 32 N, about 33 N, about 34 N, about 35 N, about 36 N, about 37 N, about 38 N, about 39 N, about 40 N, about 41 N, about 42 N, about 43 N, about 44 N, about 45 N, about 46 N, about 47 N, about 48 N, about 49 N, about 50 N, about 51 N, about 52 N, about 53 N, about 54 N, about 55 N, about 56 N, about 57 N, about 58 N, about 59 N, about 60 N, about 61 N, about 62 N, about 63 N, about 64 N, about 65 N, about 66 N, about 67 N, about 68 N, about 69 N, about 70 N, about 71 N, about 72 N, about 73 N, about 74 N, about 75 N, about 76 N, about 77 N, about 78 N, about 79 N, about 80 N, about 81 N, about 82 N, about 83 N, about 84 N, about 85 N, about 86 N, about 87 N, about 88 N, about 89 N, about 90 N, about 91 N, about 92 N, about 93 N, about 94 N, about 95 N, about 96 N, about 97 N, about 98 N, about 99 N, or about 100 N.

Clause 20. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 5 Pa to about 500 Pa, from about 15 Pa to about 50 Pa, from about 50 Pa to about 100 Pa, from about 100 Pa to about 200 Pa, from about 200 Pa to about 300 Pa, from about 300 Pa to about 350 Pa, from about 350 Pa to about 400 Pa, from about 400 Pa to about 450 Pa, or from about 450 Pa to about 500 Pa.

Clause 21. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (G'') of from about 5 Pa to about 500 Pa, from about 15 Pa to about 50 Pa, from about 50 Pa to about 100 Pa, from about 100 Pa to about 200 Pa, from about 200 Pa to about 300 Pa, from about 300 Pa to about 350 Pa, from about 350 Pa to about 400 Pa, from about 400 Pa to about 450 Pa, or from about 450 Pa to about 500 Pa.

Clause 22. The composition of any one of clauses 1 to 19, wherein the composition has Tan(δ) (G"/G') between 0 and about 0.2, between about 0.2 and about

0.4, between about 0.4 and about 0.6, between about 0.6 and about 0.8, between about 0.8 and about 1.0, or between about 1.0 and about 1.2.

Clause 23. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between 0 and about 5 Pa·s, between about 5 Pa·s and about 10 Pa·s, between about 10 Pa·s and about 15 Pa·s, between about 15 Pa·s and about 20 Pa·s, or between about 20 Pa·s and about 25 Pa·s.

Clause 24a. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 50 Pa to about 400 Pa, and an injection force (27G) between about 10 N and about 70 N. Clause 24b. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 100 Pa to about 150 Pa, and an injection force (27G) between about 40 N and about 60 N. Clause 24c. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 50 Pa to about 150 Pa, and an injection force (27G) between about 10 N and about 40 N. Clause 24d. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 250 Pa to about 350 Pa, and an injection force (27G) between about 10 N and about 30 N.

Clause 25a. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 10 Pa to about 350 Pa, and an injection force (30G) between about 5 N and about 70 N. Clause 25b. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 50 Pa to about 200 Pa, and an injection force (30G) between about 40 N and about 60 N. Clause 25c. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 200 Pa to about 350 Pa, and an injection force (30G) between about 40 N and about 70 N. Clause 25d. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (G') of from about 10 Pa to about 100 Pa, and an injection force (30G) between about 5 N and about 35 N.

Clause 26a. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (G'') of from about 25 Pa to about 350 Pa, and an injection force (27G) between about 10 N and about 70 N. Clause 26b. The composition

of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 25 Pa to about 100 Pa, and an injection force (27G) between about 40 N and about 70 N. Clause 26c. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 25 Pa to about 100 Pa, and an injection force (27G) between about 10 N and about 35 N. Clause 26d. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 150 Pa to about 350 Pa, and an injection force (27G) between about 10 N and about 60 N.

Clause 27a. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 10 Pa to about 400 Pa, and an injection force (30G) between about 5 N and about 70 N. Clause 27b. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 50 Pa to about 100 Pa, and an injection force (30G) between about 40 N and about 60 N. Clause 27c. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 10 Pa to about 75 Pa, and an injection force (30G) between about 5 N and about 35 N. Clause 27d. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 150 Pa to about 300 Pa, and an injection force (30G) between about 40 N and about 70 N.

Clause 28a. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 25 Pa to about 400 Pa, and Tan(δ) (G"/G') between 0 and about 1.2. Clause 28b. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 50 Pa to about 200 Pa, and Tan(δ) (G"/G') between 0.2 and about 0.6. Clause 28c. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 200 Pa to about 400 Pa, and Tan(δ) (G"/G') between 0 and about 0.2. Clause 28d. The composition of any one of clauses 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 25 Pa to about 400 Pa, and Tan(δ) (G"/G') between 0.8 and about 1.2.

Clause 29a. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 2.5 and about 25 Pa·s, and an

injection force (27G) between about 10 N and about 70 N. Clause 29b. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 2.5 and about 15 Pa·s, and an injection force (27G) between about 10 N and about 35 N. Clause 29c. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 2.5 and about 15 Pa·s, and an injection force (27G) between about 40 N and about 70 N. Clause 29d. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 15 and about 25 Pa·s, and an injection force (27G) between about 25 N and about 70 N.

Clause 30a. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 1 and about 20 Pa·s, and an injection force (30G) between about 5 N and about 75 N. Clause 30b. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 1 and about 5 Pa·s, and an injection force (30G) between about 5 N and about 50 N. Clause 30c. The composition of any one of clauses 1 to 19, wherein the composition has a complex viscosity (η*) between about 5 and about 17 Pa·s, and an injection force (30G) between about 40 N and about 75 N.

Clause 31a. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 5 Pa to about 400 Pa, and a storage modulus (*G'*) of from about 1 Pa to about 400 Pa. Clause 31b. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 5 Pa to about 150 Pa, and a storage modulus (*G'*) of from about 1 Pa to about 250 Pa. Clause 31c. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 5 Pa to about 150 Pa, and a storage modulus (*G'*) of from about 250 Pa to about 400 Pa. Clause 31d. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 150 Pa to about 200 Pa, and a storage modulus (*G'*) of from about 250 Pa to about 350 Pa. Clause 31e. The composition of any one of clauses 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 250 Pa to about 375 Pa, and a storage modulus (*G'*) of from about 250 Pa to about 350 Pa.

Clause 32a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 15 mg/mL, wherein the composition has a storage modulus (G') of from about 1 Pa to about 350 Pa. Clause 32b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 15 mg/mL, wherein the composition has a storage modulus (G') of from about 1 Pa to about 200 Pa. Clause 32c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 15 mg/mL, wherein the composition has a storage modulus (G') of from about 200 Pa to about 350 Pa.

Clause 33a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 18 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 350 Pa. Clause 33b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 18 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 150 Pa. Clause 33c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 18 mg/mL, wherein the composition has a storage modulus (G') of from about 150 Pa to about 350 Pa.

Clause 34a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 20 mg/mL, wherein the composition has a storage modulus (G') of from about 20 Pa to about 400 Pa. Clause 34b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 20 mg/mL, wherein the composition has a storage modulus (G') of from about 20 Pa to about 200 Pa. Clause 34c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 20 mg/mL, wherein the composition has a storage modulus (G') of from about 200 Pa to about 400 Pa.

Clause 35a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 22 mg/mL, wherein the composition has a storage modulus (G') of from about 25 Pa to about 200 Pa. Clause 35b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 22 mg/mL, wherein the composition has a storage modulus (G') of from about 25 Pa to about 100 Pa. Clause 35c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 22 mg/mL, wherein the composition has a storage modulus (G') of from about 100 Pa to about 200 Pa.

Clause 36a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 24 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 350 Pa. Clause 36b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 24 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 250 Pa. Clause 36c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 24 mg/mL, wherein the composition has a storage modulus (G') of from about 250 Pa to about 350 Pa.

Clause 37a. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 26 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 400 Pa. Clause 37b. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 26 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 200 Pa. Clause 37c. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 26 mg/mL, wherein the composition has a storage modulus (G') of from about 200 Pa to about 400 Pa.

Clause 38. The composition of any one of clauses 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 28 mg/mL, wherein the composition has a storage modulus (G') of from about 150 Pa to about 300 Pa.

Clause 39. The composition of any one of clauses 1 to 38, further comprising an imaging agent.

Clause 40. The composition of clause 39, wherein the imaging agent is selected from iodine, DOPA, and imaging nanoparticles.

Clause 41. The composition of clause 39, wherein the imaging agent is selected from a paramagnetic imaging agent and a superparamagnetic imaging agent.

Clause 42. The composition of clause 39, wherein the imaging agent is selected from NP-based magnetic resonance imaging (MRI) contrast agents, positron emission tomography (PET)/single photon emission computed tomography (SPECT) imaging agents, ultrasonically active particles, and optically active (e.g., luminescent, fluorescent, infrared) particles.

Clause 43. The composition of clause 39, wherein the imaging agent is a SPECT imaging agent, a PET imaging agent, an optical imaging agent, an MRI or MRS imaging agent, an ultrasound imaging agent, a multimodal imaging agent, an X-ray imaging agent, or a CT imaging agent.

Clause 44. A method of treatment or prevention of a disorder, disease, or condition in a subject in need thereof, the method comprising administering to the subject a composition of any one of clauses 1 to 43.

Clause 45. The method of clause 44, wherein the condition is a skin condition selected from skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle.

Clause 46. The method of clause 44 or clause 45, wherein the composition is administered into a dermal region of the subject.

Clause 47. The method of any of clauses 44 to 46, wherein the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area.

Clause 48. The method of any one of clauses 44 to 47, wherein the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection.

Clause 49. The method of any one of clauses 44 to 48, wherein the method comprises deep subcutaneous and/or deep supraperiosteal administration.

Clause 50. The method of any one of clauses 44 to 49, wherein the method comprises cheek augmentation, lip augmentation, dermal implantation, correction of perioral rhytids, and/or correction of nasolabial fold.

Clause 51. The method of clause 44, wherein the composition is injected into a tissue.

Clause 52. The method of clause 51, wherein the tissue is associated with the disorder, disease, or condition.

Clause 53. The method of clause 51 or clause 52, wherein the composition is administered into a wall of the tissue.

Clause 54. The method of any one of clauses 51 to 53, wherein the tissue comprises a portion of a wall of an internal organ.

Clause 55. The method of any one of clauses 51 to 54, wherein administration of the composition causes bulking of the tissue.

Clause 56. The method of clause 55, wherein the disorder, disease, or condition is treated or prevented by the bulking of the tissue.

Clause 57. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is selected from urinary incontinence, gastroesophageal reflux disease (GERD), vesicoureteral reflux, fecal incontinence, dental tissue defects, vocal cord tissue defects, larynx defects, and other non-dermal soft tissue defects.

Clause 58. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is urinary incontinence.

Clause 59. The method of clause 58, wherein the urinary incontinence is stress incontinence, intrinsic sphincter deficiency (ISD), stress incontinence, intrinsic sphincter deficiency (ISD), urge incontinence, overflow incontinence, or enuresis.

Clause 60. The method of clause 58 or 59, wherein the tissue is a portion of the urethra or the urethral sphincter.

Clause 61. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is gastroesophageal reflux disease (GERD).

Clause 62. The method of clause 61, wherein the tissue is a portion of the lower esophageal sphincter or the diaphragm.

Clause 63. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is vesicoureteral reflux.

Clause 64. The method of clause 63, wherein the tissue is a portion of the urethral sphincter.

Clause 65. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is fecal incontinence.

Clause 66. The method of clause 65, wherein the tissue is a portion of the rectum.

Clause 67. The method of clause 65 or clause 66, wherein the composition is administered into a region of a rectal wall.

Clause 68. The method of clause 67, wherein the region of the rectal wall is in the vicinity of the anal sphincter.

Clause 69. The method of clause 68, wherein the composition is administered into the internal sphincter.

Clause 70. The method of any one of clauses 51 to 56, wherein the disorder, disease, or condition is a vocal cord tissue defect or larynx defect.

Clause 71. The method of clause 70, wherein the vocal cord tissue defect or larynx defect is selected from glottic incompetence, unilateral vocal cord paralysis, bilateral vocal cord paralysis, paralytic dysphonia, nonparalytic dysphonia, spasmodic dysphonia, incomplete paralysis of the vocal cord ("paresis"), generally weakened vocal cords, scarring of the vocal cords, and any combination thereof.

Clause 72. The method of clause 70 or clause 71, wherein the tissue is a portion of a vocal cord or larynx.

Clause 73. The method of clause 44, further comprising administering an anticancer treatment, wherein the disorder, disease, or condition is selected from cervical cancer, rectal cancer, pulmonary tumors, mediastinum lymphoma, breast cancer, uterine cancer, pancreatic cancer, head and neck cancers, lung cancer, liver cancer, vaginal cancers, benign prostatic hyperplasia (BPH), menorrhagia, uterine fibroids, prostate

adenocarcinomas, pancreatic cancer, head and neck cancer, lung cancer, liver cancer, and vaginal cancer.

Clause 74. The method of clause 73, wherein the anticancer treatment comprises administering one or more of radiation therapy (RT), cryotherapy, drug treatment, heat and/or thermal ablation, radiofrequency and/or microwave, or cryotherapy.

Clause 75. The method of clause 74, wherein the radiation therapy comprises one or more of external beam radiotherapy, 3D conformal modulated radiotherapy, intensity modulated radiotherapy, interstitial prostate brachytherapy, interstitial prostate brachytherapy using permanent seeds, interstitial prostate brachytherapy using temporary seeds, interstitial prostate brachytherapy using high dose rate remote after loading, external radiation therapy using gamma irradiation, high energy photon beam therapy, proton beam therapy, neutron beam therapy, heavy particle beam therapy, brachytherapy, thermal radiation, or any combination thereof.

Clause 76. The method of any one of clauses 73 to 75, wherein the composition is administered between a first tissue and a second tissue, or into a space or virtual space between a first tissue and a second tissue.

Clause 77. The method of clause 76, wherein upon administration of the composition the first tissue is displaced relative to the second tissue.

Clause 78. The method of clause 76 or clause 77, wherein the space or virtual space is Denonvilliers' space or a space or virtual space adjacent to Denonvilliers' fascia.

Clause 79. The method of any one of clauses 76 to 78, wherein the first tissue receives the anticancer treatment after administration of the composition.

Clause 80. The method of clause 79, wherein the first tissue receives a substantially similar dose of anticancer treatment compared to the anticancer treatment dose the first tissue would receive in the absence of the composition.

Clause 81. The method of any one of clauses 76 to 80, wherein the second tissue receives the anticancer treatment.

Clause 82. The method of clause 81, wherein the second tissue receives a lower anticancer treatment dose compared to the anticancer treatment dose the second tissue would receive in the absence of the composition.

Clause 83. The method of any one of clauses 76 to 82, wherein the second tissue receives substantially no anticancer treatment dose.

Clause 84. The method of any of clauses 76 to 83, wherein the first tissue and the second tissue each independently comprises a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body.

Clause 85. The method of any one of clauses 76 to 83, wherein the first tissue comprises a tumor tissue, and the second tissue comprises an organ.

Clause 86. The method of any one of clauses 76 to 83, wherein the first tissue comprises an organ, and the second tissue comprises an organ.

Clause 86. The method of clause 86, wherein the first tissue comprises a portion of prostate and the second tissue comprises a portion of rectum.

Clause 87. The method of any one of clauses 44 to 86, wherein the method further comprises administering an anesthetic.

Clause 88. The method of any of clauses 44 to 87, further comprising biodegradation of the composition in the subject.

Clause 89. The method of clause 88, wherein the biodegradation is hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof.

Clause 90. The method of clause 88, wherein the composition is biodegraded by hyaluronidase enzymatic degradation.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the described embodiments, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLES

Material and Methods

Materials

Hyaluronic acid (HA) sodium salt (molecular weight 750 kDa – 1000 kDa) was acquired from Lifecore Biomedical (Chaska, MN). The injectable HA gel sold under trademark Juvéderm® Ultra Plus XC (a colorless hyaluronic acid gel that contains a small quantity of local anesthetic (lidocaine)) was acquired from Allergan (Irvine, CA). Silk fibroin was processed on site (Medford, MA). Poly(ethylene glycol) diglycidyl ether (PEGDE) and hyaluronidase (Hylenex™) were acquired from Sigma-Aldrich (St Louis, MO). Lidocaine hydrochloride was acquired from Spectrum Chemical (New Brunswick, NJ). Silk solutions of various concentrations were prepared according to the methods described above. All other chemicals and reagents were purchased from VWR (Radnor, PA) and used as received.

General Method for Silk-HA Hydrogel Preparation

Hyaluronic acid was dissolved in 0.1 N sodium hydroxide solution containing silk fibroin protein based fragments and crosslinker in amounts that varied for different hydrogel formulations. The mixtures were maintained at 55 ºC for 75 minutes to allow the crosslinking reactions to reach completion. The resulting hydrogels were then cooled to room temperature, adjusted to pH 7.4 with concentrated hydrochloric acid, and then neutralized and diluted overnight with 1x PBS. The hydrogels were then dialyzed against 1x PBS for 3 days to remove residual free crosslinker. Lidocaine hydrochloride was added to the purified hydrogels to 0.3% w/w. The final total concentration of silk fibroin protein based fragments and HA was adjusted with 1x PBS to 26 mg/ml for each hydrogel. The prepared silk-HA hydrogels were aliquot into 1-mL syringes, ready for sterilization and characterization.

Example 1: Tyndall Evaluation of Gels

In order to further support visual observations and carry out comparative performance analysis of tissue and/or dermal fillers, quantitative analysis of Tyndall effect is performed. Based on existing scientific understanding on light scattering and interaction of light with skin, two distinct approaches based on (a)

colorimetry, and (b) spectroscopy are employed to quantify Tyndall effect in skin. Based on these techniques three distinct quantitative parameters (outlined below) are defined to measure Tyndall effect in vivo.

Tyndall Effect Visual Score:

The scale has a range of 1 to 5 with increments of 0.5. A score of 1 is given to injection sites with normal skin tone and no blue discoloration. A maximum score of 5 is given to thick and pronounced blue discoloration. Three independent observers are trained on the scale before being blinded to score test samples.

Blue Component of Skin Color—"b": a chromameter is used to quantify the blue color component of light remitted from skin sites injected with the various fillers. This is achieved by using the "b" component of L-a-b color scale.

"% Blue Light" Remitted from Skin: a portable spectrophotometer is used to quantify the % blue light remitted from skin in the total visible light range. This is achieved by integrating the area under the visible light spectrum between 400-490 nm and normalizing it by the total area under the spectrum (400-700 nm).

Gels of the present disclosure and commercially available gels are injected intradermally through an appropriate needle using linear threading technique into the thighs of two months old hairless rats. The gels are implanted superficially to mimic clinical fine line procedures. Tests for Tyndall are performed 48 h after gel implantation. Before performing the Tyndall tests, the animals are humanely euthanized to improve contrast of the Tyndall effect.

A visual score of 1-5 with increments of 0.5, is used to score the injection sites. Injection sites with score of 1 show no skin discoloration, while injections sites with score of 5 show severe blue discoloration of the skin. Spectroscopic analysis are also performed on the injection sites with the aid of a chromatometer. The blue component of skin color "b", and the % of blue light remitted from skin (400-700 nm) are independently measured.

Example 2: *In vivo* Tissue Filler Testing

Tissue fillers prepared according to the foregoing description could be tested following intradermal implantation, muscle implantation, and subcutaneous injection.

For example, a dose of a tissue filler could be loaded in a syringe and injected either intradermally, intramuscularly, or subcutaneously using an appropriately sized syringe that permits flow through the needle of the tissue filler to the injection site.

Following initial injection versus a control (e.g., water and/or a marketed HA based tissue filler such as Juvederm), the injection sites may be monitored at 1 week or 2 week intervals where the patients are observed for biocompatibility concerns, including, cytotoxicity, pyrogenicity, endotoxin formation, acute system toxicity, subchronic toxicity, intradermal reactivity, genotoxicity, and skin sensitization.

In addition, the physical attributes of the tissue filler may be monitored by examining presence of Tyndalling or loss in volume, elasticity, or firmness at the injection site.

Example 3: Examination of Tissue Filler Rheology

An oscillatory parallel plate rheometer (Anton Paar Physica MCR 301) could be used to measure the rheological properties of the tissue fillers described herein. A plate diameter of 25 mm could be used at a gap height of 1 mm. Measurements could be performed at a constant temperature of 25 °C. Each measurement would consist of a frequency sweep from 1 to 10 Hz at a constant strain of 2% and a logarithmic increase of frequency followed by a strain sweep from 1 to 300% at a constant frequency of 5 Hz with a logarithmic increase in strain. The results of such analyses would provide the Storage Modulus G' and Loss Modulus G' of each tested tissue filler.

Example 4: Examination of Silk/HA Solution Opacity

Solutions of HA and silk were prepared in water or phosphate-buffered saline according to Table 18.

Table 18

Sample	Description
1	Silk MW: "Mid" Silk Conc: 0.3 mg/mL HA Conc: 22 mg/mL Solvent: Water
2	Silk MW: "Mid" Silk Conc: 0.6 mg/mL HA Conc: 22 mg/mL Solvent: Water

3	Silk MW: “Mid” Silk Conc: 3.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
4	Silk MW: “Mid” Silk Conc: 6.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
5	Silk MW: “Mid” Silk Conc: 15.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
6	Silk MW: “Mid” Silk Conc: 30.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
7	Silk MW: “Mid” Silk Conc: 45.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
8	Silk MW: “Low” Silk Conc: 0.6 mg/mL HA Conc: 22 mg/mL Solvent: Water
9	Silk MW: “Low” Silk Conc: 15.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
10	Silk MW: “Low” Silk Conc: 30.0 mg/mL HA Conc: 22 mg/mL Solvent: Water
11	Silk MW: “Low” Silk Conc: 45.0 mg/mL HA Conc: 22 mg/mL Solvent: Water

12	Silk MW: "Mid" Silk Conc: 0.6 mg/mL HA Conc: 22 mg/mL Solvent: PBS
13	Silk MW: "Mid" Silk Conc: 15.0 mg/mL HA Conc: 22 mg/mL Solvent: PBS
14	Silk MW: "Mid" Silk Conc: 30.0 mg/mL HA Conc: 22 mg/mL Solvent: PBS
15	Silk MW: "Mid" Silk Conc: 45.0 mg/mL HA Conc: 22 mg/mL Solvent: PBS

Low MW = silk molecular weights between above 0 and about 25 kDa, or as otherwise defined herein;
Mid MW = silk molecular weights of about 25 kDa to about 60 kDa, or as otherwise defined herein;

The results the solutions described in the above-table are shown in Figs. 26 and 27. The control in Figs. 26 and 27 (unlabeled flask in Fig. 26 and control syringe in Fig. 27) was a solution of HA (22 mg/mL) in water. As illustrated the Figs. 26 and 27, silk/HA solutions were homogenous and visibly opaque as compared to HA alone.

Example 5: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Hyaluronic acid may be dissolved in NaOH solution and added to a solution of silk as described herein;

Step b: Add dissolved BDDE in NaOH to Silk/HA/NaOH solution;

Step c: Cross link by mixing with heat;

Step d: Pass through a metal mesh and allow to swell in water;

Step e: Precipitate swelled gel in ethanol;

Step f: Wash with ethanol, water, and NaOH solution;

Step g: Finalize crosslinking in solution of ethanol/NaOH for about 2 hours with heating (50 °C);

Step h: Neutralize solution pH to 7;

Step i: Precipitate is washed and dried;

Step j: Resulting dry powder allowed to swell into a gel in buffered 0.9% NaCl solution; and

Step k: Gel is filled into a syringe and autoclaved provide resulting tissue filler.

Example 6: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Hyaluronic acid may be dissolved in NaOH solution;

Step b: Add silk in NaOH solution to a solution of Silk, and then add dissolved BDDE in NaOH to Silk/HA/NaOH solution;

Step c: Cross link by mixing with heat;

Step d: Pass through a metal mesh and allow to swell in water;

Step e: Precipitate swelled gel in ethanol;

Step f: Wash with ethanol, water, and NaOH solution;

Step g: Finalize crosslinking in solution of ethanol/NaOH for about 2 hours with heating (50 °C);

Step h: Neutralize solution pH to 7;

Step i: Precipitate is washed and dried;

Step j: Resulting dry powder allowed to swell into a gel in buffered 0.9% NaCl solution; and

Step k: Gel is filled into a syringe and autoclaved provide resulting tissue filler.

Example 7: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Hyaluronic acid may be dissolved in NaOH solution;

Step b: Add dissolved BDDE in NaOH to HA/NaOH solution;

Step c: Add silk solution to solution of Step b and cross link by mixing with heat;

Step d: Pass through a metal mesh and allow to swell in water;

Step e: Precipitate swelled gel in ethanol;

Step f: Wash with ethanol, water, and NaOH solution;

Step g: Finalize crosslinking in solution of ethanol/NaOH for about 2 hours with heating (50 °C);

Step h: Neutralize solution pH to 7;

Step i: Precipitate is washed and dried;

Step j: Resulting dry powder allowed to swell into a gel in buffered 0.9% NaCl solution; and

Step k: Gel is filled into a syringe and autoclaved provide resulting tissue filler.

Example 8: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Hyaluronic acid may be dissolved in NaOH solution;

Step b: Add dissolved BDDE in NaOH to HA/NaOH solution;

Step c: Cross link by mixing with heat;

Step d: Add silk solution to crosslinked HA/NaOH solution, and pass through a metal mesh and allow to swell in water;

Step e: Precipitate swelled gel in ethanol;

Step f: Wash with ethanol, water, and NaOH solution;

Step g: Finalize crosslinking in solution of ethanol/NaOH for about 2 hours with heating (50 °C);

Step h: Neutralize solution pH to 7;

Step i: Precipitate is washed and dried;

Step j: Resulting dry powder allowed to swell into a gel in buffered 0.9% NaCl solution; and

Step k: Gel is filled into a syringe and autoclaved provide resulting tissue filler.

Example 9: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Hyaluronic acid may be dissolved in NaOH solution;

Step b: Add dissolved BDDE in NaOH to HA/NaOH solution;

Step c: Cross link by mixing with heat;

Step d: Pass through a metal mesh and allow to swell in water;

Step e: Precipitate swelled gel in ethanol;

Step f: Wash with ethanol, water, and NaOH solution;

Step g: Add silk solution to material prepared in Step f and finalize crosslinking in solution of ethanol/NaOH for about 2 hours with heating (50 °C);

Step h: Neutralize solution pH to 7;

Step i: Precipitate is washed and dried;

Step j: Resulting dry powder allowed to swell into a gel in buffered 0.9% NaCl solution;

Step k: Gel is filled into a syringe and autoclaved provide resulting tissue filler.

Example 10: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Sodium hyaluronate may be mixed with NaOH solution and a solution of silk as described herein;

Step b: BDDE may be added to the solution of Step a;

Step c: The product of Step b is allowed to react;

Step d: Ammonia is added to the dialyzed mixture of Step c and the mixture is poured into a petri dish;

Step e: The product of Step d is allowed to dry into a film;

Step f: The film of Step e is divided into particles and swelled in saline;

Step g; The product of Step f is added to a syringe and autoclaved;

Step h (optional): The product of Step f can be subjected to a second, final crosslinking procedure with a solution of BDDE, or other crosslinking agent described herein, and washed.

Example 11: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Sodium hyaluronate may be mixed with NaOH solution;

Step b: A silk solution may be added to the solution of Step a and BDDE may be added;

Step c: The product of Step b is allowed to react;

Step d: Ammonia is added to the dialyzed mixture of Step c and the mixture is poured into a petri dish;

Step e: The product of Step d is allowed to dry into a film;

Step f: The film of Step e is divided into particles and swelled in saline;

Step g; The product of Step f is added to a syringe and autoclaved;

Step h (optional): The product of Step f can be subjected to a second, final crosslinking procedure with a solution of BDDE, or other crosslinking agent described herein, and washed.

Example 12: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Sodium hyaluronate may be mixed with NaOH solution;

Step b: BDDE may be added to the solution of Step a;

Step c: The product of Step b is added to a silk solution and allowed to react;

Step d: Ammonia is added to the dialyzed mixture of Step c and the mixture is poured into a petri dish;

Step e: The product of Step d is allowed to dry into a film;

Step f: The film of Step e is divided into particles and swelled in saline;

Step g; The product of Step f is added to a syringe and autoclaved;

Step h (optional): The product of Step f can be subjected to a second, final crosslinking procedure with a solution of BDDE, or other crosslinking agent described herein, and washed.

Example 13: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: Sodium hyaluronate may be mixed with NaOH solution;

Step b: BDDE may be added to the solution of Step a;

Step c: The product of Step b is allowed to react;

Step d: The product of Step c is added to a silk solution and then ammonia is added to the dialyzed mixture thereof and the mixture is poured into a petri dish;

Step e: The product of Step d is allowed to dry into a film;

Step f: The film of Step e is divided into particles and swelled in saline;

Step g; The product of Step f is added to a syringe and autoclaved;

Step h (optional): The product of Step f can be subjected to a second, final crosslinking procedure with a solution of BDDE, or other crosslinking agent described herein, and washed.

Example 14: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: A silk solution may be prepared as described herein, to which BDDE may be added in water;

Step b: HA may be added to the solution of Step a;

Step c: The mixture of Step b may be stirred (e.g., 5 minutes) and allowed to stand for about 1 day;

Step d: The resulting gel from Step c may be allowed to stand in saline for 1 week to provide the resulting tissue filler.

Example 15: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: BDDE may be added to water;

Step b: A silk solution may be added to the solution of Step a, to which HA may then be added;

Step c: The mixture of Step b may be stirred (e.g., 5 minutes) and allowed to stand for about 1 day;

Step d: The resulting gel from Step c may be allowed to stand in saline for 1 week to provide the resulting tissue filler.

Example 16: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: BDDE may be added to water;

Step b: HA may be added to the solution of Step a;

Step c: A silk solution may be added to the mixture of Step b and the resulting mixture may be stirred (e.g., 5 minutes) and allowed to stand for about 1 day;

Step d: The resulting gel from Step c may be allowed to stand in saline for 1 week to provide the resulting tissue filler.

Example 17: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: To a silk solution as described herein may be added HA dissolved (mixed for about 12 hours at 400 rpm) in NaOH solution;

Step b: The solution of Step a may be degassed;

Step c: The solution of Step b may be mixed with a crosslinking agent described herein (e.g., BDDE) at 50 °C for about 10-20 minutes;

Step d: The crosslinked gel is mixed with lidocaine HCl;

Step e: Dialysis of the adjusted crosslinked solution may be carried out for 3 days, then 2 days with PBS, then 1 day with water;

Step f: The filtered resulting product is then lyophilized to obtain solids;

Step g: The solids are dissolved in PBS and then incubated;

Step h (Optional): free HA may be added to the product of Step g;

Step i: The resulting product of Step g or h may be sterilized by steam autoclaving.

Example 18: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: HA may be dissolved (mixed for about 12 hours at 400 rpm) in NaOH solution;

Step b: A silk solution may be added to the solution of Step a and the resulting mixture may be degassed;

Step c: The solution of Step b may be mixed with a crosslinking agent described herein (e.g., BDDE) at 50 °C for about 10-20 minutes;

Step d: The crosslinked gel is mixed with lidocaine HCl;

Step e: Dialysis of the adjusted crosslinked solution may be carried out for 3 days, then 2 days with PBS, then 1 day with water;

Step f: The filtered resulting product is then lyophilized to obtain solids;

Step g: The solids are dissolved in PBS and then incubated;

Step h (Optional): free HA may be added to the product of Step g;

Step i: The resulting product of Step g or h may be sterilized by steam autoclaving.

Example 19: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: HA may be dissolved (mixed for about 12 hours at 400 rpm) in NaOH solution;

Step b: The solution of Step a may be degassed;

Step c: A silk solution may be added to the solution of Step b and the resulting mixture may be mixed with a crosslinking agent described herein (e.g., BDDE) at 50 ^{o}C for about 10-20 minutes;

Step d: The crosslinked gel is mixed with lidocaine HCl;

Step e: Dialysis of the adjusted crosslinked solution may be carried out for 3 days, then 2 days with PBS, then 1 day with water;

Step f: The filtered resulting product is then lyophilized to obtain solids;

Step g: The solids are dissolved in PBS and then incubated;

Step h (Optional): free HA may be added to the product of Step g;

Step i: The resulting product of Step g or h may be sterilized by steam autoclaving.

Example 20: Tissue Filler Preparation Method

A Silk/HA tissue filler as described herein could be prepared according to the following general method:

Step a: HA may be dissolved (mixed for about 12 hours at 400 rpm) in NaOH solution;

Step b: The solution of Step a may be degassed;

Step c: The solution of Step b may be mixed with a crosslinking agent described herein (e.g., BDDE) at 50 °C for about 10-20 minutes;

Step d: A silk solution may be added to the product of Step c and mixture may be mixed with lidocaine HCl;

Step e: Dialysis of the adjusted crosslinked solution may be carried out for 3 days, then 2 days with PBS, then 1 day with water;

Step f: The filtered resulting product is then lyophilized to obtain solids;

Step g: The solids are dissolved in PBS and then incubated;

Step h (Optional): free HA may be added to the product of Step g;

Step i: The resulting product of Step g or h may be sterilized by steam autoclaving.

Example 21: Tissue and/or Dermal Filler formulations composed of silk and hyaluronic acid cross linked with BDDE

Materials: 1,4-butanediol diglycidyl ether (BDDE; Sigma-Aldrich); sodium hyaluronate (HA; Lifecore); silk, 6 % solution (Silk Therapeutics); sodium hydroxide, 0.1 N solution (BDH); hydrochloric acid, 5 N (Ricca Chemical); phosphate buffered saline (PBS; 20x; VWR Life Science).

Formulation variables: Silk Molecular Weight: Medium and Low MW silk solution (6 %); HA Molecular Weight: 1.5 MDa and 2.2 MDa; Silk concentration: 1 % $_{v/v}$ (0.6 mg/ml), 2 % $_{v/v}$ (6 mg/ml) 5 % $_{v/v}$ (3 mg/ml) and 20 % $_{v/v}$ (12 mg/ml).

Hydrogel crosslinking: (a) add 6 % silk solution to 0.1 N sodium hydroxide; (b) gradually add HA powder to above prepared solution under overhead stir at the speed of 200-400 rpm, depending on the silk content; stir gently to avoid generating too much air bubbles; keep stirring until HA is fully dissolved; (c) add 1 % $_{w/w}$ of BDDE to the above solution; (d) heat to 50 °C and keep stirring at 100-200 rpm for 30 minutes; (e) let the crosslinked gel cool down below 30 °C; (f) add 5N hydrochloric acid to adjust pH to 7.0-7.4.

Hydrogel dialysis: (a) hydrate the dialysis cassette for 2 minutes; wipe off excessive water and measure the total mass of the empty cassette; (b) add approximately 18 g of hydrogel formulation into the dialysis cassette; measure the total mas of the

cassette after is loaded with gel; (c) suspend dialysis cassette in 2 L of 1x PBS buffer and set magnetic stir at 200 rpm; record the time when dialysis starts and change the PBS buffer after 4 hrs, 24 hrs, and 48 hrs of dialysis; collect the gel after 72 hrs.

Characterization: shear storage modulus (G') and viscosity; enzymatic degradation; BDDE residual; crosslinking density; 30-day animal study; cytotoxicity; bacterial endotoxin; turbidity.

Viscoelastic properties: A Discovery HR-1 hybrid rheometer (TA Instruments) was used to determine storage modulus (G') and complex viscosity (η) of tissue and/or dermal filler formulations. Samples were tested by swiping oscillation frequency from 0.1 Hz to 10 Hz with 10 data points per decade interval. Data were recorded and compared at 5 Hz shear rate. The G' and γ data for hydrogel formulations (after dialysis) with constant HA concentration and variable silk concentration are shown in Table 19. In this batch, 1.5 MDa molecular weight HA was used.

Table 19: Viscoelastic properties of hydrogels with constant HA concentration

Sample	HA Conc.* (mg/ml)	Silk Conc.* (mg/ml)	Silk MW	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)
C2	24	0	N/A	46.9	2.88
A	24	9.6	Medium	105.5	4.93
B	24	0.48	Low	69.7	3.62
C	24	4.8	Medium	102.7	4.82
D	24	0.48	Medium	66.4	3.59
E	24	2.4	Low	41.4	2.56
F	24	0.96	Low	42.7	2.67

*: Hydrogel absorbed PBS buffer after dialysis resulting in volume increase. The concentration of HA and silk were recalculated based on the dilution factor.

The G' and γ data for hydrogel formulations (after dialysis) with constant total concentration of 30 mg/ml of HA and silk are summarized in Table 20.

Table 20: Viscoelastic properties of hydrogels with constant total concentration

Sample	HA Conc.* (mg/ml)	HA MW (MDa)	Silk Conc.* (mg/ml)	Silk MW	% Silk	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)
XHA15M01	23.52	1.5	0.48	Low	1%	94.1	4.52

SL17122002							
XHA15M05 SL17112002	21.60	1.5	2.4	Low	5%	29.5	2.06
XHA15M20 SL17122102	14.40	1.5	9.6	Low	20%	31.7	1.63
XHA15M01 SM17121802	23.52	1.5	0.48	Medium	1%	118.1	5.55
XHA15M05 SM17111602	21.60	1.5	2.4	Medium	5%	38.4	2.35
XHA15M20 SM17112702	14.40	1.5	9.6	Medium	20%	15.6	1.06
XHA2M01 SL171121902	23.52	2.2	0.48	Low	1%	176.3	7.50
XHA2M05 SL17122002	21.60	2.2	2.4	Low	5%	85.1	4.03
XHA2M20 SL17122002	14.40	2.2	9.6	Low	20%	36.0	1.76
XHA2M01 SM17121902	23.52	2.2	0.48	Medium	1%	158.1	6.69
XHA2M05 SM17122002	21.60	2.2	2.4	Medium	5%	106.7	4.76
XHA2M20 SM17111302	14.40	2.2	9.6	Medium	20%	11.5	0.86

*: Hydrogel absorbed PBS buffer after dialysis resulting in volume increase. The concentration of HA and silk were recalculated based on the dilution factor.

Hydrogel reversibility: Hydrogels with and without silk protein were prepared and dialyzed. The final compositions were 33.3 mg/ml HA + 8 mg/ml silk for Silk-HA hydrogel and 33.3 mg/ml of HA for HA hydrogel, respectively. 1 g ± 100 g of above prepared hydrogels were added to 20 ml glass vial followed by 3 ml of 16 U/ml of hyaluronidase in 1x PBS. Samples were incubated at 37 °C for 3 days. Control samples was also prepared using HA hydrogel without adding hyaluronidase. The degradation profile is shown in Fig. 28. Control samples without hyaluronidase was not degraded during the course of 3 days incubation. Within the first 6 hours of incubation, hydrogels absorbed buffer and swelled resulting in the increase of percentage mass. The Silk-HA hydrogel and HA hydrogel were fully degraded after 3 days incubation. At the presence of silk, the hydrogel was digested faster than the pure HA hydrogel. After 12 hours of incubation, approximately 90% of the Silk-HA hydrogel was digested by enzyme.

Crosslinker (BDDE) residual: Samples listed in Table 19 were tested for BDDE residuals using GC-FID by Millennium Research Laboratories, Inc. (MRL). MRL test report MRL18JAN06 indicated that BDDE residual in all samples were none detectable, meeting the acceptance criteria of equal to or less than 2 ppm.

Crosslinking density: Samples listed in Table 19 were further fully digested by hyaluronidase and analyzed using NMR to determine the crosslinking density in term of percentage modification. The test results are listed in Table 21 (MRL test report MRL18JAN07).

Table 21: Percentage modification degree (crosslinking density) for various formulations

Sample ID	MoD (%)
XHA15M00SX17110202 (C2)	2.87
XHA15M20SM17103002 (A)	4.68
XHA15M01SL17103002 (B)	2.58
XHA15M10SM17103002 (C)	3.02
XHA15M01SM17103002 (D)	2.54
XHA15M05SL17110202 (E)	3.76

Animal study: A 30-day animal study using guinea pig model was carried out at WuXi AppTec Minneapolis, MN facility to address product safety concern. There were 2 termination time points in this study, 7 days and 30 days, to evaluate tissue response. The study was summarized in WuXi AppTec report D28195 (Project C19879). Two control samples (Juvederm Ultra Plus and Sample C2 in Table 19) and 6 formulations (Sample A – F in Table 19) were used for intradermal injection. Samples A – F and control sample C2 were steam sterilized (protocol 201707289) at Nelson Laboratories, LLC prior to injection. The study procedure in brief: twenty-four animals twelve per duration were used in this study. Each animal received six dorsal, intradermal injections using threading technique (injecting a line instead of a bolus): one control site on one side of the spine, the second control site on the contralateral side (with sides alternatively assigned by animal) and four test sites with no more than one injection of a given test article (with

right and left sides alternatively assigned among animals). Animals were observed daily throughout the study to assess general health. Animals were humanely euthanized at the scheduled termination dates. The implant sites and surrounding tissue from all animals were excised, placed in formalin, and processed to paraffin blocks followed by histopathological evaluation. The representative histology images and pathological findings were summarized in Table 22. Overall, there was no suggestion of sepsis or immunological response in any of the implant sites.

Table 22: Summary of histopathological evaluations Samples	7 Days	30 Days
Commercial Control	Fig 29	Fig 30
	The Commercial product is noted in both images as blue/gray material. There is mild granulomatous inflammation associated with the material at 7 days.	The Commercial product is noted in both images as blue/gray material. At 30 days, there is a minimal amount of inflammation with very mild fibrosis.
Product A: 24 mg/ml HA 9.6 mg/ml silk	Fig 31	Fig 32
	There is very little inflammation at 7 days. The inflammation was focal and at times hard to find. No implant material is noted.	At 30 days the inflammation is extremely difficult to find and minimal. No implant material is noted.
Product B: 24 mg/ml HA 0.48 mg/ml silk	Fig 33	Fig 34
	Product B demonstrates focal mild inflammation in the 7 days. The inflammation is chronic. This inflammation required close evaluation to identify since it was focal and minimal. No implant material is observed.	The 30-day image demonstrates even less inflammation. It was even more difficult to identify as compared to the 7 day implants. No implant material is observed.
Overall, there was no suggestion of sepsis or immunologic response in any of the implant sites.		

Bacterial endotoxin: Three post sterilization samples (Sample A, Sample E and Sample C2) were selected from 7 formulations used in animal study (listed in Table 19) for bacterial endotoxin test. The kinetic Turbidimetric method was used to determine

endotoxin level. Test results are listed in Table 23, and are below the acceptance criteria of 20 EU/ml (Nelson Labs study report 1006775-S01).

Table 23: Endotoxin test results

Sample ID	Detected Endotoxin
XHA15M20SM17103002 (A)	0.498 (EU/ml)
XHA15M00SX17110202 (C2)	< 0.400 (EU/ml)
XHA15M05SL17110202 (E)	1.56 (EU/ml)

Biocompatibility – Cytotoxicity: Four post sterilization samples (Sample A, Sample B, Sample D and Sample E) were selected from 7 formulations used in animal study (listed in Table 19) for ISO-10993-5 cytotoxicity test (ISO MEM Elution Using L-929 Mouse Fibroblast Cells). These samples represented the highest and lowest silk content of medium molecular weight silk and low molecular weight silk in tested tissue and/or dermal filler formulations. The test reports indicated that all test samples scored grade 0, meaning non-cytotoxic (Wuxi AppTec Reports D28287-1, D28287-2, D28287-3, D28287-4).

Turbidity: The pure HA hydrogel is clear under natural light. When HA was crosslinked with silk protein, the hydrogel becomes slightly turbid (cloudy) and the turbidity is dependent on the total silk concentration in the formulation. The turbidity was measured by Lambda X50S UV-Vis spectrophotometer (PerkinElmer) equipped with InGaAs integrating sphere which has the capability to collect forward scattered light in addition to standard transmitted light. The turbidity measurement of Silk-HA hydrogel is shown in Fig. 35. The black curve is the standard transmittance and the red curve was collected by the sphere showing significant forward scatter. The pure HA hydrogel without silk was used as control sample. The curves in Fig. 36 are nearly identical indicating very little scattering of the pure HA gel. The turbidity measurement suggested that the Silk-HA hydrogel has the capability of scattering lights which could eliminate Tyndall effect when used as fillers.

Conclusions: Filler formulations were developed based on constant HA concentration with various silk contents and constant total concentration. These formulations provided a broad range of storage modulus, viscosity and crosslinking

density which may lead to various applications. The silk-HA hydrogel was enzymatically reversible. The crosslinker residual after dialysis of hydrogel formulations met the acceptance criteria. Cytotoxicity test indicated that silk-HA hydrogels with of silk content ranging from 0.48 mg/ml to 9.6 mg/ml were none cytotoxic and biocompatible. The 30-day animal study demonstrated all formulations with silk content up to 9.6 mg/ml did not cause sepsis and had no immunological response.

Example 22: Tissue and/or Dermal Filler formulations composed of silk and hyaluronic acid cross linked with PEGDE (PEGDGE)

Crosslinker: Poly(ethylene glycol) diglycidyl ether (PEGDE), average molecular weight Mn=500. Reaction conditions: same as BDDE crosslinking (Example 21). The total amount of PEGDE was equivalent to BDDE in moles.

Table 24: PEGDE cross linking formulation and test results

Sample	HA Conc.* (mg/ml)	HA MW (MDa)	Silk Conc.* (mg/ml)	Silk MW	% Silk	Cross linker	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)
XHA15M05 SM17111602	21.60	1.5	2.4	Medium	10%	BDDE	38.4	2.35
XHA15M05 SM18020802P	20.45	1.5	2.27	Medium	10%	PEG-x	67.5	3.10
XHA15M05 SM18020902P	19.28	1.5	2.14	Medium	10%	PEG-x	73.5	3.40

*: Hydrogel absorbed PBS buffer after dialysis resulting in volume increase. The concentration of HA and silk were recalculated based on the dilution factor.

Example 23: Animal Study C20419

Formulations and characterization of samples for animal study C20419 are as shown in Table 25:

Table 25: Formulations and characterization of samples for animal study C20419

Sample		ID	Crosslinker	HA Conc. (mg/ml)	Silk Conc. (mg/ml)	HA MW (Da)	Silk MW	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)	Injection Force @ 30 G (N)	MoD (%)
Group 1	C3	XHA700K3M00SX180510	BDDE	24	0	700K/3M	n/a	0.2	0.1	7.41	4.87
	G	XHA700K3M05SM180510		22.8	1.2	700K/3M	Med	3.8	0.2	8.17	4.54
	H	XHA700K3M01SL180510		23.76	0.24	700K/3M	Low	0	0.1	6.95	5.42
	I	XHA700K3M05SL180510		22.8	1.2	700K/3M	Low	0.5	0.1	7.96	6.23
	K	XHA26M05SM180510		22.8	1.2	2.6M	Med	0.1	0.1	8.48	2.51
Group 2	C4	PXHA700K3M00SX180514	PEGDE	24	0	700K/3M	n/a	52.3	2.4	16.19	15.14
	L	PXHA700K3M05SM180514		22.8	1.2	700K/3M	Med	31.8	1.6	12.96	10.97
	M	PXHA700K3M01SL180514		23.76	0.24	700K/3M	Low	32.2	1.5	15.96	11.02
	N	PXHA700K3M05SL180514		22.8	1.2	700K/3M	Low	51.9	2.1	17.82	11.23
	O	PXHA26M05SM180514		22.8	1.2	2.6M	Med	18.9	1.1	10.56	17.23
Group 3	C5	Group 2 + Free HA				700K/3M	n/a	63.0	2.8	19.02	8.02
	P					700K/3M	Med	28.3	1.4	11.22	9.71
	Q					700K/3M	Low	42.7	1.9	16.80	10
	R					700K/3M	Low	83.9	3.2	20.90	10.12
	S					2.6M	Med	75.8	3.4	12.78	11.92

Figs. 37 - 46 show the results of the study. Fig. 37 is a representative histology picture of an intradermal area in a guinea pig injected with a control dermal filler. Fig. 38 is a representative histology picture of an intradermal area in a guinea pig injected with an HA dermal filler of the invention (24 mg/ml HA, PEGDE cross linked, Sample C4 – Table 25). Fig. 39 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample L – Table 25). Fig. 40 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (23.76 mg/ml HA, 0.24 mg/ml silk, PEGDE cross linked, Sample M – Table 25). Fig. 41 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample N – Table 25). Fig. 42 is a representative histology picture of an intradermal area in a guinea pig injected with a silk-HA dermal filler of the invention (22.8 mg/ml HA, 1.2 mg/ml silk, PEGDE cross linked, Sample O – Table 25).

Figs. 43 – 46 are graphical representations of histology results for Table 25 formulations 7-day post-implantation (scoring: 0 - normal; 1 - minimal; 2 - mild; 3 - moderate; and 4 – severe). Fig. 43 is a graph showing 7-day post-implantation histology results for gel degradation; BDDE crosslinked formulations are mostly degraded. Fig. 44 is a graph showing 7-day post-implantation histology results for gel migration. Fig. 45 is a graph showing 7-day post-implantation histology results for inflammation; no tissue necrosis was observed, no blood clotting was observed, and minimal collagen deposition was observed on the control formulation and some of the test formulations. Fig. 46 is a graph showing 7-day post-implantation histology results for macrophages.

Example 24: Properties of PEGDE crosslinked silk-HA hydrogels: 1) Shear storage modulus (G'), and 2) Swelling ratio during dialysis

Filler Preparation, Materials: Poly(ethylene glycol) diglycidyl ether (PEGDE), Mn = 500, Sigma-Aldrich; Sodium hyaluronate (HA), Lifecore; Silk, 6% solution, Silk Inc.; Sodium hydroxide, 0.1 N solution, BDH; Hydrochloric acid, 5 N, Ricca Chemical; Phosphate Buffered Saline (PBS), 20x, VWR Life Science.

Filler Formulation variables: Silk Molecular Weight: Medium and Low MW silk solution (6%); HA Molecular Weight: 700 KDa and 1.5 MDa; Silk concentration (Initial): 0 – 15 mg/ml.

Hydrogel crosslinking at high concentration: add 6% silk solution to 0.1 N sodium hydroxide; gradually add 100 mg/ml of mixed molecular weight HA (700 KDa / 1.5 MDa = 90/10) to the above prepared solution under gentle stirring until HA is fully dissolved; add PEGDE to the above solution; heat water bath to 40 °C and maintain the crosslinking in water bath for 45 minutes; let the crosslinked gel cool down below 30 °C; add 5N hydrochloric acid to 1x PBS, dilute the gel to 40 mg/ml and adjust the final pH to 7.0-7.4.

Hydrogel crosslinking at low concentration: add 6% silk solution to 0.1 N sodium hydroxide; gradually add 25 mg/ml of 1.5 MDa HA to above prepared solution under gentle stirring until HA is fully dissolved; add PEGDE to the above solution; heat water bath to 40 °C and maintain the crosslinking in water bath for 45 minutes; let the crosslinked gel cool down below 30 °C; add 5N hydrochloric acid to the crosslinked gel and adjust the final pH to 7.0-7.4.

Hydrogel dialysis: hydrate the dialysis cassette (20 KDa MWCO) for 2 minutes; wipe off excessive water and measure the total mass of the empty cassette; add approximately 18 g of hydrogel into dialysis cassette; measure the total mass of the cassette after loaded with gel; suspend dialysis cassette in 2 L of 1x PBS buffer and set magnetic stir at 200 rpm; collect gel after 72 hrs of dialysis.

Viscoelastic properties

A Discovery HR-1 hybrid rheometer (TA Instruments) was used to determine the storage modulus (G') of the hydrogel formulations. Samples were tested by swiping oscillation frequency from 0.1 Hz to 10 Hz with 10 data points per decade interval. Data were recorded and compared at 5 Hz shear rate. The G' of hydrogel formulations before and after dialysis with constant HA concentration and variable silk concentration are shown in Figs. 47A and 47B. For the hydrogel crosslinked by PEGDE at high initial HA concentration, the impact of silk concentration to the G' is minimal due to the relatively low ratio of silk to total HA. It may also be contributed to the mixed HA containing 90% of low molecular weight (700 KDa) which is not sensitive to the changes of silk concentration. For the hydrogel crosslinked by PEGDE at low initial HA concentration, the G' increased as more silk was added to

the formulation. The changes in silk concentration had more impact to G' when the initial HA concentration was low and also had more impact to the high molecular weight HA (1.5 MDa). No substantial impact of silk molecular weight to the G' was observed for both crosslinking procedures.

Swelling ratio during dialysis: there was no clear trend showing the amount of silk added to the hydrogel formulation had any impact to the gel swelling during dialysis for both cross-linking procedures and no substantial difference between medium molecular weight and low molecular weight silk (Figs. 48A and 48B).

The silk concentration in hydrogel formulations had minimal impact to G' if mixed HA was crosslinked by PEGDE at high initial HA concentration, but was proportional to G' if single high MW HA was crosslinked at low initial HA concentration. The molecular weight of silk in the gel formulations had no substantial difference when comparing the contribution to G' and swelling if the HA was crosslinked by PEGDE.

Example 25: Silk concentration in Silk-HA tissue and/or dermal filler formulations

Materials: silk, 6% solution, Silk, Inc.; phosphate buffered saline (PBS), 20x, VWR Life Science; crosslinked hyaluronic acid (HA) gel.

Equipment: moisture analyzer HE53, Mettler Toledo; Cary 100 UV/Vis Spectrophotometer.

Calibration Standard Curve: measure the dry content for both medium and low molecular weight 6% silk solutions to determine the actual dry content (mg/ml) of the silk solutions; create a series of standard silk solutions by diluting the 6% silk solution using 1X PBS (for example, 1 mg/ml silk, 0.75 mg/ml silk, 0.5 mg/ml silk, 0.25 mg/ml silk, and 0 mg/ml silk); measure the absorbance of each standard solution at 275 nm in a quartz cuvette - absorbance measurements can be performed with a scan from 200-800 nm, data interval of 5 nm, and an average collection of 0.1 seconds; Plot the absorbance at 275 nm against the silk concentration (mg/ml) to create a standard curve.

Measurement of Silk Concentration: dilute HA gel samples with 1X PBS such that absorbance at 275 nm is between 0 and 1.0 (for example, the samples can be diluted with a 1:12 ratio of gel to 1X PBS, i.e., 1200% dilution); perform a scan for absorbance for the silk-HA gel sample against a 1X PBS reference between 200 nm –

800 nm, measure the absorbance peak at 275 nm for each gel sample; the absorption signals for the gel samples are corrected by the difference between the absorption signal for the sample with no silk and the intercept of the calibration curve, setting the sample with no silk to have a silk concentration of 0 mg/ml; the silk concentration in the silk-HA gel samples can be calculated from the calibration curve and dilution factor.

Calibration curves were created by measuring the absorption at 275 nm for a series of standard samples with different concentrations of silk ranging from 0 mg/ml to 1 mg/ml. The calibration curves for the medium and low molecular weight silk solutions are shown in Figs. 49A and 49B. The R^2 values of 0.99947 for medium molecular weight silk and 0.99949 for low molecular weight silk demonstrate that the calibration curves are linear within the working range of 0-1 mg/ml of silk concentration. These curves can be used to determine the silk concentrations in gel samples.

Determining Silk Concentration of HA-Silk hydrogels: the absorption at 275 nm of diluted silk-HA hydrogels was measured for each sample as shown in Figs. 50A and 50B. The silk concentration of each sample was calculated with the calibration curve and dilution factor, summarized in Table 26.

Table 26 - Calculated silk concentrations for silk-HA gels with an unknown silk concentration from the calibration curve

Gel Sample	**Theoretical Silk Concentration (mg/ml)**	**Calculated Silk Concentration (mg/ml)**
XHA15M00SX17110201	0	0
XHA15M01SL17103001	0.6	0.49
XHA15M02SL17110201	1.2	1.26
XHA15M05SL17110201	3	3.08
XHA15M01SM17103001	0.6	0.57
XHA15M10SM17103001	6	6.21
XHA15M20SM17103001	12	13.83

Example 26: Silk-HA tissue and/or dermal filler formulations: Gel Opacity

Materials: crosslinked hyaluronic acid (HA) gel; phosphate buffered saline (PBS), 20x, VWR Life Science.

Equipment: Cary 100 UV/Vis Spectrophotometer.

Sample Preparation: inject about 2 mL of HA gel into a clean quartz cuvette such that there is a minimal amount of air bubbles in the sample; injection using an 18 G needle may help reduce the amount of bubbles in the sample; a blank reference sample of 1X PBS can be added to a second clean quartz cuvette (Note: for opacity measurements, a plastic cuvette can be used since the plastic cuvette does not have absorption in the visible range, 400 nm-800 nm).

Measurement of Gel Opacity: set the X-scanning range from 200 nm to 800 nm with a data interval of 5 nm and average time of 0.1 seconds; select the Y-mode to be %T for the measurement of transmitted light (Note: Absorption can also be measured and %T can be calculated from Absorption values); perform a scan on the gel sample against the 1X PBS reference standard; the data can be saved as a csv file and the spectrum can be plotted.

Gel Opacity can be measured using the UV/Vis spectrophotometer for standard transmitted light. An optically clear sample will transmit 100% of light, whereas a slightly turbid or cloudy sample may only transmit a portion of that light. Fig. 51 shows the turbidity measurement of an HA hydrogel with and without silk. The blue curve shows the % transmittance for the transmitted light for a Silk-HA gel sample with 3 mg/mL silk and 26 mg/ml HA. The red curve shows the transmitted light for a sample with no silk and 20 mg/ml HA, and shows more transmission of light than the sample with silk. The turbidity measurements suggest that the Silk-HA gel has an ability to scatter visible light more than the HA gel without silk.

Example 27: Degree of modification (MoD) of the HA hydrogel determined by NMR

Degree of Modification (MoD) is defined as the stoichiometric ratio of all linked cross-linker molecules to the moles of HA repeating units. Both cross- and mono-linked linkers are included in MoD. MoD is determined from ^{1}H NMR spectrum by integrating the signal from the N-acetyl group in HA at 2.1 ppm and the BDDE cross-linker at 1.7 ppm, or the PEGDE cross-linker at 3.0-4.5 ppm.

Prior to enzymatic degradation, the HA hydrogel was first dialyzed again PBS (1X, 2 L x 5) solution to remove the free cross-linker. A Slide-A-Lyzer dialysis cassette (MWCO 3.5 K, Thermo Scientific, Rockford, IL) was used, and the PBS solution was stirred at RT for 72 h. After the dialysis, 1 mL of the HA hydrogel solution was taken out and lyophilized with a Labconco FreeZone lyophilizer (2.5 L) to obtain the dry powder.

To prepare the NMR sample, 10 mg of the dry powder was placed into the NMR tube (5 mm, Wilmad-LabGlass) and 0.6 mL of hyaluronidase (MP Biomedicals, Solon, OH) solution in deuterium oxide (D_2O, Alfa Aesar, Ward Hill, MA) was added. The amount of the hyaluronidase was 5 U per 1 mg of HA. The NMR tube was incubated at 37 °C overnight to make all the HA degraded. The NMR spectra were recorded on a Varian MR 400 MHz Automated NMR System. The relaxation delay time is 1 s and the number of scans is 256. All the data was processed using a MestReNova software (Edition 12.0.2).

Example 28: Silk-HA 2-step cross-linking process

A silk-HA hydrogel can be formed a 2-step crosslinking process to improve the efficiency of silk binding to HA. For a given formulation, at the first step, all silk protein and a small portion of low molecular weight HA are added to NaOH solution at pH 10, and then reacted with a portion of crosslinker. Without wishing to be bound by any particular theory, it is believed that during this step, as much silk as possible reacts with the crosslinker. At the second step, NaOH solution is added to dilute the product from step-1 and increase the pH to 13. The remaining low molecular weight HA, all high molecular weight HA, and the remaining crosslinker are then added to the solution, and the crosslinking reaction is completed.

Example 29: HA hydrogel synthesis

HA hydrogel has been synthesized by using different HA molecular weight, crosslinker, reaction time, reaction temperature, HA concentration, crosslinker ratio, mixing process and stirring method. Tables 27 and 28 show the various reaction conditions employed, and the various hydrogels obtained.

Table 27

HA MW	700 k, 1.5 M, 2.2 M, 3M, or mixture with different MW at any ratio
Crosslinker	PEG500DE, and BDDE
Reaction time	30 min, 60 min, 90 min, 120 min, or 240 min
Reaction Temperature	40 °C, or 50 °C
HA concentration	30 mg/ml, 90 mg/ml, 100 mg/ml, and 140 mg/ml
Crosslinker Ratio	7 Wt.% or 10 Wt.%
Mixing process	Pre-mix HA and crosslinker together or adding crosslinker into the HA solution portion wise
Stirring	With or without mechanical stirring

Table 28

Sample	HA/ Cross linker	Cross-linker ratio (Wt.%)	HA Concentration (mg/mL)	Mixing	Stirring	Reaction time (min)	Temp.°C	G' (After Dialysis)	MoD (%)
PXHA2M00SX 18042541	2.2M/ PEG500DE	10	30	Portion wise	Y	30	40	163	13.02
PXHA2M00SX 18042543	2.2M/ PEG500DE	10	30	Portion wise	Y	60	40	106	9.55
PXHA2M00SX 18042545	2.2M/ PEG500DE	10	30	Portion wise	Y	120	40	95	11.73
PXHA2M00SX 18042547	2.2M/ PEG500DE	10	30	Portion wise	Y	240	40	10.6	15.6
PXHA2M00SX 18051041	2.2M/ PEG500DE	10	30	One pot	N	30	40	148.67	5.3
PXHA2M00SX 18051043	2.2M/ PEG500DE	10	30	One pot	N	60	40	134.61	7.88
PXHA2M00SX 18051045	2.2M/ PEG500DE	10	30	One pot	N	120	40	46.53	9.44
PXHA2M00SX 18051047	2.2M/ PEG500DE	10	30	One pot	N	240	40	28.9	11.2
BXHA700K00SX 18050141	700K/ BDDE	10	30	Dropwise	Y	30	40	42	0
BXHA700K00SX 18050143	700K/ BDDE	10	30	Dropwise	Y	60	40	38	0
BXHA700K00SX 18050145	700K/ BDDE	10	30	Dropwise	Y	120	40	15	0.54
PXHA2M00SX 18051641	2.2M/ PEG500DE	10	30	One pot/overnight	N	30	40	182.91	4.62

PXHA2M00SX 18051643	2.2M/ PEG500DE	10	30	One pot/overnight	N	60	40	129.76	8.87
BXHA700K00SX 18052941	700K/ BDDE	10	30	Portion wise	N	30	40	17.99	0
BXHA700K00SX 18052943	700K/ BDDE	10	30	Portion wise	N	60	40	33.76	0.48
BXHA2M00SX 18052941	2.2 M/ BDDE	10	30	Portion wise	N	30	40	295.6	0.52
BXHA2M00SX 18052943	2.2 M/ BDDE	10	30	Portion wise	N	60	40	222.28	0.66
BXHA15M00SX 18060851	1.5M/ BDDE	10	90	Mixed separately	N	30	50	261.76	3.26
BXHA15M00SX 18060853	1.5M/ BDDE	10	90	Mixed separately	N	60	50	196.8	3.4
BXHA15M00SX 18060855	1.5M/ BDDE	10	90	Mixed separately	N	90	50	93.6	4.84
BXHA15M00SX 18060857	1.5M/ BDDE	10	90	Mixed separately	N	120	50	72.98	4.51
PXHA15M00SX 18061351	1.5M/ PEGDE	10	90	Mixed separately	N	30	50	151.65	undergoing
PXHA15M00SX 18061351	1.5M/ PEGDE	10	90	Mixed separately	N	60	50	71.87	undergoing
BXHA15M00SX 18061551	1.5M/ BDDE	10	100	One pot	N	30	50	234.69	4.6
BXHA15M00SX 18061553	1.5M/ BDDE	10	100	One pot	N	60	50	219.43	6.1
BXHA3M00SX 18061951	3M/ BDDE	10	100	One pot	N	60	50	268.41	undergoing
BXHA3M00SX 18061953	3M/ BDDE	7	100	One pot	N	60	50	189.13	undergoing

Example 29: Silk/HA hydrogel synthesis

Silk filler is composed of crosslinked hyaluronic acid (HA) with silk fibroin fragments covalently bound to HA. The crosslinker is biocompatible and bioresorbable functionalized poly(ethylene glycol) (PEG). The crosslinker connects between HA molecules and silk fibroin to HA molecules to form injectable hydrogel. Lidocaine is also added to the formulation to reduce uncomfortableness during injection. The filler is loaded into 1-mL syringes, sterilizable, and able to inject through 30 G or 27 G needles in clinical studies.

HA induces minimal local tissue response, which does not promote collagen deposition. Silk proteins can induce transient and mild inflammatory responses as a result of implantation leading to the recruitment and activation of macrophages and fibroblasts around local implant. These transient events ultimately lead to deposition of collagen and new endogenous tissue. In fillers, this process has the potential to improve the skin's contour and reduce depressions in the skin due to scars, injury or lines.

Silk fibroin fragments may impact the Tyndall effect. The Tyndall effect refers to the scattering of light by fine particles in a colloid or suspension. The intensity of scattered light is inversely proportional to the forth power of wavelength. Because blue light has shorter wavelength, is scattered with higher intensity and therefore the scattered light appears to be blue. The Tyndall effect is sometimes observed in humans after the application of some dermal fillers. Tyndall effect is even more significant when injected into superficial skin or the skin color is pale. Hydrogel particle suspensions of HA have no UV and visible absorption. The silk dermal filler contains silk fibroin fragments and silk fibers which have UV absorption band around 275 nm and a broad absorption in the visible range. These can help mitigate or eventually eliminate Tyndall effect.

Without wishing to be bound by any particular theory, it is believed that the viscoelastic properties of silk tissue and/or dermal fillerfillers can also be controlled by covalently bound silk fibroin fragments. Existing HA dermal filler products have limited methods to control viscoelastic properties (storage modulus and loss modulus), for example by changing the concentration of crosslinked HA. Adding free HA may reduce the forced during injection but doesn't help controlling viscoelasticity as free HA will degrade fast in vivo. Silk tissue and/or dermal filler contains silk fibroin fragments covalently bound to HA. The conjugated silk fibroin fragments form a more complexed structure which alters the regular crosslinked HA 3D network. It can be controlled by

crosslinking of silk fibroin fragment with different molecular weights (molecular chain length) or different percentage of silk fibroin fragments.

The viscoelasticity and in-vivo longevity of silk tissue and/or dermal filler can also be controlled by altering the molecular weights (repeat units) of the crosslinker. Existing dermal filler products use 1,4-Butanediol diglycidyl ether (BDDE) as crosslinker. BDDE is a small molecular diepoxy lacking flexibility to control the viscoelasticity of dermal fillers, as well as degree of modification (MoD) which governs the longevity of dermal fillers in vivo. Silk filler uses a biocompatible poly(ethylene glycol) diglycidyl ether (PEGDE) as crosslinker. PEGDE is a diepoxy functionalized linear oligomer. It has longer molecular chain than BDDE and is tunable by altering the number of EO repeating unit which provides the flexibility to control hydrogel structure by changing the distance between HA molecules and HA to silk fibroin fragments. Different number of ethylene oxide (EO) repeating units changes the capability of epoxy groups accessing and reacting with HA and silk fibroin fragments which enables to control MoD.

Silk filler is an injectable hydrogel. It is composed of HA and silk fibroin fragments at a constant mass ratio of 95:5. The molecular weight of HA is about 850 KDa and the molecular weight of silk fibroin fragment is about 14 kDa. The hydrogel is crosslinked by PEGDE. The molecular weight of PEGDE is about 500 Da. The final product contains about 26 mg/mL of total HA and silk fibroin fragments, and 0.3% lidocaine in 1x PBS.

In the silk filler formulation, the HA molecules are crosslinked and silk fibroin fragments are also covalently bound to HA molecules on their hydroxyl groups through PEG bridges. The covalent conjugation of silk fibroin fragments to the PEGDE bridge is demonstrated by LC MS/MS methods. For example, the composition of fillers described herein was analyzed to determine the presence of crosslinked silk in the gel. The HA in the gel was first digested using hyaluronidase followed by a combination of proteases (Trypsin/Lys-C, Chymotrypsin, Glu-C). The mixture was then analyzed using a C18 reversed-phase (RP) column on an Ultimate3000 HPLC system with MS/MS analysis performed on a Q Exactive mass spectrometer.

As shown in Fig. 52, PEG crosslinker has primary ions with the m/z of 89.06, 133.08 and 177.11, while the primary ions of silk fragments are 136.07 and 182.08. Without wishing to be bound by any particular theory, it is believed that, at least in some

embodiments, the LC spectrum cannot clearly show free PEG fragments and/or free silk fibroin fragments. Also without wishing to be bound by any particular theory, it is believed that, at least in some embodiments, the silk in the gel might be all covalently conjugated with PEG. Also without wishing to be bound by any particular theory, it is believed that, at least in some embodiments, the MS/MS spectrum of the peak at retention time of 23.22 min (m/z 435.64, highlighted) shows strong signals of both PEG and silk fibroin fragments, which further proves that silk is crosslinked with PEG.

A hydrogel prepared as described herein, was loaded into 1-mL syringes, sterilized by superheated water, and characterized for its mechanical properties. The storage modulus (G') was measured using a TA Instruments Discovery HR-1 Rheometer equipped with cone-plate geometry. About 0.8 mL of hydrogel sample was loaded to cover entire sample plate. The G' measured at oscillation frequency of 5 Hz is about 150 Pa. The MoD is defined as the percentage of number of linked crosslinker molecules over the total number of HA disaccharide units. It can be determined by NMR using characteristic chemical shifts of crosslinker and HA. The MoD of above prepared hydrogel is about 9%. The injection force (IF) was measured using Brookfield Engineering Texture Analyzer. The sample syringe barrel was mounted on a fixture. The plunger rod was driven by a piston to extrude hydrogel through a 30 G needle at the speed of 0.2 mm/s for 10 mm travel distance. The force applied to the piston was continuously recorded. The average injection force of above prepared hydrogel is about 39 N.

A 12-month animal study using a guinea pig model is carried out (WuXi AppTec, Minneapolis, MN) to address product safety concern. There are 5 termination time points in this study, 7 days, 30 days, 90 days, 180 days, and 365 days to evaluate tissue response to the above prepared silk dermal filler. Juvederm Ultra Plus XC was used as control. The study procedure in brief: four animals per duration were used in this study. Each animal received six dorsal, intradermal injections using threading technique (injecting a line instead of a bolus): three control sites on one side of the spine and three test sample sites on the contralateral side. Animals were observed daily throughout the study to assess general health. Animals were humanely euthanized at the scheduled termination dates. The implant sites and surrounding tissue from all animals were excised, placed in formalin, and processed to paraffin blocks followed by histopathological evaluation. 7-day histopathology data are described herein (histology

images in Fig. 53A). The semi-quantitative evaluation (the lower scoring the better) showed a total score of 6.9 for the control group and a total score of 3.8 for the test group.

The pathology findings indicated at 7-day post implant, the test implant material demonstrated less reaction than the control implant. This included ulceration and diffuse migration through the muscle layer with the control material that was not observed in the test material. At 2-3 sites in test material there was minimal migration into or through the muscle layer, at a significantly lower extent compared to the control. Ulcers were not identified with the test material. The foreign body macrophage response and collagen separation were similar between the control and test implants where ulceration was not present.

In some embodiments, the pure HA hydrogel is clear under natural light. In some embodiments, when HA is crosslinked with silk fibroin fragments, the gel exhibits very faint yellowish color and silk protein fibers can be visually observed (see Fig. 54A). The gel exhibits a broad absorbance in the visible range and a distinct scattering. This is measured by a Lambda X50S UV-Vis spectrophotometer (PerkinElmer) equipped with InGaAs integrating sphere which has the capability to collect forward scattered light in addition to standard transmitted light. The turbidity measurements suggest that the Silk-HA hydrogel has the capability of scattering lights which could potentially eliminate Tyndall effect once being used as dermal filler.

In order to understand the impact of silk molecular weight on the viscoelastic properties (storage modulus G' and complex viscosity η) of the hydrogel, two samples were prepared with various molecular weights of silk fibroin fragment. Samples were prepared at a total concentration of 24 mg/ml of HA and silk, and at constant HA/silk ratio of 95:5. Medium molecular silk of about 48 kDa was added to sample A and low molecular silk of about 14 kDa was added to sample B. Both samples were crosslinked at 50 °C for 30 minutes followed by dialysis against 1x PBS for 72 hours. Samples were analyzed after dialysis. Data are shown in Table 29. Sample A crosslinked with medium molecular weight silk had lower G' and η, suggesting, without wishing to be bound by any particular theory, that longer silk fibroin fragment had more impact to HA gel structure. The impact of percentage of silk fibroin fragments in the formulation were also evaluated. Three samples with various silk content were prepared. The total concentration of HA and silk remained at 30 mg/ml. Samples were crosslinked at 50 °C

for 30 minutes followed by dialysis against 1x PBS for 72 hours. Samples were analyzed for G' and η after dialysis (Table 30). The results exhibited a decreased G' and η with the silk concentration increasing in the hydrogel. Therefore, without wishing to be bound by any particular theory, it is believed that the viscoelastic properties of the hydrogel can be controlled by varying the molecular weight and percentage of silk fibroin fragment in the formulation during the crosslinking process.

Table 29: Viscoelastic properties of hydrogels with different silk molecular weight

Sample	Total Conc. (mg/ml)	HA Conc. (mg/ml)	Silk Conc. (mg/ml)	Silk MW	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)
A	24	22.8	1.2	Medium	96.1	3.6
B	24	22.8	1.2	Low	126.2	4.4

Table 30: Viscoelastic properties of hydrogels with different silk content in the formulation

Sample	Total Conc. (mg/ml)	HA Conc. (mg/ml)	Silk Conc. (mg/ml)	Silk MW	G' at 5 Hz (Pa)	η at 5 Hz (Pa·s)
C	30	29.4	0.6	Low	176.3	7.5
D	30	27	3	Low	85.1	4.0
E	30	18	12	Low	36.0	1.8
F	26	24.7	1.3	Low	204.2	7.2
G	26	24.96	1.04	Low	151.5	5.4
H	26	25.28	0.72	Low	173.8	6.2

The silk fillers can be prepared by the following procedures.

(1) For a 10-mL batch size, add 1.167 ml of 6% low molecular weight silk solution and 385 mg of PEGDE into a beaker containing 8.833 mL of 0.1 N sodium hydroxide solution. Add 1330 mg of HA portion by portion into above prepared solution within 40 minutes. Stir gently using a spatula while adding HA to facilitate HA hydration and dissolution. Place beaker into 55 °C water batch for 75 minutes to allow crosslinking. Let the crosslinked hydrogel cool down to < 28 °C. Add 145 μl of 6 N hydrochloric acid into 5 mL of 1x PBS. Pour PBS solution into hydrogel, seal the beaker and place in 4 °C refrigerator to allow neutralization and dilution of the hydrogel overnight. Upon the PBS fully absorption by hydrogel, add another 10 mL of 1x PBS to the diluted hydrogel and place in 4 °C refrigerator to allow further dilution overnight.

Fill diluted hydrogel into 20 kDa MWCO dialysis tube and dialyze against 1x PBS (4 L) at room temperature over 72 hours. Change PBS at 6 hrs, 24 hrs and 48 hrs. After dialysis, add lidocaine and additional 1x PBS to adjust the final concentration to 26 mg/mL with 0.3% lidocaine. The hydrogel is loaded into 1-mL syringes and sterilized using superheated water. Alternatively, 0.15 N sodium hydroxide solution can be used instead of 0.1 N sodium hydroxide in the manufacturing procedure. Alternatively, 0.25 N sodium hydroxide solution can be used instead of 0.1 N sodium hydroxide in the manufacturing procedure.

(2) For a 10-mL batch size, add 1.167 ml of 6% low molecular weight silk solution and 96 mg of PEGDE into a beaker containing 8.833 mL of 0.1 N sodium hydroxide solution. Add 266 mg of HA into above prepared solution. Stir gently using a spatula until HA is fully dissolved. Place beaker into 55 °C water batch for 60 minutes to allow first step crosslinking. Let the beaker cool down to room temperature. Add 289 mg of PEGDE into beaker and stir till fully dissolve. Then add 1064 mg of HA portion by portion within 30 minutes. Stir gently using a spatula while adding HA to facilitate HA hydration and dissolution. Place beaker into 55 °C water batch for 60 minutes to allow second step crosslinking. Add 145 μL of 6 N hydrochloric acid into 5 mL of 1x PBS. Pour PBS solution into hydrogel, seal the beaker and place in 4 °C refrigerator to allow neutralization and dilution of the hydrogel overnight. Upon the PBS is fully absorbed by hydrogel, add another 10 mL of 1x PBS to the diluted hydrogel and place in 4 °C refrigerator to allow further dilution overnight. Fill diluted hydrogel into 20 kDa MWCO dialysis tube and dialyze against 1x PBS (4 L) at RT over 72 hours. Change PBS at 6 hrs, 24 hrs and 48 hrs. After dialysis, add lidocaine and additional 1x PBS to adjust the final concentration to 26 mg/mL with 0.3% lidocaine. The hydrogel is loaded into 1-mL syringes and sterilized using superheated water. Alternatively, 0.15 N sodium hydroxide solution can be used instead of 0.1 N sodium hydroxide in the manufacturing procedure. Alternatively, 0.25 N sodium hydroxide solution can be used instead of 0.1 N sodium hydroxide in the manufacturing procedure.

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While the methods of the present disclosure have been described in connection with the specific embodiments thereof, it will be understood that it is capable of further modification. Further, this application is intended to cover any variations, uses, or adaptations of the methods of the present

disclosure, including such departures from the present disclosure as come within known or customary practice in the art to which the methods of the present disclosure pertain.

Example 30. Methods for Characterization of Physicochemical Properties G', IF and MoD of the Silk-HA Hydrogels

The incorporation of silk fibroin in hyaluronic acid hydrogels, in conjunction with the use of polyethylene glycol crosslinker, represents a novel platform for the formulation of fillers. By varying HA concentration, percentage of silk and PEGDE:HA ratio, as well as the formulation reaction conditions, more than one hundred filler candidates were prepared for screening via this platform. Tests of the physicochemical and mechanical properties of the generated silk-HA hydrogels focused on determining the storage modulus (G'), degree of crosslinking or modification (MoD), injection force (IF), and spectral absorption of each hydrogel, as these properties are of particular importance in the generation of filler products with desirable characteristics.

Example 30a. Storage Modulus

The storage modulus (G') of each hydrogel was determined using a Discovery HR-1 Rheometer (TA Instruments, New Castle, DE). Measurements (three per hydrogel formulation) were performed using a cone-plate geometry at the oscillation frequency of 5 Hz.

Example 30b. Degree of Modification

NMR System Operating Procedure

Equipment: Varian INOVA 500 MHz NMR; Pipettes, 1000 µl, 200 µl and 20 µl (Eppendorf, Research Plus); Pipette Controller (VWR, Powerpette Plus, 613-4442); NMR tube (Wilmad, WG-1235-7); NMR tube caps (Kimble, 897095-0081); Water bath incubator (Benchmark Scientific, B2000-4); 20 mL glass vial (VWR, VW74515-20); Weighing boat (VWR Cat # 10770-440); Oven (Quincy Lab, 12-140AE); Lyophilizer (LabConco, Cat#700201000); Kimwipes (Kimberly-Clark Professional); Parafilm M (Bemis, PM 996); Analytical balance (Mettler Toledo, XS204 DeltaRange).

Materials: Deuterium water (Alfa Aesar, 14764); Chloroform-D (Alfa Aesar, 41389); Silk, 6% solution (Silk Medical Aesthetics, Inc.); Poly(ethylene glycol) diglycidyl ether, (SinoPEG, Technical / Medical grade); Sodium hyaluronate, 850

KDa (HTL Biotechnology, Pharmaceutical grade); Hyaluronidase (MP Biomedicals, Cat# 100740); PBS 20 x (VWR, E703-1L); Water (RICCA, Cat# 9150-5); Lidocaine HCl (Spectrum, LI103)

Methods: To determine the MoD of each hydrogel, 600 – 800 mg of hydrogel was mixed with 0.8 mL of about 275 IU/mL or about 340 IU/ml hyaluronidase in 1x PBS. The mixtures were incubated at 37 °C for 16 hr to 24 hr to allow complete digestion of crosslinked hydrogels. A 600 µl sample of the digested hydrogel solutions was air dried at 50 °C for 2 hr to 4 hr, and 10 mg of dried sample was dissolved in 600 µL of deuterated water in a NMR tube and the proton NMR spectrum was recorded on a Varian INOVA 500 MHz NMR instrument (Palo Alto, CA).

Preparation of the NMR samples

Preparation of the PEGDE sample: Take out the PEGDE sample from the freezer and leave the sample at room temperature for approximately 30 minutes to 1 hour. The PEGDE will melt and become to liquid. Use a pipette to measure 5 µl of the PEGDE and add to an NMR tube. Add 600 µl of deuterium water or chloroform-D to the NMR tube. The sample must be NMR scanned within 2 hours.

Preparation of the HA sample: Take out the HA sample from the freezer and leave the sample at room temperature for approximately 30 minutes to 1 hour. Weigh out 20 mg of HA in a 20 ml glass vial. Dilute 20x PBS to 1x PBS by adding 1 portion of 20x PBS into 19 portions of water. Weigh out 340 IU of hyaluronidase in a separate 20 ml glass vial. Add 1.1 ml of 1x PBS to the vial to dissolve the hyaluronidase. Ensure hyaluronidase is dissolved before proceeding. Add 1 ml of the hyaluronidase/PBS solution to the HA vial. Put the HA vial in a 37 °C water bath incubator and incubate for 16-24 hours. Use a pipette to measure 600 µl of the HA PBS solution and put in a weighing boat. Put the weighing boat in a 50 °C oven for 2-4 hours. Once the solvent has dried, the sample becomes a white sheet and sticks to the bottom of the weigh boat. Weigh 10 mg of the dried HA sample and put the sample into an NMR tube. Add 600 µl of deuterium water to the NMR tube. Store the NMR tube at room temperature. The sample must be NMR scanned within 1 week.

Preparation of the silk sample: Use a pipette to measure 1 ml of silk solution and add to a 20 ml glass vial. Cover the glass vial with a piece of Kimwipe and seal the Kimwipe with Parafilm. Ensure the top of the glass vial is not covered by

Parafilm. Put the vial into freezer for 4-6 hours. Take out the vial from freezer and put into the chamber of the lyophilizer. Lyophilize the sample for 24-48 hours. Take out the dried sample from the lyophilizer and weigh out 10 mg of the dried silk. Put the 10 mg of dried silk into an NMR tube. Add 600 µl of deuterium water to the NMR tube. Store the NMR tube at room temperature. The sample must be NMR scanned within 1 week.

Preparation of the Lidocaine sample: Weigh out 5 mg of lidocaine HCl sample and add into an NMR tube. Add 600 µl of deuterium water to the NMR tube. Store the NMR tube at room temperature. The sample must be NMR scanned within 1 week.

Preparation of the gel sample: Weigh out 600 - 800 mg of gel in a 20 ml glass vial. Dilute 20x PBS to 1x PBS by adding 1 portion of 20x PBS into 19 portions of water. Weigh out 340 IU of hyaluronidase in a 20 ml glass vial. Add 1 ml of 1x PBS to the vial to dissolve the hyaluronidase. Ensure hyaluronidase is dissolved before proceeding. Add 0.8 ml of hyaluronidase/PBS solution to the gel vial. Put the gel vial in a 37 °C water bath incubator and incubate for 16-24 hours. Use a pipette to measure 600 µl of the gel/PBS solution and put in a weighing boat. Put the weighing boat in a 50 °C oven for 2-4 hours. Once the solvent has dried, the sample becomes a white sheet and sticks to the bottom of the weigh boat. Weigh out 10 mg of the dried HA sample and put the sample into an NMR tube. Add 600 µl of deuterium water to the NMR tube. Store the NMR tube at room temperature. The sample must be NMR scanned within 1 week.

Running NMR tests: Run NMR proton test for the given sample and select the number of scans. For lidocaine and PEGDE, choose 64 scans. For all the other samples, choose 256 scans. Ensure correct solvent type is accounted for. Repeat as needed for multiple sample tests.

Processing NMR data: MestReNova software or an equivalent NMR software is used to load and process .fid files. The following corrections are performed for every sample: Baseline correction: To correct the baseline, a polynomial order value of 3 is applied. Phase correction: For the phase correction, all peaks should be symmetrical. Solvent peak correction: To correct the chemical shift of the solvent peak, deuterium water is 4.79 ppm and chloroform-d is 7.27 ppm. Integration: After previous corrections, the following integrations are performed for each chemical: For

PEGDE, the peaks at the chemical shifts: 2.77-2.81 ppm, 2.96-2.99 ppm, 3.33-3.38 ppm, 3.38-3.44 ppm, 3.68-3.80 ppm and 3.95-3.40 ppm, are integrated. For Lidocaine, the peaks at the chemical shifts: 1.35-1.46 ppm, 2.21-2.27 ppm, 3.34-3.48 ppm, 4.32-4.39 ppm and 7.21-7.33 ppm, are integrated. For silk, the peaks at the chemical shifts: 1.32-1.5 ppm and 3.77-4.09 ppm, are integrated. For HA, the peaks at the chemical shifts: 2.0-2.1 ppm and 3.30-4.05 ppm, are integrated. For the final gel, the peaks at the chemical shifts: 1.20-1.28 ppm, 1.35-1.48 ppm, 2.0-2.1 ppm and 3.30-4.05 ppm, are integrated. Each peak must show the chemical shift range. The integration value must be under this line.

Integration normalization: The integration values of each spectrum need to be normalized to calculate the MoD. To normalize the integration value of the peak: For PEGDE, normalize the integration of 2.77-2.81 ppm as 2. For lidocaine, normalize the integration of 1.35-1.46 ppm as 6. For silk, normalize the integration of 1.32-1.5 ppm as 2. For HA, normalize the integration of 2.0-2.1 ppm as 3. For the final gel, normalize the integration of 2.0-2.1 ppm as 3.

The Degree of Modification (MoD) of a hydrogel is defined as either of:

$$\mathrm{MoD} = \frac{\mathrm{n_{linked\ crosslinkers}}}{\mathrm{n_{HA\ disaccharides}}}$$

or

$$MoD = \frac{n_{linked\ crosslinkers}}{n_{HA\ disaccharides} + n_{SPF\ repeating\ units}}$$

depending on several variables such as concentration of SPF and/or crosslinker used during hydrogel synthesis, where n is the number of molecules, which can be determined by NMR using characteristic chemical shifts of crosslinker, HA, SPF, and/or any other optional component such as a local anesthetic.

The MoD of hydrogel samples was calculated from the NMR spectra (see for example Figs. 64 and 65) using the following equation (see also "Chemical Characterization of Hydrogels Crosslinked with Polyethylene Glycol for Soft Tissue Augmentation," Monticelli et al., Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1077-1081):

$$\mathrm{MoD\%} = \frac{\delta_{3.30\text{-}4.05} - \left(\delta_{1.20\text{-}1.28} \times \frac{1}{3}\right) - (\delta_{1.35\text{-}1.48} \times 1.25) - 11}{\mathrm{N_{PEG\text{-}H}}} \times 100\%$$

The average number of protons (N_{PEG-H}) in each PEG chain from the NMR spectrum was calculated using the equation: $N_{PEG-H} = (\delta_{3.68-3.80} \times \text{substitution\%}) + 10$, where $\delta_{3.68-3.80}$ is the integration value after normalizing the integration of 2.77-2.81 ppm as 2; "substitution%" is a measure of average numbers of glycidyl groups per linker PEGDE linker, for example a 100% substitution means that each PEGDE linker has two terminal glycidyl groups, while a number of less than 100% means that on average, not every single PEGDE linker in the sample is fully substituted with two glycidyl groups; and "10" is added for the protons in two glycidyl groups.

Without wishing to be bound by any particular theory, it is believed that the following chemical shifts in the gel NMR spectra, correspond to the following respective protons:

3.30-4.05: mix of protons from HA residues, PEG linkers, silk (SPF), and lidocaine;

1.20-1.28: two terminal methyl groups in lidocaine; and

1.35-1.48: mix of protons in silk fibroin protein fragments (SPF).

The "11" value in the numerator of the MoD equation represents the integration of HA protons in the 3.30-4.05 region of the spectra.

Example 30c. Injection Force (IF)

The injection force required to dispense each hydrogel from a 1-mL syringe equipped with 30G needle was measured using a Brookfield CT3 10K Texture Analyzer (AMETEK Brookfield, Middleboro, MA). Each sample syringe was secured in a fixture. The syringe plunger was compressed by a piston at the speed of 0.2 mm/sec for a total travel distance of 1 cm. The force applied to the piston was recorded every 0.05 second (or 0.01 mm). The average force and peak force for each sample was recorded and the overall average of 3 samples was reported.

The results for the physicochemical property characterization and the impact of silk concentration on G', IF and MoD for the silk-HA hydrogels are summarized as below. The G', IF, and MoD of multiple hydrogels formulated with the same concentration of HA and ratio of PEG crosslinker to HA (about 30 % w/w), but different concentrations of fibroin protein, were measured. Results demonstrated that both the G' and IF of the hydrogels decreased as the concentration of silk in the

formulations increased, while the MoD remained relatively unchanged (Figs. 55A-C). Importantly, these results indicate that G' can be modulated without change to MoD by varying silk concentration, enabling the optimization of these two crucial gel characteristics. That is, the silk-HA gel formulation platform allows the generation of hydrogels that vary in storage modulus (G') – important for the development of products for different indications – while maintaining characteristics that promote product longevity (high MoD) and usability (operable IF).

Based on the different mechanical properties of the various silk-HA hydrogel formulations evaluated, a silk-HA gel formulation using 5.0 % silk fibroin protein based fragments and PEG crosslinker was selected as a potential filler candidate and was evaluated in further studies, including ISO 10993 biocompatibility testing. The hydrogel formulation selected as lead candidate, AS-V1, exhibited a high MoD (8.9 ± 0.2 %) at a G' (144 ± 24 Pa), operable IF (39.2 ± 3.4 N) using a 30 gauge needle, and physiological osmolality (264 mOsmol/kg). It is composed of hyaluronic acid and silk fibroin in a 95:5 weight ratio (26 mg/mL) with PEG crosslinker at about 30 % w/w and 0.3 % w/w lidocaine by the total weight of the silk-HA hydrogel. Low molecular silk (< 28 kDa), and HA of 850 kDa was used.

In such products, gel materials that exhibit appropriate viscoelasticity and resistance to deformation ("stiffer" materials with higher G'), ease of flow during injection (low IF), and longevity or resistance to degradation in vivo (typically achieved with a higher MoD), are used to select hydrogel product candidates. The final concentrations of the hydrogel candidates range from 15 mg/mL to 26 mg/mL (silk plus HA). The hydrogel candidates exhibit mechanical properties included G' ranging from 40 – 700 Pa and IF ranging from 10 N to >100 N (Fig. 56). The MoD of these hydrogels were all similar to or higher than commercial HA-based dermal fillers.

Example 31. Optical Properties

The optical properties of silk-HA hydrogels were characterized using a Cary 7000 UV-vis-NIR (Agilent Technologies, Santa Clara, CA) equipped with a UMS integrating sphere. Three samples of each hydrogel were measured.

Injection with commercially available dermal filler products has been known to give rise to a bluing of the skin, described as a Tyndall effect, in some patients. Silk

fibroin's effects on the optical properties of HA-based hydrogels and its potential to offset the Tyndall effect was measured in two ways.

First, the refractive indices of HA-based hydrogels generated with and without silk were compared with each other and with that of a commercially available dermal filler product (Juvéderm® Ultra Plus XC). All tested hydrogel formulations were found to have refractive indices of 1.34, indicating a similar propagation of light as it interacts with the various gels and their surfaces.

Second, the absorbance of the silk-integrated dermal filler candidate (AS-V1) was evaluated and compared to a HA-based hydrogel (without silk) as well as a commercial dermal filler. AS-V1 demonstrated higher absorbance of UV and blue wavelengths of visible light than the hydrogel without silk and the commercial dermal filler (Fig. 57).

The increased absorbance of UV to blue light demonstrated by AS-V1 suggests a lower probability for causing the bluing effect in patients, and thus its potential utility in relatively superficial aesthetic corrections in pale skins.

Example 32. GLP Biocompatibility Testing in Animal Under ISO 10993

ISO 10993 based GLP animal studies for evaluation of local tissue response were performed using guinea pigs.

Albino guinea pigs (Cavia porcellus), Hartley strain (specific pathogen free), were used in these studies. All procedures were approved by the Institutional Animal Care and Use Committee. Animals were treated in accordance with NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals".

The hydrogel formulation selected for further development as a potential dermal filler product (Activated Silk Hydrogel-V1, AS-V1) was tested for biocompatibility in accordance with ISO 10993 standards set by the International Organization for Standardization for biological evaluation of medical devices, and in accordance with FDA guidance, under the category of class III medical devices for permanent implant, tissue/bone contact. The lead candidate hydrogel formulation AS-V1 demonstrated excellent characteristics in biocompatibility testing, which may lead to low risks of safety concerns and low rates of adverse event occurrence in patient populations.

Biocompatibility test results confirmed expectations built upon the demonstrated safety of all three gel components for in vivo use: (1) HA as a natural

component of the skin's viscoelastic intracellular matrix; (2) silk that has been used in many different biomedical applications throughout history, including for dermal tissue reconstruction; and (3) PEG as a biocompatible polymer. ISO 10993 biocompatibility assays on AS-V1 satisfied all acceptance criteria.

Example 33. In vitro and in vivo Reversibility Testing

Example 33a. In vitro degradation tests of silk-HA hydrogels

Approximately 1 g of each hydrogel (AS-V1 or Juvéderm® Ultra Plus XC) was placed into each of three vials along with 1 ml of PBS (0.2 M, pH 6.2) containing 150 U/ml hyaluronidase and incubated at 37 °C for 30 minutes. Following incubation, the supernatant was completely removed and the remaining weight of the gels measured. This process was repeated three more times for a total of 4 ml (600 U) of hyaluronidase over 120 min. The degree of hydrogel degradation was represented by a weight ratio (%) of the remaining hydrogel to the original hydrogel.

Example 33b. In vivo reversibility testing

Twelve animals were used in this study. Each animal received six intradermal injections dorsally, with three sites on each side of the spine as described above. Within 60 ± 30 minutes after injection of silk-HA hydrogel (test article) or Juvéderm® Ultra Plus XC (control article), reversal of the test and control materials was attempted by enzymatic degradation with hyaluronidase under the direction of a plastic surgeon. Starting with 15 units, hyaluronidase (Hylenex™, 150 U/ml) was injected intradermally and/or subcutaneously in small quantities at multiple locations along each test or control material track and gently massaged into the site. Up to 0.4 ml of hyaluronidase was injected at each test or control site at approximately 30 minute intervals. Dissolution/degradation of test or control material was assessed by macroscopic observation and palpation.

Animals were observed daily for one month to assess general health and the presence or absence of residual material. Three animals were euthanized at each of four time points after the last enzyme treatment: 65 ± 5 minutes, 24 ± 2 hours, 7 ± 0.5 days, and 30 ± 1 days after the last enzyme treatment. The implant sites and surrounding tissue were excised, formalin-fixed and paraffin embedded, sectioned, and stained with hematoxylin and eosin. Slides where evaluated by a blinded pathologist for the presence of polymorphonuclear cells, lymphocytes, plasma cells, macrophages, giant cells, tissue necrosis, overall inflammation, neovascularization,

fibrosis, fatty infiltrate, blood clotting, collagen deposition, and gel degradation and migration.

Example 33c. In vivo Reversibility Testing

Three replicates of ~1 g of each hydrogel (AS-V1 or Juvéderm Ultra Plus XC) were digested with 150 U hyaluronidase at 37 °C for 30 min. Following incubation, the remaining weight of the gels was measured. This process was repeated three more times for a total of 600 U of hyaluronidase over 120 min. The degree of *in vitro* hydrogel degradation was represented as a weight ratio (%) of the remaining hydrogel to the original hydrogel.

For *in vivo* reversibility testing, each of twelve animals received six intradermal injections dorsally, with three sites on each side of the spine as described above. Within one hour after injection of hydrogels, reversal of the test and control materials was attempted by enzymatic degradation with hyaluronidase under the direction of a plastic surgeon. Starting with 15 units, up to 60 U of hyaluronidase was injected intradermally and/or subcutaneously along each test or control material track and gently massaged into the site at ~30 minute intervals. Dissolution/degradation of test or control material was assessed by macroscopic observation and palpation.

Animals were observed daily for one month to assess general health, and three animals were euthanized at 65 ± 5 minutes, 24 ± 2 hours, 7 ± 0.5 days, and 30 ± 1 days after the last enzyme treatment. The implant sites and surrounding tissue were excised, formalin-fixed and paraffin embedded, sectioned, and stained with hematoxylin and eosin. Slides were evaluated by a blinded pathologist for the presence of polymorphonuclear cells, lymphocytes, plasma cells, macrophages, giant cells, tissue necrosis, overall inflammation, neovascularization, fibrosis, fatty infiltrate, blood clotting, collagen deposition, and gel degradation and migration.

The ability of AS-V1 to be degraded by hyaluronidase in a fashion similar to that seen with other commercial HA-based gels was assessed. The ability of HA-based gels to be degraded by hyaluronidase is a critical advantage for HA-based dermal filler products, allowing plastic surgeons to rapidly reverse injections in instances of poor outcomes or adverse events. Both in vitro and in vivo testing demonstrated that the ability of hyaluronidase to enzymatically degrade AS-V1 was not impaired. Thus the ability to “reverse” AS-V1 dermal injection, if needed, is maintained in the presence of silk. In vitro testing showed that although AS-V1 was

less degraded than Juvéderm® Ultra Plus XC gel after a single 30 min incubation with hyaluronidase, AS-V1 was degraded equivalently after incubation with enzyme for 60 min or more (Fig. 58A).

For *in vivo* testing, tissue sections taken from hyaluronidase injection sites showed nearly complete degradation ("reversal") of hydrogel material following a single 1:1 volume injection of hyaluronidase at one hour post-injection for 61% of AS-V1 and 47% of Juvéderm Ultra Plus XC injection sites (Fig. 58B). Further, the AS-V1 required fewer hyaluronidase injections to achieve full reversal than did the Juvéderm Ultra Plus XC (Fig. 58B). Thus, both *in vitro* and *in vivo* testing demonstrated that the ability of hyaluronidase to "reverse" AS-V1 dermal injection, if needed, is maintained in the presence of silk.

The in vitro results were well-correlated with the data obtained from the in vivo reversibility study. Here, three animals were treated as before, each receiving 3 intradermal injections of 0.1 mL AS-V1 and 3 injections of Juvéderm® Ultra Plus XC spaced 1 cm apart in the dorsal dermis. In tissue sections taken from hyaluronidase injection sites, nearly complete degradation ("reversal") of the hydrogel material was confirmed following a single 1:1 volume injection of hyaluronidase at 60 ± 30 minutes post-injection with both AS-V1 and Juvéderm® Ultra Plus XC (data not shown); however, some sites required up to three additional reversal injections to reach complete removal of the hydrogel. Overall, AS-V1 has a similar reversibility profile to Juvéderm® Ultra Plus XC, as demonstrated in in vivo guinea pig studies and in vitro testing settings.

The silk-HA hydrogel formulation AS-V1 demonstrated excellent characteristics in (1) durability testing, which may lead to longer-lasting treatments; and (2) reversibility testing, which should provide reassurance during use to providers and patients alike.

The results described in this example for in vivo assessment for hydrogel degradation, migration, and reversibility were also similar when comparing AS-V1 hydrogel formulation to the commercial product, indicating that the candidate silk-HA hydrogel dermal filler AS-V1 has longevity and performance characteristics similar to those of marketed products, and exhibits similar capacity for full reversibility in vivo when needed.

Example 34. Evaluation of short-term local tissue responses to AS-V1

To explore the safety of and local tissue response to AS-V1 hydrogel formulation in conditions directly relevant to its potential as an injectable dermal filler product, a comprehensive array of tests demonstrating the safety and efficacy of the AS-V1 hydrogel following intradermal injection were performed.

The local tissue response to dermal fillers following dorsal intradermal injection (implant) into guinea pigs was evaluated at time points extending up to six months post-injection per ISO 10993-6 requirements. Six animals were evaluated at each time point. The fur from the back (dorsal side) of each animal was removed, the animal was anesthetized, and the injection sites were aseptically prepared. Each animal received six intradermal injections (implants): three of the AS-V1 silk-HA hydrogel on one side of the spine and three Juvéderm® Ultra Plus XC on the contralateral side. Each injection delivered a volume of 0.1 mL per site with at least 1 cm between each injection site. The injection sites were identified with a surgical skin marker pen. Injection sites were scored for erythema and edema prior to injection; animals were observed daily for 7 days post-injection for Draize scoring (dermal irritation), and at days 3 and 4 post-injection for bruising. Animals were humanely euthanized at days 7, 30, 90 ± 1, and 180 ± 2 and 365±3 post-injection for tissue examinations. The implant sites and surrounding tissue were excised, formalin-fixed and paraffin embedded, sectioned, and stained with hematoxylin and eosin. A pathologist blinded to study conditions evaluated slides for evidence of local tissue reactions including inflammatory responses, gel degradation, gel migration and collagen deposition.

All assays were performed following injection of 0.1 mL AS-V1 into the dorsal dermis of guinea pigs, and results were compared with those obtained following injection with Juvéderm® Ultra Plus XC, an FDA-approved dermal filler composed of 1,4-butanediol diglycidyl ether (BDDE) crosslinked HA gel. AS-V1 performed similarly to or better than Juvéderm® Ultra Plus XC in all tests, at time points ranging from 1 day to 6 months post-injection.

The Draize skin irritancy test (acute irritation) was performed at day 1 through day 5 post-injection. Negligible irritation was observed, with scores of 3 or less (out of a possible 8) observed at all time points for both the AS-V1 (test article) and Juvéderm® Ultra Plus XC (FDA-approved comparator) (Fig. 60A-D), indicating

minimal unwanted tissue response following injection. In fact, the silk-HA hydrogel scored similarly on the Draize test as the Juvéderm® Ultra Plus XC, indicating that the immediate irritation which it causes for up to 5 days after injection in the guinea pig model is similar to that seen with an FDA-approved product that does not contain silk components. Further supporting the conclusion that AS-V1 causes less irritation than Juvéderm® Ultra Plus XC is the minimal post-injection bruising seen with AS-V1; this bruising is less than or equivalent to that seen in the same animals with Juvéderm® Ultra Plus XC at 3 and 4 days post-injection (Figs. 60A-B).

The testing results in this example demonstrated that the AS-V1 hydrogel caused immediate and medium-term post-injection irritation, bruising, and inflammation at levels that are similar to or lower than those seen with commercial product Juvéderm® Ultra Plus XC.

In addition, a summary toxicological assessment of AS-V1 was conducted by an independent board-certified toxicologist.

Example 35. Evaluation of Longer-Term Inflammation and Gel Performance

Additional histological assessments in guinea pigs extended the support for the biocompatibility and performance of AS-V1 up to 12 months post-injection. These assessments examined the inflammatory responses to as well as the degradation and migration of the gels in situ following intradermal injection.

Minimal inflammation was observed, with scores of approximately 4 or less (out of a possible 28) observed at all time points for both AS-V1 and comparator (Juvéderm® Ultra Plus XC) gels, indicating minimal detrimental tissue response to the products post-injection (Fig. 61A). Similar profiles were also seen for AS-V1 and Juvéderm® Ultra Plus XC for both the hydrogel degradation (Figs. 61B and 61D) and migration (Figs. 61C and 61 E) in skin tissue matrices. Here, higher scores (maximum of 4) indicate more degradation or migration of the gel; both are undesirable for dermal fillers. For degradation, AS-V1 scores remained below 1.5, indicating desirable low levels of degradation and a good in-tissue gel longevity profile. For migration, AS-V1 scores remained below 2, indicating desirable low levels of gel migration and a good in-tissue placement/location stability profile. Moreover, these results demonstrate that AS-V1 is performing on par with Juvéderm® Ultra Plus XC in intra-dermal studies in guinea pigs from both the gel migration/degradation and tissue response perspectives.

Given the comparable short-term performance profiles for AS-V1 and Juvéderm Ultra Plus XC, the long-term profiles were assessed. These assessments examined the durability, inflammatory responses, and degradation and migration of the gels in situ following intradermal injection. With regards to durability, the gel (light blueish /grey color) is clearly observed to be still integrated around the collagen matrix (pink) at 12 months post-injection (Figs. 62A-J), confirming the durability of AS-V1 and Juvéderm Ultra Plus XC for up to a year in the guinea pig model.

At 3 and 6 months post-injection, histological examination indicated the desired integration of filler gel into representative dorsal dermal tissue sections. In fact, the AS-V1 product is smoothly incorporated with the skin's collagen matrices at both time points, in contrast to the clumps of implant that appear less well incorporated with the collagen structure seen in tissues injected with Juvéderm® Ultra Plus XC (Figs. 63A-D). The lack of observed inflammatory or other undesirable tissue response pathologies indicates the favorable biocompatibility and ability to stimulate the integration of collagen by AS-V1. Similar or better performance of AS-V1 compared to Juvéderm® Ultra Plus XC in these assessments support the further development of AS-V1 as a promising dermal filler product.

Further, similar profiles for both gel degradation (Fig. 61D) and migration (Fig. 61E) in skin tissue matrices were seen for AS-V1 and Juvéderm Ultra Plus XC over the one year study. For degradation, AS-V1 scores remained low, indicating a good in-tissue gel longevity profile. For migration, AS-V1 scores remained in line with Juvéderm Ultra Plus XC, indicating desirable low levels of gel migration and a good in-tissue placement/location stability profile. At 3, 6 and 12 months post-injection, histological examination indicated the desired integration of filler gel into representative dorsal dermal tissue sections (Figs. 63A-D).

In fact, the AS-V1 product was smoothly incorporated with the skin's collagen matrices at all three time points, in contrast to the less well incorporated clumps of implant seen in tissues injected with Juvéderm Ultra Plus XC (Figs. 63A-D). Finally, the lack of observed inflammatory or other undesirable tissue response pathologies indicates the favorable biocompatibility and ability to integrate with collagen of AS-V1 (Figs. 62A-J and Figs. 63A-D).

This is confirmed in Fig. 61F, which shows that minimal inflammation was observed at all time points for both AS-V1 and comparator (Juvéderm Ultra Plus XC)

gels, indicating minimal detrimental tissue response to the products post-injection (Fig. 61F).

With respect to certain commonly seen adverse effects, there are multiple areas for which the inclusion of silk fibroin into HA-based dermal fillers may result in better product performance than current commercially available filler products. The low levels of irritation, bruising, and inflammation demonstrated by the AS-V1 hydrogel are expected to correlate to low levels of immediate and early post-injection adverse effects, such as pain, hypersensitivity, swelling, erythema, and necrosis. Further, lesion/nodule formation has been observed with some filler products, potentially as a result of a high degree of crosslinking or of using multiple sizes (molecular weights) of HA, such as occurs in the VyCross™ technologies. This can potentially be avoided with the silk-containing hydrogels described herein as a single-sized HA is used, and MoD can be easily modulated. Finally, the results indicate that the incorporation of silk protein in the dermal filler may also help avoid the undesired Tyndall effect that often occurs with other dermal filler products.

AS-V1 demonstrated a good profile across all ISO 10993 tests and demonstrated no cytotoxicity, sensitization, irritation, pyrogenicity, genotoxicity (Ames and MLA), intermediate-term local tissue inflammatory responses, or acute or subchronic systemic toxicity was observed with this product.

The ISO 10993 testing and further safety and efficacy results showed that AS-V1 performs equivalently to or better than the current market leader, Juvéderm® Ultra Plus XC, for all aspects tested to date. Further, the tests described above demonstrated that the silk-HA gel incorporated into the skin's collagen matrix more smoothly than did Juvéderm® Ultra Plus XC. At present, these results have been confirmed with 6 months post-injection data using the guinea pig model.

Example 35. Exemplary Silk-Hyaluronic Acid Tissue Fillers

HA and silk were mixed with PEGDE at the initial concentration of 90 - 140 mg/ml of total HA and silk at HA to silk ratio of 95:5 in 0.1 - 1.0 N sodium hydroxide solution. The molecular weight of HA is 850 kDa. The molecular weight of silk is Low-MW (MW < 28 kDa). For Product 1, the crosslinking reaction was carried out at 55 °C for 75 minutes. For Product 2 and 3, the crosslinking reaction was carried out at ambient temperature (20 °C) for 8-24 hours. After crosslinking, the hydrogel was neutralized and diluted to 40 - 56 mg/ml and dialyzed against 1x PBS for 3-4 days.

0.3% w/w lidocaine hydrochloride was added to dialyzed hydrogel. The final concentration of total HA and silk in the product was further diluted to 15 - 28 mg/ml (Table 31). More specifically, the following table is the current nominal settings for Product 1 and Product 2 and 3 (not design freeze)

Table 31

Formulation Product	Formulation #	Initial Conc. Total Silk + HA (mg/mL)	NaOH Conc. @ Crosslinking (N)	Silk to HA Ratio	Reaction temp. (°C)	Reaction Time	Final Conc. Total Silk + HA (mg/mL)
Product 3 (Deep)	S02-011019-01	140	0.25	5:95	20	8 hrs	24
Product 2 (Superficial)	S02-011019-03	90	0.25	5:95	20	15 hrs	20
Product 1 (NLF)	n/a	140	0.10	5:95	55	75 mins	26

In some embodiments, a deep product is indicated for deep (subcutaneous and/or supraperiosteal) injection, or tissue spacer applications. In some embodiments, the injection area maintains an improved appearance over baseline over a 12-month period. In some embodiments, the product is a reversible product, and the product can be dissolved with hyaluronidase.

In some embodiments, a superficial product is indicated for superficial injection. In some embodiments, the injection area maintains an improved appearance over baseline over a 12-month period. In some embodiments, the product is a reversible product, and the product can be dissolved with hyaluronidase.

Example 36. Preparation of Powder of Silk Fibroin Protein Fragments (SPF Powder)

Example 36a. Freeze Drying Process

Each of the 650 mL of aqueous solution of low-MW and mid-MW silk fibroin protein fragments as prepared above was added to a 1L round bottom glass bottle. The two bottles loaded with silk solutions were placed inside a freezer and were allowed stay inside the freezer overnight to provide fully frozen silk solutions. The two bottles containing frozen silk solutions were removed from the freezer. The bottles were left open and the openings were covered with Kimwipe paper tissues and were placed inside a lyophilizer. The pressure inside the lyophilizer is reduced to 0.02 mbar. The collector temperature was set at -65 °C. After 24 hours of lyophilization,

the two bottles were removed from the lyophilizer and were immediately cap to avoid the contacting the dried silk solid with moisture. The coarse powders immediately from the lyophilization were grinded with a mortar and pestle to produce fine powders of silk fibroin protein fragments with even side distribution. The further grinding/processing may be performed to produce silk solid particle with desired particle size.

The coarse solids of low-MW silk was very easy to break down using the mortar and pestle, resulting in a very fine powder. As it became smaller, the lyophilized silk revealed a lamellar-looking appearance (approximately a couple of millimeters in length and width, but extremely thin, almost see-through). These small particles are somewhat similar to mica, in the sense that they are very thin sheets that shimmer in the light (See Figs. 66A-66C).

As the solid silk were ground more and the particle size was reduced, the powder lost its shimmer. Based on the appearance and the way it tends to fly at the slightest air movement, the particle size can be between a few microns and a few hundred microns.

The solids of mid-MW silk did not crumble immediately upon grinding (as was the case for the low-MW solid silk). Other silk drying methods that could be employed include, but are not limited to, spray drying, polar drying, and thin film evaporation.

Example 36b. Thin Film Evaporation Process

Aqueous solutions of Low-MW or mid-MW silk fibroin protein fragments as prepared herein were placed inside a thin film evaporator. Water was continuously removed from silk solutions inside the thin film evaporator under reduced pressure, using gentle heating, resulting in a solid of variable particle size. The particle size can be adjusted by varying the process parameters, such as, but not limited to pressure, temperature, rotational speed of the cylinder, thickness of the liquid film in the evaporator.

Example 36c. Microparticles Prepared by Aqueous Solution Precipitation Process

Salt-out Method: A 1.0 M phosphate buffer solution was prepared and the pH value was adjusted to 8. To a gently stirring silk solution of 5.0 mg/ml concentration, phosphate buffer was added in a 1:5 ratio (v/v). Samples were reacted for 5 minutes

and then were placed inside a refrigerator to promote the precipitation of silk particles. The resulting silk solid suspension was then centrifuged to collect solid particles. The silk particles were washed three times with deionized water and dried to give solid particles of silk fibroin protein fragments (SPF powder).

PVA-assisted method: A 3.0 wt. % stock silk solution was mixed with a 5.0 wt. % solution of polyvinyl alcohol (PVA) in a 1:4 ratio (v/v). The resulting solution mixture was stirred gently for 2 hours. The solution mixture was then sonicated followed by casting to a substrate to allow formation of film. The film was reconstituted in minimal amount of D.I. water and centrifuged. The supernatant was removed and additional D.I. water was added. This process was repeated two times. After two washes, the liquid was removed from the flask to provide wet silk microparticles. Then a small volume of methanol was added to the wet microparticles in the flask (the methanol annealing). The particle suspension inside the flask was swirled. The particle suspension was then poured over a large cloth filter to isolate the microparticles (See Fig. 68).

Example 37: Exemplary Silk-Hyaluronic Acid Compositions and Methods for Making Thereof

SMA-002 procedure:

1. PEGDE was added to a clean beaker.

2. At room temperature, NaOH solution (0.25 N), same volume of silk solution and NaOH solution (0.5 N) were added to the beaker and mixed with a spatula for 30 seconds.

3. HA fibers was added to the mix can.

4. The Silk/NaOH solution prepared in step 2 was added to the mix can containing the HA fibers and stirred at 20 °C for 1 h

5. The mixture was left at 20 °C for 23 h.

6. Suitable amount of HCl and PBS (1x) solution was added to the mix can to neutralize and dilute the crosslinked gel. The mixture was stirred at 4 °C for 3 h then left at 4 °C overnight.

7. The diluted gel was stirred at 4 °C for 1 h then loaded to dialysis tubes and dialysis with PBS (1x) at room temperature for 3 days.

8. Post-dialyzed gel was transferred to the mix can. Suitable amount of lidocaine HCl/PBS solution was added to the mix can to dilute the gel to 20 mg/mL. NaOH solution was used to adjust the pH.

9. The gel with lidocaine HCl added was stirred at 4 °C for 1 h then left at 4 °C overnight.

10. The gel was ready for syringe filling.

Without wishing to be bound by any particular theory, it is believed that SMA-002 procedure generates a smooth IF curve due to: use of a higher concentration of NaOH (0.25 N) than the SMA-001 procedure (0.1 N); use of a lower initial HA concentration (75 mg/mL) than SMA-001 procedure (140 mg/mL). As a result, and without wishing to be bound by any particular theory, it is believed that the HA dissolves faster in the SMA-002 procedure and generates a homogeneous solution at the end of step 4. Compared to SMA-001 procedure, SMA-002 procedure applied either longer time or higher speed of mixing at both step 4 and step 6.

SMA-002 hydrogel with SMPs (silk microparticles) procedure:

The procedure is the same the SMA-002 manufactured with silk solution except step 8:

8. Post-dialyzed gel was transferred to the mix can. Suitable amount of lidocaine HCl/Silk microparticles/PBS solution was added to the mix can to dilute the gel to 20 mg/mL. NaOH solution was used to adjust the pH.

Note: The size of the SMPs is 30-50 μm and the final concentration of the SMPs in the hydrogel is 1 mg/mL.

Exemplary SMA-002 (superficial filler)

Sample ID	Initial conc. silk+HA (mg/ml)	Silk ratio (%)	Cross-linking time (hrs.)	Final crosslinked silk+HA (mg/ml)	Free silk added (mg/ml)	G' 5 Hz avg. (Pa)	IF 30G mean avg. (N)	pH avg.		MoD (%)	T% 500 nm
T1	80	5	24	20		93.31	25.94			11.34	
						118.93	21.93				
T2	90	20	24	20		65.28	18.92			13.01	
						82.49	18.37	7.33			74.58
T3	90	20	24	20	1	63.57	20.10			13.01	
						86.62	17.70	7.34			68.94
T4	90	20	24	20	4	69.73	18.12			13.01	
						96.25	20.26	7.25			66.41
T5	90	20	24	20	8	98.24	16.89			13.01	
						71.05	15.81	7.25			63.02
T6	90	20	24	20	Silk Microparticles (30-50 µm, 1 mg/mL)	71.18	16.50			13.01	
						80.15	14.81	7.29			17.57
C1	80	0	24	20						10.51	
						106.47	20.90				

T1-1	75	5	24	20		69.90	14.31				
						64.91	18.17				78.43
T1-2	70	5	24	20		54.36	12.01				
						54.41	14.38				
C1-1	75	0	24	20		72.50	14.20				
						60.89	20.25				78.32
C1-2	70	0	24	20		64.22	12.33				
						59.22	16.67				
S02-090221-04	120	40	24	20		133.3	26.73				
T7						89.13	18.19				58.58
T8	100	50	24	20	0	25.93	11.62	7.35			66.4
T9					2	24.47	13.75	7.28			67.6
T10					6	18.47	11.38	7.24			61.08
T11					10	22.32	11.83	7.22			34.49

T12				20		2	72.39	27.52	7.29		76.11
T13				20		6	22.33	11.40	7.34		65.71

Exemplary SMA-003 (deep)

Sample ID	Initial conc. silk+HA (mg/ml)	Silk ratio (%)	Cross-linking time (hrs.)	Final conc. silk+HA (mg/ml)	sterilized?	G' 5 Hz avg. (Pa)	IF 27G mean avg. (N)	IF 30G mean avg. (N)	
S02-250221-01	120	20	24	24		382.45	16.70	55.20	
					sterilized	310.36	16.96	44.60	
S02-250221-02	130	20	24	24		509.23	17.41	71.01	
S02-260221-01	140	5	8	24		343.96	16.49	41.52	
					sterilized	235.93	16.68	28.77	
S02-260221-02	150	5	8	24		336.34	14.55	45.96	
					sterilized	231.16	16.65	30.15	
S02-100321-01	120	5	24	24		421.61	18.96	45.35	
					sterilized	343.17	18.96	55.03	
S02-100321-02	110	5	24	24		282.93	17.28	40.88	
					sterilized	242.69	19.39	51.36	

S02-100321-03	100	5	24	24		201.94	14.86	33.90		
					sterilized	167.85	17.69	42.23		

Gen 2 process for Gen 1 gel

Sample ID	Initial conc. silk+HA (mg/ml)	Silk ratio (%)	Cross-linking time (hrs.)	Final crosslinked silk+HA (mg/ml)	Free silk added (mg/ml)	G' 5 Hz avg. (Pa)	IF 30G peak avg. (N)	IF 30G mean avg. (N)	pH avg.	MOD
S02-170321-01	70	5	24	26		116.96	26.30	24.19		
S02-170321-02	75	5	24	26		121.88	35.22	31.96		
S02-170321-03	80	5	24	26		146.55	43.18	38.50		
S02-170321-04	90	5	24	26		159.45	48.56	40.81		
S01-280421-01	80	5	24	26		178.95	24.56	23.89		
						176.35	33.59	31.75	7.51	11.9
S01-120521-01	75	5	24	26		147.80	19.74	17.17		

Example 38: The Rheological Properties of SMA Dermal Fillers

The indication of dermal filler product was primarily base on their rheological properties. The product performance, for example the ability to resist deformation, the ability to flow, the ability to hold its integrity, etc. were also assessed by each corresponding rheological parameter of the product. Leveraging the outstanding features of the silk protein, SMA's technology is able to incorporate silk and HA into a hybrid dermal filler platform and deliver a variety of prototypes with rheological properties covering a broad range. More importantly, some properties can be potentially decoupled by varying the hydrogel formulations and processes. The following figures summarized the rheological properties from more than 90 different prototypes and provided an overview of the capability of this unique technology platform and the diversity it can offer. Two different silk molecules included in these hydrogel prototypes were evaluated and summarized in this report.

The storage modulus (G') is a measure of elasticity, or the ability to store energy. For a typical dermal filler product, the injection force (IF) is generally proportional to the G'. SMA hydrogels have a wide range of G' (30 – 300 Pa) within a narrow range of IF (10 – 30 N), which is attributed to the silk-containing formulations and processes. The data in Fig. 71 is grouped by needle size, either 30G x ½" (green) or 27G x ½" (red). These data are based on samples filled in glass syringes, rather than the current SMA design that uses COC syringes that can reduce the IF by ~50%. Fig. 71: SMA Dermal Filler Injection Force (IF) vs. Storage Modulus (G').

The loss modulus (G") is a measure of viscosity, or the ability to lose energy. Similar to the G', the G" of different SMA hydrogel formulations vary in a broad range of 30 – 300 Pa in a narrow injection force range of 10 – 30 N. The data in Fig. 72 is grouped by needle size, either 30G x ½" (green) or 27G x ½" (red). These data are based on samples stored in glass syringes, rather than the current SMA design that uses COC syringes that can reduce IF by ~50%. Fig. 72: SMA Dermal Filler Injection Force (IF) vs. Loss Modulus (G").

The Tan(δ) is defined as the ratio of G"/G' and is the measure of dampening properties. In a given range of G', for example 100 – 150 Pa, different SMA hydrogel formulations showed a broad range of Tan(δ) from 0.15 – 0.55. In a certain range of

Tan(δ), for example 0.5 – 0.6, the G' of SMA hydrogels could be as low as 50 Pa and as high as 350 Pa. The data in Fig. 73 demonstrates the decoupled nature of G' and Tan(δ) in SMA hydrogels. Fig. 73: SMA Dermal Filler Storage Modulus (G') vs. Tan(δ).

The complex viscosity (η*) is a measure of resistance to flow. Typically, the higher the viscosity, the higher the injection force. Due the silk-containing formulation, the injection force could be maintained at an acceptable level even when the complex viscosities of some SMA hydrogels are higher than 10 Pa·s. The data in Fig. 74 is grouped by needle size, either 30G x ½" (green) or 27G x ½" (red). These data are based on samples filled in glass syringes, rather than the current SMA design that uses COC syringes that can reduce the IF by ~50%. Fig. 74: SMA Dermal Filler Injection Force (IF) vs. Complex Viscosity (η*).

The G" usually changes as a function of G'. Attributed to the silk in the hydrogel formulations, the G' of SMA hydrogels could be as high as greater than 350 Pa while the G" was as low as less than 50 Pa. In some other cases, the G" varies from 30 Pa to 300 Pa at the G' range of 250 – 300 Pa. The data in Fig. 75 demonstrates the decoupled nature of G' and G" in SMA hydrogels. Fig. 75: SMA Dermal Filler Storage Modulus (G') vs. Loss Modulus (G").

Many dermal filler manufacturers control the G' by adjusting the HA concentration in the product. Simply dilute the HA hydrogel can reduce the G'. SMA's Silk-HA platform made the product G' independent to the total silk and HA concentration. For each given concentration, the G' can be as low as 50 Pa or as high as 350 Pa. SMA is able to develop a dermal filler product with high G' at low concentration or with low G' at relatively high concentration. Fig. 76: SMA Dermal Filler Storage Modulus (G') vs. Silk + HA Concentration.

The SMA's unique dermal filler platform incorporates silk technology into dermal filler product, offers a versatile tool to design and develop new dermal filler products with desired mechanical and rheological properties, and greatly expands the product portfolio.

References:

1. Goldberg DJ. Breakthroughs in US dermal fillers for facial soft-tissue augmentation. J Cosmet Laser Ther. 2009;11(4):240-7.

2. Bray D, Hopkins C, Roberts DN. A review of dermal fillers in facial plastic surgery. Curr Opin Otolaryngol Head Neck Surg. 2010;18(4):295-302.

3. Liu MH, Beynet DP, Gharavi NM. Overview of Deep Dermal Fillers. Facial Plast Surg. 2019;35(3):224-9.

4. Kopera D, Ivezic-Schoenfeld Z, Federspiel IG, Grablowitz D, Gehl B, Prinz M. Treatment of facial lipoatrophy, morphological asymmetry, or debilitating scars with the hyaluronic acid dermal filler Princess((R)) FILLER. Clin Cosmet Investig Dermatol. 2018;11:621-8.

5. Cheng LY, Sun XM, Tang MY, Jin R, Cui WG, Zhang YG. An update review on recent skin fillers. Plastic and Aesthetic Research. 2016;3:92-9.

6. Fallacara A, Manfredini S, Durini E, Vertuani S. Hyaluronic Acid Fillers in Soft Tissue Regeneration. Facial Plast Surg. 2017;33(1):87-96.

7. Lee DY, Cheon C, Son S, Kim YZ, Kim JT, Jang JW, et al. Influence of Molecular Weight on Swelling and Elastic Modulus of Hyaluronic Acid Dermal Fillers. Polymer(Korea). 2015;39(6):976-80.

8. Kablik J, Monheit GD, Yu L, Chang G, Gershkovich J. Comparative physical properties of hyaluronic acid dermal fillers. Dermatol Surg. 2009;35 Suppl 1:302-12.

9. Perez-Perez L, Garcia-Gavin J, Wortsman X, Santos-Briz A. Delayed Adverse Subcutaneous Reaction to a New Family of Hyaluronic Acid Dermal Fillers With Clinical, Ultrasound, and Histologic Correlation. Dermatol Surg. 2017;43(4):605-8.

10. Altman GHD, Frank. Jakuba, Caroline. Calabro, Tara. Horan, Rebecca L. Chen, Jingsong. Lu, Helen. Richmond, John. Kaplan, David L. Silk-based biomaterials. Biomaterials 2003;24:401-16.

11. Chen J, Altman GH, Karageorgiou V, Horan R, Collette A, Volloch V, et al. Human bone marrow stromal cell and ligament fibroblast responses on RGD-modified silk fibers. J Biomed Mater Res A. 2003;67(2):559-70.

12. Li ABK, Jonathan A. Guziewicz, Nicholas A. Omenetto, Fiorenzo G. Kaplan, David L. . Silk-based stabilization of biomacromolecules. 2015.

13. Chen FM, Liu X. Advancing biomaterials of human origin for tissue engineering. Prog Polym Sci. 2016;53:86-168.

14. Rahme K, Dagher N. Chemistry Routes for Copolymer Synthesis Containing PEG for Targeting, Imaging, and Drug Delivery Purposes. Pharmaceutics. 2019;11(7).

15. Standardization IOf. ISO 10993-6:2016. Biological evaluation of medical devices — Part 6: Tests for local effects after implantation. Geneva, Switzerland: ISO; 2016. p. 29.

16. Standardization IOf. ISO 10993-1:2018. Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process. Geneva, Switzerland: ISO; 2018. p. 41.

17. Administration USFaD. Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process. 2016.

18. Raia NR, Partlow BP, McGill M, Kimmerling EP, Ghezzi CE, Kaplan DL. Enzymatically crosslinked silk-hyaluronic acid hydrogels. Biomaterials. 2017;131:58-67.

19. Yan S, Zhang Q, Wang J, Liu Y, Lu S, Li M, et al. Silk fibroin/chondroitin sulfate/hyaluronic acid ternary scaffolds for dermal tissue reconstruction. Acta Biomater. 2013;9(6):6771-82.

20. Yan S, Wang Q, Tariq Z, You R, Li X, Li M, et al. Facile preparation of bioactive silk fibroin/hyaluronic acid hydrogels. Int J Biol Macromol. 2018;118(Pt A):775-82.

21. Park SH, Cho H, Gil ES, Mandal BB, Min BH, Kaplan DL. Silk-fibrin/hyaluronic acid composite gels for nucleus pulposus tissue regeneration. Tissue Eng Part A. 2011;17(23-24):2999-3009.

22. Li AB, Kluge JA, Guziewicz NA, Omenetto FG, et al. Silk-based stabilization of biomacromolecules. J Control Release 2015; 219:416-430.

23. Altman GH, Diaz F, Jakuba C, Calabro T, et al. Silk-based biomaterials. Biomaterials 2003; 24:401-16.

CLAIMS

1. A biocompatible composition comprising silk fibroin or silk fibroin fragments, hyaluronic acid (HA), and polyethylene glycol (PEG) and/or polypropylene glycol (PPG),

wherein a portion of the HA is modified or crosslinked by one or more linker moieties comprising one or more of polyethylene glycol (PEG), polypropylene glycol (PPG), and a secondary alcohol, and

wherein a portion of the silk fibroin or silk fibroin fragments are free and/or uncrosslinked.

2. The composition of claim 1, wherein a portion of the silk fibroin or silk fibroin fragments are modified or crosslinked.

3. The composition of any one of claims 1 or 2, wherein a portion of the silk fibroin or silk fibroin fragments are crosslinked to HA.

4. The composition of any one of claims 1 to 3, wherein a portion of the silk fibroin or silk fibroin fragments are crosslinked to silk fibroin or silk fibroin fragments.

5. The tissue filler of any one of claims 1 to 4, wherein the silk fibroin or silk fibroin fragments are substantially devoid of sericin.

6. The composition of any one of claims 1 to 5, wherein a portion of silk fibroin or silk fibroin fragments have an average weight average molecular weight selected from low molecular weight, medium molecular weight, and high molecular weight.

7. The composition of any one of claims 1 to 6, wherein the silk fibroin or silk fibroin fragments have a polydispersity of between 1 and about 5.0.

8. The composition of any one of claims 1 to 6, wherein the silk fibroin or silk fibroin fragments have a polydispersity of between about 1.5 and about 3.0.

9. The composition of any one of claims 1 to 8, wherein the composition has a degree of modification (MoD) of about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15%.

10. The composition of any one of claims 1 to 9, wherein modification or cross-linking is obtained using as cross-linker a monoepoxy- or diepoxy-PEG, a monoglycidyl-, diglycidyl-, or polyglycidyl-PEG, a monoglycidyl- or diglycidyl-PEG, a monoepoxy- or diepoxy-PPG, a monoglycidyl-, diglycidyl-, or polyglycidyl-PPG, a monoglycidyl- or diglycidyl-PPG, or any combinations thereof.

11. The composition of any one of claims 1 to 10, further comprising lidocaine.

12. The composition of any one of claims 1 to 11, wherein the composition is a gel or a hydrogel.

13. The composition of any one of claims 1 to 12, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, about 30 mg/mL, about 31 mg/mL, about 32 mg/mL, about 33 mg/mL, about 34 mg/mL, about 35 mg/mL, about 36 mg/mL, about 37 mg/mL, about 38 mg/mL, about 39 mg/mL, or about 40 mg/mL.

14. The composition of any one of claims 1 to 13, wherein the ratio of HA to silk fibroin or silk fibroin fragments in the composition is about 91/9, about 92/8, about 93/7, about 94/6, about 95/5, about 96/4, about 97/3, about 18/12, about 27/3, about 29.4/0.6, about 99/1, about 92.5/7.5, about 90/10, about 80/20, about 70/30, about 60/40, or about 50/50.

15. The composition of any one of claims 1 to 13, wherein the ratio of HA to silk fibroin or silk fibroin fragments in the composition is about 50/50, about 51/49, about 52/48, about 53/47, about 54/46, about 55/45, about 56/44, about 57/43, about 58/42, about 59/41, about 60/40, about 61/39, about 62/38, about 63/37, about 64/36, about 65/35, about 66/34, about 67/33, about 68/32, about 69/31, about 70/30, about 71/29, about 72/28, about 73/27, about 74/26, about 75/25, about 76/24, about 77/23, about 78/22, about 79/21, about 80/20, about 81/19, about 82/18, about 83/17, about 84/16, about 85/15, about 86/14, about 87/13, about 88/12, about 89/11, about 90/10, about 91/9, about 92/8, about 93/7, about 94/6, about 95/5, about 96/4, about 97/3, about 98/2, or about 99/1.

16. The composition of any one of claims 1 to 17, wherein the total concentration of free and/or uncrosslinked silk fibroin or silk fibroin fragments in the composition is about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, or about 8 mg/mL.

17. The composition of any one of claims 1 to 16, wherein a portion of the free and/or uncrosslinked silk fibroin or silk fibroin fragments comprises silk microparticles having a median particle size ranging from 1.0 μm to 50.0 μm, from 1.0 μm to 25.0 μm, from 1.0 μm to 10.0 μm, from 30.0 μm to 50.0 μm, from 35.0 μm to 45.0 μm, from 35.0 μm to 55.0 μm, or from 25.0 μm to 45.0 μm.

18. The composition of any one of claims 1 to 17, wherein the composition is injectable through 30G or 27G needles, and having an injection force through a 30G needle between about 10 N and about 80 N.

19. The composition of any one of claims 1 to 17, wherein the composition is injectable through a 30G needle with an injection force of about 1 N, about 2 N, about 3 N, about 4 N, about 5 N, about 6 N, about 7 N, about 8 N, about 9 N, about 10 N, about 11 N, about 12 N, about 13 N, about 14 N, about 15 N, about 16 N, about 17 N, about 18 N, about 19 N, about 20 N, about 21 N, about 22 N, about 23 N, about 24 N, about 25 N, about 26 N, about 27 N, about 28 N, about 29 N, about 30 N, about 31 N, about 32 N, about 33 N, about 34 N, about 35 N, about 36 N, about 37 N, about 38 N,

about 39 N, about 40 N, about 41 N, about 42 N, about 43 N, about 44 N, about 45 N, about 46 N, about 47 N, about 48 N, about 49 N, about 50 N, about 51 N, about 52 N, about 53 N, about 54 N, about 55 N, about 56 N, about 57 N, about 58 N, about 59 N, about 60 N, about 61 N, about 62 N, about 63 N, about 64 N, about 65 N, about 66 N, about 67 N, about 68 N, about 69 N, about 70 N, about 71 N, about 72 N, about 73 N, about 74 N, about 75 N, about 76 N, about 77 N, about 78 N, about 79 N, about 80 N, about 81 N, about 82 N, about 83 N, about 84 N, about 85 N, about 86 N, about 87 N, about 88 N, about 89 N, about 90 N, about 91 N, about 92 N, about 93 N, about 94 N, about 95 N, about 96 N, about 97 N, about 98 N, about 99 N, or about 100 N.

20. The composition of any one of claims 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 5 Pa to about 500 Pa, from about 15 Pa to about 50 Pa, from about 50 Pa to about 100 Pa, from about 100 Pa to about 200 Pa, from about 200 Pa to about 300 Pa, from about 300 Pa to about 350 Pa, from about 350 Pa to about 400 Pa, from about 400 Pa to about 450 Pa, or from about 450 Pa to about 500 Pa.

21. The composition of any one of claims 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 5 Pa to about 500 Pa, from about 15 Pa to about 50 Pa, from about 50 Pa to about 100 Pa, from about 100 Pa to about 200 Pa, from about 200 Pa to about 300 Pa, from about 300 Pa to about 350 Pa, from about 350 Pa to about 400 Pa, from about 400 Pa to about 450 Pa, or from about 450 Pa to about 500 Pa.

22. The composition of any one of claims 1 to 19, wherein the composition has Tan(δ) (G"/G') between 0 and about 0.2, between about 0.2 and about 0.4, between about 0.4 and about 0.6, between about 0.6 and about 0.8, between about 0.8 and about 1.0, or between about 1.0 and about 1.2.

23. The composition of any one of claims 1 to 19, wherein the composition has a complex viscosity (η*) between 0 and about 5 Pa·s, between about 5 Pa·s and about 10 Pa·s, between about 10 Pa·s and about 15 Pa·s, between about 15 Pa·s and about 20 Pa·s, or between about 20 Pa·s and about 25 Pa·s.

24. The composition of any one of claims 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 50 Pa to about 400 Pa, and an injection force (27G) between about 10 N and about 70 N.

25. The composition of any one of claims 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 10 Pa to about 350 Pa, and an injection force (30G) between about 5 N and about 70 N.

26. The composition of any one of claims 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 25 Pa to about 350 Pa, and an injection force (27G) between about 10 N and about 70 N.

27. The composition of any one of claims 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 10 Pa to about 400 Pa, and an injection force (30G) between about 10 N and about 70 N.

28. The composition of any one of claims 1 to 19, wherein the composition has a storage modulus (*G'*) of from about 25 Pa to about 400 Pa, and Tan(δ) (G"/G') between 0 and about 1.2.

29. The composition of any one of claims 1 to 19, wherein the composition has a complex viscosity (η*) between about 2.5 and about 25 Pa·s, and an injection force (27G) between about 10 N and about 70 N.

30. The composition of any one of claims 1 to 19, wherein the composition has a complex viscosity (η*) between about 1 and about 20 Pa·s, and an injection force (30G) between about 5 N and about 75 N.

31. The composition of any one of claims 1 to 19, wherein the composition has a loss modulus (*G"*) of from about 5 Pa to about 400 Pa, and a storage modulus (*G'*) of from about 1 Pa to about 400 Pa.

32. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 15 mg/mL, wherein the composition has a storage modulus (G') of from about 1 Pa to about 350 Pa.

33. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 18 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 350 Pa.

34. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 20 mg/mL, wherein the composition has a storage modulus (G') of from about 20 Pa to about 400 Pa.

35. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 22 mg/mL, wherein the composition has a storage modulus (G') of from about 25 Pa to about 200 Pa.

36. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 24 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 350 Pa.

37. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is about 26 mg/mL, wherein the composition has a storage modulus (G') of from about 50 Pa to about 400 Pa.

38. The composition of any one of claims 1 to 19, wherein the total concentration of HA and silk fibroin or silk fibroin fragments in the composition is

about 28 mg/mL, wherein the composition has a storage modulus (G') of from about 150 Pa to about 300 Pa.

39. The composition of any one of claims 1 to 38, further comprising an imaging agent.

40. The composition of claim 39, wherein the imaging agent is selected from iodine, DOPA, and imaging nanoparticles.

41. The composition of claim 39, wherein the imaging agent is selected from a paramagnetic imaging agent and a superparamagnetic imaging agent.

42. The composition of claim 39, wherein the imaging agent is selected from NP-based magnetic resonance imaging (MRI) contrast agents, positron emission tomography (PET)/single photon emission computed tomography (SPECT) imaging agents, ultrasonically active particles, and optically active (e.g., luminescent, fluorescent, infrared) particles.

43. The composition of claim 39, wherein the imaging agent is a SPECT imaging agent, a PET imaging agent, an optical imaging agent, an MRI or MRS imaging agent, an ultrasound imaging agent, a multimodal imaging agent, an X-ray imaging agent, or a CT imaging agent.

44. A method of treatment or prevention of a disorder, disease, or condition in a subject in need thereof, the method comprising administering to the subject a composition of any one of claims 1 to 43.

45. The method of claim 44, wherein the condition is a skin condition selected from skin dehydration, lack of skin elasticity, skin roughness, lack of skin tautness, a skin stretch line, a skin stretch mark, skin paleness, a dermal divot, a sunken cheek, a thin lip, a retro-orbital defect, a facial fold, and a wrinkle.

46. The method of claim 44 or claim 45, wherein the composition is administered into a dermal region of the subject.

47. The method of any of claims 44 to 46, wherein the method is an augmentation, a reconstruction, treating a disease, treating a disorder, correcting a defect or imperfection of a body part, region or area.

48. The method of any one of claims 44 to 47, wherein the method is a facial augmentation, a facial reconstruction, treating a facial disease, treating a facial disorder, treating a facial defect, or treating a facial imperfection.

49. The method of any one of claims 44 to 48, wherein the method comprises deep subcutaneous and/or deep supraperiosteal administration.

50. The method of any one of claims 44 to 49, wherein the method comprises cheek augmentation, lip augmentation, dermal implantation, correction of perioral rhytids, and/or correction of nasolabial fold.

51. The method of claim 44, wherein the composition is injected into a tissue.

52. The method of claim 51, wherein the tissue is associated with the disorder, disease, or condition.

53. The method of claim 51 or claim 52, wherein the composition is administered into a wall of the tissue.

54. The method of any one of claims 51 to 53, wherein the tissue comprises a portion of a wall of an internal organ.

55. The method of any one of claims 51 to 54, wherein administration of the composition causes bulking of the tissue.

56. The method of claim 55, wherein the disorder, disease, or condition is treated or prevented by the bulking of the tissue.

57. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is selected from urinary incontinence, gastroesophageal reflux disease (GERD), vesicoureteral reflux, fecal incontinence, dental tissue defects, vocal cord tissue defects, larynx defects, and other non-dermal soft tissue defects.

58. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is urinary incontinence.

59. The method of claim 58, wherein the urinary incontinence is stress incontinence, intrinsic sphincter deficiency (ISD), stress incontinence, intrinsic sphincter deficiency (ISD), urge incontinence, overflow incontinence, or enuresis.

60. The method of claim 58 or 59, wherein the tissue is a portion of the urethra or the urethral sphincter.

61. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is gastroesophageal reflux disease (GERD).

62. The method of claim 61, wherein the tissue is a portion of the lower esophageal sphincter or the diaphragm.

63. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is vesicoureteral reflux.

64. The method of claim 63, wherein the tissue is a portion of the urethral sphincter.

65. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is fecal incontinence.

66. The method of claim 65, wherein the tissue is a portion of the rectum.

67. The method of claim 65 or claim 66, wherein the composition is administered into a region of a rectal wall.

68. The method of claim 67, wherein the region of the rectal wall is in the vicinity of the anal sphincter.

69. The method of claim 68, wherein the composition is administered into the internal sphincter.

70. The method of any one of claims 51 to 56, wherein the disorder, disease, or condition is a vocal cord tissue defect or larynx defect.

71. The method of claim 70, wherein the vocal cord tissue defect or larynx defect is selected from glottic incompetence, unilateral vocal cord paralysis, bilateral vocal cord paralysis, paralytic dysphonia, nonparalytic dysphonia, spasmodic dysphonia, incomplete paralysis of the vocal cord ("paresis"), generally weakened vocal cords, scarring of the vocal cords, and any combination thereof.

72. The method of claim 70 or claim 71, wherein the tissue is a portion of a vocal cord or larynx.

73. The method of claim 44, further comprising administering an anticancer treatment, wherein the disorder, disease, or condition is selected from cervical cancer, rectal cancer, pulmonary tumors, mediastinum lymphoma, breast cancer, uterine cancer, pancreatic cancer, head and neck cancers, lung cancer, liver cancer, vaginal cancers, benign prostatic hyperplasia (BPH), menorrhagia, uterine fibroids, prostate adenocarcinomas, pancreatic cancer, head and neck cancer, lung cancer, liver cancer, and vaginal cancer.

74. The method of claim 73, wherein the anticancer treatment comprises administering one or more of radiation therapy (RT), cryotherapy, drug treatment, heat and/or thermal ablation, radiofrequency and/or microwave, or cryotherapy.

75. The method of claim 74, wherein the radiation therapy comprises one or more of external beam radiotherapy, 3D conformal modulated radiotherapy, intensity modulated radiotherapy, interstitial prostate brachytherapy, interstitial prostate brachytherapy using permanent seeds, interstitial prostate brachytherapy using temporary seeds, interstitial prostate brachytherapy using high dose rate remote after loading, external radiation therapy using gamma irradiation, high energy photon beam therapy, proton beam therapy, neutron beam therapy, heavy particle beam therapy, brachytherapy, thermal radiation, or any combination thereof.

76. The method of any one of claims 73 to 75, wherein the composition is administered between a first tissue and a second tissue, or into a space or virtual space between a first tissue and a second tissue.

77. The method of claim 76, wherein upon administration of the composition the first tissue is displaced relative to the second tissue.

78. The method of claim 76 or claim 77, wherein the space or virtual space is Denonvilliers' space or a space or virtual space adjacent to Denonvilliers' fascia.

79. The method of any one of claims 76 to 78, wherein the first tissue receives the anticancer treatment after administration of the composition.

80. The method of claim 79, wherein the first tissue receives a substantially similar dose of anticancer treatment compared to the anticancer treatment dose the first tissue would receive in the absence of the composition.

81. The method of any one of claims 76 to 80, wherein the second tissue receives the anticancer treatment.

82. The method of claim 81, wherein the second tissue receives a lower anticancer treatment dose compared to the anticancer treatment dose the second tissue would receive in the absence of the composition.

83. The method of any one of claims 76 to 82, wherein the second tissue receives substantially no anticancer treatment dose.

84. The method of any of claims 76 to 83, wherein the first tissue and the second tissue each independently comprises a tumor tissue, a group of cells, a group of cells and interstitial matter, an organ, a portion of an organ, or an anatomical portion of a body.

85. The method of any one of claims 76 to 83, wherein the first tissue comprises a tumor tissue, and the second tissue comprises an organ.

86. The method of any one of claims 76 to 83, wherein the first tissue comprises an organ, and the second tissue comprises an organ.

86. The method of claim 86, wherein the first tissue comprises a portion of prostate and the second tissue comprises a portion of rectum.

87. The method of any one of claims 44 to 86, wherein the method further comprises administering an anesthetic.

88. The method of any of claims 44 to 87, further comprising biodegradation of the composition in the subject.

89. The method of claim 88, wherein the biodegradation is hydrolysis, proteolysis, enzymatic degradation, the action of cells in the body, or a combination thereof.

90. The method of claim 88, wherein the composition is biodegraded by hyaluronidase enzymatic degradation.