choice for patients, as it is long lasting and typically only requires 1-2 treatments with little to no follow up.

Unfortunately, permanent dermal fillers in the field often include a synthetic material that may be permanently left behind in the body, which may carry certain risks such as potential allergic reactions, potential for granulomas and/or foreign body responses.

Alternative, additional, and/or improved dermal fillers, methods and uses thereof, and methods for the production thereof, are desirable.

SUMMARY

Provided herein are dermal fillers comprising decellularized plant or fungal tissue from which cellular materials and nucleic acids of the tissue are removed; as well as methods and uses thereof for cosmetic and/or reconstructive applications. Also provided are methods for preparing such dermal fillers, and dermal filler kits.

In one aspect, the present disclosure refers to a non-resorbable dermal filler comprising decellularized plant or fungal tissue from which cellular materials and nucleic acids of the tissue are removed.

In another aspect, the present disclosure refers to use of the dermal filler as disclosed herein as a soft tissue filler, for reconstructive surgery, or both; preferably for improving cosmetic appearance of a subject in need thereof; preferably for increasing tissue volume, smoothing wrinkles, or both, in a subject in need thereof.

In another aspect, the present disclosure refers to a cosmetic method for improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, in a subject in need thereof, wherein the dermal filler as disclosed herein is to be administered or injected to a region in need thereof for improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, of the subject.

In another aspect, the present disclosure refers to a method for preparing a non-resorbable dermal filler, comprising: providing a plant or fungal tissue; decellularizing and size-reducing the plant or fungal tissue to provide particles from which cellular materials and nucleic acids of the tissue are removed; and optionally, sterilizing the particles; thereby providing the non-resorbable dermal filler.

In another aspect, the present disclosure refers to a dermal filler prepared by the method as disclosed herein; preferably wherein the dermal filler is a dermal filler as disclosed herein; preferably wherein about 0.1% to about 5%, or less, of the particles of the dermal filler have a feret diameter, or a minimum feret diameter, of less than about 20 μm; preferably wherein less than about 0.5% of the particles of the dermal filler have a feret diameter, or a minimum feret diameter, of less than 20 μm; preferably wherein less than about 0.1% to about 5% (such as less than about 0.5%), or less, of the particles of the dermal filler have a size sufficiently small so as to be phagocytosed by a cell or cells of a subject.

In another aspect, the present disclosure refers to a kit comprising any one or more of: a dermal filler as disclosed herein; a hydrogel, matrix, or carrier fluid; PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof; lidocaine or other aesthetic; one or more syringes, or another injection device; a leur-lock fitting or coupler for two or more syringes; instructions for performing a use or method as disclosed herein; one or more decellularization reagents; one or more containers, packages, or vessels; one or more buffers, water, or saline for injection; a reagent or enzyme (e.g. cellulase) for dissolving dermal filler at an undesirable region or location; or any combinations thereof.

As described herein, the present inventors have now developed dermal filler products comprising decellularized plant or fungal tissue. Data provided herein indicates dermal fillers had good biocompatibility, and were substantially non-resorbable. Methods for preparing such dermal filler products are described, which may allow for control over size and various other beneficial properties, including the avoidance of small undesirable particles below 20 μm in size. Preparations are developed having good syringability/injectability through small gauge needles,

as well as preparations compatible with carrier fluids such as hydrogels, and compatible with anesthetics. Sterilization techniques and sterilized products are also described. Particles for dermal filler products are described having desirable shapes and surface area/volume/packing properties to encourage cell invasion/growth and/or to provide good ratios of natural tissue to dermal filler in subjects.

In an example, there is provided herein a non-resorbable dermal filler comprising decellularized plant or fungal tissue from which cellular materials and nucleic acids of the tissue

are removed.

In another example of the above dermal filler, the decellularized plant or fungal tissue may be chitin-based, chitosan-based, lignocellulosic-based, or cellulose-based.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be derived from leafy structure, root, flesh, hypanthium or pulp structures of a plant.

In yet another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be derived from lettuce, carrot, apple, or pear, or any combination thereof.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be homogenized.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be dried, subjected to grinding, and optionally reconstituted or rehydrated.

In yet another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles the size of single structural cells, or smaller.

In another example of any of the above dermal filler or dermal fillers, the dermal filler may further comprise a hydrogel, matrix, or carrier fluid for the decellarized plant or fungal tissue.

In still another example of any of the above dermal filler or dermal fillers, the hydrogel, matrix, or carrier fluid may comprise PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof.

In yet another example of any of the above dermal filler or dermal fillers, the dermal filler

may be dried, or hydrated.

In another example of any of the above dermal filler or dermal fillers, the dermal filler may comprise lidocaine or other anesthetic.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having irregular 3D shapes and/or which are non-spherical, thin, flake-like structures.

In yet another example of any of the above dermal filler or dermal fillers, the flake-like structures may have a thickness of about 0.01 to about 100 μm, for example about 0.1 μm.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size sufficiently large so as not to be phagocytosed by a cell or cells; preferably, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or minimum feret diameter of at least about 20 μm.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or feret diameter within a range of about 20 μm to about 1000 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or maximum feret diameter of less than about 200 μm.

In yet another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or feret diameter distribution within a range of about 20 μm to about 200 μm.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a particle size, diameter, or feret diameter distribution having a peak within about 30 μm to about 100 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellarized plant or fungal tissue may be disassembled into particles having a mean particle size, diameter, or feret diameter within a range of about 30 μm to about 100 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or maximum feret diameter of less than about 300 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or feret diameter distribution within a range of about 100 μm to about 300 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a particle size, diameter, or feret diameter distribution having a peak within about 100 μm to about 300 μm.

In still another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having a mean particle size, diameter, or feret diameter within a range of about 100 μm to about 300 μm.

In yet another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be disassembled into particles having an average projected particle area within a range of about 200 to about 3000 μm^2 , or within a range of about 200 to about 300 μm^2; wherein the decellularized plant or fungal tissue is disassembled into particles having a surface area to volume ratio of about 0.1 to 100 μm^{-1}; wherein the decellularized plant or fungal tissue is disassembled into particles having a packing density of about 4 x 10^5 particles/mL to about 7 x 10^9 particles/mL; or any combinations thereof.

In yet another example of any of the above dermal filler or dermal fillers, the dermal filler or dermal fillers may have a viscosity of less than about 500,000 cp.

In yet another example of any of the above dermal filler or dermal fillers, the dermal filler or dermal fillers may have a viscosity within a range of about 100,000 cp to about 200,000 cp.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be sterilized.

In still another example of any of the above dermal filler or dermal fillers, the dermal filler may be sterilized.

In yet another example of any of the above dermal filler or dermal fillers, the sterilization may be by gamma sterilization.

In yet another example of any of the above dermal filler or derma fillers, the sterilization may be by autoclaving.

In another example of any of the above dermal filler or dermal fillers, the decellularized plant or fungal tissue may be mercerized.

In yet another example of any of the above dermal filler or dermal fillers, the mercerization may be by heating with sodium hydroxide and hydrogen peroxide.

In another example of any of the above dermal filler or dermal fillers, the dermal filler may be formulated for subdermal injection, deep dermal injection, subcutaneous injection (e.g. subcutaneous fat injection), or any combinations thereof.

In still another example of any of the above dermal filler or dermal fillers, the dermal filler may be provided in a syringe or injection device.

In yet another example, there is provided herein a use of any of the dermal filler or dermal fillers as described herein as a soft tissue filler, for reconstructive surgery, or both.

In another example, there is provided herein a use of any of the dermal filler or dermal fillers as described herein for improving cosmetic appearance of a subject in need thereof.

In still another example, there is provided herein a use of any of the dermal filler or dermal fillers as described herein for increasing tissue volume, smoothing wrinkles, or both, in a subject in need thereof.

In yet another example, there is provided herein a method for improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, in a subject in need thereof, said method comprising:

administering or injecting any of the dermal filler or dermal fillers as described herein to a region in need thereof;

thereby improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, of the subject.

In another example of any of the above use or uses or method or methods, native cells of the subject may infiltrate the dermal filler.

In still another example of any of the above use or uses or method or methods, the dermal filler may be non-resorbable such that the decellularized plant or fungal tissue may remain substantially intact within the subject.

In yet another example of any of the above use or uses or method or methods, the dermal filler may be degradeable through the addition of one or more enzymes thereto.

In another example of any of the above use or uses or method or methods, the plant or fungal tissue may be cellulose-based, and the one or more enzymes comprise cellulase.

In still another example of any of the above use or uses or method or methods, the cellulase may be cellulase from *Trichoderma sp*.

In yet another example, there is provided herein a method for preparing a non-resorbable dermal filler, comprising:

providing a plant or fungal tissue;

decellularizing and size-reducing the plant or fungal tissue to provide particles from which cellular materials and nucleic acids of the tissue are removed; and

optionally, sterilizing the particles;

thereby providing the non-resorbable dermal filler.

In another example of the above method, the decellularized plant or fungal tissue may be chitin-based, chitosan-based, lignocellulosic-based, or cellulose-based.

In still another example of any of the above method or methods, the decellularized plant or fungal tissue may be derived from leafy structure, root, flesh, hypanthium or pulp structures of a plant.

In yet another example of any of the above method or methods, the decellularized plant or fungal tissue may be derived from lettuce, carrot, apple, or pear, or any combination thereof.

In another example of any of the above method or methods, the size-reducing may comprise a mechanical size reduction to provide the particles, optionally where the mechanical size reduction is performed on dried, lyophilized, or freeze-dried materials.

In still another example of any of the above method or methods, the mechanical size reduction may comprise crushing, extrusion, grinding, milling, ultrasonication, electrospinning, chemical dissolution, enzymatic breakdown, or shearing the plant or fungal tissue, before or after decellularizing, to provide the particles.

In yet another example of any of the above method or methods, size-reducing may comprise drying the plant or fungal tissue, before or after decellularizing; subjecting the dried plant or fungal tissue to mechanical size reduction to provide the particles; and, optionally, reconstituting, resuspending, or rehydrating the particles.

In another example of any of the above method or methods, the size-reducing may provide particles the size of single structural cells, or smaller.

In still another example of any of the above method or methods, the method may further comprise a step of formulating the particles with a hydrogel, matrix, or carrier fluid.

In yet another example of any of the above method or methods, the hydrogel, matrix, or carrier fluid may comprise PBS, saline, hyaluronic acid (cross-linked or non-crosslinked),

alginate, collagen, pluronic acid (e.g. Pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof.

In another example of any of the above method or methods, the dermal filler may be provided in dried, or hydrated, form.

In still another example of any of the above method or methods, the method may further comprise a step of formulating with lidocaine or other anesthetic.

In yet another example of any of the above method or methods, the size-reducing may provide particles having irregular 3D shapes and/or which are non-spherical, thin, flake-like structures.

In another example of any of the above method or methods, the flake-like structures may have a thickness of about 0.01 to about 100 μm, for example about 0.1 μm.

In still another example of any of the above method or methods, the size-reducing may further comprise performing extrusion, filtering, centrifuging, or other size separation to obtain particles having a target size, particles within a target size range, or particles having a target size distribution.

In still another example of any of the above method or methods, the size-reducing may provide particles having particles having a size, diameter, or feret diameter within a range of about 20 μm to about 1000 μm.

In yet another example of any of the above method or methods, the size-reducing may provide particles having a size sufficiently large so as not to be phagocytosed by a cell or cells; preferably, the size-reducing may provide particles having a size, diameter, or minimum feret diameter of at least about 20 μm.

In another example of any of the above method or methods, the size-reducing may provide particles having a size, diameter, or maximum feret diameter of less than about 200 μm.

In still another example of any of the above method or methods, the size-reducing may provide particles having a size, diameter, or feret diameter distribution within a range of about 20 µm to about 200 µm.

In yet another example of any of the above method or methods, the size-reducing may provide particles having a particle size, diameter, or feret diameter distribution having a peak within about 30 µm to about 100 µm.

In another example of any of the above method or methods, the size-reducing may provide particles having a mean particle size, diameter, or feret diameter within a range of about 30 µm to about 100 µm.

In another example of any of the above method or methods, the size-reducing may provide particles having a size, diameter, or maximum feret diameter of less than about 300 µm.

In another example of any of the above method or methods, the size-reducing may provide particles having a size, diameter, or feret diameter distribution within a range of about 100 µm to about 300 µm.

In another example of any of the above method or methods, the size-reducing may provide particles having a particle size, diameter, or feret diameter distribution having a peak within about 100 µm to about 300 µm.

In another example of any of the above method or methods, the size-reducing may provide particles having a mean particle size, diameter, or feret diameter within a range of about 100 µm to about 300 µm.

In still another example of any of the above method or methods, the size-reducing may provide particles having an average projected particle area within a range of about 200 to about 3000 μm^2 , or within a range of about 200 to about 300 μm^2; wherein the decellularized plant or fungal tissue is disassembled into particles having a surface area to volume ratio of about 0.1 to 100 μm^{-1}; wherein the decellularized plant or fungal tissue is disassembled into particles having a packing density of about 4×10^5 particles/mL to about 7×10^9 particles/mL; or any

combinations thereof.

In yet another example of any of the above method or methods, the size-reducing may comprise grinding while simultaneously passing resulting particles through a filter to obtain particles below an upper threshold size.

In another example of any of the above method or methods, the method may further comprise filtering, differential or equilibrium centrifugation, or sieving to remove particles below a lower threshold size, obtaining particles above the lower threshold size.

In still another example of any of the above method or methods, the filtering or sieving may be performed using an automated wet-sieve.

In yet another example of any of the above method or methods, the size-reducing may comprise performing differential or equilibrium centrifugation to obtain particles having a target size, particles within a target size range, or particles having a target size distribution.

In another example of any of the above method or methods, the method may further comprise formulating the particles with a hydrogel, matrix, or carrier fluid by loading a first syringe or vessel with the particles in a fluid such as water or buffer (e.g. saline) and loading a second syringe or vessel with the hydrogel, matrix, or carrier fluid; the first and second syringes or vessels being in fluid communication; and mixing by passing the contents of the syringes or vessels back and forth between the first and second syringes or vessels.

In another example of any of the above method or methods, the method may further comprise formulating the particles with a hydrogel, matrix, or carrier fluid by mixing the particles with the hydrogel, matrix, or carrier fluid using a size exclusion mixer.

In another example of any of the above method or methods, the size exclusion mixer may be a static mixer.

In still another example of any of the above method or methods, the method may further comprise sterilizing the plant or fungal tissue, before or after decellularizing.

In yet another example of any of the above method or methods, the method may further comprise sterilizing the dermal filler.

In another example of any of the above method or methods, the sterilization may be by gamma sterilization.

In still another example of any of the above method or methods, the sterilization may be by autoclaving.

In still another example of any of the above method or methods, the sterilization may comprise a plurality of sterilization steps.

In still another example of any of the above method or methods, wherein a first sterilization step comprises a heat treatment.

In yet another example of any of the above method or methods, the method may further comprise mercerizing the plant or fungal tissue.

In another example of any of the above method or methods, the mercerizing may be performed after the decellularizing.

In still another example of any of the above method or methods, the mercerizing may comprise treatment of the decellularized plant or fungal tissue with a base and a peroxide.

In yet another example of any of the above method or methods, the base may be a hydroxide base.

In still another example of any of the above method or methods, the peroxide may be hydrogen peroxide.

In yet another example of any of the above method or methods, the mercerizing may comprise treatment of the decellularized plat or fungal tissue with an aqueous sodium hydroxide solution and hydrogen peroxide while heating.

In still another example of any of the above method or methods, the decellularized plant or

fungal tissue may be treated with the aqueous sodium hydroxide solution for a first period of time before the hydrogen peroxide is added to the reaction.

In yet another example of any of the above method or methods, the peroxide may be a 30% to 50% aqueous hydrogen peroxide stock solution.

In still another example of any of the above method or methods, the aqueous hydrogen peroxide stock solution may be used in an amount of at least about 75 mL per 500 g of decellularized plant or fungal tissue.

In still another example of any of the above method or methods, after addition of the aqueous hydrogen peroxide stock solution, the concentration of peroxide of a mixture of the decellularized plant or fungal tissue, the base, and the aqueous hydrogen peroxide stock solution may be about 1% to about 5%.

In yet another example of any of the above method or methods, the base may be a 1M sodium hydroxide solution.

In still another example of any of the above method or methods, the 1M sodium hydroxide solution may be used in an amount of about 2500 mL per 500 g of decellularized plant or fungal tissue.

In yet another example of any of the above method or methods, the method may further comprise neutralizing the pH with one or more neutralization treatments.

In still another example of any of the above method or methods, the one or more neutralization steps may comprise treatment with an aqueous HCl solution.

In yet another example of any of the above method or methods, the mercerizing may be performed with heating to about 80°C.

In still another example of any of the above method or methods, the mercerizing may be performed for about at least about 30 minutes.

In still another example of any of the above method or methods, the method may further

comprise formulating the dermal filler for subdermal injection, deep dermal injection, subcutaneous injection (e.g. subcutaneous fat injection), or any combinations thereof.

In yet another example, there is provided herein a dermal filler prepared by any of the method or methods described herein.

In another example of the above dermal filler, the dermal filler may be any of the dermal filler or dermal fillers as described herein.

In still another example of any of the above dermal filler or dermal fillers, about 0.1% to about 5%, or less, of the particles of the dermal filler may have a feret diameter, or a minimum feret diameter, of less than about 20 μm.

In yet another example of any of the above dermal filler or dermal fillers, less than about 0.5% of the particles of the dermal filler may have a feret diameter, or a minimum feret diameter, of less than 20 μm.

In yet another example of any of the above dermal filler or dermal fillers, less than about 0.1% to about 5% (such as less than about 0.5%), or less, of the particles of the dermal filler may have a size sufficiently small so as to be phagocytosed by a cell or cells of a subject.

In another example, there is provided herein a kit comprising any one or more of:

any of the dermal filler or dermal fillers as described herein;

a hydrogel, matrix, or carrier fluid;

PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof;

lidocaine or other aesthetic;

one or more syringes, or another injection device;

WHAT IS CLAIMED IS:

1. A non-resorbable dermal filler comprising decellularized plant or fungal tissue from which cellular materials and nucleic acids of the tissue are removed.

2. The dermal filler of claim 1, wherein the decellularized plant or fungal tissue is chitin-based, chitosan-based, lignocellulosic-based, or cellulose-based; preferably wherein the decellularized plant or fungal tissue is derived from leafy structure, root, flesh, hypanthium or pulp structures of a plant; preferably wherein the decellularized plant or fungal tissue is derived from lettuce, carrot, apple, or pear, or any combination thereof.

3. The dermal filler of claim 1 or 2, wherein the decellularized plant or fungal tissue is homogenized; preferably wherein the decellularized plant or fungal tissue is dried, subjected to grinding, and optionally reconstituted or rehydrated; preferably wherein the decellularized plant or fungal tissue is disassembled into particles the size of single structural cells, or smaller.

4. The dermal filler of any one of claims 1-3, wherein the dermal filler further comprises a hydrogel, matrix, or carrier fluid for the decellularized plant or fungal tissue; preferably wherein the hydrogel, matrix, or carrier fluid comprises PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof.

5. The dermal filler of any one of claims 1-4, wherein the dermal filler is dried, or hydrated; preferably wherein the dermal filler comprises lidocaine or other anesthetic.

6. The dermal filler of any one of claims 1-5, wherein the decellularized plant or fungal tissue is disassembled into particles having irregular 3D shapes and/or which are non-spherical, thin, flake-like structures; preferably wherein the flake-like structures have a thickness of about 0.01 to about 100 μm, for example about 0.1 μm; or wherein the decellularized plant or fungal tissue is disassembled into particles having a size sufficiently large so as not to be phagocytosed by a cell or cells; preferably, the decellularized plant or fungal tissue may be disassembled into particles having a size, diameter, or minimum feret diameter of at least about 20 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a size, diameter, or feret diameter within a range of about 20 μm to about 1000 μm; preferably wherein the decellularized

plant or fungal tissue is disassembled into particles having a size, diameter, or maximum feret diameter of less than about 200 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a size, diameter, or feret diameter distribution within a range of about 20 μm to about 200 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a particle size, diameter, or feret diameter distribution having a peak within about 30 μm to about 100 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a mean particle size, diameter, or feret diameter within a range of about 30 μm to about 100 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a size, diameter, or maximum feret diameter of less than about 300 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a size, diameter, or feret diameter distribution within a range of about 100 μm to about 300 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a particle size, diameter, or feret diameter distribution having a peak within about 100 μm to about 300 μm; preferably wherein the decellularized plant or fungal tissue is disassembled into particles having a mean particle size, diameter, or feret diameter within a range of about 100 μm to about 300 μm.

7. The dermal filler of any one of claims 1-6, wherein the decellularized plant or fungal tissue is disassembled into particles having an average projected particle area within a range of about 200 to about 3000 μm^2 , or within a range of about 200 to about 300 μm^2; wherein the decellularized plant or fungal tissue is disassembled into particles having a surface area to volume ratio of about 0.1 to 100 μm^{-1}; wherein the decellularized plant or fungal tissue is disassembled into particles having a packing density of about 4 x 10^5 particles/mL to about 7 x 10^9 particles/mL; or any combinations thereof; preferably wherein the dermal filler, decellularized plant or fungal tissue is sterilized; preferably wherein the sterilization is by gamma sterilization; preferably wherein the sterilization is by autoclaving; preferably wherein the sterilization is by ethanol treatment; preferably wherein the sterilization comprises a plurality of sterilization steps; preferably wherein a first sterilization step comprises a heat treatment; preferably wherein the dermal filler has a viscosity of less than about 500,000 cp; preferably wherein the dermal filler has a viscosity within a range of about 100,000 cp to about 200,000 cp, preferably wherein the decellularized plant or fungal tissue is treated by heating with sodium hydroxide and hydrogen peroxide.

8. The dermal filler of any one of claims 1-7, wherein the decellularized plant or fungal tissue is

mercerized.

9. The dermal filler of any one of claims 1-8, wherein the dermal filler is formulated for subdermal injection, deep dermal injection, subcutaneous injection (e.g. subcutaneous fat injection), or any combinations thereof, preferably wherein the dermal filler is provided in a syringe or injection device.

10. Use of the dermal filler of any one of claims 1-9 as a soft tissue filler, for reconstructive surgery, or both; preferably for improving cosmetic appearance of a subject in need thereof; preferably for increasing tissue volume, smoothing wrinkles, or both, in a subject in need thereof.

11. A cosmetic method for improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, in a subject in need thereof, wherein the dermal filler as defined in any one of claims 1-9 is to be administered or injected to a region in need thereof for improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, of the subject.

12. The use of claim 10, or the method of claim 11, wherein native cells of the subject infiltrate the dermal filler; preferably wherein the dermal filler is non-resorbable such that the decellularized plant or fungal tissue remains substantially intact within the subject; preferably wherein the dermal filler is degradable through the addition of one or more enzymes thereto; preferably wherein the plant or fungal tissue is cellulose-based, and the one or more enzymes comprise cellulase; preferably wherein the cellulase is cellulase from *Trichoderma sp*.

13. A method for preparing a non-resorbable dermal filler, comprising:

providing a plant or fungal tissue;

decellularizing and size-reducing the plant or fungal tissue to provide particles from which cellular materials and nucleic acids of the tissue are removed; and

optionally, sterilizing the particles;

thereby providing the non-resorbable dermal filler.

14. The method of claim 13, wherein the decellularized plant or fungal tissue is chitin-based, chitosan-based, lignocellulosic-based, or cellulose-based; preferably wherein the decellularized plant or fungal tissue is derived from leafy structure, root, flesh, hypanthium or pulp structures of a plant;

preferably wherein the decellularized plant or fungal tissue is derived from lettuce, carrot, apple, or pear, or any combination thereof.

15. The method of claim 13 or 14, wherein the size-reducing comprises a mechanical size reduction to provide the particles, optionally where the mechanical size reduction is performed on dried, lyophilized, or freeze-dried materials; preferably wherein the mechanical size reduction comprises crushing, extrusion, grinding, milling, ultrasonication, electrospinning, chemical dissolution, enzymatic breakdown, or shearing the plant or fungal tissue, before or after decellularizing, to provide the particles, preferably wherein size-reducing comprises drying the plant or fungal tissue, before or after decellularizing; subjecting the dried plant or fungal tissue to mechanical size reduction to provide the particles; and, optionally, reconstituting, resuspending, or rehydrating the particles; preferably wherein the size-reducing provides particles the size of single structural cells, or smaller.

16. The method of any one of claims 13-15, further comprising a step of formulating the particles with a hydrogel, matrix, or carrier fluid; preferably wherein the hydrogel, matrix, or carrier fluid comprises PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. Pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, regenerated cellulose, or any combinations thereof; preferably wherein the dermal filler is provided in dried, or hydrated, form; preferably wherein the method further comprises a step of formulating with lidocaine or other anesthetic.

17. The method of any one of claims 13-16, wherein the size-reducing provides particles having irregular 3D shapes and/or which are non-spherical, thin, flake-like structures; preferably wherein the flake-like structures have a thickness of about 0.01 to about 100 μm, for example about 0.1 μm; preferably wherein the size-reducing further comprises performing extrusion, filtering, centrifuging, or other size separation to obtain particles having a target size, particles within a target size range, or particles having a target size distribution; preferably wherein the size-reducing provides particles having a size sufficiently large so as not to be phagocytosed by a cell or cells.

18. The method of any one of claims 13-17, wherein the size-reducing provides particles having a size, diameter, or minimum feret diameter of at least about 20 μm; preferably wherein the size-reducing provides particles having a size, diameter, or feret diameter within a range of about 20 μm to about 1000 μm; preferably wherein the size-reducing provides particles having a size, diameter, or maximum feret diameter of less than about 200 μm; preferably wherein the size-reducing provides

particles having a size, diameter, or feret diameter distribution within a range of about 20 μm to about 200 μm; preferably wherein the size-reducing provides particles having a particle size, diameter, or feret diameter distribution having a peak within about 30 μm to about 100 μm; preferably wherein the size-reducing provides particles having a mean particle size, diameter, or feret diameter within a range of about 30 μm to about 100 μm; preferably wherein the size-reducing provides particles having a size, diameter, or maximum feret diameter of less than about 300 μm; preferably wherein the size-reducing provides particles having a size, diameter, or feret diameter distribution within a range of about 100 μm to about 300 μm; preferably wherein the size-reducing provides particles having a particle size, diameter, or feret diameter distribution having a peak within about 100 μm to about 300 μm; preferably wherein the size-reducing provides particles having a mean particle size, diameter, or feret diameter within a range of about 100 μm to about 300 μm.

19. The method of any one of claims 13-18, wherein the size-reducing provides particles having an average projected particle area within a range of about 200 to about 3000 μm^2 , or within a range of about 200 to about 300 μm^2; wherein the decellularized plant or fungal tissue is disassembled into particles having a surface area to volume ratio of about 0.1 to 100 μm^{-1}; wherein the decellularized plant or fungal tissue is disassembled into particles having a packing density of about 4×10^5 particles/mL to about 7×10^9 particles/mL; or any combinations thereof; preferably wherein the size-reducing comprises grinding while simultaneously passing resulting particles through a filter to obtain particles below an upper threshold size; preferably the method further comprises filtering, differential or equilibrium centrifugation, or sieving to remove particles below a lower threshold size, obtaining particles above the lower threshold size; preferably wherein the filtering or sieving is performed using an automated wet-sieve; preferably wherein the size-reducing comprises performing differential or equilibrium centrifugation to obtain particles having a target size, particles within a target size range, or particles having a target size distribution.

20. The method of any one of claims 13-19, further comprising formulating the particles with a hydrogel, matrix, or carrier fluid by loading a first syringe or vessel with the particles in a fluid such as water or buffer (e.g. saline) and loading a second syringe or vessel with the hydrogel, matrix, or carrier fluid; the first and second syringes or vessels being in fluid communication; and mixing by passing the contents of the syringes or vessels back and forth between the first and second syringes or vessels; preferably wherein the method further comprises formulating the particles with a hydrogel, matrix, or carrier fluid by mixing the particles with the hydrogel, matrix, or carrier fluid

using a size exclusion mixer; preferably wherein the size exclusion mixer is a static mixer.

21. The method of any one of claims 13-20, wherein the method further comprises sterilizing the plant or fungal tissue, before or after decellularizing; preferably wherein the method further comprises sterilizing the dermal filler; preferably wherein the sterilization is by gamma sterilization; preferably wherein the sterilization is by autoclaving; preferably wherein the sterilization comprises a plurality of sterilization steps; preferably wherein a first sterilization step comprises a heat treatment.

22. The method of any one of claims 13-21, wherein the method further comprises treating the plant or fungal tissue; preferably wherein the treating step is performed after the decellularizing step; preferably wherein the treating comprises treatment of the decellularized plant or fungal tissue with a base and a peroxide; preferably wherein the base is a hydroxide base; preferably wherein the peroxide is hydrogen peroxide; preferably wherein the treating comprises treatment of the decellularized plant or fungal tissue with an aqueous sodium hydroxide solution and hydrogen peroxide while heating; preferably wherein the treatment comprises mercerizing the decellularized plant or fungal tissue.

23. The method of any one of claims 13-22, wherein the decellularized plant or fungal tissue is treated with the aqueous sodium hydroxide solution for a first period of time before the hydrogen peroxide is added to the reaction; preferably wherein the peroxide is a 30% to 50% aqueous hydrogen peroxide stock solution; preferably wherein the aqueous hydrogen peroxide stock solution is used in an amount of at least about 75 mL per 500 g of decellularized plant or fungal tissue; preferably wherein, after addition of the aqueous hydrogen peroxide stock solution, the concentration of peroxide of a mixture of the decellularized plant or fungal tissue, the base, and the aqueous hydrogen peroxide stock solution is about 1% to about 5%; preferably wherein the base is a 1M sodium hydroxide solution; preferably wherein the 1M sodium hydroxide solution is used in an amount of about 2500 mL per 500 g of decellularized plant or fungal tissue; preferably wherein the 1M sodium hydroxide solution is used in an amount of about 2500 mL per 500 g of decellularized plant or fungal tissue.

24. The method of any one of claims 13-23, further comprising neutralizing the pH with one or more neutralization treatments; preferably wherein the one or more neutralization steps comprise treatment with an aqueous HCl solution; preferably wherein the mercerizing is performed with heating to about 80°C; preferably wherein the mercerizing is performed for at least about 30 minutes; preferably

wherein the method further comprises formulating the dermal filler for subdermal injection, deep dermal injection, subcutaneous injection (e.g. subcutaneous fat injection), or any combinations thereof.

25. A dermal filler prepared by the method of any one of claims 13-24; preferably wherein the dermal filler is a dermal filler as defined in any one of claims 1-9; preferably wherein about 0.1% to about 5%, or less, of the particles of the dermal filler have a feret diameter, or a minimum feret diameter, of less than about 20 μm; preferably wherein less than about 0.5% of the particles of the dermal filler have a feret diameter, or a minimum feret diameter, of less than 20 μm; preferably wherein less than about 0.1% to about 5% (such as less than about 0.5%), or less, of the particles of the dermal filler have a size sufficiently small so as to be phagocytosed by a cell or cells of a subject.

26. A kit comprising any one or more of:

a dermal filler as defined in any one of claims 1-9, and 25;

a hydrogel, matrix, or carrier fluid;

PBS, saline, hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, fibrin, calcium hydroxyapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, dissolved regenerated cellulose, or any combinations thereof;

lidocaine or other aesthetic;

one or more syringes, or another injection device;

a leur-lock fitting or coupler for two or more syringes;

instructions for performing a use or method as defined in any one of claims 34-42;

one or more decellularization reagents;

one or more containers, packages, or vessels;

one or more buffers, water, or saline for injection;

a reagent or enzyme (e.g. cellulase) for dissolving dermal filler at an undesirable region or

location;

or any combinations thereof.