Alternative, additional, and/or improved aerogels, hydrogels, and/or foams, as well as methods and/or uses thereof, are desirable..

SUMMARY

Provided herein are aerogels, hydrogels, and foams derived from and/or comprising decellularized plant or fungal tissue or structural cells thereof. As described in detail hereinbelow, aerogels, hydrogels, and foams have now been developed which may be derived from and/or may comprise decellularized plant or fungal tissue or structural cells thereof, and which: may comprise plant or fungal microstructures and/or architectures of interest; may be produced by readily scalable production methods; may provide for a wide range of scaffold microstructures and/or macrostructures and/or biochemistry; may provide tunable mechanical properties; may provide tunable porosity; may be biocompatible *in vitro* and/or *in vivo*; may be stable to a variety of conditions (such as cooking conditions in the case of food products); or any combinations thereof. By using single structural cells, groups of structural cells, or both, derived from a plant or fungal tissue (the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue), distributed within a carrier derived from one or more dehydrated, lyophilized, or freeze-dried hydrogels, a variety of aerogels, hydrogels, and foams have now been developed and prepared having desirable properties. In certain examples, the single structural cells, groups of structural cells, or both, may be derived from plant or fungal tissue (typically decellularized plant or fungal tissue) using mercerization treatment as described herein, which allows for reproducible and scalable production. Related methods and uses, as well as productions methods, are also described in detail herein.

In one aspect, the present disclosure refers to an aerogel or foam comprising:

single structural cells, groups of structural cells, or both, derived from a plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue;

the single structural cells, groups of structural cells, or both, being distributed within a carrier, the carrier derived from a dehydrated, lyophilized, or freeze-dried hydrogel.

In another example of the above aerogel or foam, the aerogel or foam has been rehydrated.

In still another example of any of the above aerogel or aerogels or foam or foams, the plant or fungal tissue from which the single structural cells or groups of structural cells are derived may comprise decellularized plant or fungal tissue.

In yet another example of any of the above aerogel or aerogels or foam or foams, the plant or fungal tissue may be decellularized using SDS and optionally $CaCl_2$.

In another example of any of the above aerogel or aerogels or foam or foams, the single structural cells, groups of structural cells, or both, may be derived from the plant or fungal tissue by mercerization. In certain examples, the plant or fungal tissue may be decellularized plant or fungal tissue.

In still another example of any of the above aerogel or aerogels or foam or foams, the mercerization may comprise treatment of the plant or fungal tissue using sodium hydroxide and hydrogen peroxide with heating.

In yet another example of any of the above aerogel or aerogels or foam or foams, the aerogel or foam may comprise a particle size distribution of the single structural cells with an average feret diameter within a range of about 1μm to about 1000μm, such as about 100 to about 500μm, for example about 100 to about 300μm.

In another example of any of the above aerogel or aerogels or foam or foams, the hydrogel may comprise alginate, pectin, gelatin, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, hydroxyethyl methyl cellulose , agar, pluronic acid, triblock PEO–PPO–PEO copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), dissolved or regenerated plant cellulose, dissolved cellulose-based hydrogel, hyaluronic acid, extracellular matrix proteins (e.g. collagen, gelatin, or fibronectin, or any combinations thereof), monoacrylated poly(ethylene glycol), poly(ethylene glycol) diacrylate,

poly(ethylene glycol) diacrylate (PEGDA)-co-PEGMA, poly(vinyl alcohol), poly(vinylpyrrolidone), poly(lactic-co-glycolic acid), chitosan, chitin, xanthan gum, elastin, fibrin, fibrinogen, cellulose derivatives, carrageenan, or microcrystalline cellulose, or any combinations thereof; wherein the hydrogel is optionally cross-linked.

In still another example of any of the above aerogel or aerogels or foam or foams, the aerogel or foam may comprise templated or aligned microchannels created by directional freezing; or by molding using that possess microscale features; by punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels, grooves, ridges, wells, or other structural features within and/or onto at least one surface; or any combinations thereof.

In yet another example of any of the above aerogel or aerogels or foam or foams, the plant tissue may comprise apple tissue or a pear tissue.

In another example of any of the above aerogel or aerogels or foam or foams, the aerogel or foam may comprise about 5% to about 95% m/m single structural cells, groups of structural cells, or both, when the aerogel or foam is in hydrated form.

In still another example of any of the above aerogel or aerogels or foam or foams, the hydrogel may comprise alginate, pectin, or both, and the aerogel or foam may be rehydrated with a $CaCl_2$ solution providing cross-linking.

In yet another example of any of the above aerogel or aerogels or foam or foams, the aerogel or foam may have bulk modulus within a range of about 0.1 to about 500 kPa, such as about 1 to about 200kPa.

In another example of any of the above aerogel or aerogels or foam or foams, the aerogel or foam may be rehydrated and may further comprise one or more animal cells.

In still another example of any of the above aerogel or aerogels or foam or foams, at least some cellulose and/or cellulose derivative(s) of the aerogel or foam may be cross-linked by physical cross-linking (e.g. using glycine) and/or chemical cross-linking (e.g. using citric acid in the presence of heat); wherein at least some cellulose and/or cellulose derivative(s) of the aerogel or

foam is functionalized with a linker (e.g. succinic acid) to which one or more functional moieties are optionally attached (e.g. amine-containing groups, wherein cross-linking may further optionally be achieved with one or more protein cross-linkers such as formalin, formaldehyde, glutaraldehyde, and/or transglutaminase); or any combinations thereof.

In yet another example, there is provided herein single structural cells, groups of structural cells, or both, derived from a decellularized plant or fungal tissue by mercerization of the decellularized plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue, and lacking one or more base-soluble lignin components of the plant or fungal tissue.

In yet another example, there is provided herein a method for preparing an aerogel or foam, comprising:

providing a decellularized plant or fungal tissue;

obtaining single structural cells, groups of structural cells, or both, from the decellularized plant or fungal tissue, by performing mercerization of the decellularized plant or fungal tissue and collecting the resultant single structural cells or groups of structural cells having a decellularized 3-dimensional structure;

mixing or distributing the single structural cells, groups of structural cells, or both, in a hydrogel, to provide a mixture; and

dehydrating, lyophilizing, or freeze-drying the mixture to provide the aerogel or foam.

In yet another example of the above method, the mercerization may comprise treatment of the decellularized plant or fungal tissue with a base and a peroxide, preferably sodium hydroxide or another hydroxide base as base, and hydrogen peroxide as peroxide.

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

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

In still another example of any of the above method or methods, the hydrogen peroxide for mercerization may be used in a ratio of:

about 20mL to about 5mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution;

such as:

about 20mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution;

about 10mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution; or

about 5mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution.

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

In another example of any of the above method or methods, the neutralization treatment may comprise treatment with an acid solution, preferably an aqueous HCl solution.

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

In yet another example of any of the above method or methods, the mercerization may be performed using a ratio of decellularized plant or fungal tissue : aqueous sodium hydroxide

solution (m:v, in g:L) of about 1:5 for a 1M aqueous sodium hydroxide solution, or an equivalent ratio for another aqueous sodium hydroxide solution concentration.

In another example of any of the above method or methods, the mercerization may be performed for at least about 30 minutes, preferably for about 1 hour.

In still another example of any of the above method or methods, the resultant single structural cells or groups of structural cells having a decellularized 3-dimensional structure may be collected by centrifugation.

In yet another example of any of the above method or methods, the single structural cells, groups of structural cells, or both, may be mixed or distributed in a hydrogel comprising alginate, pectin, gelatin, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, hydroxyethyl methyl cellulose, agar, pluronic acid, triblock PEO–PPO–PEO copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), dissolved or regenerated plant cellulose, dissolved cellulose-based hydrogel, hyaluronic acid, extracellular matrix proteins (e.g. collagen, gelatin, or fibronectin, or any combinations thereof), monoacrylated poly(ethylene glycol), poly(ethylene glycol) diacrylate, poly(ethylene glycol) diacrylate (PEGDA)-co-PEGMA, poly(vinyl alcohol), poly(vinylpyrrolidone), poly(lactic-co-glycolic acid), chitosan, chitin, xanthan gum, elastin, fibrin, fibrinogen, cellulose derivatives, carrageenan, or microcrystalline cellulose, or any combinations thereof; wherein the hydrogel is optionally cross-linked.

In another example of any of the above method or methods, the method may further comprise a step of performing directional freezing of the mixture to introduce templated or aligned microchannels on a surface of the mixture, within the mixture, or both; a step of molding the mixture using molds having microscale features contacting one or more surfaces of the mixture and/or the aerogel or foam resulting from dehydrating, lyophilizing, or freeze-drying of the mixture, so as to introduce templated or aligned microchannels; a step of punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels, grooves, ridges, wells, or other structural features within and/or onto at least one surface of the mixture and/or the aerogel or foam before, during, or after dehydrating, lyophilizing, or freeze-drying of the mixture;

or any combinations thereof.

In still another example of any of the above method or methods, the directional freezing may be performed by creating a thermal gradient across the mixture from one or more directions so as to form aligned ice crystals beginning from the cold side(s) of the thermal gradient.

In still another example of any of the above method or methods, a microarchitecture of the microchannels produced from directional freezing may be controlled by creating the mixture including a solvent containing varying amounts of one or more other dissolved compounds such as Sucrose, Dextrose, Trehalose, Corn Starch, Glycerol, Ethanol, Mannitol, Sodium chloride, CaCl2, Gelatine, Citric acid, PVA, PEG, Dextran, NaF, NaBr, NaI, phosphate buffer, or another such agent, which alters the structural properties of aligned ice crystals which grow from the cold side of the thermal gradient.

In yet another example of any of the above method or methods, the mixture may be directionally frozen over a period of at least about 30 minutes, preferably over a period of about 2 hours.

In another example of any of the above method or methods, the mixture may be directionally frozen by cooling to a temperature between about -190°C and about 0°C, such as at least about -15°C, preferably about -25°C.

In still another example of any of the above method or methods, the step of dehydrating, lyophilizing, or freeze-drying the mixture to provide the aerogel or foam may comprise freezing the mixture followed by lyophilizing or freeze-drying the mixture.

In yet another example of any of the above method or methods, the method may comprise a further step of cross-linking the hydrogel (such as cross-linking the hydrogel before or after freezing/lyophilisation, for example), rehydrating the aerogel or foam, or both; optionally using $CaCl_2$ solution to provide cross-linking where alginate or pectin or agar hydrogel is present.

In another example of any of the above method or methods, the method may comprise a further step of culturing animal cells on or in the aerogel or foam.

In another example, there is provided herein an aerogel or foam produced by any of the method or methods as described herein.

In still another example, there is provided herein a use of any of the aerogel or aerogels or foam or foams as described herein, for bone tissue engineering.

In yet another example, there is provided herein a use of any of the aerogel or aerogels or foam or foams as described herein, for templating or aligning growth of cells.

In another example of the above use, the cells may comprise muscle cells.

In still another example of the above use, the cells may comprise nerve cells.

In yet another example, there is provided herein a use of any of the aerogel or aerogels or foam or foams as described herein for repair of spinal cord injury.

In another example, there is provided herein a use of any of the aerogel or aerogels or foam or foams as described herein as insulation or packaging foam.

In still another example, there is provided herein a method for bone tissue engineering or repair in a subject in need thereof, comprising:

> implanting any of the aerogel or aerogels or foam or foams as described herein at an affected site of the subject in need thereof;
>
> such that the aerogel or foam promotes bone tissue generation or repair.

In another aspect, the present disclosure refers to use of an aerogel or foam as disclosed herein in the manufacture of a medicament for growing or repairing bone tissue or for treating spinal cord injury in a subject in need thereof, wherein the aerogel or foam as disclosed herein is to be implanted at an affected site of the subject in need thereof; such that the aerogel or foam promotes bone tissue generation or repair.

In yet another example, there is provided herein a method for templating or aligning growth of cells, comprising:

> culturing cells on any of the aerogel or aerogels or foam or foams as described herein, wherein the aerogel or foam comprises templated or aligned microchannels on at least one surface of the aerogel or foam, within the aerogel or foam, or both, optionally formed by directional freezing; by molding using molds having microscale features; by punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels,

grooves, ridges, wells, or other structural features within and/or onto at least one surface; or any combinations thereof;

such that the cultured cells align along the microchannels.

In another aspect, the present disclosure refers to a method for templating or aligning growth of cells, comprising: culturing cells on an aerogel or foam as disclosed herein, wherein the aerogel or foam comprises templated or aligned microchannels on at least one surface of the aerogel or foam, within the aerogel or foam, or both, optionally formed by directional or non-directional freezing; by molding using molds having microscale features; by punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels, grooves, ridges, wells, or other structural features within and/or onto at least one surface; or any combinations thereof; such that the cultured cells align along the microchannels.

In another example of the above method, the cells may comprise muscle cells or nerve cells.

In still another example, there is provided herein a method for repairing spinal cord injury in a subject in need thereof, comprising:

implanting any of the aerogel or aerogels or foam or foams as defined herein at an affected site of the subject in need thereof, wherein the aerogel or foam comprises templated or aligned microchannels optionally formed directional freezing;

such that the aerogel or foam promotes spinal cord repair by aligning growth of nerve cells along the templated or aligned microchannels.

In another example, there is provided herein a food product comprising any of the aerogel or aerogels or foam of foams as described herein.

In another aspect, the present disclosure refers to a food product comprising the aerogel or foam as disclosed herein.

In another aspect, the present disclosure refers to a food product comprising the aerogel or foam as disclosed herein, wherein the food product is a meat mimic and comprises a plurality of lines providing the appearance of fatty white lines found in tuna, salmon, or another fish-type meat.

In still another example of the above food product, the food product may be created for a cell-based or plant-based meat industry, and may utilize cellular agriculture techniques to create cultured meat products or plant-based meat products comprising or using aerogels and/or foams as described herein such as those including materials derived from decellularized plant or fungal tissues. The Examples included hereinbelow support that aerogels and/or foams as described herein may be cooked, may support mammalian cell growth, may be coloured and formed into plant-based and/or cell-based meat products. The Examples set out hereinbelow include a detailed example of a plant-based tuna fish mimic as an illustrative example.

In still another example of the above food product, the food product may comprise a dye or coloring agent.

In yet another example of any of the above food product or food products, the food product may comprise two or more aerogel or foam subunits glued together.

In another example of any of the above food product or food products, the glue may comprise agar.

In still another example of any of the above food product or food products, the aerogel or foam may comprise templated or aligned microchannels optionally formed by directional freezing, and wherein the aerogel or foam comprises muscle cells, fibroblasts, myofibroblasts, neurons, dorsal root ganglion cells, neuronal structures such as axons, neural precursor cells, neural stem cells, glial cells, endogenous stem cells, neutrophils, mesenchymal stem cells, satellite cells, myoblasts, myotubes, muscle progenitor cells, adipocytes, preadipocytes, chondrocytes, osteoblasts, osteoclasts, pre-osteoblasts, tendon progenitor cells, tenocytes, periodontal ligament stem cells, or endothelial cells, or any combinations thereof, aligned along the templated or aligned microchannels; preferably wherein the aerogel or foam comprises templated or aligned microchannels optionally formed by directional freezing, and wherein the aerogel or foam comprises muscle cells, fat cells, connective tissue cells (e.g. fibroblasts), cartilage, bone, epithelial, or endothelial cells, or any combinations thereof, aligned along the templated or aligned microchannels.

In another example, there is provided herein a use of any of the aerogel or aerogels or foam or foams as described herein in a food product.

In still another example, there is provided herein a method for preparing single structural cells, groups of structural cells, or both, from decellularized plant or fungal tissue, comprising:

providing a decellularized plant or fungal tissue;

obtaining single structural cells, groups of structural cells, or both, from the decellularized plant or fungal tissue, by performing mercerization of the decellularized plant or fungal tissue and collecting the resultant single structural cells or groups of structural cells having a decellularized 3-dimensional structure.

In yet another example of the above method, the mercerization may comprise treatment of the decellularized plant or fungal tissue with a base and a peroxide, preferably sodium hydroxide or another hydroxide base as base, and hydrogen peroxide as peroxide.

In another example of any of the above method or methods, the mercerization may comprise treatment of the decellularized plant or fungal tissue with 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 hydrogen peroxide may be added as a 30% aqueous hydrogen peroxide solution.

In still another example of any of the above method or methods, the hydrogen peroxide for mercerization may be used in a ratio of:

about 20mL to about 5mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution;

such as:

about 20mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution;

about 10mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution; or

about 5mL of 30% hydrogen peroxide solution: about 100g decellularized plant or fungal tissue : about 500mL of 1M NaOH solution.

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

In another example of any of the above method or methods, the neutralization treatment may comprise treatment with an acid solution, preferably an aqueous HCl solution.

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

performed with heating to about 80ºC.

In yet another example of any of the above method or methods, the mercerization may be performed using a ratio of decellularized plant or fungal tissue : aqueous sodium hydroxide solution (m:v, in g:L) of about 1:5 for a 1M aqueous sodium hydroxide solution, or an equivalent ratio for another aqueous sodium hydroxide solution concentration.

In still another example of any of the above method or methods, the mercerization may be performed for at least about 30 minutes, preferably for about 1 hour.

In yet another example of any of the above method or methods, the resultant single structural cells or groups of structural cells having a decellularized 3-dimensional structure may be collected by centrifugation.

In still another example, there is provided herein single structural cells, groups of structural cells, or both, prepared by any of the method or methods as described herein.

In yet another aspect, the present disclosure refers to a cellulose-based hydrogel comprising cellulose derived from decellularized plant or fungal tissue via dissolution with dimethylacetamide and lithium chloride followed by regeneration with ethanol.

In still another example, there is provided herein a method for preparing a cellulose-based hydrogel comprising:

providing a decellularized plant or fungal tissue;

dissolving cellulose of the decellularized plant or fungal tissue by treatment with dimethylacetamide (DMAc) and lithium chloride (LiCl); and

regenerating a cellulose-based hydrogel from the dissolved cellulose by solvent exchange with ethanol,

thereby providing the cellulose-based hydrogel.

In another example of the above method, the solvent exchange with ethanol may be performed

using a dialysis membrane, or by adding ethanol on top of the dissolved cellulose to promote solvent exchange.

In still another example of any of the above method or methods, the method may further comprise bleaching the cellulose-based hydrogel with hydrogen peroxide.

In yet another example, there is provided herein a cellulose-based hydrogel comprising cellulose derived from decellularized plant or fungal tissue via dissolution with: dimethylacetamide and lithium chloride, $LiClO_4$, xanthate, EDA/KSCN, H_3PO_4, NaOH/urea, $ZnCl_2$, TBAF/DMSO, NMMO, an ionic liquid (IL) (such as 1-butylpyridinium chloride and aluminum chloride, alkyl imidazolium in association with nitrate, preferably a room temperature ionic liquid), or any combinations thereof.

In still another example, there is provided herein a method for preparing a cellulose-based hydrogel comprising:

providing a decellularized plant or fungal tissue;

dissolving cellulose of the decellularized plant or fungal tissue by treatment with dimethylacetamide and lithium chloride, $LiClO_4$, xanthate, EDA/KSCN, H_3PO_4, NaOH/urea, $ZnCl_2$, TBAF/DMSO, NMMO, an ionic liquid (IL) (such as 1-butylpyridinium chloride and aluminum chloride, alkyl imidazolium in association with nitrate, preferably a room temperature ionic liquid), or any combinations thereof;

obtaining the dissolved cellulose and preparing the cellulose-based hydrogel using the dissolved cellulose.

In another example, there is provided herein a cellulose-based hydrogel prepared by any of the method or methods as described herein.

In another example of any of the aerogel or aerogels or foam or foams as described herein, the hydrogel may comprise any of the cellulose-based hydrogel or cellulose-based hydrogels as described herein.

In still another example, there is provided herein a food product comprising any of the aerogel or aerogels or foam or foams or structural cell or structural cells as described herein, wherein the food product is a meat mimic and comprises a plurality of lines providing the appearance of fatty white lines found in tuna, salmon, or another fish-type meat.

In yet another example of the above food product, the food product may be a mimic of tuna, salmon, or another fish meat.

In another example of any of the above food product or food products, the food product may contain one or more dyes or colorants providing the color of tuna, salmon, or another fish meat.

In still another example of any of the above food product or food products, the plurality of lines may be formed in cuts or channels formed in the aerogel or foam.

In yet another example of any of the above food product or food products, the plurality of lines may comprise titanium dioxide, optionally combined with agar binding agent or another such binding agent.

In another example of any of the above food product or food products, the titanium dioxide, optionally combined with agar binding agent, may be applied into cuts or channels formed in the aerogel or foam to provide the appearance of the fatty white lines found in tuna, salmon, or another fish-type meat.

In another example, there is provided herein a method for preparing a food product, the food product being a tuna, salmon, or other fish meat mimic, said method comprising:

providing any of the aerogel or aerogels or foam or foams as described herein;

optionally, dying or coloring the aerogel a color of tuna, salmon, or other fish meat;

cutting or otherwise processing the aerogel in order to form cuts or channels along the surface of the aerogel; and

applying a dye or coloring agent to the cuts or channels to provide an appearance of fatty white lines characteristic of tuna, salmon, or other fish meat.

In another aspect, the present disclosure refers to a method for preparing a food product, the food product being a tuna, salmon, or other fish meat mimic, said method comprising: providing an aerogel as disclosed herein; optionally, dying or coloring the aerogel a color of tuna, salmon, or other fish meat; cutting or otherwise processing the aerogel in order to form cuts or channels along the surface of the aerogel; and applying a dye or coloring agent to the cuts or channels to provide an appearance of fatty white lines characteristic of tuna, salmon, or other fish meat.

In another example of the above method, the dye or coloring agent applied to the cuts or channels may comprise titanium dioxide.

In still another example of any of the above method or methods, the dye or coloring agent applied to the cuts or channels may be combined with a binding agent.

In yet another example of any of the above method or methods, the binding agent may comprise agar.

In another aspect, the present disclosure refers to a food product prepared by any of the method or methods described herein.

In another example, there is provided herein a non-resorbable dermal filler comprising an aerogel, foam, structural cell, or cellulose-based hydrogel as described herein; or any combinations thereof.

In another aspect, the present disclosure refers to the aerogel, foam, or structural cell, as disclosed herein in the form of a non-resorbable dermal filler.

In yet another example, there is provided herein a dermal filler comprising single structural cells, groups of structural cells, or both, derived from a plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue, the single structural cells, groups of structural cells, or both, being derived from the plant or fungal tissue by mercerization.

In another example of any of the above dermal filler or dermal fillers, the dermal filler may further comprise a carrier fluid or gel.

In still another example of any of the above dermal filler or dermal fillers, the carrier fluid or gel may comprise water, an aqueous solution, or a hydrogel.

In yet another example of any of the above dermal filler or dermal fillers, the carrier fluid or gel may comprise a saline solution, or a collagen, hyaluronic acid, methylcellulose, and/or dissolved plant-derived decellularized cellulose-based hydrogel.

In another example of any of the above dermal filler or dermal fillers, the dermal filler may further comprise an anesthetic agent.

In still another example of any of the above dermal filler or dermal fillers, the anesthetic agent may comprise lidocaine, benzocaine, tetracaine, polocaine, epinephrine, or any combinations thereof.

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

In another example of any of the above dermal filler or dermal fillers, the dermal filler may comprise at least one of: 2% lidocaine gel; a triple anesthetic gel comprising 20% benzocaine, 6% lidocaine, and 4% tetracaine (BLTgel); 3% Polocaine; or a mixture of 2% lidocaine with epinephrine.

In another example of any of the above dermal filler or dermal fillers, the structural cells may have 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 structural cells may have a size, diameter, or maximum feret diameter of less than about 1000 μm.

In still another example of any of the above dermal filler or dermal fillers, the structural cells may have a size, diameter, or feret diameter distribution within a range of about 20 μm to about 1000 μm.

In yet another example of any of the above dermal filler or dermal fillers, the structural cells may have a particle size, diameter, or feret diameter distribution having a peak about 200 - 300 μm.

In another example of any of the above dermal filler or dermal fillers, the structural cells may have a mean particle size, diameter, or feret diameter within a range of about 200 μm to about 300 μm.

In another example of any of the above dermal filler or dermal fillers, the structural cells may have an average projected particle area within a range of about 30,000 to about 75,000 μm^2.

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 still 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 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 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 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 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 aspect, the present disclosure refers to use of a dermal filler as disclosed herein in the manufacture of a medicament 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; thereby improving cosmetic appearance, increasing tissue volume, smoothing wrinkles, or any combinations thereof, of the subject.

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

In yet another example 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 remains substantially intact within the subject.

BRIEF DESCRIPTION OF DRAWINGS

These and other features will become further understood having regard to the following Description and accompanying Drawings, wherein:

FIGURE 1 shows results of AA (apple) mercerization and discolouring in a smaller sample of AA (100g in the images), as described in Example 1. 100 g of decellularized AA (apple) material was mercerized in 500 mL of 1M NaOH at 80°C for one hour. A total of 75 mL of H_2O_2 was added throughout the mercerization process to discolour the samples (reaction formed Na_2O_2 (sodium peroxide) which is a strong oxidizer). Figure 1(A) shows AA samples in NaOH (T=0 min). Figure 1(B) shows AA samples in NaOH right after the addition of 25 mL of H_2O_2. The discolouration process started right after the addition. (T= 2 min). In Figure 1(C) the samples appear yellowish (T=10 min). In Figure 1(D) AA samples appear off-white after 60 minutes of mercerization in NaOH and the H_2O_2 additions;

FIGURE 2(A) shows the decellularized AA tissue used as the starting material for the mercerization process, and FIGURE 2(B) shows the product obtained after the mercerization, as described in Example 1. The product is shown after follow-up neutralization and centrifugation. The obtained product material shown in Figure 2(B) is very thick and viscous, resembling a sort of apple “paste”;

FIGURE 3 shows images of the apple-derived decellularized single structural cells (and some groups of structural cells comprising a small plurality of single structural cells linked together) obtained/isolated following mercerization as described in Example 1. In Figure 3, dilution and fluorescent staining of the structural cells with congo red dye revealed the microarchitecture of the cells is intact;

WHAT IS CLAIMED IS:

1. An aerogel or foam comprising:

single structural cells, groups of structural cells, or both, derived from a plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue;

the single structural cells, groups of structural cells, or both, being distributed within a carrier, the carrier derived from a dehydrated, lyophilized, or freeze-dried hydrogel.

2. The aerogel or foam of claim 1, wherein the aerogel or foam is rehydrated.

3. The aerogel or foam of claim 2, wherein the plant or fungal tissue from which the single structural cells or groups of structural cells are derived comprises decellularized plant or fungal tissue.

4. The aerogel or foam of claim 3, wherein the plant or fungal tissue is decellularized using SDS and optionally $CaCl_2$.

5. The aerogel or foam of claim 4, wherein the single structural cells, groups of structural cells, or both, are derived from the plant or fungal tissue by maceration or mercerization.

6. The aerogel or foam of claim 5, wherein the maceration or mercerization comprises treatment of the plant or fungal tissue using sodium hydroxide and hydrogen peroxide or sodium bicarbonate and hydrogen peroxide with heating.

7. The aerogel or foam of claim 6, having a particle size distribution of the single structural cells with an average feret diameter within a range of about 1µm to about 1000µm, such as about 100 to about 500µm, for example about 100 to about 300µm.

8. The aerogel or foam of claim 7, wherein the hydrogel comprises alginate, pectin, gelatin, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, hydroxyethyl methyl cellulose , agar, pluronic acid, triblock PEO–PPO–PEO copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), dissolved or

regenerated plant cellulose, dissolved cellulose-based hydrogel, hyaluronic acid, vegetable protein (e.g. pea protein), food-grade coloring (e.g. beet root), extracellular matrix proteins (e.g. collagen, gelatin, or fibronectin, or any combinations thereof), monoacrylated poly(ethylene glycol), poly(ethylene glycol) diacrylate, poly(ethylene glycol) diacrylate (PEGDA)-co-PEGMA, poly(vinyl alcohol), poly(vinylpyrrolidone), poly(lactic-co-glycolic acid), chitosan, chitin, xanthan gum, elastin, fibrin, fibrinogen, cellulose derivatives, carrageenan, or microcrystalline cellulose, or any combinations thereof; wherein the hydrogel is optionally cross-linked.

9. The aerogel or foam of claim 8, comprising templated or aligned microchannels created by directional freezing; non-directional freezing; by molding using molds having microscale and/or macroscale features (such as channels); by punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels, grooves, ridges, wells, or other structural features within and/or onto at least one surface (for example using needles); or any combinations thereof.

10. The aerogel or foam of claim 9, wherein the plant tissue comprises apple tissue, pear tissue or heart of palm tissue.

11. The aerogel or foam of claim 10, comprising about 5% to about 95% m/m, such as about 10-50% m/m (or more), single structural cells, groups of structural cells, or both, when the aerogel or foam is in hydrated form.

12. The aerogel or foam of claim 11, wherein the hydrogel comprises alginate, pectin, or both, and a) wherein the aerogel or foam is rehydrated with a $CaCl_2$ solution when the aerogel or foam is not crosslinked; or b) wherein the aerogel or foam is rehydrated with water, an aqueous solution, a buffer, a cell buffer, an alcohol, any aqueous or non-aqueous solution when the aerogel of foam is crosslinked.

13. The aerogel or foam of claim 12, wherein the aerogel or foam comprises one or more animal cells and has a bulk modulus within a range of about 0.1 to about 500 kPa, such as about 1 to about 200kPa.

14. The aerogel or foam of claim 13, wherein at least some cellulose and/or cellulose derivative(s) of the aerogel or foam is cross-linked by physical cross-linking (e.g. using glycine) and/or chemical cross-linking (e.g. using citric acid in the presence of heat); wherein at least some cellulose and/or cellulose derivative(s) of the aerogel or foam is functionalized with a linker (e.g. succinic acid) to which one or more functional moieties are optionally attached (e.g. amine-containing groups, wherein cross-linking may further optionally be achieved with one or more protein cross-linkers such as formalin, formaldehyde, glutaraldehyde, and/or transglutaminase); or any combinations thereof.

15. The aerogel of foam of claim 1, wherein the aerogel or foam comprises single structural cells, groups of structural cells, or both, derived from a decellularized plant or fungal tissue by maceration or mercerization of the decellularized plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue, and lacking one or more base-soluble lignin components of the plant or fungal tissue.

16. Use of an aerogel or foam as defined in claim 1 in the manufacture of a medicament for growing or repairing bone tissue or for treating spinal cord injury in a subject in need thereof,

wherein the aerogel or foam as defined in claim 1 is to be implanted at an affected site of the subject in need thereof;

such that the aerogel or foam promotes bone tissue generation or repair.

17. A method for templating or aligning growth of cells, comprising:

culturing cells on an aerogel or foam as defined in any one of claims 1-15, wherein the aerogel or foam comprises templated or aligned microchannels on at least one surface of the aerogel or foam, within the aerogel or foam, or both, optionally formed by directional or non-directional freezing; by molding using molds having microscale features; by punching, pressing, stamping, or otherwise forming geometric patterns, depressions, holes, channels, grooves, ridges, wells, or other structural features within and/or onto at least one surface; or any combinations thereof;

such that the cultured cells align along the microchannels.

18. The method of claim 17, further comprising using needles in the molds, preferably a 30G (gauge) needle.

19. The method of claim 17 or 18, wherein the cells comprise muscle cells or nerve cells.

20. The use of claim 16, wherein:

an aerogel or foam as defined in claim 1 is to be implanted at an affected site, wherein the aerogel or foam comprises templated or aligned microchannels;

such that the aerogel or foam promotes spinal cord repair by aligning growth of nerve cells along the templated or aligned microchannels.

21. A food product comprising the aerogel or foam as defined in claim 1.

22. The food product of claim 21, further comprising a dye or coloring agent.

23. The food product of claim 21, comprising two or more aerogel or foam subunits glued together, wherein the glues comprises agar.

24. The food product of claim 21, wherein the aerogel or foam comprises templated or aligned microchannels optionally formed by directional or non-directional freezing, and wherein the aerogel or foam comprises muscle cells, fibroblasts, myofibroblasts, neurons, dorsal root ganglion cells, neuronal structures such as axons, neural precursor cells, neural stem cells, glial cells, endogenous stem cells, neutrophils, mesenchymal stem cells, satellite cells, myoblasts, myotubes, muscle progenitor cells, adipocytes, preadipocytes, chondrocytes, osteoblasts, osteoclasts, pre-osteoblasts, tendon progenitor cells, tenocytes, periodontal ligament stem cells, or endothelial cells, or any combinations thereof, aligned along the templated or aligned microchannels; preferably wherein the aerogel or foam comprises templated or aligned microchannels optionally formed by directional or non-directional freezing, and wherein the aerogel or foam comprises muscle cells, fat cells, connective tissue cells (e.g. fibroblasts), cartilage, bone, epithelial, or endothelial cells, or any combinations thereof, aligned along the

templated or aligned microchannels.

25. A cellulose-based hydrogel comprising cellulose derived from decellularized plant or fungal tissue via dissolution with dimethylacetamide and lithium chloride followed by regeneration with ethanol.

26. The cellulose-based hydrogel of claim 25, comprising cellulose derived from decellularized plant or fungal tissue via dissolution with: dimethylacetamide and lithium chloride, $LiClO_4$, xanthate, EDA/KSCN, H_3PO_4, NaOH/urea, $ZnCl_2$, TBAF/DMSO, NMMO, an ionic liquid (IL) (such as 1-butylpyridinium chloride and aluminum chloride, alkyl imidazolium in association with nitrate, preferably a room temperature ionic liquid), or any combinations thereof.

27. A food product comprising the aerogel or foam as defined in claim 1, wherein the food product is a meat mimic and comprises a plurality of lines providing the appearance of fatty white lines found in tuna, salmon, or another fish-type meat.

28. The food product of claim 27, wherein the food product is a mimic of tuna, salmon, or another fish meat.

29. The food product of claim 27, wherein the food product contains one or more dyes or colorants providing the color of tuna, salmon, or another fish meat.

30. The food product of claim 27, wherein the plurality of lines are formed in cuts or channels formed in the aerogel or foam, wherein the plurality of lines comprise titanium dioxide or beetroot, optionally combined with agar or sodium alignate as a binding agent.

31. The food product of claim 30, wherein the titanium dioxide, optionally combined with agar or sodium alignate as a binding agent, is applied into cuts or channels formed in the aerogel or foam to provide the appearance of the fatty white lines found in tuna, salmon, or another fish-type meat.

32. A method for preparing a food product, the food product being a tuna, salmon, or other fish meat mimic, said method comprising:

providing an aerogel as defined in any one of claims 1-15;

optionally, dying or coloring the aerogel a color of tuna, salmon, or other fish meat;

cutting or otherwise processing the aerogel in order to form cuts or channels along the surface of the aerogel; and

applying a dye or coloring agent to the cuts or channels to provide an appearance of fatty white lines characteristic of tuna, salmon, or other fish meat.

33. The method of claim 32, wherein the dye or coloring agent applied to the cuts or channels comprises or beetroot.

34. The method of claim 32 or 33, wherein the dye or coloring agent applied to the cuts or channels is combined with a binding agent.

35. The method of claim 34, wherein the binding agent comprises agar or sodium alignate.

36. A food product prepared by the method of any one of claims 32-35.

37. The aerogel, foam, or structural cell, as defined in claim 1 in the form of a non-resorbable dermal filler.

38. The dermal filler of claim 37, wherein the filler comprises single structural cells, groups of structural cells, or both, derived from a plant or fungal tissue, the single structural cells or groups of structural cells having a decellularized 3-dimensional structure lacking cellular materials and nucleic acids of plant or fungal tissue, the single structural cells, groups of structural cells, or both, being derived from the plant or fungal tissue by maceration or mercerization.

39. The dermal filler of claim 38, wherein the dermal filler further comprises a carrier fluid or gel.

40. The dermal filler of claim 39, wherein the carrier fluid or gel comprises water, an aqueous solution such as a saline solution or a hydrogel such as a collagen, hyaluronic acid,

methylcellulose, and/or dissolved plant-derived decellularized cellulose-based hydrogel.

41. The dermal filler of claim 40, further comprising an anesthetic agent such as lidocaine, benzocaine, tetracaine, polocaine, epinephrine, or any combinations thereof.

42. The dermal filler of claim 41, wherein the dermal filler comprises PBS (saline), hyaluronic acid (cross-linked or non-crosslinked), alginate, collagen, pluronic acid (e.g. pluronic F 127), agar, agarose, or fibrin, calcium hydroxylapatite, Poly-L-lactic acid, autologous fat, silicone, dextran, methylcellulose, or any combinations thereof.

43. The dermal filler of claim 42, wherein the dermal filler comprises, at stock concentrations, of at least one of: 2% lidocaine gel; a triple anesthetic gel comprising 20% benzocaine, 6% lidocaine, and 4% tetracaine (BLTgel); 3% Polocaine; or a mixture of 2% lidocaine with epinephrine.

44. The dermal filler of claim 43, wherein the structural cells have a size, diameter, or feret diameter distribution within a range from at least about 20 μm to about 1000 μm and an average projected particle area within a range of about 30,000 to about 75,000 μm^2.

45. The dermal filler of claim 44, wherein the structural cells have a particle size, diameter, or feret diameter distribution having a peak about 200 - 300 μm or a mean particle size, diameter, or feret diameter within a range of about 200 μm to about 300 μm.

46. The dermal filler of claim 45, wherein the dermal filler is sterilized.

47. The dermal filler of claim 46, wherein the sterilization is by gamma sterilization.

48. The dermal filler of claim 46, wherein the dermal filler is formulated for subdermal injection, deep dermal injection, subcutaneous injection (e.g. subcutaneous fat injection), or any combinations thereof and is provided in a syringe or an injection device.

49. Use of a dermal filler as defined in any one of claims 37-48 in the manufacture of a medicament 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 37-48 is to be administered or injected to a region in need thereof;

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

50. The use of claim 49, wherein native cells of the subject infiltrate the dermal filler.

51. The use of claim 50, or the dermal filler of claim 44, wherein the dermal filler is non-resorbable such that the decellularized plant or fungal tissue remains substantially intact within the subject.

52. The aerogel or foam of claim 8, wherein the cross-linking is performed by extruding the hydrogel from a mold into a cross-linker bath for continuous feed crosslinking.

53. The aerogel or foam of claim 52, wherein the hydrogel has a desired shape.

54. The dermal filler of claim 42, wherein the dermal filler comprises a final concentration of 0.3% to 2% lidocaine gel.