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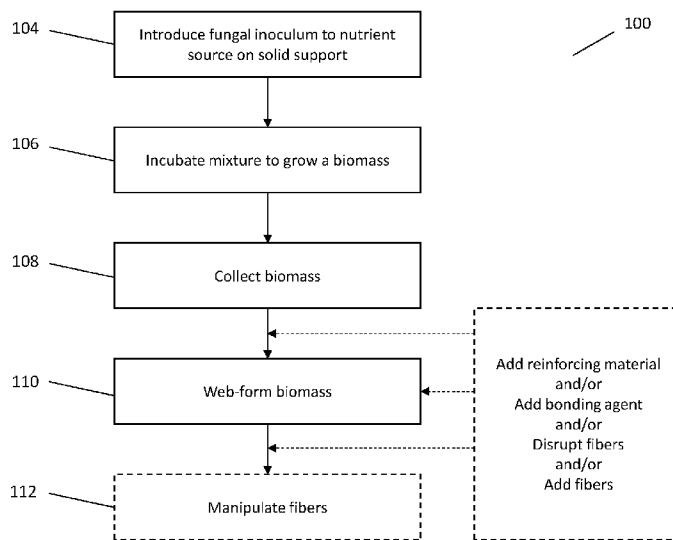


FIG. 20

(57) Abstract: Provided herein are mycelium materials and methods for production thereof. In some embodiments, a mycelium material includes: a mycelium material including one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction and/or a bonding agent may be combined with the mycelium material. Methods of producing a mycelium material are also provided.



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## **A COMPOSITE MATERIAL WITH ENHANCED RESISTANCE, AND METHODS FOR PRODUCTION THEREOF**

### **FIELD**

[0001] The present disclosure generally relates to various mycelium materials having a grown mycelium component and methods for production thereof to provide favorable mechanical and aesthetic qualities.

### **BACKGROUND**

[0002] Due to its bioefficiency, strength, and low environmental footprint, mycelium is of increasing interest in the next generation of sustainable materials. To this end, various applications have discussed methods of growing networks of enmeshed mycelium both on its own and as a composite material (e.g. enmeshed with particles, fibers, networks of fibers, solid matrix bonding agent, or nonwoven lamina). However, the mycelium materials currently undergoing development have poor mechanical qualities, including susceptibility to delamination and tearing under stress, and non-uniform aesthetic qualities. What is needed, therefore, are improved mycelium materials with favorable mechanical properties, aesthetic properties, and other advantages, as well as materials and methods for making improved mycelium materials.

### **SUMMARY**

[0003] Provided herein, according to some embodiments, are various mycelium materials and methods for production thereof to provide mycelium materials and composite mycelium materials with favorable mechanical and aesthetic qualities, and related advantages.

[0004] In one aspect, provided herein is a composite mycelium material. In one aspect, provided herein are composite mycelium materials, comprising: a mycelium material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and a bonding agent.

[0005] In some embodiments, the mycelium material has been generated on a solid or liquid substrate.

[0006] In some embodiments, the one or more disrupted fibers has a length of 0.1 mm to 5 mm. In some embodiments, the one or more disrupted fibers is manipulated by needle punching, felting, hydroentangling, or needling. In some embodiments, the one or more fibers are needled.

**[0007]** In some embodiments, the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix. In some embodiments, the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.

**[0008]** In some embodiments, the bonding agent is a vinyl acetate-ethylene (VAE) copolymer. In some embodiments, the bonding agent is a vinyl acetate-acrylic copolymer.

**[0009]** In some embodiments, the composite mycelium material further comprises a reinforcing material. In some embodiments, the reinforcing material is entangled within the composite mycelium material. In some embodiments, the reinforcing material comprises a base material. In some embodiments, the base material is positioned on one surface of the composite mycelium material. In some embodiments, the base material is positioned within the composite mycelium material. In some embodiments, the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

**[0010]** In some embodiments, the composite mycelium material further comprises a dye. In some embodiments, the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye. In one aspect, the dye is a reactive dye. In some aspects, the composite mycelium material is colored with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material. In other aspects, the dye is present throughout the interior of the composite mycelium material.

**[0011]** In some embodiments, the composite mycelium material further comprises a plasticizer. In some embodiments, the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecanyl succinic anhydride, octadecanyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane. In some embodiments, the composite mycelium material is flexible.

**[0012]** In some embodiments, the composite mycelium material comprises a mechanical property. In some embodiments, the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or

a peel resistance. In one aspect, the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

**[0013]** In some embodiments, the composite mycelium material comprises an additive. In one aspect, the additive comprises a lyocell fiber.

**[0014]** In another aspect, provided herein are composite mycelium materials, comprising: a mycelium material comprising one or more fibers oriented in a z-direction; and a fiber additive.

**[0015]** In some embodiments, the mycelium material has been generated on a solid or liquid substrate.

**[0016]** In some embodiments, the one or more fibers has a length of 0.1 mm to 5 mm.

**[0017]** In some embodiments, wherein the one or more fibers is manipulated by needle punching, felting, hydroentangling, or needling. In some embodiments, the one or more fibers are needled.

**[0018]** In some embodiments, the mycelium material comprises a bonding agent comprising an adhesive, a resin, a crosslinking agent, and/or a matrix. In some embodiments, the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive. In some embodiments, the bonding agent is a vinyl acetate-ethylene (VAE) copolymer. In some embodiments, the bonding agent is a vinyl acetate-acrylic copolymer.

**[0019]** In some embodiments, the composite mycelium material further comprises a reinforcing material. In some embodiments, the reinforcing material is entangled within the composite mycelium material. In some embodiments, the reinforcing material comprises a base material. In some embodiments, the base material is positioned on one surface of the composite mycelium material. In some embodiments, the base material is positioned within the composite mycelium material. In some embodiments, the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

**[0020]** In some embodiments, the one or more fibers is disrupted by a mechanical action. In some embodiments, the mechanical action comprises blending the one or more fibers. In some embodiments, the one or more fibers is disrupted by chemical treatment. In some embodiments, the chemical treatment comprises contacting the one or more fibers with a base or other chemical agent in an amount sufficient to cause a disruption.

[0021] In some embodiments, the base comprises alkaline peroxide.

[0022] In some embodiments, the composite mycelium material further comprises a dye. In one aspect, the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye. In one particular aspect, the dye is a reactive dye. In some aspects, the composite mycelium material is colored with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material. In some other aspects, the dye is present throughout the interior of the composite mycelium material.

[0023] In some embodiments, the composite mycelium material further comprises a plasticizer. In some embodiments, the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecenyl succinic anhydride, octadecenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

[0024] In some embodiments, the composite mycelium material is flexible.

[0025] In some embodiments, the composite mycelium material comprises a mechanical property. In some embodiments, the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance. In some embodiments, the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm. In some embodiments, the fiber additive comprises an abaca fiber. In some embodiments, the fiber additive comprises a lyocell fiber.

[0026] In some aspects, provided herein are systems, comprising; a mycelium composition comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; an apparatus having one or more projections; a solution; and a bonding agent.

[0027] In some embodiments, the mycelium composition comprises a solid content of 1-10%. In some embodiments, the mycelium composition comprises a solid content of 3-7%. In some embodiments, the mycelium composition comprises a solid content of 4-6%. In some embodiments, the mycelium composition comprises a solid content of 1-6%.

[0028] In some embodiments, the solution comprises water. In some embodiments, the one or more projections comprises needles. In some embodiments, the needles comprise a

diameter of 0.05-1 mm. In some embodiments, the needles comprise a diameter of 0.07-0.5 mm. In some embodiments, the apparatus comprises a needle density of 1-120 needle punctures per square cm.

**[0029]** In some embodiments, the one or more projections comprises water. In some embodiments, the one or more projections is positioned perpendicular to the mycelium composition. In some embodiments, the one or more projections subjects pressure to the mycelium composition, thus orienting the one or more disrupted fibers in the z-direction.

**[0030]** Provided herein are composite mycelium materials, comprising: a mycelium material comprising one or more disrupted fibers oriented in a z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and a lyocell fiber.

**[0031]** In some other aspects, provided herein are composite mycelium materials, comprising: a mycelium material comprising one or more disrupted fibers oriented in a z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and an abaca fiber.

**[0032]** In some aspects, provided herein are methods of producing a composite mycelium material, the method comprising: generating a mycelium material comprising one or more disrupted fibers; adding a solution to the mycelium material to produce a mycelium composition; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; adding a bonding agent to the mycelium composition; and draining the solution; thus producing the composite mycelium material.

**[0033]** In some other aspects, provided herein are methods, comprising: generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and needling the mycelium material.

**[0034]** In other aspects, provided herein are methods, comprising: generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and manipulating the mycelium material.

**[0035]** In one aspect, provided herein are methods, comprising: generating a mycelium composition comprising a mycelium material, a solution, and a bonding agent; and manipulating the mycelium composition.

**[0036]** Also provided herein are composite fibrous materials, comprising: a fibrous material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and a bonding agent.

**[0037]** Provided herein are methods of producing a composite mycelium material, the method comprising: generating a mycelium material; and manipulating the mycelium

composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction.

**[0038]** In one aspect, provided herein are methods of producing a composite mycelium material, the method comprising: generating a mycelium material comprising one or more disrupted fibers; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; and adding a bonding agent to the mycelium composition.

**[0039]** In some embodiments, the mycelium material has been generated on a solid or liquid substrate.

**[0040]** In some embodiments, the one or more fibers has a length of 0.1 mm to 5 mm.

**[0041]** In some embodiments, the manipulating comprises needle punching, felting, hydroentangling, or needling. In some embodiments, the manipulating comprises needling.

**[0042]** In some embodiments, the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix. In some embodiments, the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive. In some embodiments, the bonding agent is a vinyl acetate-ethylene (VAE) copolymer. In some embodiments, the bonding agent is a vinyl acetate-acrylic copolymer.

**[0043]** In some embodiments, the method comprises further adding a reinforcing material. In some embodiments, the reinforcing material is entangled within the composite mycelium material. In some embodiments, the reinforcing material comprises a base material. In some embodiments, the base material is positioned on one surface of the composite mycelium material. In some embodiments, the base material is positioned within the composite mycelium material. In some embodiments, the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

**[0044]** In some embodiments, the method comprises further adding a dye. In some embodiments, the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye. In some embodiments, the dye is a reactive dye.

**[0045]** In some embodiments, the method comprises further coloring the composite mycelium material with the dye, wherein the color of the composite mycelium material is



substantially uniform on one or more surfaces of the composite mycelium material. In some embodiments, the dye is present throughout the interior of the composite mycelium material.

[0046] In one aspect, the method comprises further adding a plasticizer. In some embodiments, the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecenyl succinic anhydride, octadecenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

[0047] In some embodiments, the composite mycelium material is flexible.

[0048] In some embodiments, the composite mycelium material comprises a mechanical property. In some embodiments, the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance. In one aspect, the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

[0049] In one aspect, the method comprises further adding an additive. In some embodiments, the additive comprises a lyocell fiber

## **BRIEF DESCRIPTION OF THE FIGURES**

[0100] **FIG. 1** depicts a schematic diagram of methods of producing a composite mycelium material according to some embodiments described herein.

[0101] **FIG. 2** depicts a scanning electron microscope (SEM) micrograph at 40X of a composite mycelium material with no fiber additives subjected to a manual peel. A scale bar of 2 mm is included.

[0102] **FIG. 3** depicts an SEM micrograph at 60X of a composite mycelium material with 20% 10 mm lyocell fibers subjected to a manual peel. A scale bar of 1 mm is included.

[0103] **FIG. 4** depicts an SEM micrograph at 180X of a composite mycelium material with no fiber additives subjected to a manual peel. A close-up image of the tip of the peel is also shown at 3000X. Scale bars of 500  $\mu\text{m}$  and 30  $\mu\text{m}$  are included, respectively.

[0104] **FIG. 5** depicts an SEM micrograph at 40X of a needled composite mycelium material with 15% abaca fibers subjected to a manual peel. A scale bar of 2 mm is included.

[0105] **FIG. 6** depicts a photograph of multiple panels of needled composite mycelium material.

[0106] **FIG. 7** depicts an SEM micrograph at 200X of a needled composite mycelium material with 15% abaca fibers subjected to a manual peel. A scale bar of 500  $\mu\text{m}$  is included.

[0107] **FIG. 8** depicts an SEM micrograph at 40X of a needled composite mycelium material with 15% abaca fibers subjected to a manual peel. A scale bar of 2 mm is included.

[0108] **FIG. 9** depicts an SEM micrograph at 40X of a needled composite mycelium material with 15% lyocell fibers subjected to a manual peel. A scale bar of 2 mm is included.

[0109] **FIG. 10** compares the average peel force (N/cm) of composite mycelium materials with fiber additives that have not been needled with that of needled composite mycelium materials with fiber additives.

[0110] **FIG. 11** depicts the average peel force (N/cm) of composite mycelium materials with 15% abaca fibers that have been needled with twisted and/or crown needles.

[0111] **FIG. 12** depicts the maximum peel force (N/cm) of composite mycelium materials with 10% and 15% lyocell fibers that have been needled with twisted and/or crown needles.

[0112] **FIG. 13** depicts the average peel force (N/cm) of composite mycelium materials with 10% and 15% lyocell fibers that have been needled with twisted and/or crown needles.

[0113] **FIG. 14** depicts the maximum single edge force (N/cm) of composite mycelium materials with 10% and 15% lyocell fibers that have been needled with twisted and/or crown needles.

[0114] **FIG. 15** depicts the average single edge force (N/cm) of composite mycelium materials with 10% and 15% lyocell fibers that have been needled with twisted and/or crown needles.

[0115] **FIG. 16** depicts an image of mycelium slurry after being needled with a handheld tool as in some embodiments described herein.

[0116] **FIG. 17** depicts the average peel force (N/cm) of composite mycelium material samples without fiber additives that have been needled with twisted and crown needles.

[0117] **FIG. 18** depicts the average single edge force (N/cm) of composite mycelium material samples without fiber additives that have been needled with twisted and crown needles.

[0118] **FIG. 19** depicts maximum single edge force (N/cm) of composite mycelium material samples without fiber additives that have been needled with twisted and crown needles

[0119] **FIG. 20** depicts a flowchart of a method of producing a material comprising mycelium.

[0120] **FIG. 21** depicts a flowchart of a method for converting raw mycelium material into a processed material.

## DETAILED DESCRIPTION

### Definitions

[0121] The details of various embodiments of the disclosure are set forth in the description below. Other features, objects, and advantages of the disclosure will be apparent from the description. Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. The terms “a” and “an” includes plural references unless the context dictates otherwise. Generally, nomenclatures used in connection with, and techniques of, biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0122] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0123] The term “hyphae” refers to a morphological structure of a fungus that is characterized by a branching filamentous shape.

[0124] The term “hyphal” refers to an object having a component thereof comprised of hyphae.

[0125] The term “mycelium” refers to a structure formed by one or more fibers. Mycelium is a distinct and separate structure from a fruiting body of a fungus or sporocarp.

[0126] The terms “cultivate” and “cultivated” refer to the use of defined techniques to deliberately grow a fungus or other organism.

[0127] The term “cultivated mycelium material” or “mycelium material” refers to material that includes one or more masses of cultivated mycelium, or includes solely of cultivated mycelium. In some embodiments, the one or more masses of cultivated mycelium is disrupted as described herein. In most cases, the cultivated mycelium material has been generated on a solid or liquid substrate, as described below.

[0128] The term “composite mycelium material” refers to any material including cultivated mycelium material combined with another material, such as a bonding agent or a supporting material as described herein, such as a crosslinking agent, natural adhesive, or a synthetic adhesive. In some embodiments, the mycelium comprises a supporting material. Suitable supporting materials include, but are not limited to, a mass of contiguous, disordered fibers

(e.g. non-woven fibers), a perforated material (e.g. metal mesh, perforated plastic), a mass of discontinuous particles (e.g. pieces of woodchip) or any combination thereof. In specific embodiments, the supporting material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit, a woven, and a non-woven textile. In some embodiments, the mycelium comprises a reinforcing material. A reinforcing material is a supporting material that is entangled within a mycelium or composite mycelium material. In some embodiments, the mycelium comprises a base material. A base material is a supporting material that is positioned on one or more surfaces of the mycelium or composite mycelium material.

**[0129]** The term “incorporate” refers to any substance, e.g., cultivated mycelium material, composite mycelium material, or a bonding agent, that can be combined with or contacted with another substance. In a specific embodiment, a mycelium or composite mycelium material can be combined with, contacted with, or incorporated into a supporting material, e.g., woven, twisted, wound, folded, entwined, entangled, or braided together, to produce a mycelium material that has become incorporated with the supporting material. In another embodiment, one or more bonding agents may be incorporated within the cultivated mycelium material to be bonded, either in its disrupted or undisrupted state, e.g., embedded throughout the material, or added as a thin coating layer, such as by spraying, saturation, dipping, nip rolling, coating, and the like, to produce a mycelium material.

**[0130]** As used herein, the term “disrupted” or “manipulate” with respect to one or more fibers refer to one or more fibers of which one or more disruptions have been applied. A “disruption” or “manipulation” as described herein, may be mechanical or chemical, or a combination thereof. In some embodiments, the one or more fibers is disrupted by a mechanical action. A “mechanical action” as used herein refers to a manipulation of or relating to machinery or tools. Exemplary mechanical actions include, but are not limited to, blending, chopping, impacting, compacting, bounding, shredding, grinding, compressing, high-pressure, shearing, laser cutting, hammer milling, needling, needle felting, needle punching, and waterjet forces. In some embodiments, a mechanical action may include applying a physical force, e.g., in one or more directions such that the at least some of the fibers are aligned in parallel in one or more directions, wherein the physical force is applied repeatedly. In some other embodiments, the one or more fibers is disrupted by chemical treatment. “Chemical treatment” as used herein refers to contacting the mycelium material or composite mycelium material with a chemical agent, e.g., a base or other chemical agent, in an amount sufficient to cause a disruption. In various embodiments, a combination of mechanical actions and chemical treatments may be used herein. The amount of mechanical

action (for example, the amount of pressure) and/or chemical agent applied, the period of time for which the mechanical action and/or chemical treatment is applied, and the temperature at which the mechanical action and/or chemical agent is applied, depends, in part, on the components of the cultivated mycelium material or composite mycelium material, and are selected to provide an optimal disruption on the cultivated mycelium material or composite mycelium material.

**[0131]** The term “plasticizer” as used herein refers to any molecule that interacts with a structure to increase mobility of the structure.

**[0132]** The term “processed mycelium material” as used herein refers to a mycelium that has been post-processed by any combination of treatments with preserving agents, plasticizers, finishing agents, dyes, and/or protein treatments.

**[0133]** The term “web” as used herein refers to a mycelium material or composite mycelium material that has been disrupted, converted into a slurry, and arranged in a formation (e.g. drylaid, airlaid and/or wetlaid).

**[0134]** The term “z-direction” as used herein refers to fibers disposed outside of the plane of orientation of a web or panel. The term “is oriented in a z-direction” as used herein refers to fibers that become oriented during forming of a mycelium web resulting from processing of a composite mycelium material.

**[0135]** The term “needled” or “needling” as used herein refers to an action of subjecting one or more projections to a mycelium material or composite mycelium material. The one or more projections may be oriented in an angular or perpendicular direction. In some embodiments, the needling comprises hydraulically needling, pricking, piercing, or penetrating.

**[0136]** The term “fibrous” as used herein refers to any combination of pulp fibers, synthetic fibers, natural fibers, biocomponent fibers, continuous filaments or mixtures thereof.

**[0137]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosed subject matter belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the disclosed subject matter, the preferred methods and materials are now described. All publications mentioned herein are incorporated by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

**[0138]** Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between

the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the aspects of the present disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the aspects of the present disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the aspects of the present disclosure.

[0139] Certain ranges are presented herein with numerical values being preceded by the term “about.” The term “about” is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

[0140] Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice of the present disclosure and will be apparent to those of skill in the art. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

### **Mycelium Compositions and Methods of Production**

[0141] Provided herein are mycelium materials and composite mycelium materials and scalable methods of producing the mycelium materials and composite mycelium materials. In some or most embodiments, the composite mycelium materials include a mycelium material having one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction, and a bonding agent. In some or most embodiments, the composite mycelium materials include a mycelium material comprising one or more fibers oriented in a z-direction; and a bonding agent. In some or most embodiments, the composite mycelium materials include a mycelium material comprising one or more disrupted fibers oriented in a z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and a lyocell fiber. In some or most embodiments, the composite mycelium materials include a mycelium material comprising one or more disrupted fibers oriented in a z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and an abaca fiber. In some other embodiments, the composite fibrous material includes a fibrous material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction;

and a bonding agent. Methods of producing the mycelium material and composite mycelium material are also provided.

**[0142]** Exemplary patents and applications discussing methods of growing mycelium include, but are not limited to: WIPO Patent Publication No. 1999/024555; G.B. Patent No. 2,148,959; G.B. Patent No. 2,165,865; U.S. Patent No. 5,854,056; U.S. Patent No. 2,850,841; U.S. Patent No. 3,616,246; U.S. Patent No. 9,485,917; U.S. Patent No. 9,879,219; U.S. Patent No. 9,469,838; U.S. Patent No. 9,914,906; U.S. Patent No. 9,555,395; U.S. Patent Publication No. 2015/0101509; U.S. Patent Publication No. 2015/0033620, all of which are incorporated herein by reference in their entirety. U.S. Patent Publication No. 2018/0282529, published on October 4, 2018 discusses various mechanisms of solution-based post-processing mycelium material to produce a material that has favorable mechanical characteristics for processing into a textile or leather alternative. WIPO Patent Publication No. WO2020237201 and U.S. Patent Publication No. 2020-0392341 are also incorporated herein by reference in their entirety.

**[0143]** As shown in **FIG. 1**, exemplary methods of producing mycelium materials according to some embodiments described herein include cultivating mycelium material, optionally disrupting mycelium material, optionally adding a bonding agent, optionally incorporating additional materials such as a support material, and combinations thereof. In various embodiments, traditional paper milling equipment may be adapted or used to perform some, or all, of the steps presented herein. In such embodiments, the mycelium material is produced using traditional paper milling equipment.

**[0144]** A description of an embodiment with several components in communication with each other does not imply that all such components are required. To the contrary, a variety of optional components may be described to illustrate a wide variety of possible embodiments of one or more aspects of the present disclosure and in order to more fully illustrate one or more aspects of the present disclosure. Similarly, although process steps, method steps, algorithms or the like may be described in sequential order, such processes, methods, and algorithms may generally be configured to work in alternate orders, unless specifically stated to the contrary. In other words, any sequence or order of steps that may be described herein does not, in and of itself, indicate a requirement that the steps be performed in that order. The steps of described processes may be performed in any order practical. Further, some steps may be performed simultaneously despite being described or implied as occurring non-simultaneously (e.g., because one step is described after the other step). Moreover, the illustration of a process by its depiction in a drawing does not imply that the illustrated

process is exclusive of other variations and modifications thereto, does not imply that the illustrated process or any of its steps are necessary to one or more embodiments, and does not imply that the illustrated process is preferred. Also, steps are generally described once per embodiment, but this does not mean they must occur once, or that they may only occur once each time a process, method, or algorithm is carried out or executed. Some steps may be omitted in some embodiments or some occurrences, or some steps may be executed more than once in a given embodiment or occurrence.

#### *Cultivated Mycelium Material*

[0145] Embodiments of the present disclosure include various types of cultivated mycelium materials or fibrous materials. Depending on the particular embodiment and requirements of the material sought, various known methods of cultivating mycelium may be used. Any fungus that can be cultivated as mycelium may be used. Suitable fungus species for use include but are not limited to: *Agaricus arvensis*; *Agrocybe brasiliensis*; *Amylomyces rouxii*; *Amylomyces sp.*; *Armillaria mellea*; *Aspergillus nidulans*; *Aspergillus niger*; *Aspergillus oryzae*; *Ceriporia lacerata*; *Coprinus comatus*; *Fibroporia vaillantii*; *Fistulina hepatica*; *Flammulina velutipes*; *Fomitopsis officinalis*; *Ganoderma sessile*; *Ganoderma tsugae*; *Hericium erinaceus*; *Hypholoma capnoides*; *Hypholoma sublaterium*; *Inonotus obliquus*; *Lactarius chrysorrheus*; *Macrolepiota procera*; *Morchella angusticeps*; *Myceliophthora thermophila*; *Neurospora crassa*; *Penicillium camembertii*; *Penicillium chrysogenum*; *Penicillium rubens*; *Phycomyces blakesleeanus*; *Pleurotus djamor*; *Pleurotus ostreatus*; *Polyporus squamosus*; *Psathyrella aquatica*; *Rhizopus microspores*; *Rhizopus oryzae*; *Schizophyllum commune*; *Streptomyces venezuelae*; *Stropharia rugosoannulata*; *Thielavia terrestris*; and *Ustilago maydis*. In some embodiments, the fungus used includes *Ganoderma sessile*, *Neurospora crassa*, and/or *Phycomyces blakesleeanus*.

[0146] In some embodiments, the strain or species of fungus may be bred to produce cultivated mycelium material with specific characteristics, such as a dense network of hyphae, a highly-branched network of hyphae, hyphal fusion within the network of hyphae, and other characteristics that may alter the properties of the cultivated mycelium material. In some embodiments, the strain or species of fungus may be genetically modified to produce cultivated mycelium material with specific characteristics.

[0147] In most embodiments, the cultivated mycelium may be grown by first inoculating a solid or liquid substrate with an inoculum of the mycelium from the selected species of fungus. In some embodiments, the substrate is pasteurized or sterilized prior to inoculation to prevent contamination or competition from other organisms. For example, a standard method



of cultivating mycelium includes inoculating a sterilized solid substrate (e.g. grain) with an inoculum of mycelium. Other standard methods of cultivating mycelium include inoculating a sterilized liquid medium (e.g. liquid potato dextrose) with an inoculum of mycelium or a pure cultured spawn. In some embodiments, the solid and/or liquid substrate will include lignocellulose as a carbon source for mycelium. In some embodiments, the solid and/or liquid substrate will contain simple or complex sugars as a carbon source for the mycelium.

**[0148]** Referring now to **FIG. 20**, a method 100 for producing a mycelium material is illustrated. The method 100 includes inoculating a nutrient source on a solid support 104, and incubating the mixture to grow a biomass of mycelium at 106, collecting the cultivated biomass of mycelium at 108, web-forming the biomass of mycelium at 110 to form a hyphal network, and manipulating fibers in the hyphal network at 112.

**[0149]** At step 106, the inoculated nutrient source is incubated to promote growth of the mycelium biomass. The conditions of the nutrient source and solid support can be selected to promote growth of a mycelium biomass having a plurality of fibers having sufficient morphological characteristics for entanglement in a downstream process. Exemplary morphological characteristics include a minimum length of hyphae branches, a desired density of the hyphae network, a desired degree of branching of the hyphae, a desired aspect ratio, and/or a desired degree of hyphal fusion of the hyphae network. According to one aspect of the present disclosure, the conditions of the solid support in the incubating step at 106 are selected to promote growth of a biomass of mycelium having a plurality of fibers having a length of at least about 0.1 mm. For example, the hyphae can have a length of from about 0.1 mm to about 5 mm, about 0.1 mm to about 4 mm, about 0.1 mm to about 3 mm, about 0.1 mm to about 2 mm, about 0.1 mm to about 1 mm, about 1 mm to about 5 mm, about 1 mm to about 4 mm, about 1 mm to about 3 mm, about 1 mm to about 2 mm, about 2 mm to about 5 mm, about 2 mm to about 4 mm, or about 2 mm to about 3 mm.

**[0150]** The incubation step 106 can occur under aerobic conditions in the presence of oxygen. Optionally, the solid support can be sealed into a chamber during all or a portion of the incubation step. In some examples, oxygen may be introduced into the chamber. The incubation temperature can be selected based on the specific fungal species. In some examples, the temperature of the chamber during incubation is from about 20°C to about 40°C, about 25°C to about 40°C, about 30°C to about 40°C, about 35°C to about 40°C, about 20°C to about 35°C, about 25°C to about 35°C, about 30°C to about 35°C, about 20°C to about 30°C, or about 25°C to about 30°C.

**[0151]** The incubation step 106 is configured to promote the growth of a biomass of mycelium that includes a plurality of fibers. The incubation step 106 can be ended when the cultivated biomass of mycelium is collected at step 108. The incubation step 106 may be ended at a predetermined time or when a predetermined concentration of mycelium biomass is reached. There may be some continued growth of the mycelium after the cultivated biomass is collected at step 108. Optionally, the mycelium biomass may be treated to stop growth of the mycelium.

**[0152]** At step 108 the cultivated mycelium biomass is collected. The collected biomass can be made into a slurry by adding the dry mycelium biomass to an aqueous solution. At step 108 a concentration of the collected biomass of mycelium in such a slurry may be adjusted based on the subsequent web-forming process at step 110. In some examples, the cultivated biomass of mycelium is in the form of slurry. The concentration of the biomass of mycelium may be adjusted by increasing a volume of the slurry or concentrating the mycelium biomass by removing at least a portion of the liquid from the slurry. In some examples, the concentration of the mycelium biomass may be adjusted to a concentration of from about 10 g/L to about 30 g/L, about 10 g/L to about 25 g/L, or about 10 g/L to about 20 g/L. In other examples, the cultivated biomass of mycelium may be collected and dried.

**[0153]** In some aspects, a bonding agent can optionally be added to the cultivated biomass of mycelium before, during, or after the web-forming process at step 110. The bonding agent can be added before, during, or after collecting the cultivated biomass of mycelium and/or adjusting the concentration of the cultivated biomass of mycelium. The bonding agent can include any adhesive, resin, cross-linking agent, or polymeric matrix material described herein and combinations thereof.

**[0154]** In some aspects, the plurality of fibers can optionally be disrupted, before, during, or after the web-forming process at step 110. The plurality of fibers can be disrupted according to any of the mechanical and/or chemical methods described herein for disrupting mycelium fibers. For example, prior to the web-forming process at step 110, the fibers can mechanically be disrupted by a mechanical action such as blending, chopping, impacting, compacting, bounding, shredding, grinding, compressing, high-pressure waterjet, or shearing forces. The fibers can be disrupted before, during, or after adjusting the concentration of the cultivated biomass of mycelium.

**[0155]** In some aspects, the collected biomass of mycelium can optionally be combined with pulp fibers, synthetic fibers, natural fibers, biocomponent fibers, continuous filaments and/or mixtures thereof, before, during, or after the web-forming process at step 110. In one aspect,

the fibers can be combined with the mycelium before, during, or after disrupting the plurality of fibers. The fibers can have any suitable dimension. Non-limiting examples of suitable fibers include cellulosic fibers, cotton fibers, rayon fibers, Lyocell fibers, TENCEL™ fibers, polypropylene fibers, and combinations thereof. In some embodiments, these fibers may be fibers made from polyester, polyamides and polyolefins such as, for example, one or more of polyethylene, polypropylene, polybutene, ethylene copolymers, propylene copolymers and butene copolymers. In one aspect, the one or more fibers can have a length of less than about 25 mm, less than about 20 mm, less than about 15 mm, or less than about 10 mm. For example, the one or more fibers can have a length of from about 1 mm to about 25 mm, about 1 mm to about 20 mm, about 1 mm to about 15 mm, about 1 mm to about 10 mm, about 1 mm to about 5 mm, about 5 mm to about 25 mm, about 5 mm to about 20 mm, about 5 mm to about 15 mm, about 5 mm to about 10 mm, about 10 mm to about 25 mm, about 10 mm to about 20 mm, or about 10 mm to about 15 mm. The fibers may be combined with the mycelium in a desired concentration. In one example, the fibers may be combined with the mycelium in an amount of from about 1 wt% to about 25 wt%, about 1 wt% to about 20 wt%, about 1 wt% to about 15 wt%, about 1 wt% to about 10 wt%, about 1 wt% to about 5 wt%, about 5 wt% to about 25 wt%, about 5 wt% to about 20 wt%, about 5 wt% to about 15 wt%, about 5 wt% to about 10 wt%, about 10 wt% to about 25 wt%, about 10 wt% to about 20 wt%, or about 10 wt% to about 15 wt%.

**[0156]** At step 110, the biomass of mycelium collected in step 108 can be treated according to a web-forming process to form a hyphal network. The web-forming process can include any of the wet-lay, dry array, or air-lay techniques described herein. The fibers of the web formed in step 110 can optionally be chemically and/or thermally bonded using any of the bonding agents described herein.

**[0157]** Optionally, the web-forming at step 110 can include laying the fibers on a supporting material. As described herein, in some aspects, the supporting material is a reinforcing material. Non-limiting examples of a suitable supporting material include a woven fiber, a mass of contiguous, disordered fibers (e.g., non-woven fibers), perforated material (e.g., a metal mesh or perforated plastic), a mass of discontinuous particles (e.g., pieces of woodchip), a cheesecloth, a fabric, a knot fiber, a scrim, and a textile. The fibers can be combined with, contacted with, and/or incorporated into the supporting material. For example, in some aspects, the fibers can be woven, twisted, would, folded, entwined, entangled, and/or braided together with the supporting material to form a mycelium material, as described herein. In some aspects, the fibers can be laid on the supporting material before,

during, and/or after adding a chemical bonding agent. In some aspects, a reinforcing material can be combined with the fibers before, during, or after the web-forming step 110.

**[0158]** At step 112, the hyphal network formed at step 110 can undergo a manipulation process to entangle the plurality of fibers in the hyphal network. The entanglement process can include needle punching (also referred to as felting) and/or hydroentangling. When a supporting material is present, the entanglement process optionally includes entangling at least a portion of the plurality of fibers with the supporting material. The entanglement process can form mechanical interactions between fibers and optionally between fibers and a supporting material (when present). In some embodiments, the fibers are not entangled with a supporting material.

**[0159]** In some aspects, the manipulation at step 112 is achieved through a needle punching or needle felting process in which one or more needles are passed into and out of the hyphal network. Movement of the needles in and out of the hyphal network facilitate entangling the fibers and optionally orienting the fibers. A needle punch having an array of needles can be used to punch the hyphal network at a plurality of locations with each pass of the needle array. The number of needles, spacing of needles, shape of the needles, and size of the needles (i.e., needle gauge) can be selected to provide the desired degree of entanglement of the hyphal network. For example, the needles may be barbed and have any suitable shape, non-limiting examples of which include a pinch blade, a star blade, and a conical blade. The number of needle punches per area and the punching rate can also be selected to provide the desired degree of entanglement of the hyphal network. The parameters of the needle punching or needle felting process can be selected at least based in part on the fungal species, the morphology and dimensions of the fibers forming the hyphal network, the desired degree of entanglement, and/or end-use applications of the mycelium material.

**[0160]** In some aspects, the manipulating at step 112 is achieved through a hydroentanglement process. The hydroentanglement process directs high pressure liquid jets into the hyphal network to facilitate entangling the fibers. The liquid may be any suitable liquid, an example of which includes water. The entanglement process can include a spinneret having an array of holes configured to direct a stream of liquid at a specific location in the hyphal network. The diameter of the holes can be selected to provide a jet of liquid having the desired diameter to direct at the hyphal network. Additional aspects of the spinneret, such as the number of holes in the array and the spacing of the holes in the array can be selected to provide the desired degree of entanglement of the hyphal network. The hyphal network and the spinneret may move relative to one another such that the liquid jets

are directed at the hyphal network in a pattern. For example, the spinneret may move relative to the hyphal network in a generally “Z” or “N” shaped pattern to provide multiple passes of the spinneret over the hyphal network. The number of passes and the application pattern can be selected to provide the desired degree of entanglement of the hyphal network. The parameters of the hydroentanglement process can be selected based at least in part on the fungal species, the morphology and dimensions of the fibers forming the hyphal network, the desired degree of entanglement, and/or end-use applications of the mycelium material. In some examples, the hydroentanglement process occurs in phases in which a portion of the mycelium material is web-formed (e.g., wet-laying), the hydroentanglement process proceeds, and then a second portion of the mycelium material is web-formed on top of the first portion and the hydroentanglement process is repeated. This process of web-forming a portion of the mycelium material and hydroentangling the web-formed portion can be repeated any number of times until a final thickness of material is web-formed.

**[0161]** The liquid pressure, the diameter of the openings in the spinneret, and/or the flow rate of liquid can be selected to provide the desired degree of entanglement of the hyphal network and optionally entanglement of the hyphal network and a supporting material. For example, the liquid pressure during the hydroentanglement process can be at least 100 psi, at least 200 psi, at least 300 psi, at least 400 psi, at least 500 psi, at least 600 psi, at least 700 psi, at least 800 psi, at least 900 psi, or at least 1000 psi. In some examples, the liquid jet pressure is from about 700 to about 900 psi. In some examples, the diameter of the openings in the spinneret is at least about 10 microns, at least about 30 microns, at least about 50 microns, at least about 70 microns, at least about 90 microns, at least about 110 microns, at least about 130 microns, or at least about 150 microns. For example, the diameter of the openings in the spinneret can be from about 10 microns to about 150 microns, from 20 microns to about 70 microns, about 30 microns to about 80 microns, about 40 microns to about 90 microns, about 50 microns to about 100 microns, about 60 microns to about 110 microns, or about 70 microns to about 120 microns. In some examples, the openings have a diameter of about 50 microns. The flow rate of liquid can be from about 100 mL/min. to about 300 mL/min. in some examples. In some examples, the belt speed during the entanglement process is about 1 meter/minute.

**[0162]** In some aspects, the manipulating at step 112 is achieved through a needling process. In some embodiments, the needles may comprise barbs such as twisted needles or crown needles. In some embodiments, the needles may be barbless. In some or most embodiments, the manipulating step occurs when the mycelium material or composite mycelium material is wet. In some or most embodiments, the manipulating step occurs at a wet stage. In some or

most embodiments, the manipulating step occurs when the mycelium material or composite mycelium material is in a slurry. In such embodiments, the one or more fibers have mobility. In some embodiments, the needles penetrate the mycelium material or composite mycelium material but does not go through the mycelium material or composite mycelium material. In some embodiments, the individual holes or orifices do not go through the entire width of the mycelium material or composite mycelium material. For example, the needling may not puncture the foraminous surface (i.e. forming cloth) on which the wet-laid web is formed.

**[0163]** Without intending to be bound by any particular theory, the one or more fibers may attach to the one or more needles as it penetrates the mycelium material or composite mycelium material. Without intending to be bound by any particular theory, the one or more needles may break large bubbles in the material into smaller ones. In some embodiments, the one or more needles reduces the overall porous content of the mycelium material or composite mycelium material. In some embodiments, the peel of the mycelium material or composite mycelium material has an increased surface area as a result of the manipulation.

**[0164]** In some embodiments, the length of the needles is greater than the length of the fibers. In some embodiments, the length of the needles is less than the length of the fibers. In some embodiments, the diameter of the needles is greater than the diameter of the fibers.

**[0165]** In some embodiments, the water content of the slurry is modulated or controlled. In some other embodiments, the drainage rate of water content is modulated or controlled.

**[0166]** In some embodiments, the mass of fibers in the z-direction after the manipulation step comprises 2-70% of the mycelium material. In some embodiments, the mass of fibers in the z-direction after the manipulation step comprises at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the mycelium material.

**[0167]** In some aspects, the mycelium material or composite mycelium material is hydraulically needled. That is, conventional hydraulic entangling equipment may be operated at conditions which impart relatively low energies (e.g., 0.001 to 0.03 hp-hr/lb) to the web. Water jet treatment equipment which may be adapted to the process of the present invention may be found, for example, in U.S. Patent No. 3,485,706 to Evans, the disclosure of which is hereby incorporated by reference. The hydraulic needling process of the present invention may be carried out with any appropriate working fluid such as, for example, water. The working fluid flows through a manifold which evenly distributes the fluid to a series of individual holes or orifices. These holes or orifices may be from about 0.003 to about 0.015 inch in diameter. In the hydraulic needling process, the working fluid passes through the

orifices at a pressures ranging from about 50 to about 1500 pounds per square inch gage (psig) to form fluid streams which impact the mycelium material or composite mycelium material, typically with much less energy than found in conventional hydraulic entangling processes. More entangling energy may be required for high basis weight materials, nonwoven fibrous webs containing large proportions of staple length fibers, or fibers having a stiffer modulus.

**[0168]** In some other embodiments, the mycelium material or composite mycelium material may be manipulated by other means, including, but not limited to, mechanical manipulation such as pricking, folding, felting, needle punching, wet felting, pulling, magnetic guiding, and crimping.

**[0169]** After completion of the manipulation process at 112, the mycelium material or composite mycelium material comprises one or more fibers oriented in the z-direction. In some embodiments, the mycelium material or composite mycelium material may have z-direction components in the form of ridges or peaks on at least one face. The ridges or peaks formed may be regularly spaced or irregular in spacing and shape.

**[0170]** The mycelium material can be processed according to any of the post-processing methods and/or treatments described herein. Non-limiting examples of post-processing methods and treatments include treatment with a plasticizer, treatment with a tannin and/or dye, treatment with a preservative, treatment with a protein source, treatment with a coating and/or finishing agent, a drying process, a rolling or pressing process, and treatment in an embossing process.

**[0171]** In various embodiments, the solid or liquid substrate may be supplemented with one or more different nutritional sources. The nutritional sources may contain lignocellulose, simple sugars (e.g. dextrose, glucose), complex sugars, agar, malt extract, a nitrogen source (e.g. ammonium nitrate, ammonium chloride, amino acids) and other minerals (e.g. magnesium sulfate, phosphate). In some embodiments, one or more of the nutritional sources may be present in lumber waste (e.g. sawdust including from hardwoods, beeches, and hickory) and/or agricultural waste (e.g. livestock feces, straw, corn stover). Once the substrate has been inoculated and, optionally, supplemented with one or more different nutritional sources, cultivated mycelium may be grown. Methods of growing mycelium have been well established in the art. Exemplary methods of growing mycelium include but are not limited to U.S. Patent No. 5,854,056; U.S. Patent No. 4,960,413; and U.S. Patent No. 7,951,388.

**[0172]** In some embodiments, the growth of the cultivated mycelium will be controlled to prevent the formation of fruiting bodies. Various methods of preventing fruiting body formation as discussed in detail in U.S. Patent Publication No. 2015/0033620; U.S. Patent No. 9,867,337; and U.S. Patent No. 7,951,388. In other embodiments, the cultivated mycelium may be grown so that it is devoid of any morphological or structural variations. Depending on the embodiment sought, growing conditions such as exposure to light (e.g. sunlight or a growing lamp), temperature, carbon dioxide may be controlled during growth.

**[0173]** In some embodiments, the cultivated mycelium may be grown on an agar medium. Nutrients may be added to the agar/water base. Standard agar media commonly used to cultivate mycelium material include, but are not limited to, a fortified version of Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Oatmeal Agar (OMA), and Dog Food Agar (DFA).

**[0174]** In most embodiments, the cultivated mycelium material may be grown as a solid mass and may later be disrupted. Cultivated mycelium material that is disrupted may be a live mat, preserved, or otherwise treated to kill the mycelium (i.e., stop mycelium growth) as described below.

**[0175]** In some embodiments, cultivated mycelium material may be grown to include elongate fibers defining fine filaments that interconnect with one another, and further may interconnect with various supporting materials provided in a growing procedure, as further described below. The fine filaments may be analyzed using an optical magnifying or imaging device to determine if a grown length of the fine filaments is adequate to support sufficient network interconnection between the fine filaments and various additives. The fine filaments should not only be of a sufficient length, but also flexible to provide adequate interconnection therebetween.

**[0176]** In some embodiments, cultivated mycelium material may be processed using a dry array, a wet-lay, or an air-lay technique. In dry-lay or dry array, an inert or growing mycelium network of fibers may be pulled apart and detangled to expand the volume of the network. Similarly, in a wet-lay technique, an inert or growing mycelium network of fibers may be saturated in a liquid medium to detangle and expand the volume of the network. Further, in an air-lay technique, an inert or growing mycelium network of fibers may be suspended in air to create a web that expands the volume of the network. After such a technique, the expanded network can be compressed to provide a dense or compacted network. The web can be densified to include an overall density profile of at least 6gm per



cubic meter. A compacted web can be embossed with a replicated leather pattern for providing a leather alternative material.

[0177] In some embodiments, the method comprises a step of web-forming the collected biomass of the mycelium. In some embodiments, the step of web-forming the collected biomass of mycelium comprises depositing the biomass of mycelium on a supporting material.

[0178] In some embodiments, the supporting material comprises a woven fiber, a non-woven fiber, a mesh, a perforated plastic, woodchips, a cheesecloth, a fabric, a knot fiber, a scrim, a textile, or combinations thereof.

[0179] In some embodiments, the entangling the plurality of fibers comprises entangling at least a portion of the plurality of fibers with the supporting material.

[0180] In some embodiments, the method further comprises combining a reinforcing material with the biomass of mycelium one of prior to the web-forming step, during the web-forming step, or after the web-forming step. In some embodiments, web-forming comprises wet-laying, air-laying, or dry-laying.

[0181] In some embodiments, the method further comprises combining one of natural fibers, synthetic fibers, or a combination thereof with the biomass of mycelium one of prior to the web-forming step, during the web-forming step, or after the web-forming step.

[0182] In some embodiments, the fibers have a length of less than 25 millimeters.

#### *Disrupted Cultivated Mycelium Material*

[0183] Various types of cultivated mycelium material including one or fibers may be disrupted at a variety of points during the production process, thus generating one or more disrupted fibers. In such embodiments, the cultivated mycelium material comprises one or more disrupted fibers. The cultivated mycelium material may be disrupted before or after adding a bonding agent. In one aspect, the cultivated mycelium material may be disrupted at the same time as adding a bonding agent. Exemplary embodiments of disruptions include, but are not limited to, mechanical action, chemical treatment, or a combination thereof. For example, the one or more fibers may be disrupted by both a mechanical action and chemical treatment, a mechanical action alone, or chemical treatment alone.

[0184] In some embodiments, the one or more fibers is disrupted by a mechanical action. Mechanical actions may include blending, chopping, impacting, compacting, bounding, shredding, grinding, compressing, high-pressure, waterjet, and shearing forces. In some embodiments, the mechanical action includes blending the one or more fibers. Exemplary methods of achieving such a disruption include use of a blender, a mill, a hammer mill, a

drum carder, heat, pressure, liquid such as water, a grinder, a beater, and a refiner. In an exemplary production process, a cultivated mycelium material is mechanically disrupted by a conventional unit operation, such as homogenization, grinding, coacervation, milling, jet milling, waterjet and the like.

**[0185]** According to a further aspect, the mechanical action includes applying a physical force to the one or more fibers such that at least some of the fibers are aligned in a particular formation, e.g., aligned in a parallel formation, or along or against the stress direction. The physical force can be applied to one or more layers of a cultivated mycelium material or composite mycelium material. Such disrupted mycelia material can typically be constructed with layers with varying orientation. Exemplary physical forces include, but are not limited to, pulling and aligning forces. Exemplary methods of achieving such a disruption include use of rollers and drafting equipment. In some embodiments, a physical force is applied in one or more directions such that the at least some of the fibers are aligned in parallel in one or more directions, wherein the physical force is applied repeatedly. In such embodiments, the physical force may be applied at least two times, e.g., at least three times, at least four times, or at least five times.

**[0186]** In some other embodiments, the one or more fibers is disrupted by chemical treatment. In such embodiments, the chemical treatment includes contacting the one or more fibers with a base or other chemical agent sufficient to cause a disruption including, but not limited to alkaline peroxide, beta-glucanase, surfactants, acids, and bases such as sodium hydroxide and sodium carbonate (or soda ash). The pH of the cultivated mycelium material in solution can be monitored for the purpose of maintaining the optimal pH.

**[0187]** In some embodiments, the disruptions described herein generate one or more disrupted fibers, e.g., sub-networks. As used herein, a “sub-network” refers to discrete masses of fibers that are produced after disruption, e.g., a mechanical action or chemical treatment. A sub-network may come in a wide assortment of shapes, e.g., sphere-, square-, rectangular-, diamond-, and odd-shaped sub-networks, etc., and each sub-network may come in varied sizes. The cultivated mycelium material may be disrupted sufficiently to produce one or more disrupted fibers, e.g., sub-networks, having a size in the desired ranges. In many instances, the disruption can be controlled sufficiently to obtain both the size and size distribution of the sub-network within a desired range. In other embodiments, where more precise size distributions of sub-networks are required, the disrupted cultivated mycelium material can be further treated or selected to provide the desired size distribution, e.g. by sieving, aggregation, or the like. For example, a sub-network may have a size represented by, e.g.,

length, of about 0.1 mm to about 5 mm, inclusive, e.g., of about 0.1 mm to about 2 mm, about 1 mm to about 3 mm, about 2 mm to about 4 mm, and about 3 mm to about 5 mm. In some embodiments, a sub-network may have a size represented by a length of about 2 mm. The “length” of a sub-network is a measure of distance equivalent to the most extended dimension of the sub-network. Other measurable dimensions include, but are not limited to, length, width, height, area, and volume.

**[0188]** In various embodiments, physical force may be used to create new physical interactions (i.e. re-entangle) between the one or more fibers after disruption. Various known methods of creating entanglements between fiber may be used, including methods of creating non-woven materials by creating mechanical interactions between fibers. In some embodiments described below, hydroentanglement may be used to create mechanical interactions between the fibers after the fibers have disrupted.

*Preserved Cultivated Mycelium Material*

**[0189]** Once the cultivated mycelium material has been grown, it may be optionally separated from the substrate in any manner known in the art, and optionally subjected to post-processing in order to prevent further growth by killing the mycelium and otherwise rendering the mycelium imputrescible, referred to herein as “preserved mycelium material”. Suitable methods of generating preserved mycelium material can include drying or desiccating the cultivated mycelium material (e.g. pressing the cultivated mycelium material to expel moisture) and/or heat treating the cultivated mycelium material.

**[0190]** In a specific embodiment, the cultivated mycelium material is pressed at 190,000 pounds force to 0.25 inches for 30 minutes. The cultivated mycelium material can be pressed by at least 100, 1000, 10,000, 100,000, 110,000, 120,000, 130,000, 140,000, 150,000, 160,000, 170,000, 180,000, 190,000, 200,000, or 300,00 or more pounds force. The cultivated mycelium material can be pressed to at least 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.4, 0.41, 0.42, 0.43, 0.44, 0.45, 0.46, 0.47, 0.48, 0.49, 0.5, 0.51, 0.52, 0.53, 0.54, 0.55, 0.56, 0.57, 0.58, 0.59, 0.6, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.7, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.8, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, or 1 inch or more. The cultivated mycelium material can be pressed to at least 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.4, 0.41, 0.42, 0.43, 0.44, 0.45, 0.46, 0.47, 0.48, 0.49, 0.5, 0.51, 0.52, 0.53, 0.54, 0.55, 0.56, 0.57, 0.58, 0.59, 0.6, 0.61, 0.62, 0.63, 0.64,

0.65, 0.66, 0.67, 0.68, 0.69, 0.7, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.8, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, or 1 centimeter or more. The cultivated mycelium material can be pressed for at least 1 min, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min, or 60 min or more.

**[0191]** Suitable methods of drying organic matter to render it imputrescible are well known in the art. In one specific embodiment, the cultivated mycelium material is dried in an oven at a temperature of 100°F or higher. In another specific embodiment, the cultivated mycelium material is heat pressed.

**[0192]** In other instances, living or dried cultivated mycelium material is processed using one or more solutions that function to remove waste material and water from the mycelium. In some embodiments, the solutions include a solvent such as ethanol, methanol or isopropyl alcohol. In some embodiments, the solutions include a salt such as calcium chloride.

Depending on the embodiments, the cultivated mycelium material may be submerged in the solution for various durations of time with and without pressure. In some embodiments the cultivated mycelium material may be submerged in several solutions consecutively. In a specific embodiment, the cultivated mycelium material may first be submerged in one or more first solutions including an alcohol and a salt, then submerged in a second solution including alcohol. In another specific embodiment, the cultivated mycelium material may first be submerged in one or more first solutions including an alcohol and a salt, then submerged in a second solution including water. After treatment with solution, the cultivated mycelium material may be pressed using a hot or cold process and/or dried using various methods including air drying and/or vacuum drying. U.S. Patent Publication No.

2018/0282529, the entirety of which is incorporated herein by reference, describes these embodiments in detail.

**[0193]** In one aspect, the cultivated mycelium material may be fixated by adjusting pH using an acid such as formic acid. In specific embodiments, the pH will be at least 2, 3, 4 or 5. In some embodiments, the pH of the cultivated mycelium material will be adjusted to an acidic pH of 3 in order to fix the cultivated mycelium material using various agents such as formic acid. In specific embodiments, the pH will be adjusted to a pH less than 6, 5, 4 or 3 in order to fix the cultivated mycelium material. In one embodiment, the pH will be adjusted to a pH of 5.5.

*Bonding Agents*

[0194] Various aspects of the present disclosure include a bonding agent. A “bonding agent” as used herein may include any suitable agent that provides added strength and/or other properties such as additional softness, strength, durability, and compatibility. A bonding agent may be an agent that reacts with some portion of the cultivated mycelium material, enhances the treatment of the cultivated mycelium material, co-treated with the cultivated mycelium material or treated separately, but as a network with the cultivated mycelium material, to produce a composite mycelium material. In some aspects, a bonding agent is added prior to the disruption. In other aspects, a bonding agent is added after the disruption. In some other aspects, a bonding agent is added while the sample is being disrupted. Bonding agents include an adhesive, a resin, a crosslinking agent, and/or a matrix. A composite mycelium material described herein includes cultivated mycelium material and bonding agents that may be water-based, 100% solids, UV and moisture cure, two-component reactive blend, pressure sensitive, self-crosslinking hot melt, and the like.

[0195] In some embodiments, the bonding agent is selected from the group including a natural adhesive or a synthetic adhesive. In such embodiments, the natural adhesive may include a natural latex-based adhesive. In specific embodiments, the natural latex-based adhesive is leather glue or weld. The bonding agents may include anionic, cationic, and/or non-ionic agents. In one aspect, the bonding agents may include crosslinking agents.

[0196] In some embodiments, the bonding agent has a particle size of less than or equal to 1  $\mu\text{m}$ , a sub-zero glass transition temperature, or a self-crosslinking function. In some embodiments, the bonding agent has a particle size of less than or equal to 1  $\mu\text{m}$ , a sub-zero glass transition temperature, and a self-crosslinking function. In some embodiments, the bonding agent has a particle size of less than or equal to 1  $\mu\text{m}$ . In some embodiments, the bonding agent has a sub-zero glass transition temperature. In some embodiments, the bonding agent has a self-crosslinking function. In some embodiments, the bonding agent has a particle size of less than or equal to 500 nanometers. Specific exemplary bonding agents include vinyl acetate ethylene copolymers such as Dur-O-Set® Elite Plus, Dur-O-Set® Elite 22, and Dur-O-Set® E230.

[0197] In some embodiments, the bonding agent has a glass transition temperature of -100- -10°C, -100- -90°C, -90- -80°C, -80- -70°C, -70- -60°C, -60- -50°C, -50- -40°C, -40- -30°C, -30- -20°C, -20- -10°C, -10- -10°C, -30- -25°C, -25- -20°C, -20- -15°C, -15- -10°C, -10- -5°C, -5- -0°C, -90°C, -80°C, -70°C, -60°C, -50°C, -40°C, -35°C, -30°C, -25°C, -20°C, -15°C, -

10°C, -5°C, or 0°C. In some embodiments, the bonding agent has a glass transition temperature of -15°C.

**[0198]** Other exemplary bonding agents include, but are not limited to transglutaminase, polyamide-epichlorohydrin resin (PAE), citric acid, genipin, alginate, vinyl acetate-ethylene copolymers, and vinyl acetate-acrylic copolymers. In some embodiments, the binder is polyamide-epichlorohydrin resin (PAE). In some embodiments, the binder is a vinyl acetate-ethylene copolymer. In some embodiments, the binder is a vinyl acetate-acrylic copolymer.

**[0199]** In some embodiments, the bonding agent includes one or more reactive groups. For example, the bonding agent reacts with active hydrogen containing groups such as amine, hydroxyl, and carboxyl groups. In a specific embodiment, the bonding agent crosslinks one or more fibers via the one or more reactive groups. In some instances, amines are present on chitin, and hydroxyl and carboxyl groups are present on the polysaccharides and proteins surrounding the chitin. In a specific embodiment, PAE includes cationic azetidinium groups. In such embodiments, the cationic azetidinium groups on PAE act as reactive sites in the polyamideamine backbone, and react with active hydrogen containing groups such as amine, hydroxyl, and carboxyl groups, in the one or more fibers.

**[0200]** Further examples of bonding agents include, but are not limited to, citric acid in combination with sodium hypophosphite or monosodium phosphate or sodium dichloroacetate, alginate in combination with sodium hypophosphite or monosodium phosphate or sodium dichloroacetate, epoxidized soybean oil, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), polyamide epichlorohydrin resin (PAE), and ammonium persulfate. Some examples of bonding agents include epoxies, isocyanates, sulfur compounds, aldehydes, anhydrides, silanes, aziridines, and azetidinium compounds and compounds with all such functional groups. Possible formaldehyde-containing bonding agents include formaldehyde, phenol formaldehyde, urea formaldehyde, melamine urea formaldehyde, melamine formaldehyde, phenol resorcinol and any combinations of them.

**[0201]** Additional examples of suitable bonding agents include latex materials, such as butadiene copolymers, acrylates, vinyl-acrylics, styrene-acrylics, styrene-butadiene, nitrile-butadiene, polyvinyl acetates, olefin containing polymers, e.g., vinyl acetate-ethylene copolymers, vinyl ester copolymers, halogenated copolymers, e.g., vinylidene chloride polymers. Latex-based agents, when used, can contain functionality. Any kind of latex can be used, including acrylics. Representative acrylics include those formed from ethyl acrylate, butyl acrylate methyl (meth)acrylate, carboxylated versions thereof, glycosylated versions thereof, self-crosslinking versions thereof (for example, those including N-methyl

acrylamide), and copolymers and blends thereof, including copolymers with other monomers such as acrylonitrile. Natural polymers such as starch, natural rubber latex, dextrin, lignin, cellulosic polymers, saccharide gums, and the like can also be used. In addition, other synthetic polymers, such as epoxies, urethanes, phenolics, neoprene, butyl rubber, polyolefins, polyamides, polypropylene, polyesters, polyvinyl alcohol, and polyester amides can also be used. The term “polypropylene” as used herein includes polymers of propylene or polymerizing propylene with other aliphatic polyolefins, such as ethylene, 1-butene, 1-pentene, 3-methyl-1-butene, 4-methyl-1-pentene, 4-methyl-1-hexene, 5-methyl-1-hexene and mixtures thereof. In specific embodiments, bonding agents include, but are not limited to, natural adhesives (e.g. natural latex-based adhesives such as leather glue or weld, latex, soy protein-based adhesives), synthetic adhesives (polyurethane), neoprene (PCP), acrylic copolymer, styrene-butadiene copolymer, ethylene-vinyl acetate-b, nitrocellulose, polyvinyl acetate (PVA), and vinyl acetate ethylene (VAE). In other embodiments, the bonding agent is VAE.

**[0202]** In one aspect, one or more bonding agents may be incorporated within the cultivated mycelium material to be bonded, either in its disrupted or undisrupted state, e.g., embedded throughout the material, or added as a thin coating layer, such as by spraying, dipping, rolling, coating, and the like, to produce a composite mycelium material. In one other aspect, one or more bonding agents may be incorporated at the same time the disruption occurs. Any suitable method of bonding may be used according to the present disclosure. Bonding of the surfaces may occur on drying, and a strong cured bond can be developed. The bonding of one or more bonding agents may include the use of open or closed-cell foam materials like urethane, olefinic rubber, and vinyl foam materials, as well as textiles, metal and fabrics in various lamination arrangements.

**[0203]** A bonded assembly (i.e., a laminate) may be prepared by uniformly applying the aqueous adhesive to the cultivated mycelium material. In some embodiments, the lamina includes two successive layers. In some embodiments, the lamina includes three successive layers. Various coating methods may be used such as spraying, roll coating, saturation, and the like. The coated substrate can be dried before bonding.

**[0204]** A composite mycelium material may be chemically bonded by impregnating the composite mycelium material with a chemical binder to link fibers to one another, including linking cellulosic fibers to one another. Non-limiting examples of suitable binders include gum arabic, vinyl acetate-ethylene (VAE), and adhesives. Examples of suitable adhesive include S-10, available from US Adhesives, U.S.A., and Bish’s Original Tear Mender Instant

Fabric & Leather Adhesive, available from Tear Mender, U.S.A. One example of a suitable VAE-based binder is Dur-O-Set® Elite 22, which is available from Celanese Emulsions, U.S.A. One other example of a suitable VAE-based binder is Dur-O-Set® Elite Plus, which is available from Celanese Emulsions, U.S.A. One other example of a suitable VAE-based binder is Dur-O-Set® E230, which is available from Celanese Emulsions, U.S.A. Another exemplary binder includes X-LINK® 2833, available from Celanese Emulsions, U.S.A., and which is described as a self-crosslinking vinyl acetate acrylic. In a web of interconnected fibers, a chemical binder will have to saturate the web to diffuse through the web and reach the core of the network. Thus, a composite mycelium material may be immersed in a binder solution to fully impregnate the material. A spray application of a chemical binder may also be provided to a composite mycelium material. A spray application of a chemical binder may be aided by capillary action for dispersal, or may be aided by a vacuum application to draw the chemical binder through the material. A coater may also be used for coating a composite mycelium material.

**[0205]** A composite mycelium material may be bonded using a thermal bonding technique, wherein an additive is provided along with the composite mycelium material. This additive may be a “meltable” material that melts at a known heat level. The cellulosic material of the composite mycelium material does not melt, such that the composite mycelium material along with the additive can be heated to the additive’s melting point. As melted, the additive can disperse within the composite mycelium material and then be cooled to harden the overall material.

**[0206]** The present disclosure is not limited to the above lists of suitable bonding agents. Other bonding agents are known in the art. The role of a bonding agent, regardless of type, is to, in part, provide several reactive sites per molecule. The type and amount of bonding agent used in the present disclosure depend on what properties are desired. In various embodiments, an effective amount of bonding agent may be used. As used herein, an “effective amount” with respect to a bonding agent refers to the amount of agent that is sufficient to provide added strength and/or other properties such as additional softness, strength, durability, and compatibility.

**[0207]** The bonding agent can be added to cultivated mycelium material that has been pressed, had one or more fibers disrupted, and/or hydroentangled. The bonding agent can be added to cultivated mycelium material before disruption of the one or more fibers or pressing. The bonding agent can be added to cultivated mycelium material during disruption of the one



or more fibers or pressing. The bonding agent can be added to cultivated mycelium material after disruption of the one or more fibers or pressing.

**[0208]** In some embodiments, a pressed cultivated mycelium material is contacted with a bonding agent. In some embodiments, a disrupted cultivated mycelium material is contacted with a bonding agent. In some embodiments, the bonding agent is added before the fibers are disrupted. In some embodiments, the bonding agent is added during the disruption of the one or more fibers. In some embodiments, the bonding agent is added after the fibers are disrupted. In some embodiments, the bonding agent is added before the cultivated mycelium material is pressed. In some embodiments, the bonding agent is added during the pressing of the cultivated mycelium material. In some embodiments, the bonding agent is added after the cultivated mycelium material is pressed.

### **Supporting Materials**

**[0209]** According to one aspect, the cultivated mycelium material or composite mycelium material may further include a supporting material, e.g., to form a bonded assembly, i.e., a laminate. As used herein, the term “supporting material” refers to any material, or combination of one or more materials, that provide support to the cultivated mycelium material or composite mycelium material. In some embodiments, the support material is a scaffold. In some embodiments, the support material is a scrim.

**[0210]** In some embodiments, the supporting material is entangled within the cultivated mycelium material or composite mycelium material, e.g., a reinforcing material. In some other embodiments, the supporting material is positioned on a surface of the cultivated mycelium material or composite mycelium material, e.g., a base material. In some embodiments, the supporting material includes, but is not limited to, a mesh, a cheesecloth, a fabric, a plurality of fibers, a knit textile, a woven textile, a non-woven textile, a knit fiber, a woven fiber, a non-woven fiber, a film, a surface spray coating, and a fiber additive. In some embodiments, a knit textile is a knit fiber. In some embodiments, a woven textile is a woven fiber. In some embodiments, a non-woven textile is a non-woven fiber. In some embodiments, the supporting material may be constructed in whole or in part of any combination of synthetic fiber, natural fiber (e.g. lignocellulosic fiber), metal, or plastic. The supporting material may be entangled, in part, within the cultivated mycelium material or composite mycelium material, e.g., using known methods of entanglement like felting or needle punching. In some aspects, the supporting material is not entangled within the cultivated mycelium material or composite mycelium material. Various methods known in the art may be used to form a laminate as described herein. In some other embodiments, the

supporting material includes a base material that is, e.g., applied to a top or bottom surface of a cultivated mycelium material or composite mycelium material. The supporting material may be attached through any means known in the art, including, but not limited to, chemical attachment, e.g., a suitable spray coating material, in particular, a suitable adhesive, or alternatively, e.g., due to their inherent tackiness.

**[0211]** A laminate according to the present disclosure may include at least one supporting material. If more than one supporting material is used, the cultivated mycelium material or composite mycelium material can include an inner layer of a sandwich of multiple layers, with the inner layer, e.g., being a supporting material such as a knit or woven or scaffold. In this instance, the supporting material would be embedded within the cultivated mycelium material or composite mycelium material.

**[0212]** Supporting materials as used herein can include scaffolds or textiles. A “scaffold” as used herein refers to any material known in the art that is distinct from the cultivated mycelium material and provides support to the cultivated mycelium material or composite mycelium material. A “scaffold” may be embedded within the cultivated mycelium material or composite mycelium material or layered on, under, or within the cultivated mycelium material or composite mycelium material. In the present disclosure, all kinds and types of scaffolds may be used, including, but not limited to films, textiles, scrims, and polymers. A “textile” as used herein refers to a type of scaffold that may be any woven, knitted, or non-woven fibrous structure. Where multiple layers are included in the cultivated mycelium material or composite mycelium material, the two or more layers may include a scaffold; or in other embodiments, the two or more layers may include a cheesecloth. Useful scaffolds include woven and non-woven scaffolds, directional and non-directional scaffolds, and orthogonal and non-orthogonal scaffolds. Useful scaffolds may include conventional scaffolds, which include a plurality of yarns oriented in the machine direction, or along the length of the scaffold, and a plurality of yarns oriented in the cross-machine direction, or across the width of the scaffold. These yarns may be referred to as the warp yarns and weft yarns, respectively. Numerous yarns can be employed including, but not limited to, fibrous materials and polymers. For example, the yarns can include, but are not limited to, fiberglass, aluminum, or aromatic polyamide polymers. In one embodiment, the scaffold includes fiberglass yarns. The scaffolds may be adhered together or locked into position using conventional bonding agents such as cross-linkable acrylic resins, polyvinyl alcohol, or similar adhesives. The scaffolds may also be mechanically entangled by or manipulated by employing techniques such as, but not limited to, needle punching. In yet another

embodiment, the scaffolds can be locked into place by weaving. A combination of supporting materials may be used according to the present disclosure.

**[0213]** In some embodiments, supporting materials may be incorporated into a cultivated mycelium material or composite mycelium material as described herein according to methods known in the art, including but not limited to the methods described in U.S. Patent No. 4,939,016 and U.S. Patent No. 6,942,711, the entirety of which are incorporated herein by reference. For example, supporting materials may be incorporated into a cultivated mycelium material or composite mycelium material via hydroentanglement. In such embodiments, supporting materials may be incorporated into a cultivated mycelium material or composite mycelium material before or after adding a bonding agent and/or a crosslinking agent. In some embodiments, a liquid such as water directed to the cultivated mycelium material or composite mycelium material through one or more pores for hydroentanglement can pass through the cultivated mycelium material or composite mycelium material. In some embodiments, the liquid is a high-pressure liquid. In some embodiments, the pressure and water flow may vary depending, in part, on the type of supporting material and pore size. In various embodiments, the water pressure is at least 100 psi, e.g., at least 200 psi, at least 300 psi, at least 400 psi, at least 500 psi, at least 600 psi, at least 700 psi, at least 800 psi, at least 900 psi, and at least 1000 psi. In various embodiments, the water pressure is about 100 psi to about 5000 psi, inclusive, e.g., about 200 psi to about 1000 psi, about 300 psi to about 2000 psi, about 400 psi to about 3000 psi, about 500 psi to about 4000 psi, and about 600 psi to about 5000 psi. In some embodiments, the water pressure is about 750 psi. In various embodiments, the one or more pores has a diameter of at least 10 microns, e.g., at least 30 microns, at least 50 microns, at least 70 microns, at least 90 microns, at least 110 microns, at least 130 microns, and at least 150 microns. In various embodiments, the one or more pores has a diameter of about 10 microns to about 150 microns, inclusive, e.g., about 20 microns to about 70 microns, about 30 microns to about 80 microns, about 40 microns to about 90 microns, about 50 microns to about 100 microns, about 60 microns to about 110 microns, and about 70 microns to about 120 microns. In some embodiments, the one or more pores has a diameter of about 50 microns.

**[0214]** The cultivated mycelium material or composite mycelium material may also include auxiliary agents that are used in foam materials. Auxiliary agents or additives include crosslinking agents, processing aids (e.g., drainage aid), dispersing agent, flocculent, viscosity reducers, flame retardants, dispersing agents, plasticizers, antioxidants, compatibility agents, fillers, pigments, UV protectors, EDC (1-ethyl-3-(3-

dimethylaminopropyl)carbodiimide hydrochloride), ammonium persulphate (APS), NHS (N-hydroxysuccinimide), fibers such as abaca fibers, microfibrillated cellulose (MFC), and the like. It is further contemplated that a foaming agent can be used to introduce a chemical bonding agent to a composite mycelium material. Such a foaming agent can make a web of composite mycelium material more porous by introducing air to the web.

### **Plasticizers**

[0215] Various plasticizers may be applied to the cultivated mycelium material or composite mycelium material to alter the mechanical properties of the cultivated mycelium material or composite mycelium material. In such embodiments, the cultivated mycelium material or composite mycelium material further includes a plasticizer. U.S. Patent No. 9,555,395 discusses adding a variety of humectants and plasticization agents. Specifically, the U.S. Patent No. 9,555,395 discusses using glycerol, sorbitol, triglyceride plasticizers, oils such as linseed oils, castor oils, drying oils, ionic and/or nonionic glycols, and polyethylene oxides. U.S. Patent Publication No. 2018/0282529 further discusses treating cultivated mycelium material or composite mycelium material with plasticizers such as glycerol, sorbitol or another humectant to retain moisture and otherwise enhance the mechanical properties of the cultivated mycelium material or composite mycelium material such as the elasticity and flexibility of the cultivated mycelium material or composite mycelium material. In such embodiments, the cultivated mycelium material or composite mycelium material is flexible.

[0216] Other similar plasticizers and humectants are well-known in the art, such as polyethylene glycol and fatliquors obtained by emulsifying natural oil with a liquid that is immiscible with oil (e.g. water) such that the micro-droplets of oil may penetrate the material. Various fatliquors contain emulsified oil in water with the addition of other compounds such as ionic and non-ionic emulsifying agents, surfactants, soap, and sulfate. Fatliquors may include various types of oil such as mineral, animal and plant-based oils. Appropriate fatliquors include, but are not limited to, Truposol® LEX fatliquor (Trumpler, Germany), Trupon® DXV fatliquor (Trumpler, Germany), Diethyloxyster dimethyl ammonium chloride (DEEDMAC), Downy fabric softener, sorbitol, m-erythritol, Tween 20 and Tween 80.

### **Tannins and Dyes**

[0217] In various embodiments of the present disclosure, it may be ideal to impart color to the cultivated mycelium material or composite mycelium material. As discussed in U.S. Patent Publication No. 2018/0282529, tannins may be used to impart a color to cultivated

mycelium material, composite mycelium material, or preserved composite mycelium material.

**[0218]** As cultivated mycelium material and/or composite mycelium material includes, in part, of chitin, it lacks the functional sites that are abundant in protein-based materials. Therefore, it may be necessary to functionalize the chitin in the cultivated mycelium material or composite mycelium material in order to create binding sites for acid and direct dyes. Methods of functionalizing chitin are discussed above.

**[0219]** Various dyes may be used to impart color to the cultivated mycelium material or composite mycelium material such as acid dyes, direct dyes, disperse dyes, sulfur dyes, synthetic dyes, reactive dyes, pigments (e.g. iron oxide black and cobalt blue) and natural dyes. In some embodiments, the cultivated mycelium material or composite mycelium material is submerged in an alkaline solution to facilitate dye uptake and penetration into the material prior to application of a dye solution. In some embodiments, the cultivated mycelium material or composite mycelium material is pre-soaked in ammonium chloride, ammonium hydroxide, and/or formic acid prior to application of a dye solution to facilitate dye uptake and penetration into the material. In some embodiments, tannins may be added to the dye solution. In various embodiments, the cultivated mycelium material or composite mycelium material may be preserved as discussed above before dye treatment or pre-treatment.

**[0220]** Depending on the embodiment, the dye solution may be applied to the cultivated mycelium material or composite mycelium material using different application techniques. In some embodiments, the dye solution may be applied to the one or more exterior surfaces of the cultivated mycelium material or composite mycelium material. In other embodiments, the cultivated mycelium material or composite mycelium material may be submerged in the dye solution.

**[0221]** In addition to pre-soaking with various solutions, agents may be added to the dye solution to facilitate dye uptake and penetration into the material. In some embodiments, ammonium hydroxide and/or formic acid with an acid or direct dye to facilitate dye uptake and penetration into the material. In some embodiments, an ethoxylated fatty amine is used to facilitate dye uptake and penetration into the processed material.

**[0222]** In various embodiments, a plasticization agent is added after or during the addition of the dye. In various embodiments, the plasticization agent may be added with the dye solution. In specific embodiments, the plasticization agent may be coconut oil, vegetable glycerol, or a sulfited or sulfated fatliquor.

**[0223]** In some embodiments, the dye solution may be maintained at a basic pH using a base such as ammonium hydroxide. In specific embodiments, the pH will be at least 9, 10, 11 or 12. In some embodiments, the pH of the dye solution will be adjusted to an acidic pH in order to fix the dye using various agents such as formic acid. In specific embodiments, the pH will be adjusted to a pH less than 6, 5, 4 or 3 in order to fix the dye.

**[0224]** In various methods, the cultivated mycelium material, composite mycelium material, and/or preserved composite mycelium material may be subject to mechanical working or agitation while the dye solution is being applied in order to facilitate dye uptake and penetration into the material. In some embodiments, subjecting the cultivated mycelium material, composite mycelium material, and/or preserved composite mycelium material to squeezing or other forms of pressure while in a dye solution enhanced dye uptake and penetration. In some embodiments, the cultivated mycelium material, composite mycelium material, and/or preserved composite mycelium material may be subject to sonication.

**[0225]** Using the methods described herein, the cultivated mycelium material or composite mycelium material may be dyed or colored such that the color of the processed cultivated mycelium material or composite mycelium material is substantially uniform. In some embodiments, the cultivated mycelium material or composite mycelium material is colored with the dye and the color of the cultivated mycelium material or composite mycelium material is substantially uniform on one or more surfaces of the cultivated mycelium material or composite mycelium material. Using the methods described above, the cultivated mycelium material or composite mycelium material may be dyed or colored such that dye and color is not just present in the surfaces of the cultivated mycelium material or composite mycelium material but instead penetrated through the surface to the inner core of the material. In such embodiments, the dye is present throughout the interior of the cultivated mycelium material or composite mycelium material.

**[0226]** In various embodiments of the present disclosure, the cultivated mycelium material or composite mycelium material may be dyed so that the cultivated mycelium material or composite mycelium material is colorfast. Colorfastness may be measured using various techniques such as ISO 11640:2012: Tests for Color Fastness – Tests for color fastness – Color fastness to cycles of to-and-fro rubbing or ISO 11640:2018 which is an update of ISO 11640:2012. In a specific embodiment, colorfastness will be measured according to the above using a Grey Scale Rating as a metric to determine rub fastness and change to sample. In some embodiments, the cultivated mycelium material or composite mycelium material will

demonstrate strong colorfastness indicated by a Grey Scale Rating of at least 3, at least 4 or at least 5.

### **Protein Sources**

[0227] In various embodiments, it may be beneficial to optionally treat the cultivated mycelium material or composite mycelium material with one or more protein sources that are not naturally occurring in the cultivated mycelium material or composite mycelium material (i.e. exogenous protein sources). In some embodiments, the one or more proteins are from a species other than a fungal species from which the cultivated mycelium material is generated. In some embodiments, the cultivated mycelium material or composite mycelium material may be optionally treated with a plant protein source such as pea protein, rice protein, hemp protein and soy protein. In some embodiments, the protein source will be an animal protein such as an insect protein or a mammalian protein. In some embodiments, the protein will be a recombinant protein produced by a microorganism. In some embodiments, the protein will be a fibrous protein such as silk or collagen. In some embodiments, the protein will be an elastomeric protein such as elastin or resilin. In some embodiments, the protein will have one or more chitin-binding domains. Exemplary proteins with chitin-binding domains include resilin and various bacterial chitin-binding proteins. In some embodiments, the protein will be an engineered or fusion protein including one or more chitin-binding domains. Depending on the embodiment, the cultivated mycelium material or composite mycelium material may be preserved, as described above, before treatment or treated without prior preservation.

[0228] In a specific embodiment of the disclosure, the cultivated mycelium material or composite mycelium material is submerged in a solution including the protein source. In a specific embodiment, the solution including the protein source is aqueous. In other embodiments, the solution including the protein source includes a buffer such as a phosphate buffered saline.

[0229] In some embodiments, the solution including the protein source will include an agent that functions to crosslink the protein source. Depending on the embodiment, various known agents that interact with functional groups of amino acids can be used. In a specific embodiment, the agent that functions to crosslink the protein source is transglutaminase. Other suitable agents that crosslink amino acid functional groups include tyrosinases, genipin, sodium borate, and lactases. In other embodiments, traditional tanning agents may be used to crosslink proteins including chromium, vegetable tannins, tanning oils, epoxies, aldehydes and syntans. As discussed above, due to toxicity and environmental concerns with

chromium, PAE other minerals may be used such as aluminum, titanium, zirconium, iron and combinations thereof with and without chromium.

[0230] In various embodiments, treatment with a protein source may occur before, after or concurrently with preserving the cultivated mycelium material or composite mycelium material, plasticizing the cultivated mycelium material or composite mycelium material and/or dyeing the cultivated mycelium material or composite mycelium material. In some embodiments, treatment with a protein source may occur before or during preservation of the cultivated mycelium material or composite mycelium material using a solution including alcohol and salt. In some embodiments, treatment with a protein source occurs before or concurrently with dyeing the cultivated mycelium material or composite mycelium material. In some of these embodiments, the protein source is dissolved in the dye solution. In a specific embodiment, the protein source will be dissolved in a basic dye solution optionally including one or more agents to facilitate dye uptake.

[0231] In some embodiments, a plasticizer will be added to the dye solution including the dissolved protein source to concurrently plasticize the cultivated mycelium material or composite mycelium material. In a specific embodiment, the plasticizer may be a fatliquor. In a specific embodiment, a plasticizer will be added to a protein source that is dissolved in a basic dye solution including one or more agents to facilitate dye uptake.

### **Coating and Finishing Agents**

[0232] After a cultivated mycelium material or composite mycelium material has been processed using any combination of methods as described above, the cultivated mycelium material or composite mycelium material may be treated with a finishing agent or coating. Various finishing agents common to the leather industry such as proteins in binder solutions, nitrocellulose, synthetic waxes, natural waxes, waxes with protein dispersions, oils, polyurethane, acrylic polymers, acrylic resins, emulsion polymers, water-resistant polymers and various combinations thereof may be used. In a specific embodiment, a finishing agent including nitrocellulose may be applied to the cultivated mycelium material or composite mycelium material. In another specific embodiment, a finishing agent including conventional polyurethane finish will be applied to the cultivated mycelium material or composite mycelium material. In various embodiments, one or more finishing agents will be applied to the cultivated mycelium material or composite mycelium material sequentially. In some instances, the finishing agents will be combined with a dye or pigment. In some instances, the finishing agents will be combined with a handle modifier (i.e. feel modifier or touch) including one or more of natural and synthetic waxes, silicone, paraffins, saponified fatty



substances, amides of fatty acids, amides esters, stearic amides, emulsions thereof, and any combination of the foregoing. In some instances, the finishing agents will be combined with an antifoam agent. In some embodiments, an external element or force is applied to the cultivated mycelium material or composite mycelium material. In such embodiments, the external element or force includes heating and/or pressing. In some embodiments, the external element or force is hot pressing. In some embodiments, an external force is applied to the cultivated mycelium material or composite mycelium material. In such embodiments, the external force includes heating and/or pressing. In some embodiments, the external force is hot pressing.

### **Processed Mycelium Material**

[0233] In various embodiments of the present disclosure, the cultivated mycelium material or composite mycelium material is sonicated, perforated, or vacuum-processed. Perforation may include needle-punching, air-punching, or water-punching.

[0234] In various embodiments of the present disclosure, the cultivated mycelium material or composite mycelium material may be mechanically processed and/or chemically processed in different ways both in solution (i.e. dye solution, protein solution or plasticizer) and after the cultivated mycelium material or composite mycelium material has been removed from the solution. In such embodiments, the method includes mechanically processing and/or chemically processing the cultivated mycelium material or composite mycelium material, wherein a processed mycelium material is produced.

[0235] While the cultivated mycelium material or composite mycelium material is in a solution or dispersion it may be agitated, sonicated, squeezed or pressed to ensure uptake of the solution. The degree of mechanical processing will depend on the specific treatment being applied and the level of fragility of the cultivated mycelium material or composite mycelium material at its stage in processing. Squeezing or pressing of the cultivated mycelium material or composite mycelium material may be accomplished by hand wringing, mechanical wringing, a platen press, a lino roller or a calendar roller.

[0236] Similarly, as discussed above, the cultivated mycelium material or composite mycelium material may be pressed or otherwise worked to remove solution from the composite mycelium material after it is removed from solution. Treating with a solution and pressing the material may be repeated several times. In some embodiments, the material is pressed at least two times, at least three times, at least four times, or at least five times.

[0237] Once the cultivated mycelium material or composite mycelium material is fully dried (e.g. using heat, pressing or other desiccation techniques described above), the cultivated

mycelium material or composite mycelium material may be subject to additional mechanical- and/or chemical-processing. Depending on the technique used to treat the cultivated mycelium material or composite mycelium material and the resultant toughness of the cultivated mycelium material or composite mycelium material, different types of mechanical processing may be applied including but not limited to sanding, brushing, plating, staking, tumbling, vibration and cross-rolling.

**[0238]** In some embodiments, the cultivated mycelium material or composite mycelium material may be embossed with any heat source or through the application of chemicals. In some embodiments, the cultivated mycelium material or composite mycelium material in solution may be subjected to additional chemical processing, such as, e.g., being maintained at a basic pH using a base such as ammonium hydroxide. In specific embodiments, the pH will be at least 9, 10, 11 or 12. In some embodiments, the pH of the cultivated mycelium material or composite mycelium material in solution will be adjusted to an acidic pH in order to fix the composite mycelium material using various agents such as formic acid. In specific embodiments, the pH will be adjusted to a pH less than 6, 5, 4 or 3 in order to fix the cultivated mycelium material or composite mycelium material.

**[0239]** Finishing, coating and other steps may be performed after or before mechanical processing and/or chemical processing of the dried cultivated mycelium material or composite mycelium material. Similarly, final pressing steps, including ornamental steps such as embossing or engraving, may be performed after or before mechanical processing and/or chemical processing of the dried cultivated mycelium material or composite mycelium material.

**[0240]** **FIG. 21** illustrates a flow chart of a method 200 for converting raw mycelium material into a crust material that can be treated according to a desired finishing process (e.g., finishing coatings, ornamental steps, final pressing steps) based on the end-use application of the material. The raw mycelium material can be dried, refrigerated, or frozen material made according to any of the processes described herein. The raw material may optionally be split on the top and/or bottom to provide a mycelium panel having the desired thickness. Splitting can also provide a smoother surface at the cut. The crust material can be dyed, plasticized, dried and/or otherwise post-processed as described herein.

**[0241]** Still referring to **FIG. 21**, at step 202 a pre-finishing treatment solution can be prepared based on the dimensions and mass of the mycelium material. In one example, the pre-finishing treatment solution can be prepared at a volume of about 6 mL per gram of wet mycelium material or 20 mL per gram of dried mycelium material. The pre-finishing

treatment solution can include one or more dyes, tannins, and/or plasticizers (e.g. fatliquors) in a suitable solvent, such as water. In one example, the pre-finishing treatment solution includes one or more dyes and/or tannins and one or more fatliquors. The amount of dye added can be based on the particular type of dye and the desired color of the resulting product. An exemplary pre-finishing treatment solution includes: one or more acid dyes at a concentration to produce the desired color; about 25 g/L vegetable tannins; about 6.25 g/L Truposol® LEX fatliquor (Trumpler, Germany); and about 18 g/L to about 19 g/L Trupon® DXV fatliquor (Trumpler, Germany).

**[0242]** At step 204, the pre-finishing treatment solution can be applied to the mycelium material through a combination of soaking and pressing processes. In one example, the material is soaked in the pre-finishing treatment solution for a predetermined period of time (e.g., 1 minute) and then moved through a pressing system. An example of a suitable pressing system includes moving the soaked material through a pair of rollers that are spaced to provide the desired degree of pressing to the material with each pass between the rollers. The material can be pushed and/or pulled through the rollers. The rate at which the material is passed through the rollers can vary. According to one aspect of the present disclosure, the soaking and pressing process at step 204 can be repeated one or more times (e.g., 1, 2, 3, 4, 5 or more times).

**[0243]** Following the pre-finishing treatment application at 204, the material can proceed to a fixation process 206. The fixation process 206 includes adjusting the pH of the pre-finishing treatment solution to a pH suitable for fixing the dyes. In one example, the fixation process is an acid fixing process that includes decreasing the pH of the pre-finishing treatment solution. Non-limiting examples of acids suitable for acid fixing include acetic acid and formic acid. For example, acetic acid can be used to decrease the pH of the exemplary pre-finishing treatment solution described above to a pH of  $3.15 \pm 1.0$ .

**[0244]** At step 210, the mycelium material can be soaked in the pH adjusted pre-finishing treatment solution and flattened in a manner similar to that described above with regard to step 204. The soaking and pressing process at step 210 can be repeated one or more times (e.g., 1, 2, 3, 4, 5 or more times).

**[0245]** Step 212 includes a final, extended soak of the material in the pH adjusted pre-finishing treatment solution. The material can be inverted about halfway through the extended soak period. The extended soak period can be from about 30 minutes to 1 hour or more. When the extended soak time period is complete, at 214 the material can be processed

through a final pressing process. The final pressing process can be the same or different than that described above with regard to steps 204 and 210.

[0246] Following the fixation process 206, at step 216 the material can be dried with or without heating. The material can be held generally vertically, horizontally, or any orientation therebetween during the drying step 216. The material may optionally be restrained during the drying step. For example, one or more clamps may be used to restrain all or a portion of the material during drying. In some examples, the drying step 216 is conducted at ambient conditions.

### **Systems of Mycelium Compositions**

[0247] Provided herein are systems, including a mycelium composition comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; an apparatus having one or more projections; a solution; and a bonding agent.

[0248] In some or most embodiments, the mycelium composition comprises a solid content of 1-10%. In some or most embodiments, the mycelium composition comprises a solid content of 3-7%. In some or most embodiments, the mycelium composition comprises a solid content of 4-6%. In some or most embodiments, the mycelium composition comprises a solid content of 1-6%. In some embodiments, the mycelium composition comprises a solid content of at least 1%, of at least 2%, of at least 3%, of at least 4%, of at least 5%, of at least 6%, of at least 7%, of at least 8%, of at least 9%, or of at least 10%.

[0249] In some or most embodiments, the mycelium composition comprises a hydration content of 1-10%. In some or most embodiments, the mycelium composition comprises a hydration content of 3-7%. In some or most embodiments, the mycelium composition comprises a hydration content of 4-6%. In some or most embodiments, the mycelium composition comprises a hydration content of 1-6%. In some embodiments, the mycelium composition comprises a hydration content of at least 1%, of at least 2%, of at least 3%, of at least 4%, of at least 5%, of at least 6%, of at least 7%, of at least 8%, of at least 9%, or of at least 10%.

[0250] In some embodiments, the solution comprises water. In some embodiments, the one or more projections comprises needles. In some embodiments, the needles comprise a diameter of 0.05-1 mm. In some embodiments, the needles comprise a diameter of 0.07-0.5 mm. In some embodiments, the apparatus comprises a needle density of 1-120 needle punctures per square cm. In such embodiments, the apparatus may be a handheld tool or automated.

[0251] In some embodiments, the one or more projections comprises metal or the like. In some embodiments, the one or more projections comprises water. In some embodiments, the one or more projections is positioned perpendicular to the mycelium composition. In some embodiments, the one or more projections subjects pressure to the mycelium composition, thus orienting the one or more disrupted fibers in the z-direction. In some embodiments, the needles may have barbs. In some embodiments, the needles may be barbless.

### **Mechanical Properties of Composite Mycelium Material**

[0252] Various methods of the present disclosure may be combined to provide processed cultivated or composite mycelium material that has a variety of mechanical properties. In such embodiments, the mycelium material includes a mechanical property, e.g., a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, and/or a slit tear strength. Other mechanical properties include, but are not limited to, elasticity, stiffness, yield strength, ultimate tensile strength, ductility, hardness, toughness, creep resistance, and other mechanical properties known in the art.

[0253] In various embodiments, the processed mycelium material may have a thickness that is less than 1 inch, less than 1/2 inch, less than 1/4 inch or less than 1/8 inch. In some embodiments, the composite mycelium material has a thickness of about 0.5 mm to about 3.5 mm, inclusive, e.g., about 0.5 mm to about 1.5 mm, about 1 mm to about 2.5 mm, and about 1.5 mm to about 3.5 mm. The thickness of the material within a given piece of material may have varying coefficients of variance. In some embodiments, the thickness is substantially uniform to produce a minimal coefficient of variance.

[0254] In some embodiments, the mycelium material can have a peel resistance or a T-peel resistance force of at least 1 N/cm, at least 5 N/cm, at least 6.25 N/cm, at least 10 N/cm, at least 12 N/cm, at least 15 N/cm, at least 20 N/cm, at least 25 N/cm, at least 30 N/cm, at least 35 N/cm, at least 40 N/cm, at least 45 N/cm, at least 50 N/cm. In some embodiments, the composite mycelium material can have an average T peel value of at about 8-12 N/cm or about 10-12 N/cm. In some embodiments, the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

[0255] In some embodiments, the mycelium material can have an initial modulus of at least 20 MPa, at least 25 MPa, at least 30 MPa, at least 40 MPa, at least 50MPa, at least 60 MPa, at least 70 MPa, at least 80 MPa, at least 90 MPa, at least 100 MPa, at least 110 MPa, at least 120 MPa, at least 150 MPa, at least 175 MPa, at least 200 MPa, at least 225 MPa, at least 250 MPa, at least 275 MPa, or at least 300 MPa. In some embodiments, the mycelium material may have an initial modulus of about 0.5 MPa to about 300 MPa, inclusive, for example

about 0.5 MPa to about 10 MPa, about 1 MPa to about 20 MPa, about 10 MPa to about 30 MPa, about 20 MPa to about 40 MPa, about 30 MPa to about 50 MPa, about 40 MPa to about 60 MPa, about 50 MPa to about 70 MPa, about 60 MPa to about 80 MPa, about 70 MPa to about 90 MPa, about 80 MPa to about 100 MPa, about 90 MPa to about 150 MPa, about 100 MPa to about 200 MPa, and about 150 MPa to about 300 MPa. In specific embodiments, the mycelium material has an initial modulus of 0.8 MPa. In one aspect, the mycelium material has an initial modulus of 1.6 MPa. In another aspect, the mycelium material has an initial modulus of 97 MPa.

**[0256]** In some embodiments, the mycelium material can have a wet tensile strength of about 0.05 MPa to about 50 MPa, inclusive, e.g., about 1 MPa to about 5 MPa, about 5 MPa to about 20 MPa, about 10 MPa to about 30 MPa, about 15 MPa to about 40 MPa, and about 20 MPa to about 50 MPa. In specific embodiments, the mycelium material may have a wet tensile strength of about 5 MPa to about 20 MPa. In one aspect, the mycelium material has a wet tensile strength of about 7 MPa. In a specific embodiment, the wet tensile strength will be measured by ASTM D638.

**[0257]** In some embodiments, the mycelium material can have a breaking strength (“ultimate tensile strength”) of at least 1.1 MPa, at least 6.25 MPa, at least 10 MPa, at least 12 MPa, at least 15 MPa, at least 20 MPa, at least 25 MPa, at least 30 MPa, at least 35 MPa, at least 40 MPa, at least 45 MPa, at least 50 MPa.

**[0258]** In some embodiments, the mycelium material has an elongation at the break of less than 2%, less than 3%, less than 5%, less than 20%, less than 25%, less than 50%, less than 77.6%, or less than 200%. For example, the mycelium material may have an elongation at the break of about 1% to about 200%, inclusive, e.g., about 1% to about 25%, about 10% to about 50%, about 20% to about 75%, about 30% to about 100%, about 40% to about 125%, about 50% to about 150%, about 60% to about 175%, and about 70% to about 200%.

**[0259]** In some embodiments, the initial modulus, ultimate tensile strength, and elongation at the break are measured using ASTM D2209 or ASTM D638. In a specific embodiment, the initial modulus, ultimate tensile strength, and elongation at the break are measured using a modified version ASTM D638 that uses the same sample dimension as ASTM D638 with the strain rate of ASTM D2209.

**[0260]** In some embodiments, the mycelium material can have a single stitch tear strength of at least 15N, at least 20N, at least 25N, at least 30N, at least 35N, at least 40N, at least 50N, at least 60N, at least 70N, at least 80N, at least 90N, at least 100N, at least 125N, at least

150N, at least 175N, or at least 200N. In a specific embodiment, the tongue tear strength will be measured by ASTM D4786.

**[0261]** In some embodiments, the mycelium material can have a double stitch tear strength of at least 20N, at least 40N, at least 60N, at least 80N, at least 100N, at least 120N, at least 140N, at least 160N, at least 180N, or at least 200N. In a specific embodiment, the tongue tear strength will be measured by ASTM D4705.

**[0262]** In some embodiments, the mycelium material can have a tongue tear strength (also referred to as slit tear strength) of at least 1.8N, at least 15N, at least 25N, at least 35N, at least 50N, at least 75N, at least 100N, at least 150N, or at least 200N, as measured by ISO-3377. In a specific embodiment, the tongue tear strength will be measured by ASTM D4704. In some embodiments, the mycelium material may have a slit tear strength of at least 1N, at least 20N, at least 40N, at least 60N, at least 80N, at least 100N, at least 120N, at least 140N, at least 160N, at least 180N, or at least 200N, as measured by ISO-3377-2. In one aspect, the mycelium material has a slit tear strength of about 1N to about 200N, inclusive, e.g., about 10N to about 30N, about 20N to about 40N, about 30N to about 50N, about 40N to about 60N, about 50N to about 70N, about 60N to about 80N, about 70N to about 90N, about 80N to about 100N, about 90N to about 110N, about 100N to about 120N, about 110N to about 130N, about 120N to about 140N, about 130N to about 150N, about 140N to about 160N, about 150N to about 170N, about 160N to about 180N, about 170N to about 190N, and about 180N to about 200N, as measured by ISO-3377-2.

**[0263]** In some embodiments, the mycelium material has a flexural modulus (Flexure) of at least 0.2 MPa, at least 1 MPa, at least 5 MPa, at least 20 MPa, at least 30 MPa, at least 50 MPa, at least 80 MPa, at least 100MPa, at least 120MPa, at least 140MPa, at least 160MPa, at least 200MPa, at least 250MPa, at least 300MPa, at least 350MPa, at least 380MPa. In a specific embodiment, the compression will be measured by ASTM D695. In some embodiments, the mycelium material has a flexural modulus of about 5-10 MPa. In some embodiments, the mycelium material has a flexural modulus of about 10-20 MPa. In some embodiments, the mycelium material has a flexural modulus of about 20-30 MPa. In some embodiments, the mycelium material has a flexural modulus of about 30-40 MPa. In some embodiments, the mycelium material has a flexural modulus of about 10-11 MPa. In some embodiments, the mycelium material has a flexural modulus of about 10 MPa.

**[0264]** In various embodiments of the present disclosure, the mycelium material has different absorption properties measured as a percentage mass increase after soaking in water. In some embodiments, the percent mass increase after soaking in water for 1 hour is less than 1%, less

than 5%, less than 25%, less than 50%, less than 74%, or less than 92%. In a specific embodiment, the percent mass increase after soaking in water after 1 hour is measured using ASTM D6015.

### **Methods of Producing a Mycelium Material**

[0265] Also provided is a method of producing a mycelium material as described herein. According to one embodiment of the disclosure, a mycelium material can be produced by generating a cultivated mycelium material including one or more fibers; disrupting the cultivated mycelium material including the one or more fibers; and adding a bonding agent to the cultivated mycelium material (e.g., by contacting the disrupted cultivated mycelium material with a solution comprising a bonding agent); thus producing the composite mycelium material. In some embodiments, the cultivated mycelium material includes one or more disrupted fibers. In some embodiments, the one or more disrupted fibers has a length. In such embodiments, the one or more disrupted fibers has a length of about 0.1 mm to about 5 mm.

[0266] In some aspects, provided herein is a method of producing a composite mycelium material as described herein. According to one embodiment of the disclosure, a mycelium material can be produced by generating a mycelium material comprising one or more disrupted fibers; adding a solution to the mycelium material to produce a mycelium composition; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; adding a bonding agent to the mycelium composition; and draining the solution; thus producing the composite mycelium material; thus, producing the composite mycelium material. According to one embodiment of the disclosure, a method includes, generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and needling the mycelium material. According to one embodiment of the disclosure, a method includes, generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and manipulating the mycelium material. According to one embodiment of the disclosure, a method includes, generating a mycelium composition comprising a mycelium material, a solution, and a bonding agent; and manipulating the mycelium material. According to one embodiment of the disclosure, a method of producing a composite mycelium material includes, generating a mycelium material; and manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction. According to one embodiment of the disclosure, a method of producing a composite mycelium material



includes, generating a mycelium material comprising one or more disrupted fibers; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; and adding a bonding agent to the mycelium composition.

**[0267]** In another aspect, a mycelium material can be produced by generating a cultivated mycelium material; pressing the cultivated mycelium material; and adding a bonding agent to the cultivated mycelium material (e.g., by contacting the pressed cultivated mycelium material with a solution comprising a bonding agent), thus producing the composite mycelium material.

**[0268]** In some embodiments, the generating comprises generating cultivated mycelium material on a solid or liquid substrate. In some embodiments, the method further comprises incorporating a supporting material into the mycelium material. In some embodiments, the supporting material is a reinforcing material. In some embodiments, the reinforcing material is entangled within the composite mycelium material. In some embodiments, the reinforcing material is a base material. In some embodiments, the disrupting comprises disrupting the one or more fibers by a mechanical action. In some embodiments, the method further comprises adding one or more proteins that are from a species other than a fungal species from which the cultivated mycelium material is generated. In some embodiments, the method further comprises adding a dye to the cultivated mycelium material or the mycelium material. In some embodiments, the method further comprises adding a plasticizer to the cultivated mycelium material or the mycelium material. In some embodiments, the method further comprises adding a tannin to the cultivated mycelium material or the mycelium material. In some embodiments, the method further comprises adding a finishing agent to the mycelium material. In some embodiments, the method further comprises determining a mechanical property of the mycelium material, wherein the mechanical property includes, but is not limited to, wet tensile strength, initial modulus, elongation percentage at the break, thickness, slit tear strength, elasticity, stiffness, yield strength, ultimate tensile strength, ductility, hardness, toughness, creep resistance, and the like. For example, the mycelium material has a wet tensile strength of about 0.05 MPa to about 50 MPa, an initial modulus of about 0.5 MPa to about 300 MPa, an elongation percentage at the break of about 1% to about 200%, a thickness of about 0.5 mm to about 3.5 mm, and/or a slit tear strength of about 1 N to about 200 N.

## EXAMPLES

[0269] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

### Materials and Methods

[0270] The following material and methods were used.

#### *Composite Mycelium Material Samples*

[0271] For each of the samples described below, components were blended together in a blender (Vitamix or Blendtec Pro 800). The concentration of the slurry ranged from 0.5 - 2.5 w/v% depending on the experiment. The mycelium was blended to produce a uniform slurry, typically on setting 5 for 90 seconds. After blending, the slurry was sieved on a 500-micron sieve to remove fines and soluble components. The sieved mycelium was then resuspended back to 1 w/v%, stirred manually to disperse, and sieved/resuspended two more times. After the final resuspension, sodium dodecyl sulfate (SDS) solution in DI water was added and the slurry was blended on setting 5 for 10 seconds to create foam. The final concentration of sodium dodecyl sulfate in the slurry was typically 0.002 w/v%. The foam remained trapped in the slurry and helped to create porosity in the web after wetlaying.

[0272] The resulting slurry was poured into a mold resting on a forming cloth that let water pass through. A vacuum was applied for about 1-5 minutes. The vacuum level was maintained at 75 mbar for all conditions. The blended mycelium was peeled off and water was added to resuspend the mycelium. The slurry was then transferred to a 4 L glass beaker before adding 30% v/v hydrogen peroxide to bring the final concentration to 2.5%. After overnight bleaching, the samples were quenched with 300  $\mu$ L of Sigma Aldrich Catalase or Novozymes Catalase, washed with water, and drained. For all samples, 26 g of Elite-Plus

binder and 16 g of StarSoft Bis-45 was added. The slurry composition was filtered and mycelium solids were recovered. Water was added to make the total slurry volume of 3 L and the solids were then wetlaid on a forming cloth. Prior to wetlaying the material, the slurry was subjected to needling in some embodiments.

[0273] The following samples were used:

[0274] 15% abaca web: 29.8 g of biomass was used, in addition to 5.3 g of Abaca. A vacuum was applied for 2 minutes. 2000 g of liquid had drained after 2 minutes of vacuum. After needling, another 200-300 g of liquid drained out.

[0275] 10% lyocell web: 1mm lyocell was used for this sample. 3.5 g lyocell and 21.2 g of prebleached biomass was used. A vacuum was applied for 60 seconds and about 2.5 kg of liquid was drained. At the end of needling another 100-200 g of liquid drained from the web.

[0276] 15% lyocell web: 1mm lyocell was used for this sample. 20g of biomass and 5.3 g of lyocell was used. A vacuum was applied for 60 seconds and about 2.5 kg of liquid was drained. At the end of needling, another 100-200 g of liquid drained from the web.

[0277] 20% lyocell web: 1mm lyocell was used for this sample. 18.8 of biomass and 7.0 g of lyocell was used. A vacuum was applied for 60 seconds and about 2.5 kg of liquid was drained. At the end of needling, another 100-200 g of liquid drained from the web.

#### *Fiber Additives*

[0278] Abaca: 2-inch pieces were cut out from a F30 Premium Abaca sheet and weighed. Pieces were added into a blender and 700 mL of water was added. The mixture was blended for 90 seconds before adding the desired amount of biomass. The mixture was blended for another 10 seconds.

[0279] Lyocell: A certain amount of lyocell fiber was added to 1 L water. The mixture was blended for 5 seconds and transferred to a 4 L beaker. The mixture was stirred at 300 rpm for 5 minutes. Bleached biomass was resuspended in 500 mL water and blended for 15 seconds. Mycelium slurry was added to the lyocell slurry and stirred at 300 rpm for 5 minutes.

[0280] Microfibrillated cellulose: A certain amount of F29 MFC (PC110A) was weighed and added to a blender, before adding 700 mL water. The mixture was blended for 90 seconds before adding the desired amount of biomass. The mixture was blended for another 10 seconds.

#### *Needling of the Slurry*

[0281] A tool with needle projections was applied 400-600 times on each web, where it was pressed down vertically and lifted back up, moving it along the surface rough about 1 cm at a time. Care was taken not to move the needle tool horizontally when it was immersed in

slurry. After needling, vacuum was applied for a total of 4 minutes. A forming cloth was applied to the surface and the surface was smoothed with a flat object.

[0282] After pouring the slurry, vacuum was applied to dewater it. The vacuum was shut off when a consistency of 3.5% to 6.5% was reached. The moist web was then needled thoroughly, with the needles pushing through the whole thickness until they impacted the forming cloth. The needling density was 30-200 needles per cm<sup>2</sup>. The needles used included barbed twisted needles, crown needles, and barbless needles.

[0283] For some samples, the drainage during needling was stopped by blocking the forming cloth.

#### *Wet laying*

[0284] A bench scale wetlay apparatus was constructed to produce webs from slurry. The apparatus consisted of a 150 mm diameter Buchner funnel lined with a forming cloth, attached to a 4 L vacuum flask. The vacuum flask was attached to a vacuum pump (Vacuubrand VARIO PC 3001 Select) which allowed control over the vacuum setpoint with a precision of 1 torr. A 3-way valve in between the vacuum pump and the flask controlled whether the flask was under vacuum or vented to atmosphere. Wetlaying was accomplished by pouring the slurry into the Buchner funnel and subsequent vacuum filtration at a defined vacuum level (typically 600 torr) until no more water was observed dripping out of the funnel (typically 90 seconds). The resulting web had a moisture content between 75-80 wt%. The web was then manually peeled from the forming cloth and dried at 45 °C with convection until its mass reached a steady state (typically several hours).

#### *Pressing*

[0285] Two aluminum plates were pre-heated to 90°C on a manual heated press (Carver 4120). Each sample was placed between the aluminum plates and pressed for 2 minutes at 40kN.

#### *Curing*

[0286] The two aluminum plates were pre-heated to 120°C on a manual heated press (Carver 4120). Each panel was placed directly on the aluminum plate before being pressed for 30 minutes at 20kN. After pressing, each sample was placed in a convection oven for 30 minutes at 90°C with the flap set to 100%.

#### *Hot-pressing of mycelium materials*

[0287] The mechanical properties of mycelium materials such as the resistance to tear and delamination can be improved by the addition of a binder and also by hot-pressing. Hot-

pressing increases the areal density and fuses the mycelium fibers together which results in improved mechanical properties.

[0288] Prior to testing, the samples were conditioned at 65% relative humidity for 24 hours. Parallel samples were also incubated with 0.5% S-10 vinyl acetate-ethylene prior to hot pressing. T-peel testing was performed according to ASTM D1876 and the slit tear properties were tested according to ISO 3377-2 using universal tensile tester.

#### *Lamination*

[0289] Lamination, scrim incorporation, and heat pressing to compress the material and smooth the surface could be performed concurrently. To laminate two crusted webs together, a thin layer of DUR-O-SET Elite 22 VAE was first applied to one side of each web using a brush or roller. The typical application was approximately 9 mg/cm<sup>2</sup> on a wet basis. After application, the binder was exposed to ambient conditions for roughly 30 seconds. The webs were then pressed together (binder side inward) using a manual heated press (Carver 4120) at 80°C for 1 minute to a final thickness (controlled by placing shims in between the press platens) ranging from 1 - 3 mm. A scrim could be incorporated into the material (typically 95 gsm woven cotton) by placing it in between the two webs before pressing. After pressing, the laminate was dried at 45°C with convection until its mass reached a steady state (typically several hours) to remove residual water from the binder before curing.

#### *Hydroentanglement of mycelium materials*

[0290] Hydroentanglement of mycelia webs was performed to some samples. Samples were hydroentangled with 50 micrometer water jets with 1000 psi. Each sample was hydroentangled with multiple passes in both the vertical and horizontal direction.

#### *Wet Tensile Testing*

[0291] The standard test method for tensile testing of composite mycelium materials was performed according to the ASTM D638 protocol. Samples were conditioned at 65 ± 2%RH for 24 hours. In some embodiments, samples were soaked in water for 1 hour at room temperature prior to testing. ASTM standard dies such as an ASTM D638 type IV dogbone was used to punch out samples. Each sample's thickness, width, and mass were measured. The appropriate tensile test method was then run on a universal testing machine from Zwick (zwickLine Materials Testing Machine Z5.0 TH).

#### *Peel Resistance (T-Peel)*

[0292] The methods for T-peel testing of mycelium materials broadly followed ASTM D1876 using a Zwick ProLine universal testing system. The main output metric was the maximum peel force per unit of bond line length, among other metrics. Samples were

conditioned at  $6565 \pm 2\%$  RH for 24 h following ASTM D1610. Samples were prepared by applying adhesive backings and cutting specimens to size. Each specimen's width and thickness were taken. The testing system was configured by installing the load cell and grips on the Zwick ProLine. The appropriate test method was then run on the Zwick ProLine.

#### *Slit Tear Test*

[0293] The standard test method for slit tear testing of composite mycelium materials was performed according to the ISO 3377-2 protocol, using the universal testing system from Zwick. Samples were conditioned at  $65 \pm 2\%$  RH for 24 h. In some embodiments, samples were equilibrated at 65% relative humidity for 16 h at room temperature prior to testing. The ISO 3377-2 die was used to cut out 1"x2" specimens with a center slit. Each specimen's thickness and mass were measured. The appropriate slit tear test method was then run on the universal mechanical tester from Zwick.

#### *Scanning Electron Microscopy (SEM) Imaging and Fourier Transform (FT) Analysis*

[0294] Scanning electron microscopy (SEM) used a focus electron beam to assess the morphology of materials through the secondary electrons. The electron beam was scanned in a raster pattern to collect micrographs at scales between 1 mm and 10 nm or between 10X and 100,000X magnification. The SEM method used low vacuum (1 to 10 torr), avoiding the need for dehydrating or sputter coating biological samples.

[0295] SEM micrographs were then cropped to a square size and analyzed using Fourier transform (FT). The FT of an image represented a sum of complex exponentials of varying magnitudes (i.e. intensity), frequencies, and phase angle. The resulting frequency domain revealed the periodicity in the image as a function of the angle. Because aligned fibers gave rise to a periodicity orthogonal to the fiber axis, the frequency domain was used to quantify the preferential fiber alignment. The polar coordinate frequency domain image was then transformed into Cartesian coordinates to extract the profile of the azimuthal distribution. The azimuthal distribution was then fitted with a Gaussian peak to calculate the full-width at half-maximum and the maximum angular position.

#### **Example 1: SEM micrographs of composite mycelium materials**

[0296] Composite mycelium material that has not been needled has a relatively flat, layer-by-layer structure as shown in **FIG. 2**. Only a few fibers appear to bridge the peel gap as a result of a manual peel. Crack and peel propagation tend to follow the path of least resistance. Mycelium fibers and additive fibers of composite mycelium material samples tend to lie in the x/y plane. **FIG. 3** shows an SEM micrograph of a sample with 20% 10 mm lyocell fibers

subjected to a manual peel. **FIG. 4** shows an SEM micrograph of a composite mycelium material sample without fiber additives subjected to a manual peel. The tip of the peel has been enlarged for illustration purposes. As shown in the figure, the layers of the mycelium material appear to lie in the x/y plane.

[0297] After the composite mycelium material samples were needled, images were taken to show that there were small bubbles and a wavy texture in the cross section, as shown in **FIG. 5** and **FIG. 6**. There were additional fibers in the z-direction, bridging the gap between the peeled surfaces, as shown in **FIG. 7** and **FIG. 8**. The samples depicted were needled, composite mycelium material samples with 15% abaca fibers. **FIG. 9** shows an SEM micrograph of a needled composite mycelium material with 15% lyocell fibers subjected to a manual peel. This sample portrayed a wavy texture and a peel that followed along the wavy layers. There were bubbles present in the samples, but the orientation of the bubbles was modified as a result of the needling. Additional disruption was introduced by needling the composite mycelium material samples at the slurry stage. There were noticeably more fibers oriented in the z-direction plane, as compared to that in samples not subjected to any needling.

#### **Example 2: Tensile properties of needled composite mycelium material samples**

As shown in the following results, by incorporating a step of needling, the resulting average peel force (N/cm) of mycelium samples that have been needled was significantly higher than that of mycelium samples that were not needled, as shown in **FIG. 10**. The average peel force (N/cm) of composite mycelium materials with 15% abaca fibers was around 4 N/cm compared to around 11 N/cm for needled composite mycelium materials with 15% abaca fibers. The average peel force (N/cm) of composite mycelium materials with 15% lyocell fibers was around 4 N/cm compared to around 10 N/cm for needled composite mycelium materials with 15% lyocell fibers.

##### *Needling of 15% Abaca Webs*

[0298] Samples with 15% abaca fibers were produced as described above. Both twisted and crown needles were used. Peel force of the needled composite mycelium materials increased compared to that of composite mycelium materials without fiber additives or needling. As shown in **FIG. 11**, the average peel force (N/cm) for 15% abaca fiber samples was above 10 N/cm using either twisted or crown needles, as compared to about 4 N/cm for 15% abaca fiber samples that were not subjected to needling.

##### *Needling of 10% and 15% Lyocell Webs*

[0299] Samples with 10% and 15% Lyocell fibers were produced as described above. The slurry was needled after 60-90 seconds of vacuum because slurry with lyocell fibers drained faster than slurry with abaca fibers. Around 2.5 kg of liquid drained during the first vacuum of all samples prior to needling. Webs lost another 100-200 g of liquid during needling. Four categories were compared: (1) 10% lyocell mycelium samples needled with twisted needles; (2) 10% lyocell mycelium samples needled with crown needles; (3) 15% lyocell mycelium samples needled with crown needles; and (4) control (10% lyocell, not needled). The hand feel and aesthetics of needled lyocell webs had a favorable drape and a soft texture.

[0300] As shown in **FIG. 12**, the maximum peel force (N/cm) for 10% lyocell samples needled with twisted needles, 10% lyocell samples needled with crown needles, and 15% lyocell samples needled with crown needles were compared. The maximum peel force for all samples was above 15 N/cm. As shown in **FIG. 13**, the average peel force (N/cm) was above 10 N/cm for the same samples. Not shown in the graph is the peel resistance of about 4 N/cm for control samples (composite mycelium material with 10% lyocell fibers).

[0301] As shown in **FIG. 14**, the maximum single edge force (N/cm) for 10% lyocell samples needled with twisted needles, 10% lyocell samples needled with crown needles, and 15% lyocell samples needled with crown needles were compared. The maximum single edge force for all samples was above 11 N/cm. As shown in **FIG. 15**, the average single edge force (N/cm) was above 7 N/cm for the same samples. Not shown in the graph is the single edge force of 1 N/cm for control samples (composite mycelium material with 10% lyocell fibers).

### **Example 3: Needling of the composite mycelium material with different needle types**

[0302] The following three types of needles were used in this experiment: twisted needles, 46-gauge crown needles, and 40-gauge barbless needles, all supplied by Groz-Beckert. For the twisted needle, the point to the first barb was 3.18 mm, the total barb depth was 0.040 mm, the barb to spacing was 2.1 mm and the barb angle was 21 degrees. For the crown needles, there were 3 barb needles with all barbs around 2.0 mm from the tip. The total barb depth of the crown needles was 0.024 mm and the bar angle was 18 degrees. The needling tool used included a plate with 100 needles arranged in a 10x10 cm array. A baseline slurry was prepared as described above. The needling tool was pressed down in the slurry until the needles touched the forming cloth, as shown in **FIG. 16**. The surface was smoothed at the top with the handheld tool. Needling of the samples significantly increased the peel force of the materials.



[0303] As shown in **FIG. 17**, the average peel force (N/cm) of composite mycelium material samples without fiber additives that were needled was about 7.3 N/cm, which was about 2.5 times higher than the average peel force of composite mycelium material samples that were not needled.

[0304] As shown in **FIG. 18**, the average single edge force (N/cm) of composite mycelium material samples without fiber additives that were needled was about 1.8 N/cm, which was slightly higher than the average peel force of composite mycelium material samples that were needled. Such samples that were not needled had a single edge tear force of about 1 N/cm.

[0305] As shown in **FIG. 19**, the maximum single edge force (N/cm) of composite mycelium material samples without fiber additives that were not needled was about 9 N/cm. There were no apparent significant differences in mechanical properties of composite mycelium materials that were needled with twisted needles, crown needles, and/or barbless needles.

**Additional aspects of the present disclosure include:**

[0306] According to a first aspect of the present disclosure, a composite mycelium material, includes: a mycelium material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and a bonding agent.

[0307] According to a second aspect of the present disclosure, the composite mycelium material of aspect 1, wherein the mycelium material has been generated on a solid or liquid substrate.

[0308] According to a third aspect of the present disclosure, the composite mycelium material of aspects 1 or 2, wherein the one or more fibers has a length of 0.1 mm to 5 mm.

[0309] According to a fourth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 3, wherein the one or more disrupted fibers is manipulated by needle punching, felting, hydroentangling, or needling.

[0310] According to a fifth aspect of the present disclosure, the composite mycelium material of aspect 4, wherein the one or more fibers are needled.

[0311] According to a sixth aspect of the present disclosure, composite mycelium material of any one of aspects 1 to 5, wherein the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix.

[0312] According to a seventh aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 6, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.

[0313] According to an eighth aspect of the present disclosure, the composite mycelium material of aspect 7, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.

[0314] According to a ninth aspect of the present disclosure, the composite mycelium material of aspect 7, wherein the bonding agent is a vinyl acetate-acrylic copolymer.

[0315] According to a tenth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 9, wherein the composite mycelium material further comprises a reinforcing material.

[0316] According to an eleventh aspect of the present disclosure, the composite mycelium material of aspect 10, wherein the reinforcing material is entangled within the composite mycelium material.

[0317] According to a twelfth aspect of the present disclosure, the composite mycelium material of aspect 10, wherein the reinforcing material comprises a base material.

[0318] According to a thirteenth aspect of the present disclosure, the composite mycelium material of aspect 12, wherein the base material is positioned on one surface of the composite mycelium material.

[0319] According to a fourteenth aspect of the present disclosure, the composite mycelium material of aspect 12, wherein the base material is positioned within the composite mycelium material.

[0320] According to a fifteenth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 14, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

[0321] According to a sixteenth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 15, wherein the composite mycelium material further comprises a dye.

[0322] According to a seventeenth aspect of the present disclosure, the composite mycelium material of aspect 16, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye.

[0323] According to an eighteenth aspect of the present disclosure, the composite mycelium material of aspect 16, wherein the dye is a reactive dye.

[0324] According to a nineteenth aspect of the present disclosure, the composite mycelium material of any one of aspects 16 to 18, wherein the composite mycelium material is colored with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.

**[0325]** According to a twentieth aspect of the present disclosure, the composite mycelium material of any one of aspects 16 to 18, wherein the dye is present throughout the interior of the composite mycelium material.

**[0326]** According to a twenty-first aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 20, wherein the composite mycelium material further comprises a plasticizer.

**[0327]** According to a twenty-second aspect of the present disclosure, the composite mycelium material of aspect 21, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecanyl succinic anhydride, octadecanyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

**[0328]** According to a twenty-third aspect of the present disclosure, the composite mycelium material of aspect 22, wherein the composite mycelium material is flexible.

**[0329]** According to a twenty-fourth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 23, wherein the composite mycelium material comprises a mechanical property.

**[0330]** According to a twenty-fifth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 24, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.

**[0331]** According to a twenty-sixth aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 25, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

**[0332]** According to a twenty-seventh aspect of the present disclosure, the composite mycelium material of any one of aspects 1 to 26, wherein the composite mycelium material comprises an additive.

**[0333]** According to a twenty-eighth aspect of the present disclosure, the composite mycelium material of aspect 27, wherein the additive comprises a lyocell fiber.

**[0334]** According to a twenty-ninth aspect of the present disclosure, a composite mycelium material, includes a mycelium material comprising one or more fibers oriented in a z-direction; and a fiber additive.

**[0335]** According to a thirtieth aspect of the present disclosure, the composite mycelium material of aspect 29, wherein the mycelium material has been generated on a solid or liquid substrate.

**[0336]** According to a thirty-first aspect of the present disclosure, the composite mycelium material of aspects 29 or 30, wherein the one or more fibers has a length of 0.1 mm to 5 mm.

**[0337]** According to a thirty-second aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 31, wherein the one or more fibers is manipulated by needle punching, felting, hydroentangling, or needling.

**[0338]** According to a thirty-third aspect of the present disclosure, the composite mycelium material of aspect 32, wherein the one or more fibers are needled.

**[0339]** According to a thirty-fourth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 33, wherein the mycelium material comprises a bonding agent comprising an adhesive, a resin, a crosslinking agent, and/or a matrix.

**[0340]** According to a thirty-fifth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 34, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.

**[0341]** According to a thirty-sixth aspect of the present disclosure, the composite mycelium material of aspect 35, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.

**[0342]** According to a thirty-seventh aspect of the present disclosure, the composite mycelium material of aspect 35, wherein the bonding agent is a vinyl acetate-acrylic copolymer.

**[0343]** According to a thirty-eighth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 37, wherein the composite mycelium material further comprises a reinforcing material.

**[0344]** According to a thirty-ninth aspect of the present disclosure, the composite mycelium material of aspect 38, wherein the reinforcing material is entangled within the composite mycelium material.

**[0345]** According to a fortieth aspect of the present disclosure, the composite mycelium material of aspect 38, wherein the reinforcing material comprises a base material.

[0346] According to a forty-first aspect of the present disclosure, the composite mycelium material of aspect 40, wherein the base material is positioned on one surface of the composite mycelium material.

[0347] According to a forty-second aspect of the present disclosure, the composite mycelium material of aspect 40, wherein the base material is positioned within the composite mycelium material.

[0348] According to a forty-third aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 42, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

[0349] According to a forty-fourth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 43, wherein the one or more fibers is disrupted by a mechanical action.

[0350] According to a forty-fifth aspect of the present disclosure, the composite mycelium material of aspect 44, wherein the mechanical action comprises blending the one or more fibers.

[0351] According to a forty-sixth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 43, wherein the one or more fibers is disrupted by chemical treatment.

[0352] According to a forty-seventh aspect of the present disclosure, the composite mycelium material of aspect 46, wherein the chemical treatment comprises contacting the one or more fibers with a base or other chemical agent in an amount sufficient to cause a disruption.

[0353] According to a forty-eighth aspect of the present disclosure, the composite mycelium material of aspect 47, wherein the base comprises alkaline peroxide.

[0354] According to a forty-ninth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 48, wherein the composite mycelium material further comprises a dye.

[0355] According to a fiftieth aspect of the present disclosure, the composite mycelium material of aspect 49, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dy.

[0356] According to a fifty-first aspect of the present disclosure, the composite mycelium material of aspect 50, wherein the dye is a reactive dye.

[0357] According to a fifty-second aspect of the present disclosure, the composite mycelium material of any one of aspects 49 to 51, wherein the composite mycelium material is colored

with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.

**[0358]** According to a fifty-third aspect of the present disclosure, the composite mycelium material of any one of aspects 49 to 51, wherein the dye is present throughout the interior of the composite mycelium material.

**[0359]** According to a fifty-fourth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 53, wherein the composite mycelium material further comprises a plasticizer.

**[0360]** According to a fifty-fifth aspect of the present disclosure, the composite mycelium material of aspect 54, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecenyl succinic anhydride, octacedenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

**[0361]** According to a fifty-sixth aspect of the present disclosure, the composite mycelium material of aspect 55, wherein the composite mycelium material is flexible.

**[0362]** According to a fifty-seventh aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 56, wherein the composite mycelium material comprises a mechanical property.

**[0363]** According to a fifty-eighth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 57, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.

**[0364]** According to a fifty-ninth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 58, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

**[0365]** According to a sixtieth aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 59, wherein the fiber additive comprises an abaca fiber.

**[0366]** According to a sixty-first aspect of the present disclosure, the composite mycelium material of any one of aspects 29 to 59, wherein the fiber additive comprises an lyocell fiber.

**[0367]** According to a sixty-second aspect of the present disclosure, a system, includes a mycelium composition comprising one or more disrupted fibers, wherein the one or more

disrupted fibers is oriented in a z-direction; an apparatus having one or more projections; a solution; and a bonding agent.

[0368] According to a sixty-third aspect of the present disclosure, the system of aspect 62, wherein the mycelium composition comprises a solid content of 1-10%.

[0369] According to a sixty-fourth aspect of the present disclosure, the system of aspect 62, wherein the mycelium composition comprises a solid content of 3-7%.

[0370] According to a sixty-fifth aspect of the present disclosure, the system of aspect 62, wherein the mycelium composition comprises a solid content of 4-6%.

[0371] According to a sixty-sixth aspect of the present disclosure, the system of aspect 62, wherein the mycelium composition comprises a solid content of 1-6%.

[0372] According to a sixty-seventh aspect of the present disclosure, the system of any one of aspects 62 to 66, wherein the solution comprises water.

[0373] According to a sixty-eighth aspect of the present disclosure, the system of any one of aspects 62 to 67, wherein the one or more projections comprises needles.

[0374] According to a sixty-ninth aspect of the present disclosure, the system of any one of aspects 62 to 68, wherein the needles comprise a diameter of 0.05-1 mm.

[0375] According to a seventieth aspect of the present disclosure, the system of aspect 69, wherein the needles comprise a diameter of 0.07-0.5 mm.

[0376] According to a seventy-first aspect of the present disclosure, the system of aspect 62, wherein the apparatus comprises a needle density of 1-120 needle punctures per square cm.

[0377] According to a seventy-second aspect of the present disclosure, the system of aspect 62, wherein the one or more projections comprises water.

[0378] According to a seventy-third aspect of the present disclosure, the system of any one of aspects 62 to 72, wherein the one or more projections is positioned perpendicular to the mycelium composition.

[0379] According to a seventy-fourth aspect of the present disclosure, the system of any one of aspects 62 to 72, wherein the one or more projections subjects pressure to the mycelium composition, thus orienting the one or more disrupted fibers in the z-direction.

[0380] According to a seventy-fifth aspect of the present disclosure, a composite mycelium material, including a mycelium material comprising one or more disrupted fibers oriented in a z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and a lyocell fiber.

[0381] According to a seventy-sixth aspect of the present disclosure, a composite mycelium material, including a mycelium material comprising one or more disrupted fibers oriented in a

z-direction; a bonding agent comprising a vinyl acetate-ethylene copolymer; and an abaca fiber.

**[0382]** According to a seventy-seventh aspect of the present disclosure, a method of producing a composite mycelium material, the method including: generating a mycelium material comprising one or more disrupted fibers; adding a solution to the mycelium material to produce a mycelium composition; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; adding a bonding agent to the mycelium composition; and draining the solution; thus producing the composite mycelium material.

**[0383]** According to a seventy-eighth aspect of the present disclosure, a method, including generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and needling the mycelium material.

**[0384]** According to a seventy-ninth aspect of the present disclosure, a method, including generating a mycelium material; contacting the mycelium material with a solution comprising a bonding agent and a solution; and manipulating the mycelium material.

**[0385]** According to an eightieth aspect of the present disclosure, a method, including generating a mycelium composition comprising a mycelium material, a solution, and a bonding agent; and manipulating the mycelium composition.

**[0386]** According to an eighty-first aspect of the present disclosure, a composite fibrous material, including a fibrous material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and a bonding agent.

**[0387]** According to an eighty-second aspect of the present disclosure, a method of producing a composite mycelium material, the method including generating a mycelium material; and manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction.

**[0388]** According to an eighty-third aspect of the present disclosure, a method of producing a composite mycelium material, generating a mycelium material comprising one or more disrupted fibers; manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; and adding a bonding agent to the mycelium composition.

**[0389]** According to an eighty-fourth aspect of the present disclosure, the method of aspect 83, wherein the mycelium material has been generated on a solid or liquid substrate.

**[0390]** According to an eighty-fifth aspect of the present disclosure, the method of aspects 83 or 84, wherein the one or more fibers has a length of 0.1 mm to 5 mm.



**[0391]** According to an eighty-sixth aspect of the present disclosure, the method of any one of aspects 83 to 85, wherein the manipulating comprises needle punching, felting, hydroentangling, or needling.

**[0392]** According to an eighty-seventh aspect of the present disclosure, the method of any one of aspects 83 to 86, wherein the manipulating comprises needling.

**[0393]** According to an eighty-eighth aspect of the present disclosure, the method of any one of aspects 83 to 87, wherein the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix.

**[0394]** According to an eighty-nine aspect of the present disclosure, the method of any one of aspects 83 to 88, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.

**[0395]** According to a ninetieth aspect of the present disclosure, the method of aspect 89, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.

**[0396]** According to a ninety-first aspect of the present disclosure, the method of aspect 89, wherein the bonding agent is a vinyl acetate-acrylic copolymer.

**[0397]** According to a ninety-second aspect of the present disclosure, the method of any one of aspects 83 to 91, further adding a reinforcing material.

**[0398]** According to a ninety-third aspect of the present disclosure, the method of aspect 92, wherein the reinforcing material is entangled within the composite mycelium material.

**[0399]** According to a ninety-fourth aspect of the present disclosure, the method of aspect 92, wherein the reinforcing material comprises a base material.

**[0400]** According to a ninety-fifth aspect of the present disclosure, the method of aspect 94, wherein the base material is positioned on one surface of the composite mycelium material.

**[0401]** According to a ninety-sixth aspect of the present disclosure, the method of aspect 94, wherein the base material is positioned within the composite mycelium material.

**[0402]** According to a ninety-seventh aspect of the present disclosure, the method of any one of aspects 83 to 96, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.

**[0403]** According to a ninety-eighth aspect of the present disclosure, the method of any one of aspects 83 to 97, further adding a dye.

[0404] According to a ninety-ninth aspect of the present disclosure, the method of aspect 98, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye.

[0405] According to a one hundredth aspect of the present disclosure, the method of aspect 98, wherein the dye is a reactive dye.

[0406] According to a one hundredth first aspect of the present disclosure, the method of any one of aspects 98 to 100, further coloring the composite mycelium material with the dye, wherein the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.

[0407] According to a one hundredth second aspect of the present disclosure, the method of any one of aspects 98 to 100, wherein the dye is present throughout the interior of the composite mycelium material.

[0408] According to a one hundredth third aspect of the present disclosure, the method of any one of aspects 83 to 102, further adding a plasticizer.

[0409] According to a one hundredth fourth aspect of the present disclosure, the method of aspect 103, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecenyl succinic anhydride, octadecenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

[0410] According to a one hundredth fifth aspect of the present disclosure, the method of aspect 104, wherein the composite mycelium material is flexible.

[0411] According to a one hundredth sixth aspect of the present disclosure, the method of any one of aspects 83 to 105, wherein the composite mycelium material comprises a mechanical property.

[0412] According to a one hundredth seventh aspect of the present disclosure, the method of any one of aspects 83 to 106, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.

[0413] According to a one hundredth eighth aspect of the present disclosure, the method of any one of aspects 83 to 107, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.

[0414] According to a one hundredth ninth aspect of the present disclosure, the method of any one of aspects 83 to 108, further adding an additive.

[0415] According to a one hundredth tenth aspect of the present disclosure, the method of aspect 109, wherein the additive comprises a lyocell fiber.

[0416] It will be understood by one having ordinary skill in the art that construction of the described disclosure and other components is not limited to any specific material. Other exemplary embodiments of the disclosure disclosed herein may be formed from a wide variety of materials, unless described otherwise herein.

[0417] It is also important to note that the construction and arrangement of the elements of the disclosure as shown in the exemplary embodiments is illustrative only. Although only a few embodiments of the present innovations have been described in detail in this disclosure, those skilled in the art who review this disclosure will readily appreciate that many modifications are possible (e.g., variations in sizes, dimensions, structures, shapes and proportions of the various elements, values of parameters, mounting arrangements, use of materials, colors, orientations, etc.) without materially departing from the novel teachings and advantages of the subject matter recited. Accordingly, all such modifications are intended to be included within the scope of the present disclosure. Other substitutions, modifications, changes, and omissions may be made in the design, operating conditions, and arrangement of the desired and other exemplary embodiments without departing from the spirit of the present disclosure.

[0418] It will be understood that any described processes or steps within processes described herein may be combined with other disclosed processes or steps to form structures within the scope of the present disclosure. The exemplary structures and processes disclosed herein are for illustrative purposes and are not to be construed as limiting.

**CLAIMS**

1. A composite mycelium material, comprising:
  - a. a mycelium material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and
  - b. a bonding agent.
2. The composite mycelium material of claim 1, wherein the mycelium material has been generated on a solid or liquid substrate.
3. The composite mycelium material of any one of claims 1-2, wherein the one or more fibers has a length of 0.1 mm to 5 mm.
4. The composite mycelium material of any one of claims 1-3, wherein the one or more disrupted fibers is manipulated by needle punching, felting, hydroentangling, or needling.
5. The composite mycelium material of claim 4, wherein the one or more fibers are needled.
6. The composite mycelium material of any one of claims 1-5, wherein the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix.
7. The composite mycelium material of any one of claims 1-6, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.
8. The composite mycelium material of claim 7, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.
9. The composite mycelium material of claim 7, wherein the bonding agent is a vinyl acetate-acrylic copolymer.
10. The composite mycelium material of any one of claims 1-9, wherein the composite mycelium material further comprises a reinforcing material.
11. The composite mycelium material of claim 10, wherein the reinforcing material is entangled within the composite mycelium material.

12. The composite mycelium material of claim 10, wherein the reinforcing material comprises a base material.
13. The composite mycelium material of claim 12, wherein the base material is positioned on one surface of the composite mycelium material.
14. The composite mycelium material of claim 12, wherein the base material is positioned within the composite mycelium material.
15. The composite mycelium material of any one of claims 1-14, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.
16. The composite mycelium material of any one of claims 1-15, wherein the composite mycelium material further comprises a dye.
17. The composite mycelium material of claim 16, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye.
18. The composite mycelium material of claim 16, wherein the dye is a reactive dye.
19. The composite mycelium material of any one of claims 16-18, wherein the composite mycelium material is colored with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.
20. The composite mycelium material of any one of claims 16-18, wherein the dye is present throughout the interior of the composite mycelium material.
21. The composite mycelium material of any one of claims 1-20, wherein the composite mycelium material further comprises a plasticizer.
22. The composite mycelium material of claim 21, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecenyl succinic anhydride, octacedenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.

23. The composite mycelium material of claim 22, wherein the composite mycelium material is flexible.
24. The composite mycelium material of any one of claims 1-23, wherein the composite mycelium material comprises a mechanical property.
25. The composite mycelium material of any one of claims 1-24, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.
26. The composite mycelium material of any one of claims 1-25, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.
27. The composite mycelium material of any one of claims 1-26, wherein the composite mycelium material comprises an additive.
28. The composite mycelium material of claim 27, wherein the additive comprises a lyocell fiber.
29. A composite mycelium material, comprising:
- a. a mycelium material comprising one or more fibers oriented in a z-direction;  
and
  - b. a fiber additive.
30. The composite mycelium material of claim 29, wherein the mycelium material has been generated on a solid or liquid substrate.
31. The composite mycelium material of any one of claims 29-30, wherein the one or more fibers has a length of 0.1 mm to 5 mm.
32. The composite mycelium material of any one of claims 29-31, wherein the one or more fibers is manipulated by needle punching, felting, hydroentangling, or needling.
33. The composite mycelium material of claim 32, wherein the one or more fibers are needled.
34. The composite mycelium material of any one of claims 29-33, wherein the mycelium material comprises a bonding agent comprising an adhesive, a resin, a crosslinking agent, and/or a matrix.

- 35.** The composite mycelium material of any one of claims 29-34, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.
- 36.** The composite mycelium material of claim 35, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.
- 37.** The composite mycelium material of claim 35, wherein the bonding agent is a vinyl acetate-acrylic copolymer.
- 38.** The composite mycelium material of any one of claims 29-37, wherein the composite mycelium material further comprises a reinforcing material.
- 39.** The composite mycelium material of claim 38, wherein the reinforcing material is entangled within the composite mycelium material.
- 40.** The composite mycelium material of claim 38, wherein the reinforcing material comprises a base material.
- 41.** The composite mycelium material of claim 40, wherein the base material is positioned on one surface of the composite mycelium material.
- 42.** The composite mycelium material of claim 40, wherein the base material is positioned within the composite mycelium material.
- 43.** The composite mycelium material of any one of claims 29-42, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.
- 44.** The composite mycelium material of any one of claims 29-43, wherein the one or more fibers is disrupted by a mechanical action.
- 45.** The composite mycelium material of claim 44, wherein the mechanical action comprises blending the one or more fibers.
- 46.** The composite mycelium material of any one of claims 29-43, wherein the one or more fibers is disrupted by chemical treatment.

47. The composite mycelium material of claim 46, wherein the chemical treatment comprises contacting the one or more fibers with a base or other chemical agent in an amount sufficient to cause a disruption.
48. The composite mycelium material of claim 47, wherein the base comprises alkaline peroxide.
49. The composite mycelium material of any one of claims 29-48, wherein the composite mycelium material further comprises a dye.
50. The composite mycelium material of claim 49, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye.
51. The composite mycelium material of claim 50, wherein the dye is a reactive dye.
52. The composite mycelium material of any one of claims 49-51, wherein the composite mycelium material is colored with the dye and the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.
53. The composite mycelium material of any one of claims 49-51, wherein the dye is present throughout the interior of the composite mycelium material.
54. The composite mycelium material of any one of claims 29-53, wherein the composite mycelium material further comprises a plasticizer.
55. The composite mycelium material of claim 54, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethoxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecanyl succinic anhydride, octacedenyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.
56. The composite mycelium material of claim 55, wherein the composite mycelium material is flexible.
57. The composite mycelium material of any one of claims 29-56, wherein the composite mycelium material comprises a mechanical property.



- 58.** The composite mycelium material of any one of claims 29-57, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.
- 59.** The composite mycelium material of any one of claims 29-58, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.
- 60.** The composite mycelium material of any one of claims 29-59, wherein the fiber additive comprises an abaca fiber.
- 61.** The composite mycelium material of any one of claims 29-59, wherein the fiber additive comprises a lyocell fiber.
- 62.** A system, comprising;
- a. a mycelium composition comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction;
  - b. an apparatus having one or more projections;
  - c. a solution; and
  - d. a bonding agent.
- 63.** The system of claim 62, wherein the mycelium composition comprises a solid content of 1-10%.
- 64.** The system of claim 62, wherein the mycelium composition comprises a solid content of 3-7%.
- 65.** The system of claim 62, wherein the mycelium composition comprises a solid content of 4-6%.
- 66.** The system of claim 62, wherein the mycelium composition comprises a solid content of 1-6%.
- 67.** The system of any one of claims 62-66, wherein the solution comprises water.
- 68.** The system of claim 62-67, wherein the one or more projections comprises needles.
- 69.** The system of any one of claims 62-68, wherein the needles comprise a diameter of 0.05-1 mm.
- 70.** The system of claim 69, wherein the needles comprise a diameter of 0.07-0.5 mm.

71. The system of claim 62, wherein the apparatus comprises a needle density of 1-120 needle punctures per square cm.
72. The system of claim 62, wherein the one or more projections comprises water.
73. The system of any one of claims 62-72, wherein the one or more projections is positioned perpendicular to the mycelium composition.
74. The system of any one of claims 62-72, wherein the one or more projections subjects pressure to the mycelium composition, thus orienting the one or more disrupted fibers in the z-direction.
75. A composite mycelium material, comprising:
- a. a mycelium material comprising one or more disrupted fibers oriented in a z-direction;
  - b. a bonding agent comprising a vinyl acetate-ethylene copolymer; and
  - c. a lyocell fiber.
76. A composite mycelium material, comprising:
- a. a mycelium material comprising one or more disrupted fibers oriented in a z-direction;
  - b. a bonding agent comprising a vinyl acetate-ethylene copolymer; and
  - c. an abaca fiber.
77. A method of producing a composite mycelium material, the method comprising:
- a. generating a mycelium material comprising one or more disrupted fibers;
  - b. adding a solution to the mycelium material to produce a mycelium composition;
  - c. manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction;
  - d. adding a bonding agent to the mycelium composition; and
  - e. draining the solution; thus producing the composite mycelium material.
78. A method, comprising:
- a. generating a mycelium material;

- b. contacting the mycelium material with a solution comprising a bonding agent and a solution; and
- c. needling the mycelium material.

**79.** A method, comprising:

- a. generating a mycelium material;
- b. contacting the mycelium material with a solution comprising a bonding agent and a solution; and
- c. manipulating the mycelium material.

**80.** A method, comprising:

- a. generating a mycelium composition comprising a mycelium material, a solution, and a bonding agent; and
- b. manipulating the mycelium composition.

**81.** A composite fibrous material, comprising:

- a. a fibrous material comprising one or more disrupted fibers, wherein the one or more disrupted fibers is oriented in a z-direction; and
- b. a bonding agent.

**82.** A method of producing a composite mycelium material, the method comprising:

- a. generating a mycelium material; and
- b. manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction.

**83.** A method of producing a composite mycelium material, the method comprising:

- a. generating a mycelium material comprising one or more disrupted fibers;
- b. manipulating the mycelium composition comprising one or more disrupted fibers such that the one or more disrupted fibers is oriented in a z-direction; and
- c. adding a bonding agent to the mycelium composition.

**84.** The method of claim 83, wherein the mycelium material has been generated on a solid or liquid substrate.

- 85.** The method of any one of claims 83-84, wherein the one or more fibers has a length of 0.1 mm to 5 mm.
- 86.** The method of any one of claims 83-85, wherein the manipulating comprises needle punching, felting, hydroentangling, or needling.
- 87.** The method of any one of claims 83-86, wherein the manipulating comprises needling.
- 88.** The method of any one of claims 83-87, wherein the bonding agent comprises an adhesive, a resin, a crosslinking agent, and/or a matrix.
- 89.** The method of any one of claims 83-88, wherein the bonding agent is selected from the group consisting of a vinyl acetate-ethylene (VAE) copolymer, a vinyl acetate-acrylic copolymer, a polyamide-epichlorohydrin resin (PAE), a copolymer, transglutaminase, citric acid, genipin, alginate, gum arabic, latex, a natural adhesive, and a synthetic adhesive.
- 90.** The method of claim 89, wherein the bonding agent is a vinyl acetate-ethylene (VAE) copolymer.
- 91.** The method of claim 89, wherein the bonding agent is a vinyl acetate-acrylic copolymer.
- 92.** The method of any one of claims 83-91, further adding a reinforcing material.
- 93.** The method of claim 92, wherein the reinforcing material is entangled within the composite mycelium material.
- 94.** The method of claim 92, wherein the reinforcing material comprises a base material.
- 95.** The method of claim 94, wherein the base material is positioned on one surface of the composite mycelium material.
- 96.** The method of claim 94, wherein the base material is positioned within the composite mycelium material.
- 97.** The method of any one of claims 83-96, wherein the reinforcing material is selected from the group consisting of a mesh, a cheesecloth, a fabric, a knit fabric, a woven fabric, and a non-woven fabric.
- 98.** The method of any one of claims 83-97, further adding a dye.

- 99.** The method of claim 98, wherein the dye is selected from the group consisting of an acid dye, a direct dye, a synthetic dye, a natural dye, and a reactive dye.
- 100.** The method of claim 98, wherein the dye is a reactive dye.
- 101.** The method of any one of claims 98-100, further coloring the composite mycelium material with the dye, wherein the color of the composite mycelium material is substantially uniform on one or more surfaces of the composite mycelium material.
- 102.** The method of any one of claims 98-100, wherein the dye is present throughout the interior of the composite mycelium material.
- 103.** The method of any one of claims 83-102, further adding a plasticizer.
- 104.** The method of claim 103, wherein the plasticizer is selected from the group consisting of oil, glycerin, fatliquor, sorbitol, diethyloxyester dimethyl ammonium chloride, Tween 20, Tween 80, m-erythritol, water, glycol, triethyl citrate, water, acetylated monoglycerides, epoxidized soybean oil, aliphatic chain compound, 2-octenyl succinic anhydride (OSA), 2-dodecyl succinic anhydride, octadecyl succinic anhydride, stearic anhydride, 3-Chloro-2-hydroxypropyldimethyldodecylammonium chloride, heptanoic anhydride, butyric anhydride, chlorohydrin, and siloxane.
- 105.** The method of claim 104, wherein the composite mycelium material is flexible.
- 106.** The method of any one of claims 83-105, wherein the composite mycelium material comprises a mechanical property.
- 107.** The method of any one of claims 83-106, wherein the mechanical property comprises a wet tensile strength, an initial modulus, an elongation percentage at the break, a thickness, a slit tear strength, and/or a peel resistance.
- 108.** The method of any one of claims 83-107, wherein the peel resistance comprises an average T peel value of 5-20 N/cm and a max T peel value of 10-30 N/cm.
- 109.** The method of any one of claims 83-108, further adding an additive.
- 110.** The method of claim 109, wherein the additive comprises a lyocell fiber.

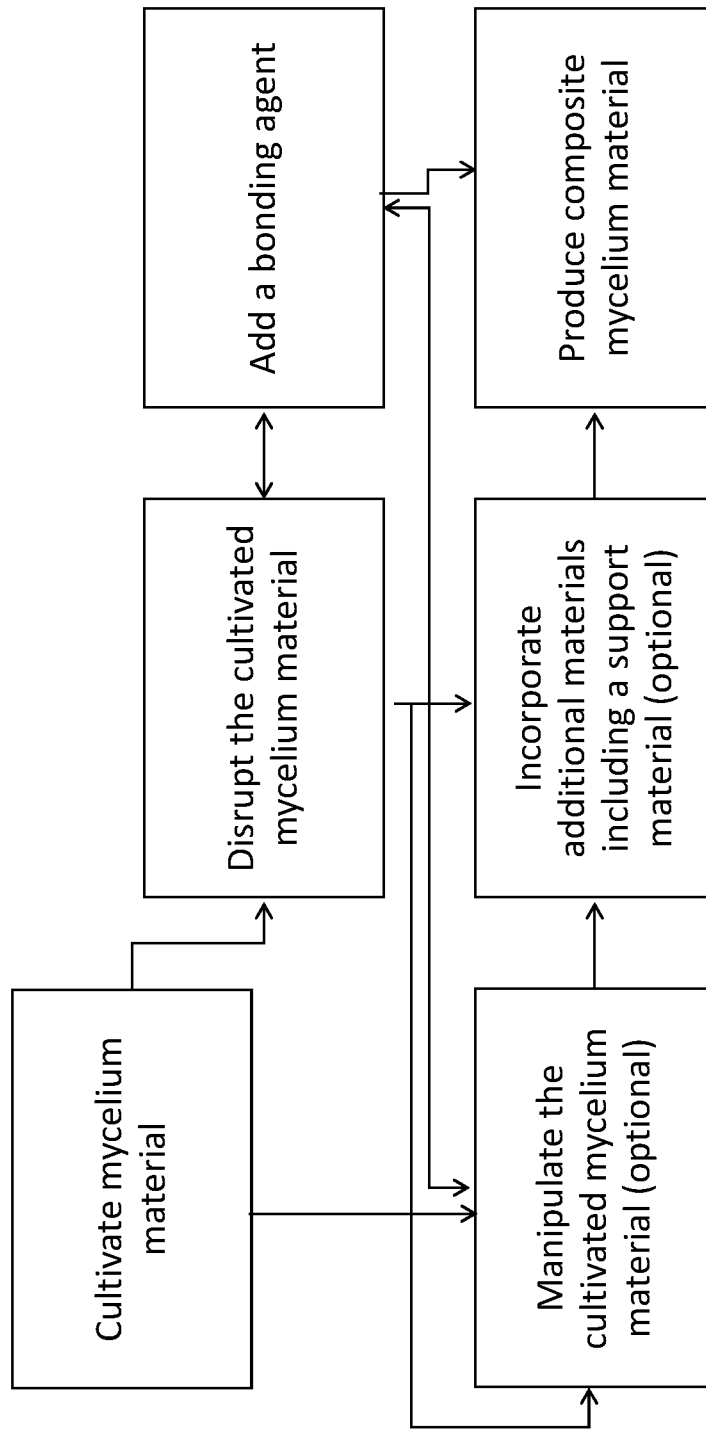


FIG. 1

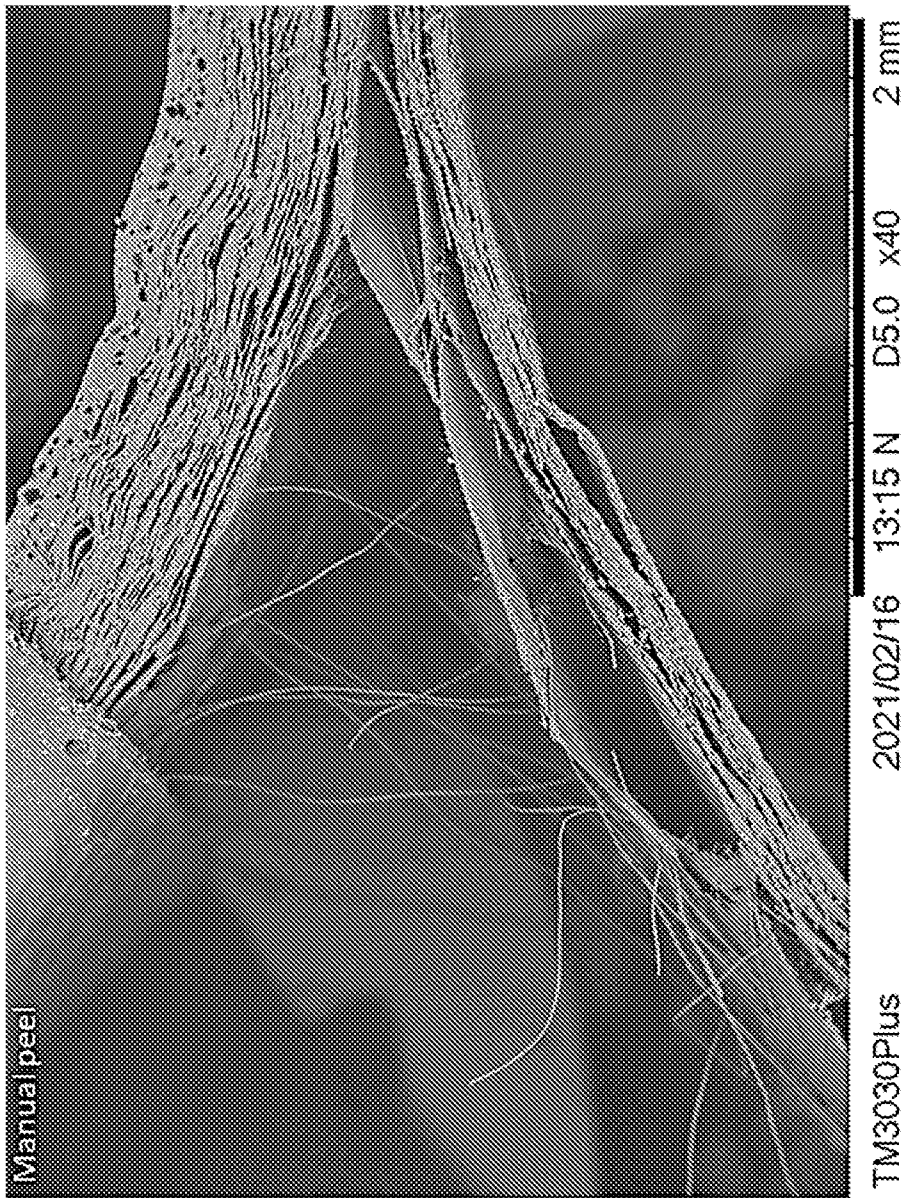


FIG. 2

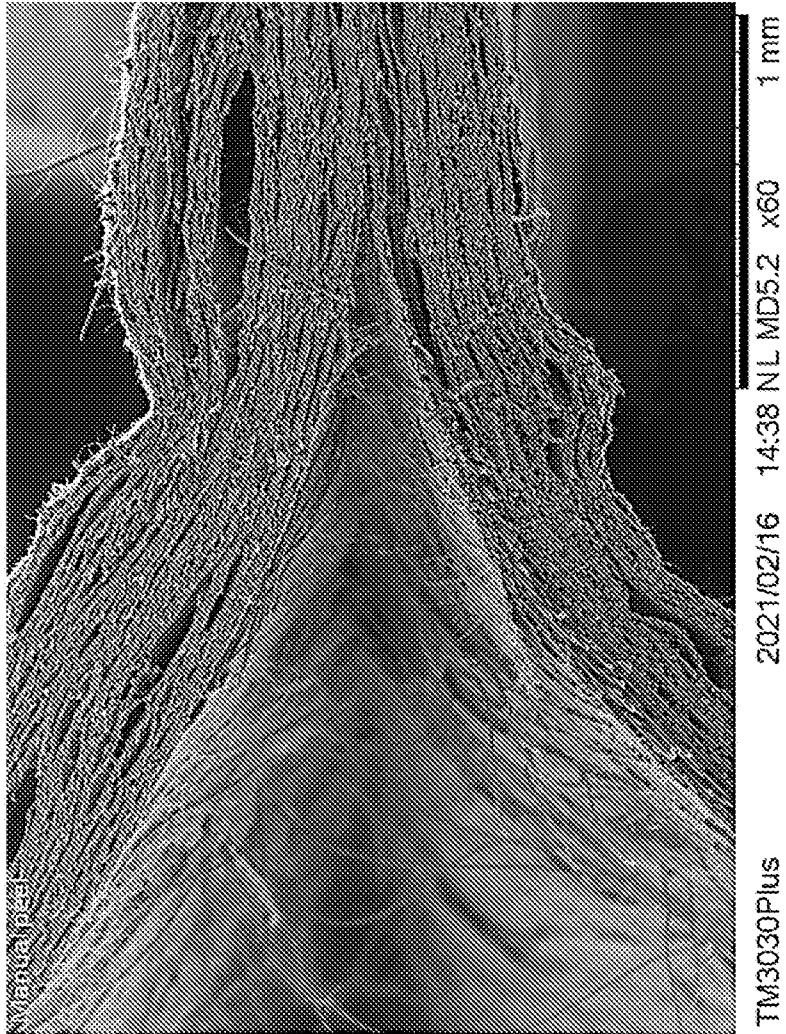


FIG. 3



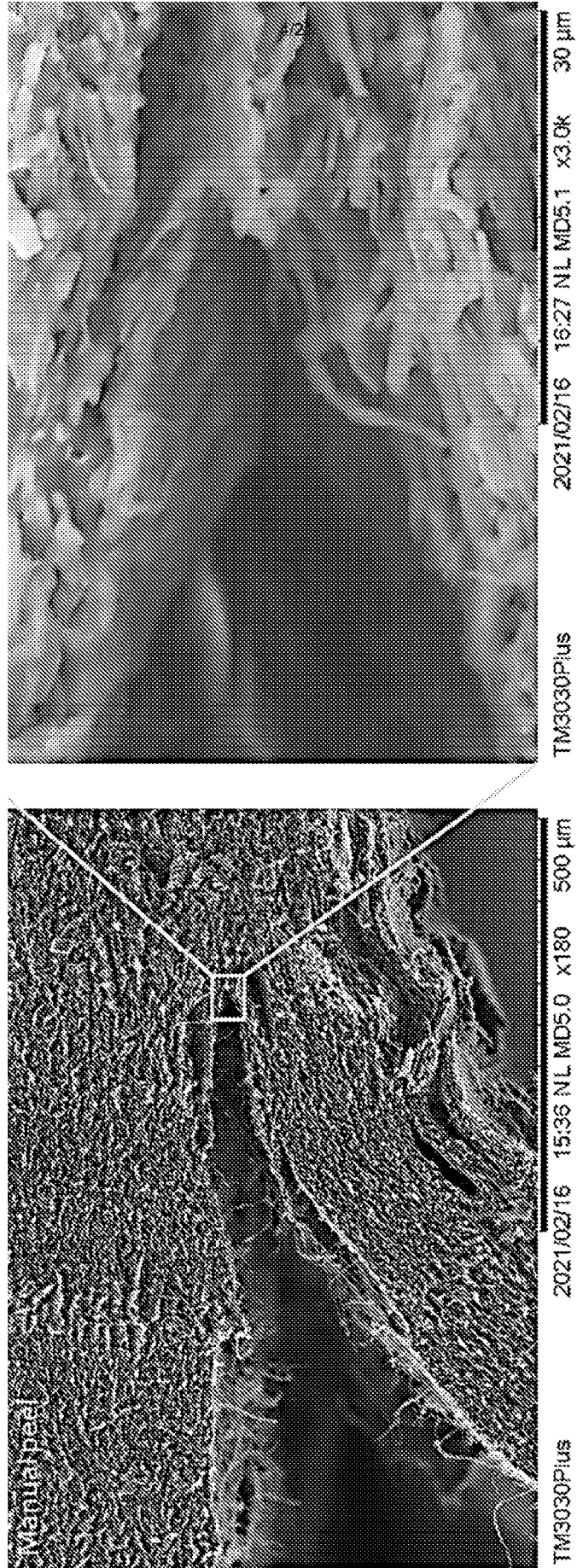


FIG. 4

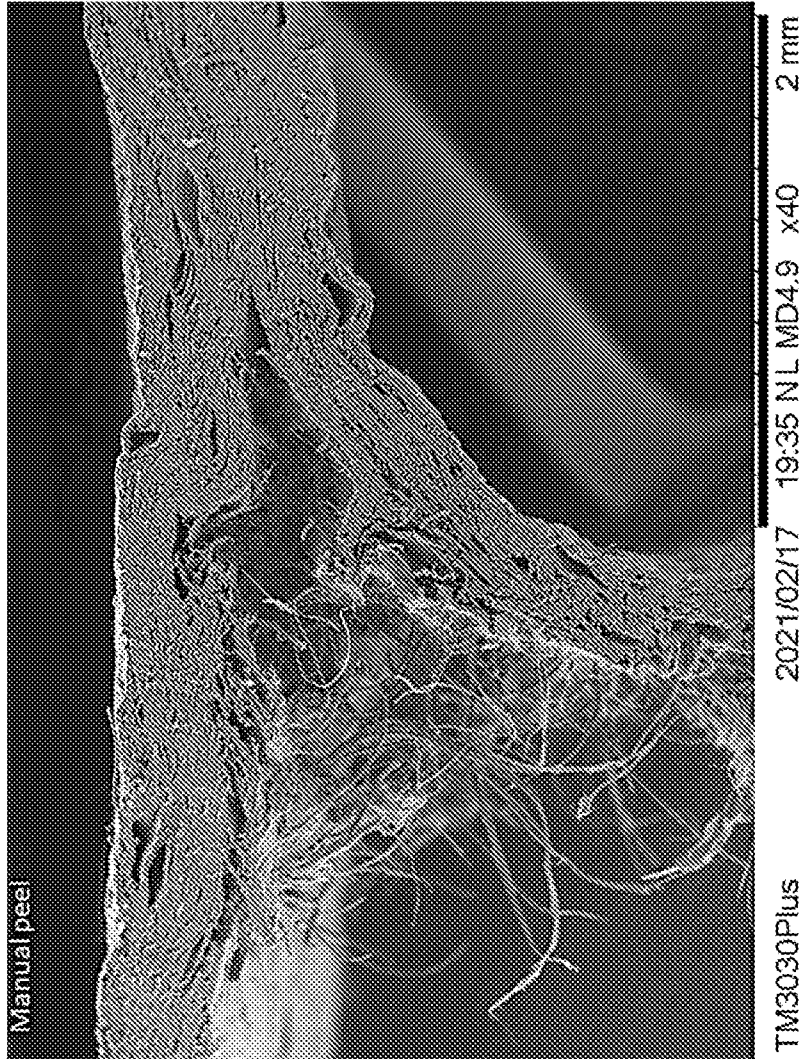


FIG. 5

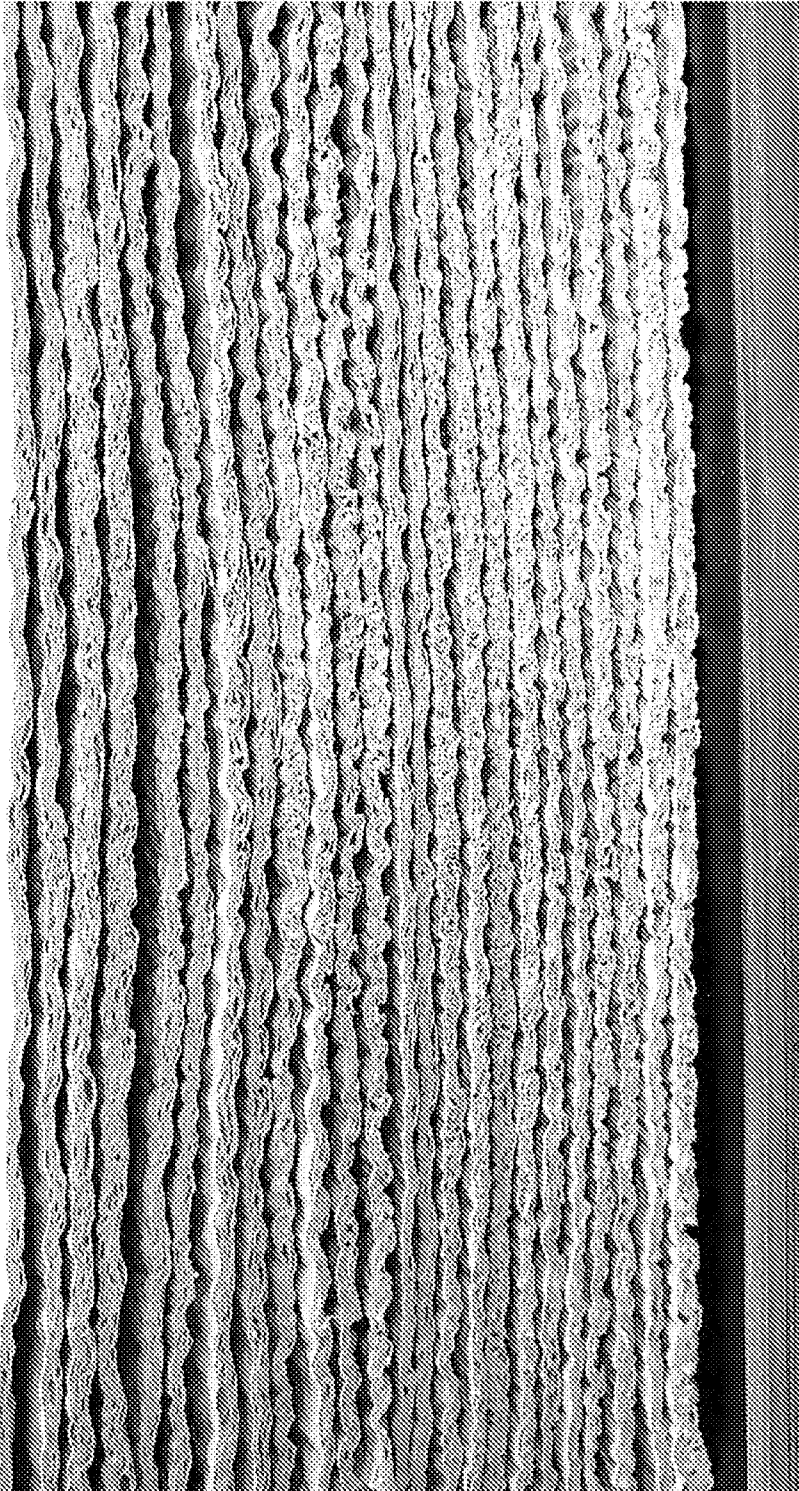


FIG. 6

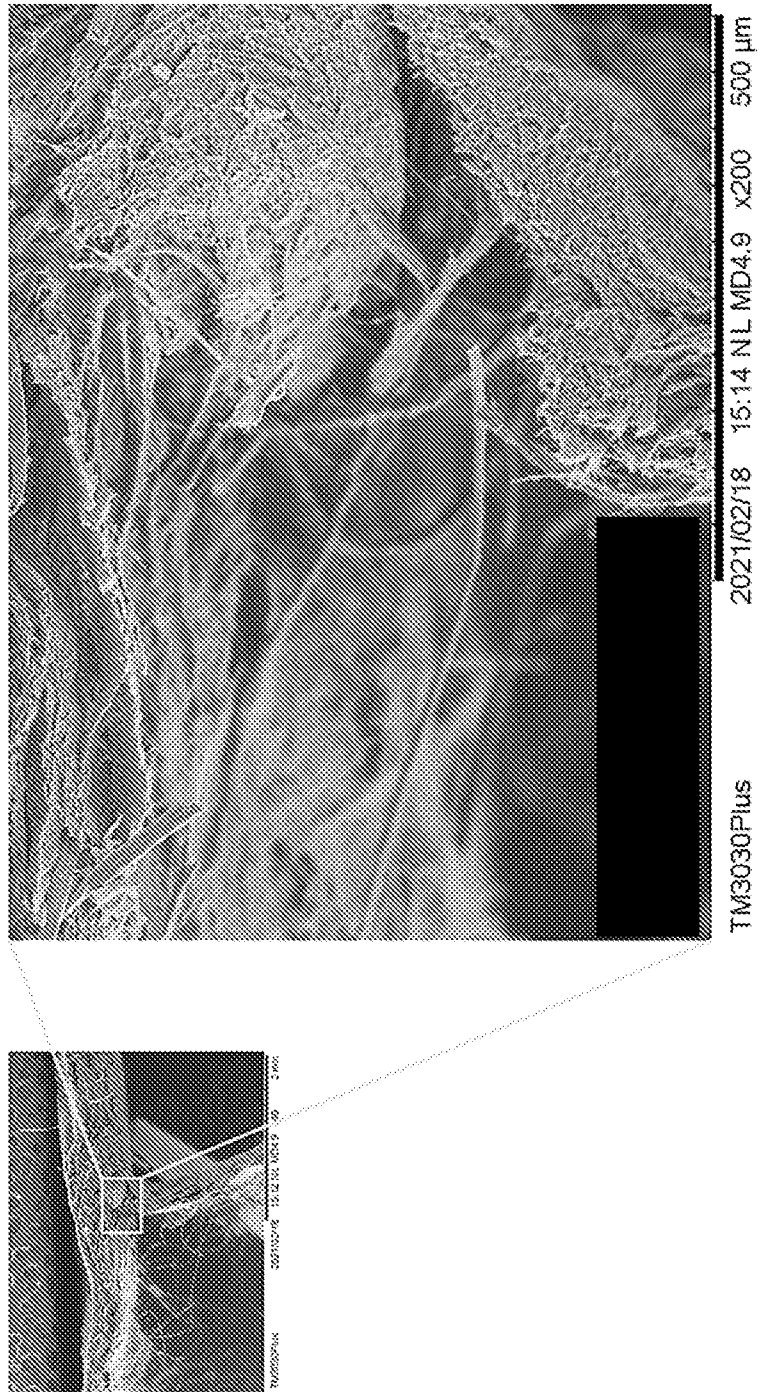


FIG. 7

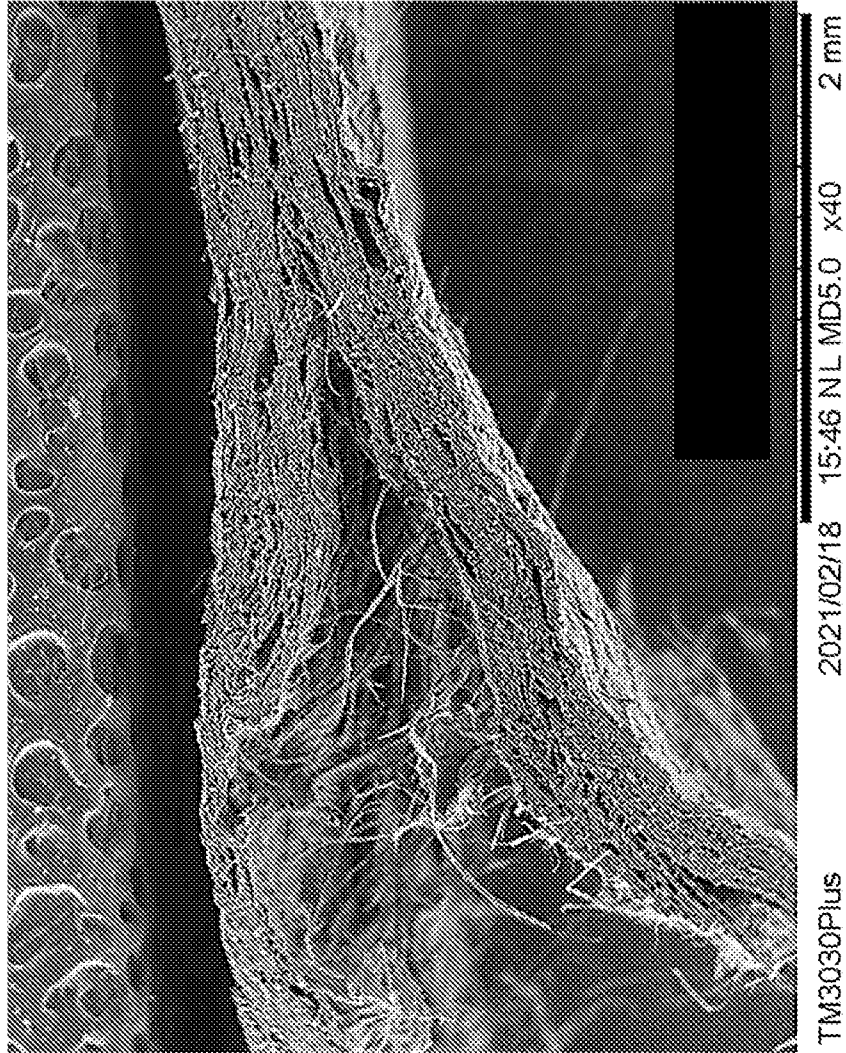


FIG. 8



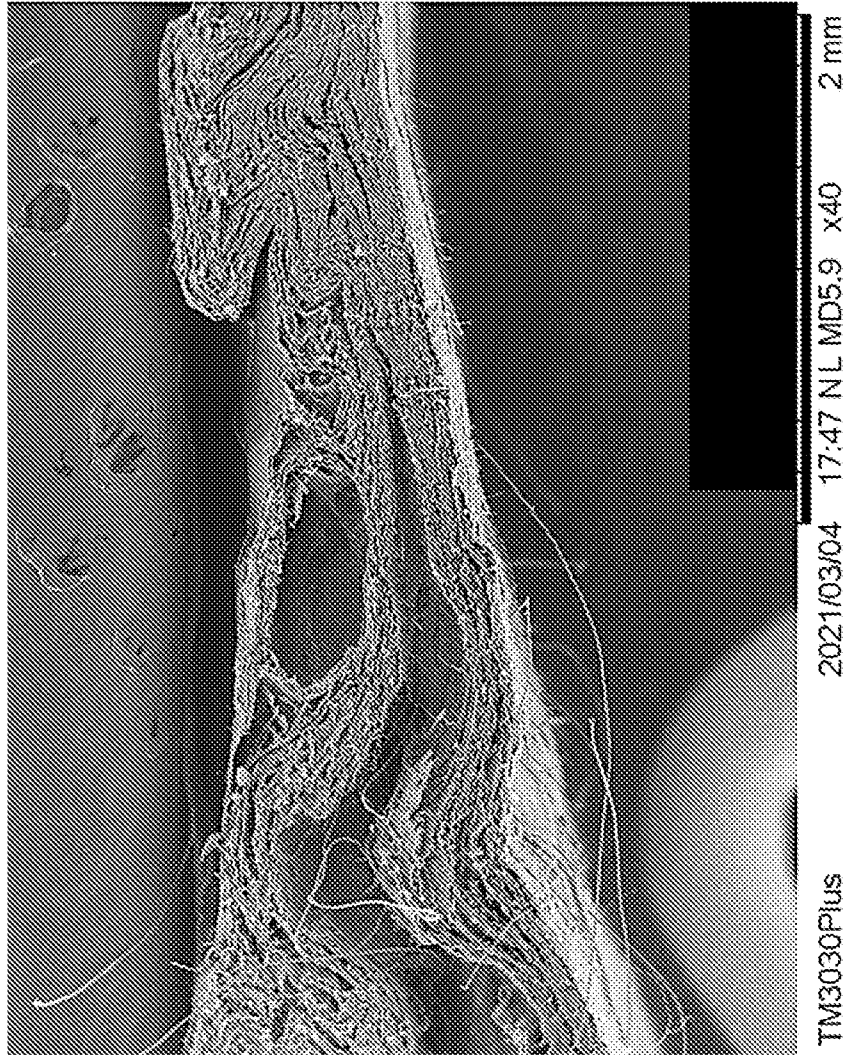


FIG. 9

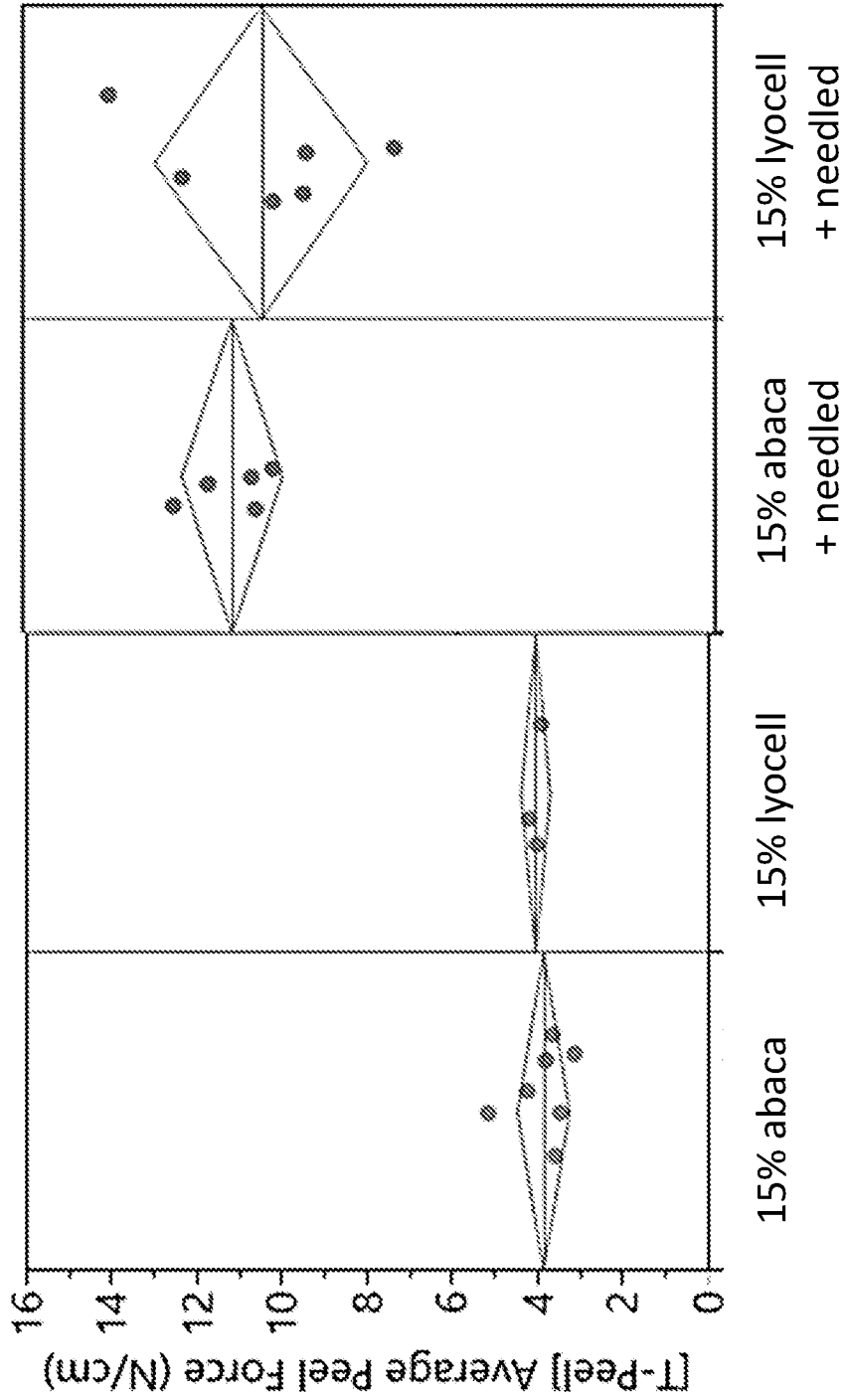


FIG. 10

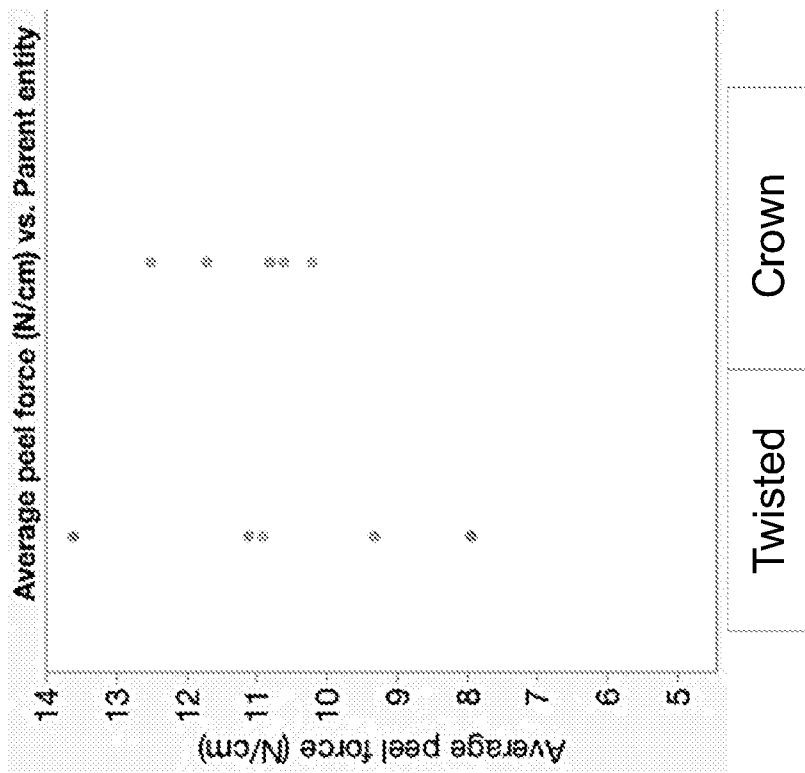


FIG. 11



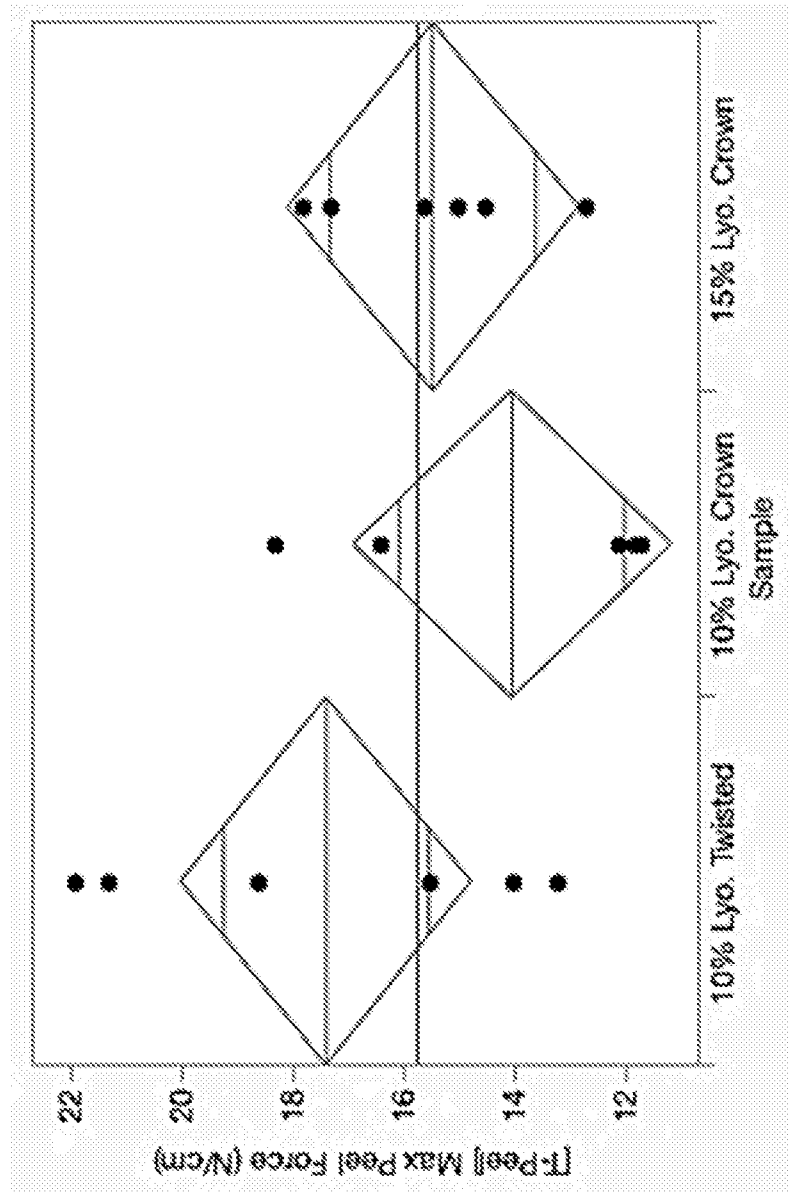


FIG. 12

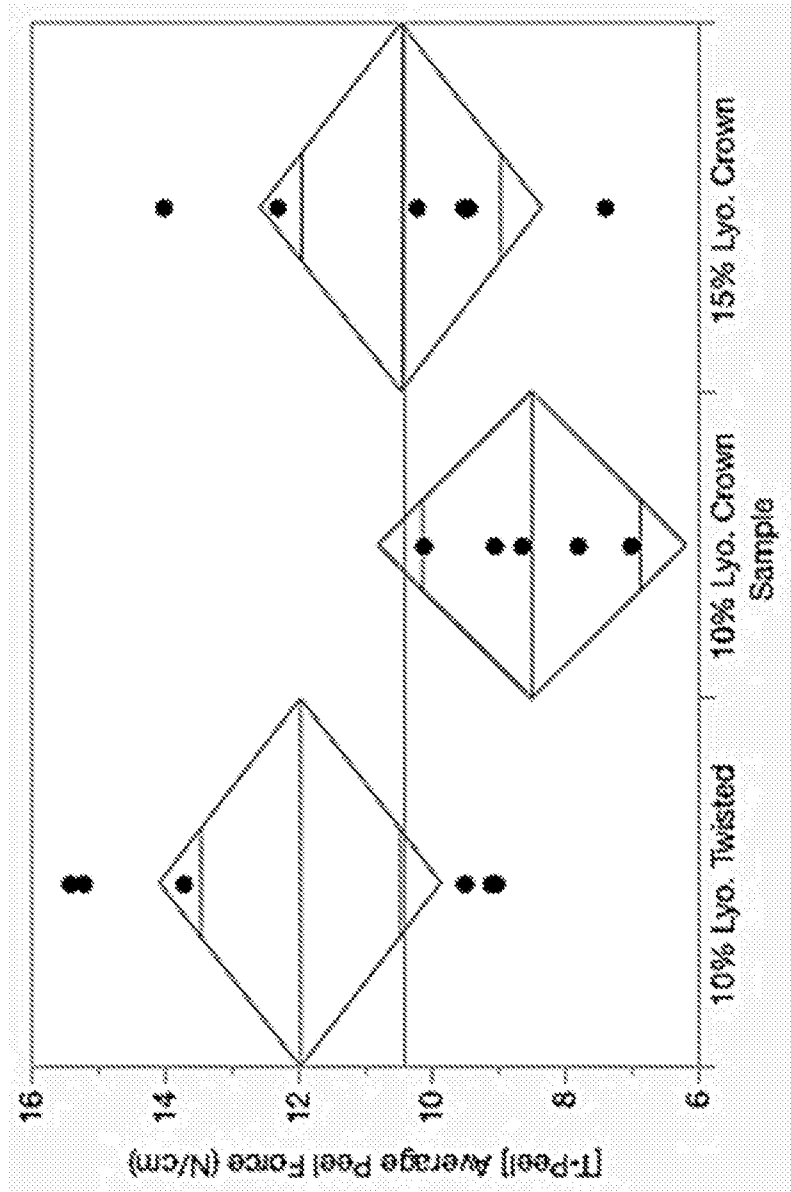


FIG. 13

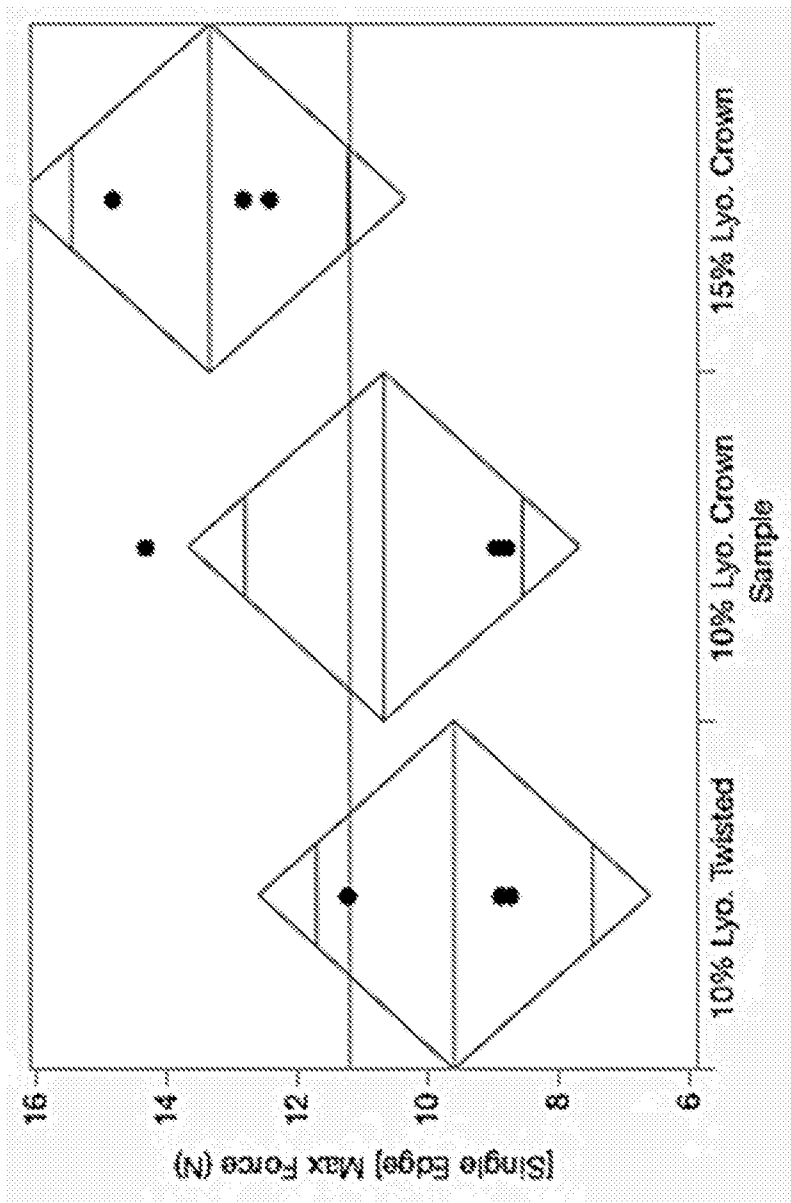


FIG. 14

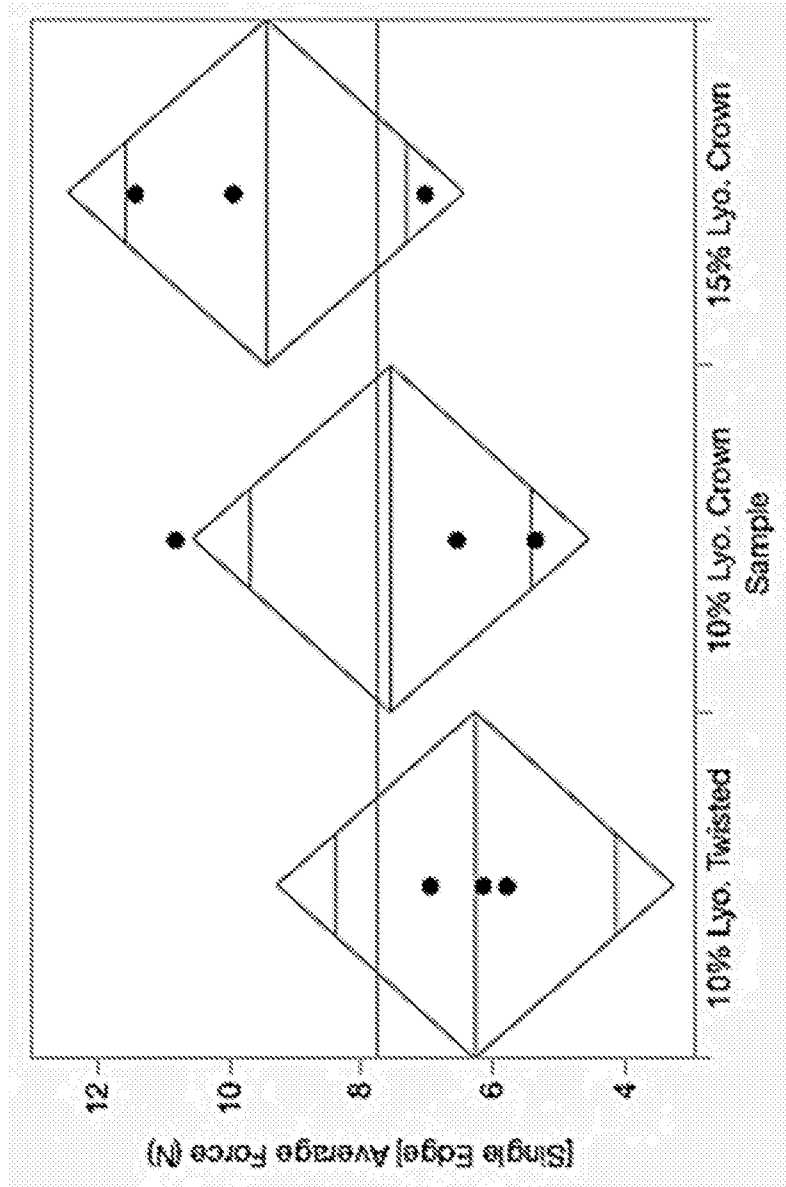


FIG. 15

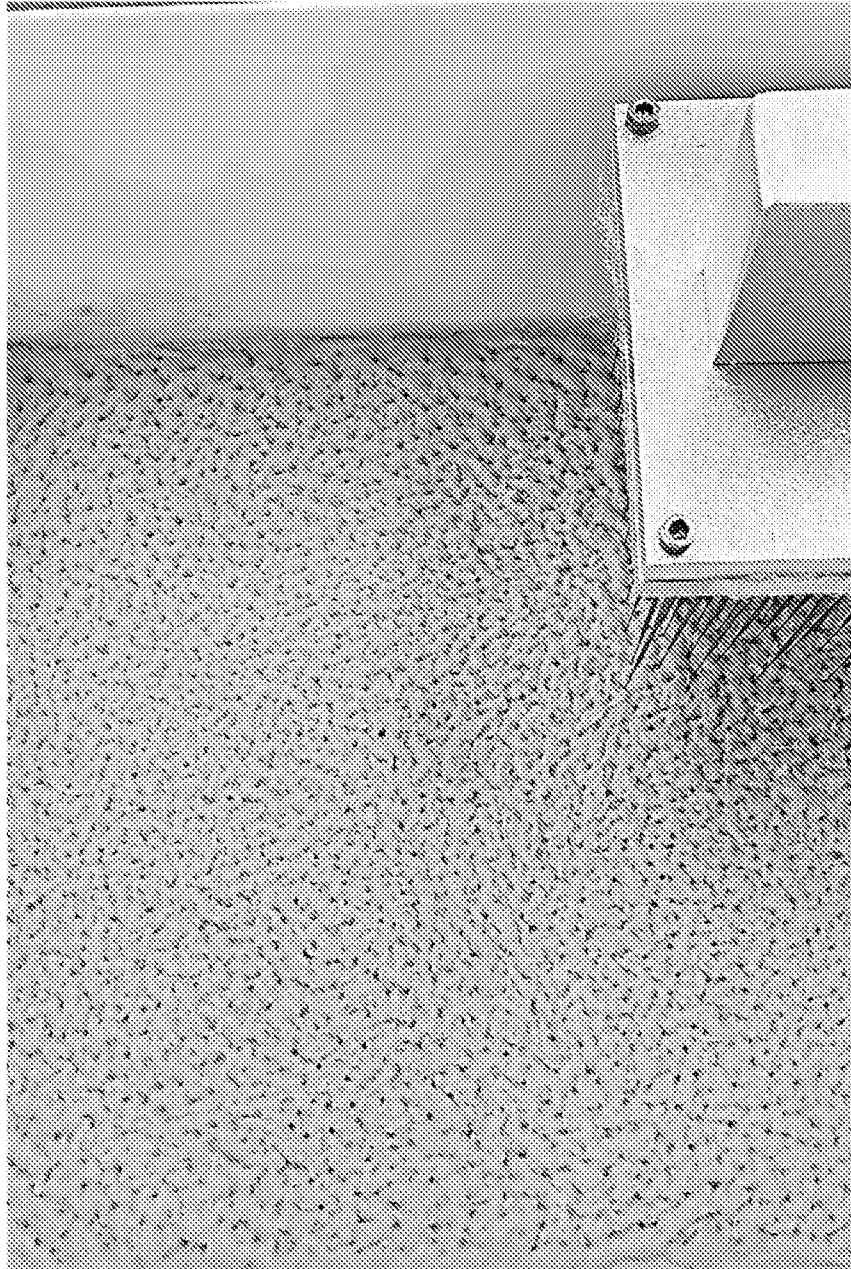


FIG. 16

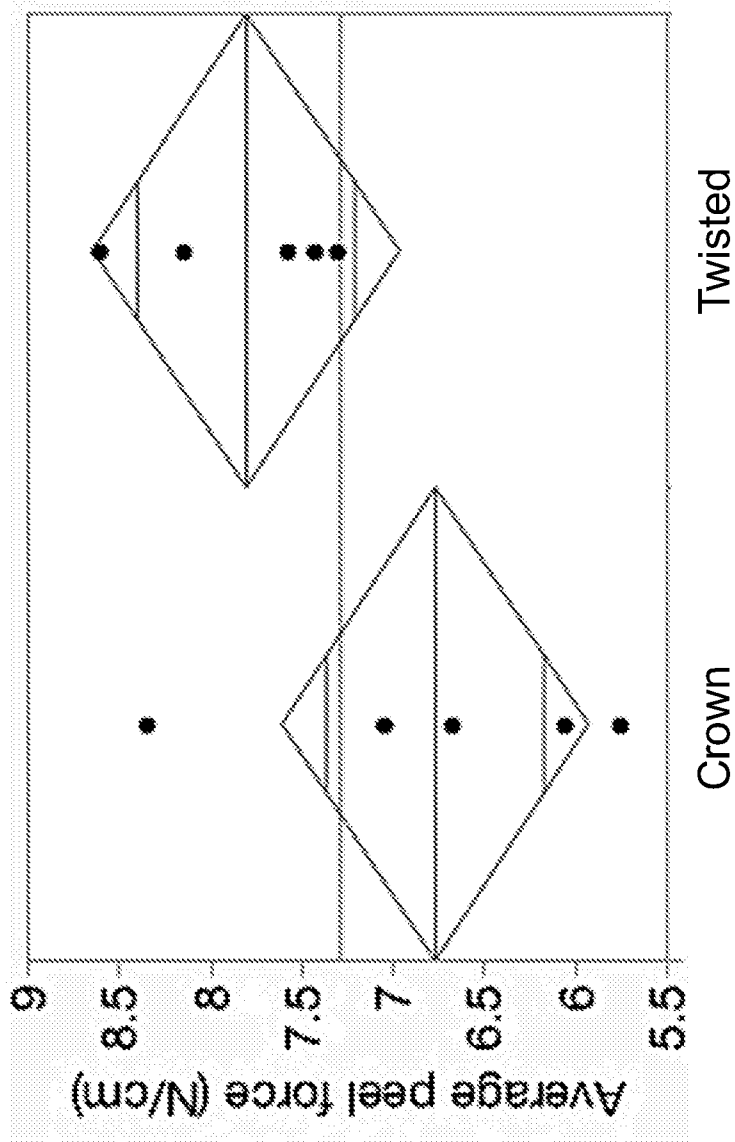


FIG. 17

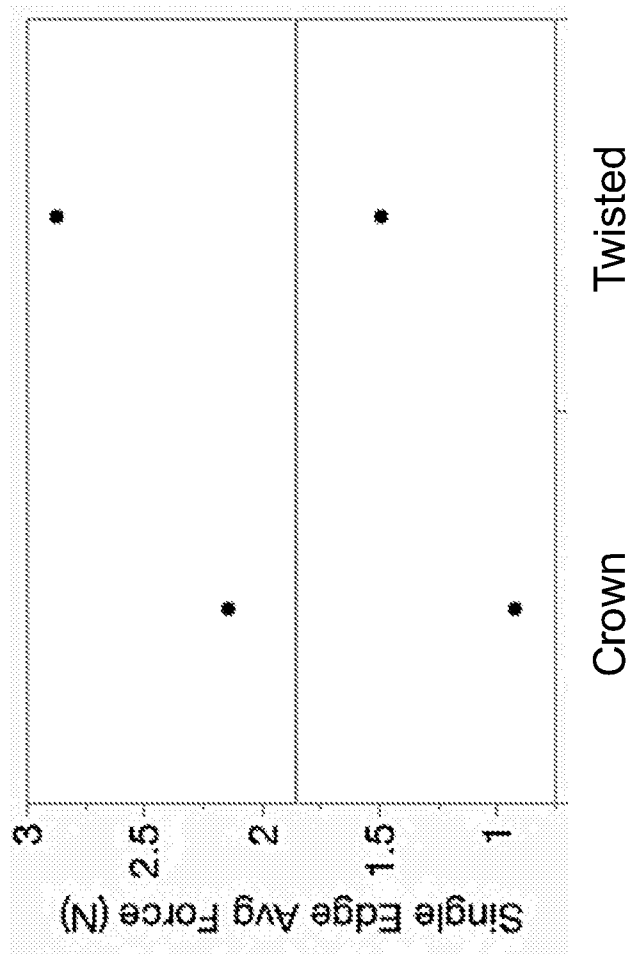


FIG. 18

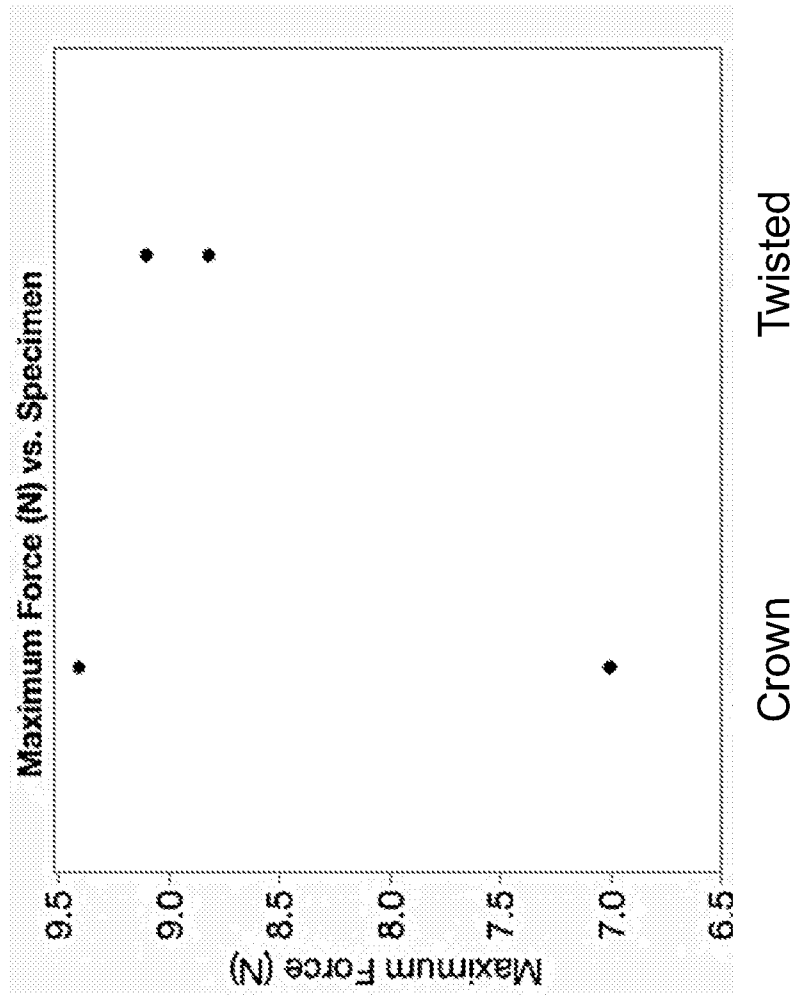


FIG. 19



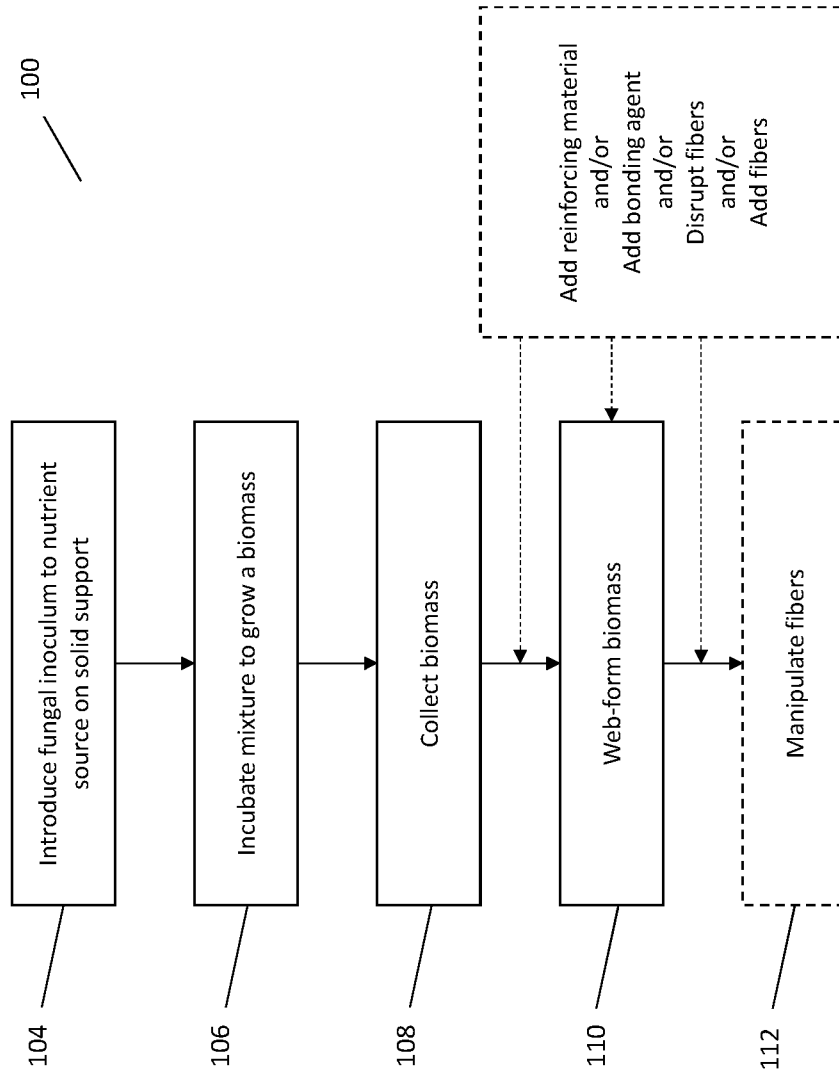


FIG. 20

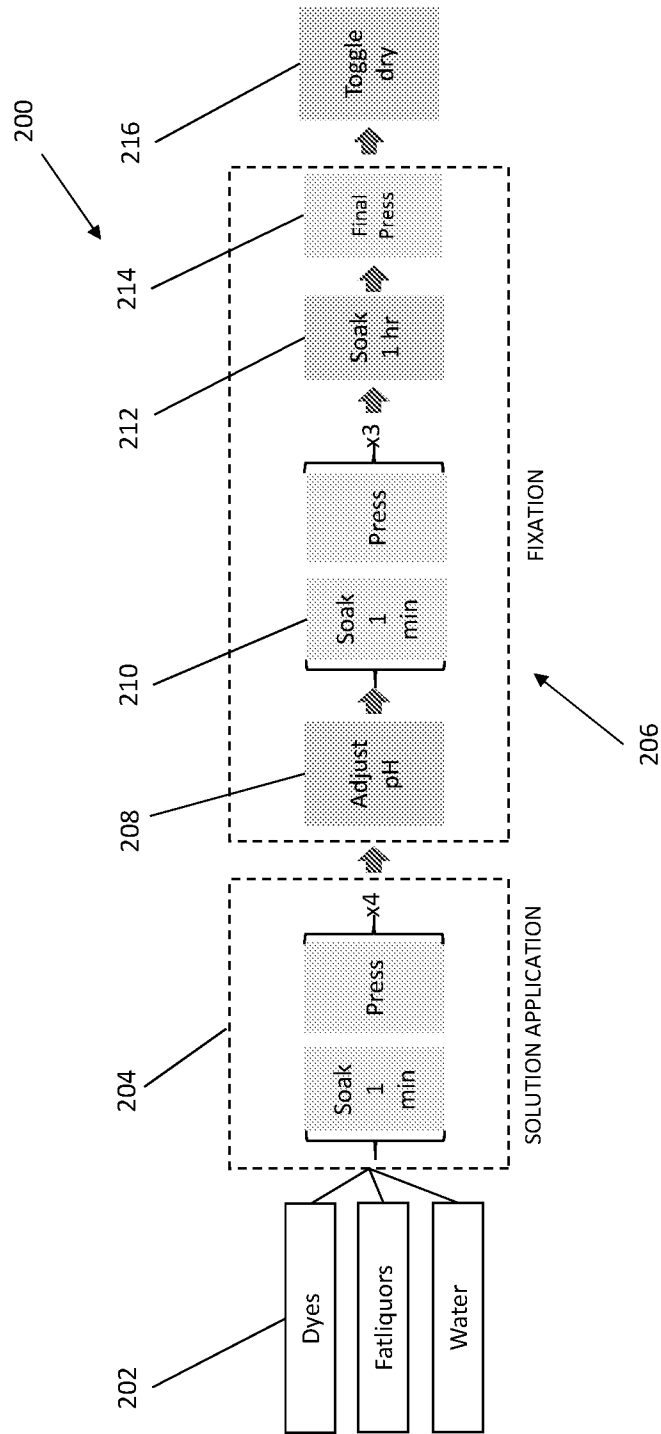


FIG. 21

# INTERNATIONAL SEARCH REPORT

International application No <b>PCT/US2022/025973</b>
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b>				
<b>INV.</b>	<b>D04H1/4266</b>	<b>C08J5/04</b>		
	<b>D04H1/64</b>	<b>D04H1/70</b>		
	<b>D04H3/12</b>			
	<b>D04H1/488</b>	<b>D04H1/49</b>		
	<b>D04H3/015</b>	<b>D04H3/105</b>		
	<b>D04H3/11</b>			
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols) <b>D04H C08J</b>				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) <b>EPO-Internal, WPI Data</b>				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
<b>X</b>	<b>US 2020/392341 A1 (SMITH MATTHEW JORDAN [US] ET AL) 17 December 2020 (2020-12-17) cited in the application the whole document</b> -----	<b>1-110</b>		
<b>E</b>	<b>WO 2022/115541 A1 (BOLT THREADS INC [US]) 2 June 2022 (2022-06-02) claims 1-71</b> -----	<b>1-110</b>		
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <span style="margin-left: 100px;"><input checked="" type="checkbox"/> See patent family annex.</span>				
* Special categories of cited documents : <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;">               "A" document defining the general state of the art which is not considered to be of particular relevance                "E" earlier application or patent but published on or after the international filing date                "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)                "O" document referring to an oral disclosure, use, exhibition or other means                "P" document published prior to the international filing date but later than the priority date claimed             </td> <td style="width: 50%; border: none; vertical-align: top;">               "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art                "&amp;" document member of the same patent family             </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family			
Date of the actual completion of the international search		Date of mailing of the international search report		
<b>25 July 2022</b>		<b>09/08/2022</b>		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer  <b>Demay, Stéphane</b>		

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/025973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
<b>US 2020392341</b>	<b>A1</b>	<b>17-12-2020</b>	
		<b>AU 2020279832 A1</b>	<b>06-01-2022</b>
		<b>CA 3137693 A1</b>	<b>26-11-2020</b>
		<b>CN 114127278 A</b>	<b>01-03-2022</b>
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		<b>US 2021292706 A1</b>	<b>23-09-2021</b>
		<b>WO 2020237201 A1</b>	<b>26-11-2020</b>
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<b>WO 2022115541</b>	<b>A1</b>	<b>02-06-2022</b>	<b>NONE</b>
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