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(54) Title: COLLAGEN-INFUSED COMPOSITE MATERIALS AND METHODS OF MANUFACTURING THE SAME

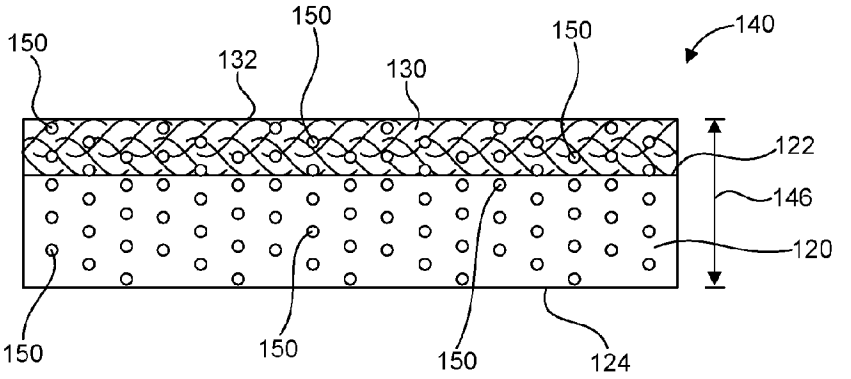

FIG. 1B

(57) Abrégé/Abstract:
The present disclosure provides a collagen-infused composite material comprising an optionally functionalized armature including a base substrate and one or more and non-woven substrates. The non-woven substrate(s) can be directly coupled to a topmost surface and/or bottommost surface of the base substrate. The material properties and/or collagen infusion capacities of the base substrate and the non-woven substrate(s) can be tailored to create a collagen-infused composite material with characteristics that mimic those of a natural leather. In some embodiments, the base substrate can be spacer fabric. In some embodiments, the armature can be functionalized to facilitate the crosslinking the collagen to the armature during one or more tanning processes.

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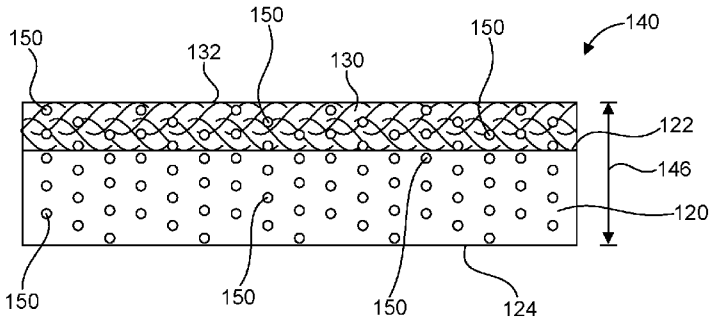

FIG. 1B

(57) Abstract: The present disclosure provides a collagen-infused composite material comprising an optionally functionalized armature including a base substrate and one or more and non-woven substrates. The non-woven substrate(s) can be directly coupled to a topmost surface and/or bottommost surface of the base substrate. The material properties and/or collagen infusion capacities of the base substrate and the non-woven substrate(s) can be tailored to create a collagen-infused composite material with characteristics that mimic those of a natural leather. In some embodiments, the base substrate can be spacer fabric. In some embodiments, the armature can be functionalized to facilitate the crosslinking the collagen to the armature during one or more tanning processes.

WO 2020/186169 A1

[Continued on next page]

WO 2020/186169 A1

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COLLAGEN-INFUSED COMPOSITE MATERIALS AND METHODS OF MANUFACTURING THE SAME

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FIELD

[0002] The present disclosure relates to collagen-infused composite materials. In particular, the present disclosure relates to collagen-infused composite materials that can have the look, the feel, aesthetic properties, and/or mechanical properties similar to natural leather, and that can be used to make goods and articles previously prepared from natural leather.

BACKGROUND

[0003] Leather is used in a vast variety of applications, such as in furniture, upholstery, clothing, shoes, luggage, handbags and accessories, and in automotive applications. The global trade value for leather is high, and there is a continuing and increasing demand for leather products. However, there are variety of costs, constraints, and social concerns associated with producing natural leather. Natural leathers are produced from the skins of animals that require raising livestock. This requires enormous amounts of feed, pastureland, water, and fossil fuels. It produces air and waterway pollution, including production of greenhouse gases like methane. It also raises social concerns about the treatment of animals. In recent years, there has also been a fairly well documented decrease in the availability of traditional high quality hides. For at least these reasons, alternative means to meet the demand for leather are desirable.

BRIEF SUMMARY

[0004] A first embodiment (1) of the present disclosure is directed to a collagen-infused composite material comprising an armature, the armature comprising a spacer fabric comprising a topmost surface and a bottommost surface opposite the topmost surface, a first non-woven substrate disposed over the topmost surface of the spacer fabric, and a second non-woven substrate disposed over the bottommost surface of the spacer fabric such that the spacer fabric is disposed between the second non-woven substrate and the first non-woven substrate; and collagen infused into the spacer fabric, the first non-woven substrate, and the second non-woven substrate.

[0005] In a second embodiment (2), the first non-woven substrate according to the first embodiment (1) is disposed on the topmost surface of the spacer fabric.

[0006] In a third embodiment (3), the second non-woven substrate according to the first embodiment (1) or the second embodiment (2) is disposed on the bottommost surface of the spacer fabric.

[0007] In a fourth embodiment (4), the collagen according to any of embodiments (1)–(3) couples the first non-woven substrate to the spacer fabric and couples the second non-woven substrate to the spacer fabric.

[0008] In a fifth embodiment (5), the first non-woven substrate according to any of embodiments (1)–(4) is directly coupled to the spacer fabric.

[0009] In a sixth embodiment (6), the first non-woven substrate according to the sixth embodiment (6) is directly coupled to the spacer fabric by a plurality of needle-punched entanglements between the first non-woven substrate and the spacer fabric.

[0010] In a seventh embodiment (7), the collagen infused into the first non-woven substrate according to any of embodiments (1)–(6) defines a collagen layer on a top surface of the first non-woven substrate, the collagen layer being continuously distributed over the top surface of the first non-woven substrate.

[0011] In an eighth embodiment (8), the collagen layer on the top surface of the first non-woven substrate according to the seventh embodiment (7) comprises a rough top surface, and wherein the rough top surface has a surface area per square inch of at least 10% greater than 1 in^2.

[0012] In a ninth embodiment (9), the composite material of according to the eighth embodiment (8) further comprises a coloring agent, where the rough top surface

comprises a plurality of peaks and a plurality of valleys, and where a color of the peaks is different than a color of the valleys. In a tenth embodiment (10), the color of the peaks is darker than the color of the valleys. In an eleventh embodiment (11), the coloring agent comprises a dye.

[0013] In a twelfth embodiment (12), the dye according to the eleventh embodiment (11) is a fiber reactive dye or an acid dye.

[0014] In a thirteenth embodiment (13), at least one of the topmost surface of the spacer fabric or the bottommost surface of the spacer fabric according to any of embodiments (1)–(12) is defined by a woven fabric layer or a knitted fabric layer.

[0015] In a fourteenth embodiment (14), the armature according to any of embodiments (1)–(13) is functionalized.

[0016] In a fifteenth embodiment (15), the composite material of any of embodiments (1)–(8), (13), or (14) further comprises a coloring agent.

[0017] In a sixteenth embodiment (16), the coloring agent of the fifteenth embodiment (15) comprises a dye.

[0018] In a seventeenth embodiment (17), the dye of the sixteenth embodiment (16) is a fiber reactive dye or an acid dye.

[0019] An eightieth embodiment (18) of the present disclosure is directed to a collagen-infused composite material comprising an armature comprising a base substrate having a first collagen infusion capacity and a first tear strength, the base substrate comprising a topmost surface and a bottommost surface opposite the topmost surface, and a non-woven substrate having a second collagen infusion capacity and a second tear strength, the non-woven substrate disposed over the topmost surface of the base substrate; and collagen infused into the base substrate and the non-woven substrate, where the collagen is infused into the non-woven substrate at a first collagen density and the collagen is infused into the base substrate at a second collagen density less than the first collagen density.

[0020] In a ninetieth embodiment (19), the first collagen infusion capacity of the eighteenth embodiment (18) is less than the second collagen infusion capacity.

[0021] In a twentieth embodiment (20), the base substrate according to the eighteenth embodiment (18) or the nineteenth embodiment (19) comprises at least one of: a woven fabric layer or a knitted fabric layer.

[0022] In twenty-first embodiment (21), the first tear strength according to any of embodiments (18)–(20) is greater than the second tear strength.

[0023] A twenty-second embodiment (22) of the present disclosure is directed to a method for manufacturing a composite material, the method comprising preparing a collagen solution; infusing the collagen solution into an armature to form a collagen-infused armature, the armature comprising a first non-woven substrate, a second non-woven substrate, and a spacer fabric, where the first non-woven substrate is directly coupled to a topmost surface of the spacer fabric and where the second non-woven substrate is directly coupled to a bottommost surface of the spacer fabric; fibrillating the infused collagen solution; and drying the collagen-infused armature.

[0024] In a twenty-third embodiment (23), the first non-woven substrate according to the twenty-second embodiment (22) is directly coupled to the topmost surface of the spacer fabric by a plurality of needle-punched entanglements between the first non-woven substrate and the spacer fabric, and the second non-woven substrate according to the twenty-second embodiment (22) is directly coupled to the bottommost surface of the spacer fabric by a plurality of needle-punched entanglements between the second non-woven substrate and the spacer fabric.

[0025] In a twenty-fourth embodiment (24), the method according to the twenty-second embodiment (22) or the twenty-third embodiment (23) further comprises adding a coloring agent before drying the collagen-infused armature.

[0026] In a twenty-fifth embodiment (25), the collagen-infused armature according to the twenty-fourth embodiment (24) comprises a rough top surface defined by a collagen layer, the rough top surface comprises a plurality of peaks and a plurality of valleys and a color of the coloring agent on the peaks is different than a color of the coloring agent in the valleys.

[0027] In a twenty-sixth embodiment (26), the coloring agent according to the twenty-fifth embodiment (25) comprises a dye.

[0028] In a twenty-seventh embodiment (27), infusing the collagen solution into the armature in the method according to any of embodiments (22)–(26) comprises filtering the collagen solution through the armature.

[0029] In a twenty-eighth embodiment (28), infusing the collagen solution into the armature in the method according to any of embodiments (22)–(27) comprises soaking the armature in a bath comprising the collagen solution.

[0030] In a twenty-ninth embodiment (29), the method according to any of embodiments (22)–(28) further comprises functionalizing the armature before infusing the collagen solution into the armature.

[0031] In a thirtieth embodiment (30), the method according to any of embodiments (22)–(29) further comprises tanning the collagen-infused armature.

[0032] In a thirty-first embodiment (31), tanning the collagen-infused armature in the method of the thirtieth embodiment (30) is performed before drying the collagen infused armature.

[0033] In a thirty-second embodiment (32), the method according to the thirtieth embodiment (30) or the thirty-first embodiment (31) further comprises retanning the tanned collagen-infused armature.

[0034] In a thirty-third embodiment (33), retanning the tanned collagen-infused armature in the method according to the thirty-second embodiment (32) is performed before drying the collagen infused armature.

[0035] In a thirty-fourth embodiment (34), the armature according to any of embodiments (30)–(33) is functionalized such that tanning the collagen infused armature results in the formation of chemical crosslinks between the collagen molecules and the functionalized armature.

[0036] A thirty-fifth embodiment (35) of the present disclosure is directed to a composite material comprising collagen and an armature having a thickness of about 0.5 mm to about 50 mm. In a thirty-sixth embodiment (36), the thickness is about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm.

[0037] In a thirty-seventh embodiment (37), the collagen of the thirty-fifth embodiment (35) or the thirty-sixth embodiment (36) is recombinant collagen.

[0038] In a thirty-eighth embodiment (38), the armature according to any of embodiments (35)–(37) comprises a cellulosic material.

[0039] In a thirty-ninth embodiment (39), the armature according to any of embodiments (35)–(38) comprises multiple layers.

[0040] In a fortieth embodiment (40), the armature according to the thirty-ninth embodiment (39) comprises two, three, four, five, six, seven, eight, nine or ten layers.

[0041] In a forty-first embodiment (41), the armature according to the fortieth embodiment (40) comprises two or three layers.

[0042] In a forty-second embodiment (42), the armature according to the forty-first embodiment (41) comprises three layers, the collagen is infused into at least two of the three layers, and collagen is disposed on at least an outermost surface of at least one of the three layers.

[0043] In a forty-third embodiment (43), the collagen and the armature according to any of embodiments (35)–(42) are tanned or dyed.

[0044] In a forty-fourth embodiment (44), the armature according to any of embodiments (35)–(43) comprises one or more materials selected from the group consisting of cotton, linen, silk, wool, kenaf, flax, cashmere, angora, bamboo, bast, hemp, soya, seacell, milk or milk proteins, spider silk, chitosan, mycelium, cellulose including bacterial cellulose, wood, polyester, nylon, an aromatic polyamide, a polyethylene, a polypropylene, a polypropylene/polyethylene copolymer, rayon, lyocell, viscose, antimicrobial yarn (A.M.Y.), Sorbtek, nylon, Lycra®, spandex, Elastane®, polyester-polyurethane copolymers, aramids, carbon fibers, fullerenes, glass, silicon, minerals, metals, and combinations thereof.

[0045] In a forty-fifth embodiment (45), the metals according to the forty-fourth embodiment (44) are metal alloys are selected from the group consisting of iron, steel, lead alloys, gold alloys, silver alloys, platinum alloys, copper alloys, zinc alloys, and titanium alloys.

[0046] In a forty-sixth embodiment (46), the armature according to any of embodiments (35)–(45) is functionalized.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] The accompanying figures, which are incorporated herein, form part of the specification and illustrate embodiments of the present disclosure. Together with the description, the figures further serve to explain the principles of and to enable a person

skilled in the relevant art(s) to make and use the disclosed embodiments. These figures are intended to be illustrative, not limiting. Although the disclosure is generally described in the context of these embodiments, it should be understood that it is not intended to limit the scope of the disclosure to these particular embodiments. In the drawings, like reference numbers indicate identical or functionally similar elements.

[0048] FIG. 1A shows a cross-section of an armature according to some embodiments.

[0049] FIG. 1B shows a cross-section of a collagen-infused composite material according to some embodiments.

[0050] FIG. 1C shows a cross-section of an armature according to some embodiments.

[0051] FIG. 1D shows a cross-section of a collagen-infused composite material according to some embodiments.

[0052] FIG. 2 shows a cross-section of a spacer fabric according to some embodiments.

[0053] FIG. 3A shows a cross-section of an armature including a spacer fabric according to some embodiments.

[0054] FIG. 3B shows a cross-section of a collagen-infused composite material including a spacer fabric according to some embodiments.

[0055] FIG. 4A is a photograph of a collagen-infused composite material according to some embodiments. FIG. 4B is a schematic representation of a surface of the collagen-infused composite material in FIG. 4A.

[0056] FIG. 5 is a block diagram illustrating a method of manufacturing a collagen-infused composite material according to some embodiments.

DETAILED DESCRIPTION

[0057] The following examples are illustrative, but not limiting, of the present disclosure. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in the field, and which would be apparent to those skilled in the art, are within the spirit and scope of the disclosure.

Definitions

[0058] The indefinite articles “a” and “an” to describe an element or component means that one or at least one of these elements or components is present. Although these articles are conventionally employed to signify that the modified noun is a singular noun,

as used herein the articles "a" and "an" also include the plural, unless otherwise stated in specific instances. Similarly, the definite article "the," as used herein, also signifies that the modified noun can be singular or plural, again unless otherwise stated in specific instances.

[0059] As used in the claims, "comprising" is an open-ended transitional phrase. A list of elements following the transitional phrase "comprising" is a non-exclusive list, such that elements in addition to those specifically recited in the list can also be present. As used in the claims, "consisting essentially of" or "composed essentially of" limits the composition of a material to the specified materials and those that do not materially affect the basic and novel characteristic(s) of the material. As used in the claims, "consisting of" or "composed entirely of" limits the composition of a material to the specified materials and excludes any material not specified.

[0060] Where a range of numerical values is recited herein, comprising upper and lower values, unless otherwise stated in specific circumstances, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the claims be limited to the specific values recited when defining a range. Further, when an amount, concentration, or other value or parameter is given as a range, one or more preferred ranges or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether such pairs are separately disclosed. Finally, when the term "about" is used in describing a value or an end-point of a range, the disclosure should be understood to include the specific value or end-point referred to. Whether or not a numerical value or end-point of a range recites "about," the numerical value or end-point of a range is intended to include two embodiments: one modified by "about," and one not modified by "about."

[0061] As used herein, the term "about" refers to a value that is within ± 10% of the value stated. For example, about 3 kPa can include any number between 2.7 kPa and 3.3 kPa.

[0062] As used herein, the term "armature" refers to a framework configured to be infused with collagen, for example, fibrillated collagen. In some embodiments, the armature can be a multi-layered framework configured to be infused with collagen. In

some embodiments, and as described elsewhere herein, the armature can be optionally functionalized.

[0063] As used herein, the term "non-woven substrate" refers to a fibrous substrate comprising interlocked individual fibers. The fibers can be interlocked by, for example, mechanical, thermal, and/or chemical means. A non-woven substrate can include interlocked staple fibers and/or long filament fibers. Fibers of a non-woven substrate are neither woven nor knitted. Non-woven substrates can include, for example, hydroentangled fibers, needle-punched fibers, or thermally bonded fibers. Generally, a woven substrate provides superior mechanical properties (e.g., strength) compared to a non-woven substrate.

[0064] As used herein, the term "collagen density" refers to the mass of collagen in grams per unit volume in a specified component (e.g., substrate or layer).

[0065] As used herein, the term "collagen infusion capacity" defines a component's (e.g., substrate's or layer's) ability to accept collagen. A collagen infusion capacity is a measure of percent weight change of a component resulting from a collagen infusion process. A "collagen infusion capacity" is not necessarily the maximum amount of collagen that can be infused into a component. A collagen infusion capacity value described herein is measured using the following "Water Absorption Test." The water absorption capacity value calculated in the "Water Absorption Test" is the collagen infusion capacity of the component.

[0066] For the "Water Absorption Test," the following steps are performed. (1) The mass of a dry test specimen (e.g., substrate or layer) is measured. (2) The test specimen is immersed into a beaker containing deionized water. If testing more than one specimen simultaneously, multiple specimens of the same sample can be placed within the same beaker but should be separated from each other. (3) The beaker with the specimen(s) inside is subject to a vacuum of about 5 kPa (kilopascals) and held at this vacuum for 2 minutes. A vacuum oven, dome, or chamber can be used to produce and hold the vacuum on the beaker. (4) After 2 minutes, the vacuum is released and the beaker is restored to atmospheric pressure. (5) Steps 3 and 4 are repeated two more times. (6) The specimen(s) are removed from the water and the final wet mass of the specimen(s) is measured. (7) The water absorption and water absorption capacity of each individual specimen are calculated using the following equations:

$$\text{Water Absorpotion (g)} = \text{Wet Mass (g)} - \text{Dry Mass (g)}$$

$$\text{Water Absorption Capacity (\%)} = \frac{\text{Water Absorption (g)}}{\text{Dry Mass (g)}}$$

[0067] As used herein, the term "fibrillation" refers to a process of producing collagen fibrils. Fibrillation can be induced by raising the pH of a solution comprising the collagen or by adjusting the salt concentration of the collagen solution.

[0068] As used herein, the term "crosslinking" refers to formation (or reformation) of chemical bonds between collagen molecules and/or an armature, which can optionally be functionalized.

[0069] As used herein, the term "rough" refers to surfaces having an uneven or irregular surface that is not smooth or level.

[0070] As used herein, the terms "lubricating" and "fat liquoring" refer to a process of applying a lubricant to a collagen infused armature. Exemplary lubricants include, but are not limited to, a fat, other hydrophobic compounds, or any material that modulates or controls fibril-fibril bonding during dehydration.

[0071] As used herein, the terms "dehydrating" and "dewatering" refer to a process of removing water from a mixture containing collagen fibrils and water, such as an aqueous solution, suspension, gel, or hydrogel containing fibrillated collagen.

[0072] Water can be removed by filtration, evaporation, freeze-drying, solvent exchange, vacuum drying, convection drying, heating, irradiating or microwaving, or by other known methods for removing water. In addition, chemical crosslinking of collagen is known to remove bound water from collagen by consuming hydrophilic amino acid residues such as lysine, arginine, and hydroxylysine among others. The inventors have found that, in some embodiments, acetone can quickly dehydrate collagen fibrils and can also remove water bound to hydrated collagen molecules.

[0073] As used herein, the phrase "disposed on" means that a first component (e.g., layer or substrate) is in direct contact with a second component. A first component "disposed on" a second component can be deposited, formed, placed, or otherwise applied directly onto the second component. In other words, if a first component is disposed on a second component, there are no components between the first component and the second component. A surface treatment, such as a surface functionalization treatment, is not considered a component disposed between a first component and a second component.

[0074] As used herein, the phrase "disposed over" means other components (e.g., layers or substrates) may or may not be present between a first component and a second component.

[0075] As used herein, a first component described as "coupled to" a second component means that the components are coupled to each other, either directly or indirectly, via a mechanical attachment and/or a thermal bond. A first component described as "directly coupled to" a second component means that the components are coupled to each other via direct mechanical attachment and/or a direct thermal bond between the material(s) of the components. Exemplary direct coupling processes include, hydroentanglement, needle-punching, and thermal bonding.

[0076] As used herein, a "spacer fabric" means a textile structure comprising two outer fabric layers connected by a layer of spacer yarns. The spacer yarns define a distance between interior surfaces of the opposing outer fabric layers. The outer fabric layers can include one or more woven fabric layers or one or more knitted fabric layers. Spacer yarns can be mono-filament yarns.

[0077] The designation "C_{x-y}" wherein x and y are non-zero integers, when used to modify a particular group refers to the number of carbon atoms in the group being modified. For example, a C_{1-10} alkyl group refers to an alky group having from one to ten carbon atoms and a C_{2-10} alkenyl group refers to an alkenyl group having from two to ten carbon atoms.

[0078] As used herein, term "alkyl" includes straight-chain or branched alkyl groups having one to ten carbon atoms. Exemplary alkyl groups include, but are not limited to methyl, ethyl, *n*-propyl, *i*-propyl, and the various butyl, pentyl, hexyl, heptyl, octyl, nonyl, and decyl isomers. Exemplary C_{1-6} alkyl groups include, but are not limited to, methyl, ethyl, *n*-propyl, *i*-propyl, and the different butyl, pentyl, and hexyl isomers.

[0079] As used herein the term "alkenyl" includes straight-chain and branched alkenes. Exemplary alkenyl groups include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, and the different butenyl, pentenyl and hexenyl isomers. Alkenyl also includes, for example, polyenes such as 1,2-propadienyl and 2,4-hexadienyl.

[0080] As used herein the term "alkynyl" includes straight-chain and branched alkynes. Exemplary alkynyl groups include, but are not limited to, ethynyl, 1-propynyl,

2-propynyl, and the different butynyl, pentynyl and hexynyl isomers. Alkynyl can also include moieties comprised of multiple triple bonds such as 2,5-hexadiynyl.

[0081] As used herein the term "alkoxy" refers to the group "alkyl-O-" wherein alkyl has the definition provide above. Exemplary alkoxy groups include, but are not limited to, methoxy, ethoxy, *n*-propyloxy, *i*-propyloxy, and the different butoxy, pentoxy and hexyloxy isomers.

[0082] As used herein the term "cyclic alkyl" refers to cyclic alkyl groups having at least three carbon atoms. Exemplary cyclic alky groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

[0083] The phrase "cyclic alkenyl" refers to cyclic alkenyl groups having at least three carbon atoms. Exemplary cyclic alkenyl groups include, but are not limited to, cyclopentenyl and cyclohexenyl as well as cyclic groups with more than one double bond such as 1,3- or 1,4-cyclohexadienyl.

[0084] As used herein, the term "heterocycle" refers to a ring or ring system wherein at least one of the atoms forming the backbone or the ring or ring system is other than carbon. Unless otherwise indicated, a heterocycle can be a saturated, partially unsaturated, or fully unsaturated ring. When a fully unsaturated heterocyclic ring or ring system satisfies Hückel's rule, then the ring or ring system is also called a "heteroaryl." Suitable heteroatoms include, but are not limited to, oxygen, sulfur, phosphorous, and nitrogen. A heterocycle or heteroaryl group can contain from one to four heteroatoms and the ring can be attached to another substituent through any appropriate atom, including a heteroatom, in the ring or ring system.

[0085] As used herein, the term "aryl" refers to a carbocyclic ring or ring system that satisfies Hückel's rule.

[0086] The term "carbocyclic" denotes a ring or ring system wherein the atoms in the ring or ring system backbone are only carbon atoms.

[0087] The term "ring system" denotes two or more covalently bonded rings including, but not limited to, polycyclic ring systems (including fused ring systems and bridged ring systems) and spiro ring systems.

[0088] As used herein, a "collagen fiber" is composed of collagen fibrils that are tightly packed and exhibit a high degree of alignment in the direction of the fiber. A collagen fiber can vary in diameter from more than about 1 μm to more than about 10 μm. For

example, a collagen fiber can have a diameter or more than about 1 μm, more than about 2 μm, more than about 3 μm, more than about 4 μm, more than about 5 μm, more than about 6 μm, more than about 7 μm, more than about 8 μm, more than about 9 μm, more than about 10 μm, more than about 11 μm, or more than about 12 μm.

[0089] As used herein "collagen" refers to the family of at least 28 distinct naturally occurring collagen types including, but not limited to collagen types I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, and XX. The term collagen as used herein also refers to collagen prepared using recombinant techniques. The term collagen includes collagen, collagen fragments, collagen-like proteins, triple helical collagen, alpha chains, monomers, gelatin, trimers and combinations thereof. Recombinant expression of collagen and collagen-like proteins is known in the art (*see, e.g.,* Bell, EP 1232182B1, Bovine collagen and method for producing recombinant gelatin; Olsen, *et al.*, U.S. Patent No. 6,428,978 and VanHeerde, *et al.*, U.S. Patent No. 8,188,230, incorporated by reference herein in their entireties) Unless otherwise specified, collagen of any type, whether naturally occurring or prepared using recombinant techniques, can be used in any of the embodiments described herein. That said, in some embodiments, the composite materials described herein can be prepared using Bovine Type I collagen.

[0090] Collagens are characterized by a repeating triplet of amino acids, -(Gly-X-Y)n-, so that approximately one-third of the amino acid residues in collagen are glycine. X is often proline and Y is often hydroxyproline. Thus, the structure of collagen may consist of three intertwined peptide chains of differing lengths. Different animals may produce different amino acid compositions of the collagen, which may result in different properties (and differences in the resulting leather). Collagen triple helices (also called monomers or tropocollagen) may be produced from alpha-chains of about 1050 amino acids long, so that the triple helix takes the form of a rod of about approximately 300 nm long, with a diameter of approximately 1.5 nm. In the production of extracellular matrix by fibroblast skin cells, triple helix monomers may be synthesized and the monomers may self-assemble into a fibrous form. These triple helices may be held together by electrostatic interactions (including salt bridging), hydrogen bonding, Van der Waals interactions, dipole-dipole forces, polarization forces, hydrophobic interactions, and covalent bonding. Triple helices can be bound together in bundles called fibrils, and

fibrils can further assemble to create fibers and fiber bundles. In some embodiments, fibrils can have a characteristic banded appearance due to the staggered overlap of collagen monomers. This banding can be called "D-banding." The bands are created by the clustering of basic and acidic amino acids, and the pattern is repeated four times in the triple helix (D-period). (*See, e.g.,* Covington, A., *Tanning Chemistry: The Science of Leather* (2009)) The distance between bands can be approximately 67 nm for Type 1 collagen. These bands can be detected using diffraction Transmission Electron Microscope (TEM), which can be used to access the degree of fibrillation in collagen. Fibrils and fibers typically branch and interact with each other throughout a layer of skin. Variations of the organization or crosslinking of fibrils and fibers can provide strength to a material disclosed herein. In some embodiments, protein is formed, but the entire collagen structure is not triple helical. In certain embodiments, the collagen structure can be about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% triple helical.

[0091] Regardless of the type of collagen, all are formed and stabilized through a combination of physical and chemical interactions including electrostatic interactions (including salt bridging), hydrogen bonding, Van der Waals interactions, dipole-dipole forces, polarization forces, hydrophobic interactions, and covalent bonding often catalyzed by enzymatic reactions. For Type I collagen fibrils, fibers, and fiber bundles, its complex assembly is achieved in vivo during development and is critical in providing mechanical support to the tissue while allowing for cellular motility and nutrient transport.

[0092] Various distinct collagen types have been identified in vertebrates, including bovine, ovine, porcine, chicken, and human collagens. Generally, the collagen types are numbered by Roman numerals, and the chains found in each collagen type are identified by Arabic numerals. Detailed descriptions of structure and biological functions of the various different types of naturally occurring collagens are generally available in the art; *see, e.g.*, Ayad *et al.* (1998) The Extracellular Matrix Facts Book, Academic Press, San Diego, CA; Burgeson, R E., and Nimmi (1992) "Collagen types: Molecular Structure and Tissue Distribution" in Clin. Orthop. 282:250-272; Kielty, C. M. et al. (1993) "The

Collagen Family: Structure, Assembly And Organization In The Extracellular Matrix," Connective Tissue And Its Heritable Disorders, Molecular Genetics, And Medical Aspects, Royce, P. M. and B. Steinmann eds., Wiley-Liss, NY, pp. 103-147; and Prockop, D.J- and K.I. Kivirikko (1995) "Collagens: Molecular Biology, Diseases, and Potentials for Therapy," *Annu. Rev. Biochem.*, 64:403-434.).

[0093] Type I collagen is the major fibrillar collagen of bone and skin, comprising approximately 80-90% of an organism's total collagen. Type I collagen is the major structural macromolecule present in the extracellular matrix of multicellular organisms and comprises approximately 20% of total protein mass. Type I collagen is a heterotrimeric molecule comprising two α1(I) chains and one α2(I) chain, encoded by the COL1A1 and COL1A2 genes, respectively. Other collagen types are less abundant than type I collagen, and exhibit different distribution patterns. For example, type II collagen is the predominant collagen in cartilage and vitreous humor, while type III collagen is found at high levels in blood vessels and to a lesser extent in skin.

[0094] Type II collagen is a homotrimeric collagen comprising three identical α1(II) chains encoded by the COL2A1 gene. Purified type II collagen may be prepared from tissues by, methods known in the art, for example, by procedures described in Miller and Rhodes (1982) *Methods In Enzymology* 82:33-64.

[0095] Type III collagen is a major fibrillar collagen found in skin and vascular tissues. Type III collagen is a homotrimeric collagen comprising three identical α1(III) chains encoded by the COL3A1 gene. Methods for purifying type III collagen from tissues can be found in, for example, Byers et al. (1974) *Biochemistry* 13:5243-5248; and Miller and Rhodes, *supra*.

[0096] In certain embodiments, the collagen can be Col3 alpha. In some embodiments, the collagen can be encoded by a sequence that is about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% identical to a naturally occurring Col3 alpha chain sequence. In other embodiments, the collagen can be encoded by a sequence that is about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99% identical to SEQ ID NO: 4. In particular embodiments, the collagen is encoded by SEQ ID NO: 4. Sequence identity or similarity can be determined using a similarity matrix such as BLOSUM45, BLOSUM62 or BLOSUM80 where BLOSUM45 can be used for closely related sequences, BLOSUM62

for midrange sequences, and BLOSUM80 for more distantly related sequences. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity or similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. Typically, a representative BLASTP setting uses an Expect Threshold of 10, a Word Size of 3, BLOSUM 62 as a matrix, and Gap Penalty of 11 (Existence) and 1 (Extension) and a conditional compositional score matrix adjustment. Other common settings are known to those of ordinary skill in the art.

[0097] Type IV collagen is found in basement membranes in the form of sheets rather than fibrils. Most commonly, type IV collagen contains two α1(IV) chains and one α2(IV) chain. The particular chains comprising type IV collagen are tissue-specific. Type IV collagen may be purified using, for example, the procedures described in Furuto and Miller (1987) *Methods in Enzymology*, 144:41-61, Academic Press.

[0098] Type V collagen is a fibrillar collagen found in, primarily, bones, tendon, cornea, skin, and blood vessels. Type V collagen exists in both homotrimeric and heterotrimeric forms. One form of type V collagen is a heterotrimer of two α1(V) chains and one α2(V) chain. Another form of type V collagen is a heterotrimer of α1(V), α2(V), and α3(V) chains. A further form of type V collagen is a homotrimer of α1(V). Methods for isolating type V collagen from natural sources can be found, for example, in Elstow and Weiss (1983) *Collagen Rel. Res.* 3:181-193, and Abedin et al. (1982) *Biosci. Rep.* 2:493-502.

[0099] Type VI collagen has a small triple helical region and two large non-collagenous remainder portions. Type VI collagen is a heterotrimer comprising α1(VI), α2(VI), and α3(VI) chains. Type VI collagen is found in many connective tissues. Descriptions of how to purify type VI collagen from natural sources can be found, for example, in Wu et al. (1987) *Biochem. J.* 248:373-381, and Kielty et al. (1991) *J. Cell Sci.* 99:797-807.

[0100] Type VII collagen is a fibrillar collagen found in particular epithelial tissues. Type VII collagen is a homotrimeric molecule of three α1(VII) chains. Descriptions of how to

purify type VII collagen from tissue can be found in, for example, Lunstrum *et al.* (1986) *J. Biol. Chem.* 261:9042-9048, and Bentz *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:3168-3172. Type VIII collagen can be found in Descemet's membrane in the cornea. Type VIII collagen is a heterotrimer comprising two α1(VIII) chains and one α2(VIII) chain, although other chain compositions have been reported. Methods for the purification of type VIII collagen from nature can be found, for example, in Benya and Padilla (1986) *J. Biol. Chem.* 261:4160-4169, and Kapoor *et al.* (1986) *Biochemistry* 25:3930-3937.

[0101] Type IX collagen is a fibril-associated collagen found in cartilage and vitreous humor. Type IX collagen is a heterotrimeric molecule comprising α1(IX), α2(IX), and α3 (IX) chains. Type IX collagen has been classified as a FACIT (Fibril Associated Collagens with Interrupted Triple Helices) collagen, possessing several triple helical domains separated by non-triple helical domains. Procedures for purifying type IX collagen can be found, for example, in Duance, *et al.* (1984) *Biochem. J.* 221:885-889; Ayad *et al.* (1989) *Biochem. J.* 262:753-761; and Grant *et al.* (1988) The Control of Tissue Damage, Glauert, A. M., ed., Elsevier Science Publishers, Amsterdam, pp. 3-28.

[0102] Type X collagen is a homotrimeric compound of α1(X) chains. Type X collagen has been isolated from, for example, hypertrophic cartilage found in growth plates. (*See, e.g.*, Apte *et al.* (1992) *Eur J Biochem* 206 (1):217-24.)

[0103] Type XI collagen can be found in cartilaginous tissues associated with type II and type IX collagens, and in other locations in the body. Type XI collagen is a heterotrimeric molecule comprising α1(XI), α2(XI), and α3(XI) chains. Methods for purifying type XI collagen can be found, for example, in Grant *et al., supra.*

[0104] Type XII collagen is a FACIT collagen found primarily in association with type I collagen. Type XII collagen is a homotrimeric molecule comprising three α1(XII) chains. Methods for purifying type XII collagen and variants thereof can be found, for example, in Dublet *et al.* (1989) *J. Biol. Chem.* 264:13150-13156; Lunstrum *et al.* (1992) *J. Biol. Chem.* 267:20087-20092; and Watt *et al.* (1992) *J. Biol. Chem.* 267:20093-20099.

[0105] Type XIII is a non-fibrillar collagen found, for example, in skin, intestine, bone, cartilage, and striated muscle. A detailed description of type XIII collagen may be found, for example, in Juvonen *et al.* (1992) *J. Biol. Chem.* 267: 24700-24707.

[0106] Type XIV is a FACIT collagen characterized as a homotrimeric molecule comprising α1(XIV) chains. Methods for isolating type XIV collagen can be found, for example, in Aubert-Foucher *et al.* (1992) *J. Biol. Chem.* 267:15759-15764,and Watt *et al., supra*.

[0107] Type XV collagen is homologous in structure to type XVIII collagen. Information about the structure and isolation of natural type XV collagen can be found, for example, in Myers *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10144-10148; Huebner *et al.* (1992) *Genomics* 14:220-224; Kivirikko *et al.* (1994) *J. Biol. Chem.* 269:4773-4779; and Muragaki, J. (1994) *Biol. Chem.* 264:4042-4046.

[0108] Type XVI collagen is a fibril-associated collagen, found, for example, in skin, lung fibroblast, and keratinocytes. Information on the structure of type XVI collagen and the gene encoding type XVI collagen can be found, for example, in Pan *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6565-6569; and Yamaguchi *et al.* (1992) *J. Biochem.* 112:856-863.

[0109] Type XVII collagen is a hemidesmosal transmembrane collagen, also known at the bullous pemphigoid antigen. Information on the structure of type XVII collagen and the gene encoding type XVII collagen can be found, for example, in Li *et al.* (1993) *J. Biol. Chem.* 268(12):8825-8834; and McGrath *et al.* (1995) *Nat. Genet.* 11(1):83-86.

[0110] Type XVIII collagen is similar in structure to type XV collagen and can be isolated from the liver. Descriptions of the structures and isolation of type XVIII collagen from natural sources can be found, for example, in Rehn and Pihlajaniemi (1994*) Proc. Natl. Acad. Sci USA* 91:4234-4238; Oh *et al.* (1994) *Proc. Natl. Acad. Sci USA* 91:4229-4233; Rehn *et al.* (1994) *J. Biol. Chem.* 269:13924-13935; and Oh *et al.* (1994) *Genomics* 19:494-499.

[0111] Type XIX collagen is believed to be another member of the FACIT collagen family, and has been found in mRNA isolated from rhabdomyosarcoma cells. Descriptions of the structures and isolation of type XIX collagen can be found, for example, in Inoguchi *et al.* (1995) *J. Biochem.* 117:137-146; Yoshioka *et al.* (1992) *Genomics* 13:884-886; and Myers *et al.*, *J. Biol. Chem.* 289:18549-18557 (1994).

[0112] Type XX collagen is a newly found member of the FACIT collagenous family, and has been identified in chick cornea. (*See, e.g.*, Gordon *et al.* (1999) *FASEB Journal* 13:A1119; and Gordon *et al.* (1998), *IOVS* 39:S1128.)

[0113] **Prokaryotic expression**. In prokaryotic systems, such as bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the expressed polypeptide. For example, when large quantities of the animal collagens and gelatins are to be produced, such as for the generation of antibodies, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the E. coli expression vector pUR278 (Ruther et al. (1983) EMBO J. 2:1791), in which the coding sequence may be ligated into the vector in frame with the lac Z coding region so that a hybrid AS-lacZ protein is produced; pIN vectors (Inouye et al. (1985) Nucleic Acids Res. 13:3101-3109 and Van Heeke et al. (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety. A recombinant collagen can comprise collagen molecules that have not been post-translationally modified, e.g., not glycosylated or hydroxylated, or can comprise one or more post-translational modifications, for example, modifications that facilitate fibrillation and formation of unbundled and randomly oriented fibrils of collagen molecules. A recombinant collagen molecule can comprise a fragment of the amino acid sequence of a native collagen molecule that can form trimeric collagen fibrils or a modified collagen molecule or truncated collagen molecule having an amino acid sequence at least 70, 80, 90, 95, 96, 97, 98, or 99% identical or similar to a native collagen amino acid sequence (or to a fibril forming region thereof or to a segment substantially comprising [Gly-X-Y]n) , such as those of bovine collagen, described by SEQ ID NOS: 1, 2 or 3 and by amino acid sequences of Col1A1, Col1A2, and Col1A3, described by Accession Nos. NP_001029211.1 (https://_www.ncbi.nlm.nih.gov/protein/77404252, last accessed February 9, 2017), NP_776945.1 (https://_www.ncbi.nlm.nih.gov/protein/27806257 last accessed February 9, 2017) and NP_001070299.1 (https://_www.ncbi.nlm.nih.gov/protein/116003881 last accessed February 9, 2017) which are incorporated by reference. (These links have been inactivated by inclusion of an underline after the double slash.)

[0114] Such recombinant or modified collagen molecules will generally comprise the repeated -(Gly-X-Y)n- sequence described herein.

[0115] BLASTN can be used to identify a polynucleotide sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, or 99% sequence identity to a reference polynucleotide such as a polynucleotide encoding a collagen polypeptide or encoding the amino acid sequences of SEQ ID NOS: 1, 2, or 3. A representative BLASTN setting optimized to find highly similar sequences uses an Expect Threshold of 10 and a Wordsize of 28, max matches in query range of 0, match/mismatch scores of 1/-2, and linear gap cost. Low complexity regions may be filtered or masked. Default settings of a Standard Nucleotide BLAST are described by and incorporated by reference to https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (last accessed January 27, 2017).

[0116] BLASTP can be used to identify an amino acid sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, or 99% sequence identity, or similarity to a reference amino acid, such as a collagen amino acid sequence, using a similarity matrix such as BLOSUM45, BLOSUM62, or BLOSUM80, where BLOSUM45 can be used for closely related sequences, BLOSUM62 for midrange sequences, and BLOSUM80 for more distantly related sequences. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity or similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. A representative BLASTP setting that uses an Expect Threshold of 10, a Word Size of 3, BLOSUM 62 as a matrix, and Gap Penalty of 11 (Existence) and 1 (Extension) and a conditional compositional score matrix adjustment. Other default settings for BLASTP are described by and incorporated by reference to the disclosure available at:

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (last accessed January 27, 2017).

[0117] **Yeast expression.** In some embodiments, collagen molecules can be produced in a yeast expression system. In yeast, a number of vectors containing constitutive or inducible promoters known in the art may be used. Ausubel et al., supra, Vol. 2, Chapter 13; Grant et al. (1987) Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Ed. Wu & Grossman, Acad. Press, N.Y. 153:516-544; Glover (1986) DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; Bitter (1987) Heterologous Gene Expression in Yeast, in Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y. 152:673-684; and The Molecular Biology of the Yeast Saccharomyces, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II (1982).

[0118] Collagen can be expressed using host cells, for example, from the yeast Saccharomyces cerevisiae. This particular yeast can be used with any of a large number of expression vectors. Commonly employed expression vectors are shuttle vectors containing the 2P origin of replication for propagation in yeast and the Col E1 origin for E. coli, for efficient transcription of the foreign gene. A typical example of such vectors based on 2P plasmids is pWYG4, which has the 2P ORI-STB elements, the GAL1-10 promoter, and the 2P D gene terminator. In this vector, an Ncol cloning site is used to insert the gene for the polypeptide to be expressed, and to provide the ATG start codon. Another expression vector is pWYG7L, which has intact 2αORI, STB, REP1 and REP2, and the GAL1-10 promoter, and uses the FLP terminator. In this vector, the encoding polynucleotide is inserted in the polylinker with its 5' ends at a BamHI or Ncol site. The vector containing the inserted polynucleotide is transformed into S. cerevisiae either after removal of the cell wall to produce spheroplasts that take up DNA on treatment with calcium and polyethylene glycol or by treatment of intact cells with lithium ions.

[0119] Alternatively, DNA can be introduced by electroporation. Transformants can be selected, for example, using host yeast cells that are auxotrophic for leucine, tryptophan, uracil, or histidine together with selectable marker genes such as LEU2, TRP1, URA3, HIS3, or LEU2-D.

[0120] In one embodiment, polynucleotides encoding collagen are introduced into host cells from the yeast Pichia. Species of non-Saccharomyces yeast such as Pichia pastoris appear to have special advantages in producing high yields of recombinant protein in

scaled up procedures. Additionally, a Pichia expression kit is available from Invitrogen Corporation (San Diego, CA).

[0121] There are a number of methanol responsive genes in methylotrophic yeasts such as Pichia pastoris, the expression of each being controlled by methanol responsive regulatory regions, also referred to as promoters. Any of such methanol responsive promoters are suitable for use. Examples of specific regulatory regions include the AOX1 promoter, the AOX2 promoter, the dihydroxyacetone synthase (DAS), the P40 promoter, and the promoter for the catalase gene from P. pastoris, etc.

[0122] In other embodiments, the methylotrophic yeast Hansenula polymorpha is used. Growth on methanol results in the induction of key enzymes of the methanol metabolism, such as MOX (methanol oxidase), DAS (dihydroxyacetone synthase), and FMHD (formate dehydrogenase). These enzymes can constitute up to 30-40% of the total cell protein. The genes encoding MOX, DAS, and FMDH production are controlled by strong promoters induced by growth on methanol and repressed by growth on glucose. Any or all three of these promoters can be used to obtain high-level expression of heterologous genes in H. polymorpha. Therefore, in one aspect, a polynucleotide encoding animal collagen or fragments or variants thereof is cloned into an expression vector under the control of an inducible H. polymorpha promoter. If secretion of the product is desired, a polynucleotide encoding a signal sequence for secretion in yeast is fused in frame with the polynucleotide. In a further embodiment, the expression vector preferably contains an auxotrophic marker gene, such as URA3 or LEU2, which can be used to complement the deficiency of an auxotrophic host.

[0123] The expression vector is then used to transform H. polymorpha host cells using techniques known to those of skill in the art. A useful feature of H. polymorpha transformation is the spontaneous integration of up to 100 copies of the expression vector into the genome. In most cases, the integrated polynucleotide forms multimers exhibiting a head-to-tail arrangement. The integrated foreign polynucleotide has been shown to be mitotically stable in several recombinant strains, even under non-selective conditions. This phenomena of high copy integration further ads to the high productivity potential of the system.

[0124] **Fungal Expression**. Filamentous fungi can also be used to produce the present polypeptides. Vectors for expressing and/or secreting recombinant proteins in filamentous

fungi are well-known, and one of skill in the art could use these vectors to express the recombinant animal collagens.

[0125] **Plant Expression.** In one aspect, an animal collagen is produced in a plant or plant cells. In cases where plant expression vectors are used, the expression of sequences encoding the collagens can be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al. (1984) Nature 310:511-514), or the coat protein promoter of TMV (Takamatsu et al. (1987) EMBO J. 6:307-311) can be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al. (1984) EMBO J. 3:1671-1680; Broglie et al. (1984) Science 224:838-843) or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al. (1986) Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells by a variety of methods known to those of skill in the art, such as by using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach & Weissbach, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463 (1988); Grierson & Corey, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9 (1988); Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins, Owen and Pen eds., John Wiliey & Sons, 1996; Transgenic Plants, Galun and Breiman eds, Imperial College Press, 1997; and Applied Plant Biotechnology, Chopra, Malik, and Bhat eds., Science Publishers, Inc., 1999.

[0126] Plant cells do not naturally produce sufficient amounts of post-translational enzymes to efficiently produce stable collagen. Therefore, where hydroxylation is desired, plant cells used to express animal collagens are supplemented with the necessary post-translational enzymes to sufficiently produce stable collagen. In a preferred embodiment, the post-translational enzyme is prolyl 4-hydroxylase.

[0127] Methods of producing the present animal collagens in plant systems can be achieved by providing a biomass from plants or plant cells, wherein the plants or plant cells comprise at least one coding sequence is operably linked to a promoter to effect the expression of the polypeptide, and the polypeptide is then extracted from the biomass. Alternatively, the polypeptide can be non-extracted, e.g., expressed into the endosperm.

[0128] Plant expression vectors and reporter genes are generally known in the art. See, e.g., Gruber et al. (1993) in Methods of Plant Molecular Biology and Biotechnology,

CRC Press. Typically, the expression vector comprises a nucleic acid construct generated, for example, recombinantly or synthetically, and comprising a promoter that functions in a plant cell, wherein such promoter is operably linked to a nucleic acid sequence encoding an animal collagen or fragments or variants thereof, or a post-translational enzyme important to the biosynthesis of collagen.

[0129] Promoters drive the level of protein expression in plants. To produce a desired level of protein expression in plants, expression can be under the direction of a plant promoter. Promoters suitable for use are generally available in the art. See, e.g., PCT Publication No. WO 91/19806. Examples of promoters that can be used include non-constitutive promoters or constitutive promoters. These promoters include, but are not limited to, the promoter for the small subunit of ribulose-1,5-bis-phosphate carboxylase; promoters from tumor-inducing plasmids of Agrobacterium tumefaciens , such as the RUBISCO nopaline synthase (NOS) and octopine synthase promoters; bacterial T-DNA promoters such as mas and ocs promoters; and viral promoters such as the cauliflower mosaic virus (CaMV) 19S and 35S promoters or the figwort mosaic virus 35S promoter.

[0130] Polynucleotide sequences can be placed under the transcriptional control of a constitutive promoter, directing expression of the collagen or post-translational enzyme in most tissues of a plant. In some embodiments, the polynucleotide sequence is under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The double stranded caulimorvirus family has provided the single most important promoter expression for transgene expression in plants, in particular, the 35S promoter. See, e.g., Kay et al. (1987) Science 236:1299. Additional promoters from this family, such as the figwort mosaic virus promoter, etc., have been described in the art, and can also be used. See, e.g., Sanger et al. (1990) Plant Mol. Biol. 14:433-443; Medberry et al. (1992) Plant Cell 4:195-192; and Yin and Beachy (1995) Plant J. 7:969-980.

[0131] The promoters used in polynucleotide constructs for expressing collagen can be modified, if desired, to affect their control characteristics. For example, the CaMV promoter can be ligated to the portion of the RUBISCO gene that represses the expression of RUBISCO in the absence of light, to create a promoter that is active in leaves, but not in roots. The resulting chimeric promoter can be used.

[0132] Constitutive plant promoters having general expression properties known in the art can be used with the expression vectors. These promoters are abundantly expressed in

most plant tissues and include, for example, the actin promoter and the ubiquitin promoter. See, e.g., McElroy et al. (1990) Plant Cell 2:163-171; and Christensen et al. (1992) Plant Mol. Biol. 18:675-689.

[0133] Alternatively, a polypeptide can be expressed in a specific tissue, cell type, or under more precise environmental conditions or developmental control. Promoters directing expression in these instances are known as inducible promoters. In the case where a tissue-specific promoter is used, protein expression is particularly high in the tissue from which extraction of the protein is desired. Depending on the desired tissue, expression can be targeted to the endosperm, aleurone layer, embryo (or its parts as scutellum and cotyledons), pericarp, stem, leaves tubers, roots, etc. Examples of known tissue-specific promoters include the tuber-directed class I patatin promoter, the promoters associated with potato tuber ADPGPP genes, the soybean promoter of β-conglycinin (7S protein) which drives seed-directed transcription, and seed-directed promoters from the zein genes of maize endosperm. See, e.g., Bevan et al. (1986) Nucleic Acids Res. 14: 4625-38; Muller et al. (1990) Mol. Gen. Genet. 224:136-46; Bray (1987) Planta 172: 364-370; and Pedersen et al. (1982) Cell 29:1015-26.

[0134] Collagen polypeptides can be produced in seed by way of seed-based production techniques using, for example, canola, corn, soybeans, rice and barley seed. In such a process, for example, the product is recovered during seed germination. See, e.g., PCT Publication Numbers WO 9940210; WO 9916890; WO 9907206;U.S. Patent No. 5,866,121; U.S. Patent No. 5,792,933; and all references cited therein. Promoters that can be used to direct the expression of the polypeptides can be heterologous or non-heterologous. These promoters can also be used to drive expression of antisense nucleic acids to reduce, increase, or alter concentration and composition of the present animal collagens in a desired tissue.

[0135] Other modifications that can be made to increase and/or maximize transcription of the present polypeptides in a plant or plant cell are standard and known to those in the art. For example, a vector comprising a polynucleotide sequence encoding a recombinant animal collagen, or a fragment or variant thereof, operably linked to a promoter can further comprise at least one factor that modifies the transcription rate of collagen or related post-translational enzymes, including, but not limited to, peptide export signal sequence, codon usage, introns, polyadenylation, and transcription termination sites.

Methods of modifying constructs to increase expression levels in plants are generally known in the art. See, e.g. Rogers et al. (1985) J. Biol. Chem. 260:3731; and Cornejo et al. (1993) Plant Mol Biol 23:567-58. In engineering a plant system that affects the rate of transcription of the present collagens and related post-translational enzymes, various factors known in the art, including regulatory sequences such as positively or negatively acting sequences, enhancers and silencers, as well as chromatin structure can affect the rate of transcription in plants. At least one of these factors can be utilized when expressing a recombinant animal collagen, including but not limited to the collagen types described above.

[0136] The vectors comprising the present polynucleotides will typically comprise a marker gene which confers a selectable phenotype on plant cells. Usually, the selectable marker gene will encode antibiotic resistance, with suitable genes including at least one set of genes coding for resistance to the antibiotic spectinomycin, the streptomycin phophotransferase (SPT) gene coding for streptomycin resistance, the neomycin phophotransferase (NPTH) gene encoding kanamycin or geneticin resistance, the hygromycin resistance, genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular, the sulfonylurea-type herbicides; e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations, genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phophinothricin or basta; e.g. the bar gene, or other similar genes known in the art. The bar gene encodes resistance to the herbicide basta, the nptII gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

[0137] Typical vectors useful for expression of foreign genes in plants are well-known in the art, including, but not limited to, vectors derived from the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens. These vectors are plant-integrating vectors that upon transformation, integrate a portion of the DNA into the genome of the host plant. See e.g., Rogers et al. (1987) Meth In Enzymol. 153:253-277; Schardl et al. (1987) Gene 61:1-11; and Berger et al., Proc. Natl. Acad. Sci. U.S.A. 86:8402-8406.

[0138] Vectors comprising sequences encoding the present polypeptides and vectors comprising post-translational enzymes or subunits thereof may be co-introduced into the desired plant. Procedures for transforming plant cells are available in the art, for example,

direct gene transfer, in vitro protoplast transformation, plant virus-mediated transformation, liposome-mediated transformation, microinjection, electroporation, Agrobacterium mediated transformation, and particle bombardment. See e.g., Paszkowski et al. (1984) EMBO J. 3:2717-2722; U.S. Patent No. 4,684,611; European Application No. 0 67 553; U.S. Patent No. 4,407,956; U.S. Patent No. 4,536,475; Crossway et al. (1986) Biotechniques 4:320-334; Riggs et al. (1986) Proc. Natl. Acad. Sci USA 83:5602-5606; Hinchee et al. (1988) Biotechnology 6:915-921; and U.S. Patent No. 4,945,050.) Standard methods for the transformation of, e.g., rice, wheat, corn, sorghum, and barley are described in the art. See, e.g., Christou et al. (1992) Trends in Biotechnology 10: 239 and Lee et al. (1991) Proc. Nat'l Acad. Sci. USA 88:6389. Wheat can be transformed by techniques similar to those employed for transforming corn or rice. Furthermore, Casas et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11212, describe a method for transforming sorghum, while Wan et al. (1994) Plant Physiol. 104: 37, teach a method for transforming barley. Suitable methods for corn transformation are provided by Fromm et al. (1990) Bio/Technology 8:833 and by Gordon-Kamm et al., supra.

[0139] Additional methods that can be used to generate plants that produce animal collagens are established in the art. See, e.g., U.S. Patent No. 5,959,091; U.S. Patent No. 5,859,347; U.S. Patent No.5,763,241; U.S. Patent No. 5,659,122; U.S. Patent No. 5,593,874; U.S. Patent No. 5,495,071; U.S. Patent No. 5,424,412; U.S. Patent No. 5,362,865; U.S. Patent No. 5,229,112; U.S. Patent No. 5,981,841; U.S. Patent No. 5,959,179; U.S. Patent No. 5,932,439; U.S. Patent No. 5,869,720; U.S. Patent No. 5,804,425; U.S. Patent No. 5,763,245; U.S. Patent No. 5,716,837; U.S. Patent No. 5,689,052; U.S. Patent No. 5,633,435; U.S. Patent No. 5,631,152; U.S. Patent No.5,627,061; U.S. Patent No. 5,602,321; U.S. Patent No. 5,589,612; U.S. Patent No. 5,510,253; U.S. Patent No. 5,503,999; U.S. Patent No. 5,378,619; U.S. Patent No. 5,349,124; U.S. Patent No. 5,304,730; U.S. Patent No. 5,185,253; U.S. Patent No. 4,970,168; European Publication No. EPA 00709462; European Publication No. EPA 00578627; European Publication No. EPA 00531273; European Publication No. EPA 00426641; PCT Publication No. WO 99/31248; PCT Publication No. WO 98/58069; PCT Publication No. WO 98/45457; PCT Publication No. WO 98/31812; PCT Publication No. WO 98/08962; PCT Publication No. WO 97/48814; PCT Publication No. WO 97/30582; and PCT Publication No. WO 9717459.

[0140] **Insect Expression**. Another alternative expression system for collagen is an insect system. Baculoviruses are very efficient expression vectors for the large-scale production of various recombinant proteins in insect cells. The methods as described in Luckow et al. (1989) Virology 170:31-39 and Gruenwald, S. and Heitz, J. (1993) Baculovirus Expression Vector System: Procedures & Methods Manual, Pharmingen, San Diego, CA, can be employed to construct expression vectors containing a collagen coding sequence for collagen and the appropriate transcriptional/translational control signals. For example, recombinant production of proteins can be achieved in insect cells, by infection of baculovirus vectors encoding the polypeptide. The production of recombinant collagen , collagen-like or collagenous polypeptides with stable triple helices can involve the co-infection of insect cells with three baculoviruses, one encoding the animal collagen to be expressed and one each encoding the α subunit and β subunit of prolyl 4-hydroxylase. This insect cell system allows for production of recombinant proteins in large quantities. In one such system, Autographa californica nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. This virus grows in Spodoptera frugiperda cells. Coding sequences for collagen or collagen-like polypeptides may be cloned into non-essential regions (for example the polyhedron gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus; e.g., viruses lacking the proteinaceous coat coded for by the polyhedron gene. These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed; see, e.g., Smith et al. (1983) J. Virol. 46:584; and U.S. Patent No. 4,215,051. Further examples of this expression system may be found in, for example, Ausubel et al. above.

[0141] **Animal Expression**. In animal host cells, a number of expression systems can be utilized. In cases where an adenovirus is used as an expression vector, polynucleotide sequences encoding collagen or collagen-like polypeptides can be ligated to an adenovirus transcription/ translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the encoded polypeptides in infected hosts. See, e.g., Logan & Shenk, Proc.

Natl. Acad. Sci. USA 81:3655-3659 (1984). Alternatively, the vaccinia 7.5 K promoter may be used; see, e.g., Mackett et al. (1982) Proc. Natl. Acad. Sci. USA 79:7415-7419; Mackett et al. (1982) J. Virol. 49:857-864; and Panicali et al. (1982) Proc. Natl. Acad. Sci. USA 79:4927-4931.

[0142] A preferred expression system in mammalian host cells is the Semliki Forest virus. Infection of mammalian host cells, for example, baby hamster kidney (BHK) cells and Chinese hamster ovary (CHO) cells can yield very high recombinant expression levels. Semliki Forest virus is a preferred expression system as the virus has a broad host range such that infection of mammalian cell lines will be possible. More specifically, Semliki Forest virus can be used in a wide range of hosts, as the system is not based on chromosomal integration, and thus provides an easier way of obtaining modifications of the recombinant animal collagens in studies aiming at identifying structure function relationships and testing the effects of various hybrid molecules. Methods for constructing Semliki Forest virus vectors for expression of exogenous proteins in mammalian host cells are described in, for example, Olkkonen et al. (1994) Methods Cell Biol 43:43-53.

[0143] Non-human Transgenic animals can also be used to express the polypeptides. Such systems can be constructed by operably linking a polynucleotide to a promoter, along with other required or optional regulatory sequences capable of effecting expression in mammary glands. Likewise, required or optional post-translational enzymes can be produced simultaneously in the target cells employing suitable expression systems. Methods of using non-human transgenic animals to recombinantly produce proteins are known in the art. See, e.g., U.S. Patent No. 4,736,866; U.S. Patent No. 5,824,838; U.S. Patent No. 5,487,992; and U.S. Patent No. 5,614,396.

[0144] In some embodiments, collagen can be obtained by cell culture techniques including from cells grown in a bioreactor.

[0145] In some embodiments, collagen can be obtained via recombinant DNA techniques. Constructs encoding non-human collagen can be introduced into host organisms to produce non-human collagen. For instance, collagen can be produced with yeast, such as Hansenula polymorpha, Saccharomyces cerevisiae, Pichia pastoris, and the like as the host. Further, in recent years, bacterial genomes have been identified that provide the signature (Gly-Xaa-Yaa)n repeating amino acid sequence that is characteristic

of triple helix collagen. For example, gram-positive bacterium Streptococcus pyogenes contains two collagen-like proteins, Scl1 and Scl2 that now have well characterized structure and functional properties. Thus, it would be possible to obtain constructs in recombinant E. coli systems with various sequence modifications of either Scl1 or Scl2 for establishing large-scale production methods. Collagen can also be obtained through standard peptide synthesis techniques. Collagen obtained from any of the techniques mentioned can be further polymerized. Collagen dimers and trimers are formed from self-association of collagen monomers in solution.

[0146] The references cited in the sections above which describe the production of recombinant collagens are each incorporated by reference.

DESCRIPTION

[0147] The present disclosure provides collagen-infused composite materials, and methods of making collagen-infused composite materials, that have a look and feel, as well as mechanical properties, similar to natural leather. The collagen-infused composite materials can have, among other things, haptic properties, aesthetic properties, mechanical/performance properties, manufacturability properties, and/or thermal properties similar to natural leather. Mechanical/performance properties that can be similar to natural leather include, but are not limited to, tensile strength, tear strength, elongation at break, resistance to abrasion, internal cohesion, water resistance, and the ability to retain color when rubbed (color fastness). Haptic properties that can be similar to natural leather include, but are not limited to, softness, rigidity, coefficient of friction, and compression modulus. Aesthetic properties that can be similar to natural leather include, but are not limited to, dye-ability, embossing, aging, color, color depth, and color patterns. Manufacturing properties that can be similar to natural leather include, but are not limited to, the ability to be stitched, cut, skived, and split. Thermal properties that can be similar to natural leather include, but are not limited to, heat resistance and resistance to stiffening or softening over a significantly wide temperature range, for example 25 °C to 100 °C.

[0148] Physical properties of the collagen-infused composite materials described herein can be tailored by one or more of: the type of collagen, the amount or concentration of collagen, the degree of fibrillation, the degree of crosslinking, the degree of dehydration,

and the degree of lubrication. Composite materials described herein can provide strong, flexible and/or substantially uniform properties.

[0149] Embodiments described herein can include infusing an aqueous mixture including collagen or collagen-like proteins into an armature. Infusing collagen or collagen-like proteins into an armature as described herein can enable the fabrication of a new class of materials with enhanced functionality compared to natural leather.

[0150] Many leather applications require a durable product that does not rip or tear, even when the leather has been stitched together. Typical products that include stitched leather and require durable leather include automobile steering wheel covers, automobile seats, furniture, sporting goods, sport shoes, sneakers, watchstraps, and the like. There is a need to increase the durability of leather-like materials to improve performance in these products.

[0151] Collagen-infused composites described herein can allow for a thick and uniform grain-like material with tunable mechanical properties through control of continuous and dispersed phases. The top grain surface of natural leather is often regarded as desirable due to its soft texture and smooth surface. The natural grain is a highly porous network of collagen fibrils. The strength of the collagen fibril, microscale porosity, and density of the natural fibrils in the grain allow tanning agent penetration to stabilize and lubricate the fibrils, producing a soft, smooth and stable material. While the aesthetic of the grain is very desirable, the strength and tear resistance of the grain is often a limitation for practical application of the grain alone. Therefore, the grain is often backed with corium, its naturally reinforcing collagen layer, or can be backed artificially with laminar layers of synthetic materials. Collagen-infused composites described herein can mimic the top grain surface of natural leather while providing desirable mechanical properties.

[0152] In addition to enhanced mechanical properties, fabrication approaches described herein can also enable aesthetic functionality. For example, a collagen-infused composite described herein can further comprise a photoluminescent material. In traditional tanning, smaller nanoparticles to single molecules such as dyes are used to produce uniform coloration and aesthetic in natural leather. Since incorporation of dyes and aesthetic features relies on penetration of these molecules into the hide or skin, patterned features with controlled spatial organizations have not been possible with natural leather. Photoluminescence features incorporated into collagen-infused composite materials

described herein can provide unique functionality, including brand logos, personalization, aesthetically pleasing patterns, and anti-counterfeit technology.

[0153] In some embodiments, the materials described herein can be used to produce composite materials with patterned photoluminescence features. In order to visualize the pattern, the light emitted from the embedded photoluminescent molecule must penetrate through the thickness of a material. Recent studies have shown that light penetration into collagen-rich materials, such as skin, is highly wavelength dependent and decreases exponentially through the thickness of the material. Therefore, variables such as the emission wavelength of the embedded photoluminescent material and the distance of the photoluminescent material from the surface of a collagen-infused composite material can influence whether or not photoluminescent features are visible by eye. Likewise, the intensity of the embedded photoluminescent material can influence whether or not features are detectable by readers other than the eye, such as light emitting scanners for example.

[0154] Although surface patterns of traditional leather materials are limited by natural variations in the skin surface of the animal, or by the ability to emboss patterns onto the grain surface of leather, the present composite material can further comprise a three-dimensional object. In order to achieve unique patterns with deep surface features, three-dimensional objects can be embedded into a collagen-infused composite material.

[0155] Methods for producing collagen-infused composite materials are also disclosed herein and can involve fibrillating an isolated or purified solution or suspension of collagen molecules to produce collagen fibrils, crosslinking the fibrils, dehydrating the fibrils, and/or lubricating the fibrils.

[0156] In contrast to natural leathers which exhibit heterogeneous internal collagen structures, collagen-infused composite materials described herein can exhibit a substantially uniform internal structure characterized by unbundled and randomly-oriented collagen fibrils throughout its volume.

[0157] Collagen-infused composite materials described herein can be used in any way that natural leather is used and can be similar in appearance and feel to real leather, while having compositional, functional, and/or aesthetic features that differentiate it from natural leather. For example, unlike natural leather, a collagen-infused composite material need not contain potentially allergenic non-collagen proteins or components found in a

natural leather, a collagen-infused composite material can exhibit a similar flexibility and strength in all directions (non-anisotropy) due to substantial non-alignment of its collagen fibrils, and aesthetically can have a smooth grain texture on both sides. In some embodiments, a collagen-infused composite can exhibit uniformity of properties including uniform thickness and consistency, uniform distribution of lubricants, cross-linkers, dyes, uniform non-anisotropic strength, stretch, flexibility, and/or resistance to piping (the tendency for natural leather to separate or split parallel to a plane of a sheet). By selecting the content of collagen and processing conditions, a collagen-infused composite can be "tuned" to a particular thickness, consistency, flexibility, softness, drape, surface texture, and/or other functionality. Laminated, layered, or composite products can comprise a collagen-infused composite material.

[0158] Some embodiments described herein are directed to a process to prepare a collagen-infused composite material. In some embodiments, collagen can be dissolved or suspended in an acidic aqueous solution, an armature is infused with the collagen solution or suspension, and the collagen is fibrillated. In some embodiments, fibrillation can be performed by raising the pH to about neutral by, for example, adding a buffer solution.

[0159] In some embodiments, collagen can be free of other leather components such as elastin or non-structural animal proteins. In some embodiments, the content of actin, keratin, elastin, fibrin, albumin, globulin, mucin, mucinoids, noncollagen structural proteins, and/or noncollagen nonstructural proteins in a collagen-infused composite material can range from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 to 10% by weight of the composite material. In some embodiments, a content of actin, keratin, elastin, fibrin, albumin, globulin, mucin, mucinoids, noncollagen structural proteins, and/or noncollagen nonstructural proteins can be incorporated into a collagen-infused composite material in amounts ranging from about 0.1% to about 20%, about 0.1% to about 19%, about 0.1% to about 18%, about 0.1% to about 17%, about 0.1% to about 16%, about 0.1% to about 15%, about 0.1% to about 14%, about 0.1% to about 13%, about 0.1% to about 12%, about 0.1% to about 11%, about 0.1% to about 10%, about 0.1% to about 9%, about 0.1% to about 8%, about 0.1% to about 7%, about 0.1% to about 6%, about 0.1% to about 5%, about 0.1% to about 4%, about 0.1% to about 3%, about 0.1% to about 2%, or about 0.1% to about 1%, by weight of the composite material. Such components can be introduced during or after fibrillation, cross-linking, dehydration, or lubrication.

[0160] Embodiments described herein are directed to a composite material including collagen and an armature. The armature can be a single-layer material or a multi-layer material. The single-layer material can be, for example, a non-woven layer, a woven layer, or a knitted layer as described herein. The multi-layer material can include any number of layers, such as any number of non-woven layers, woven layers, or knitted layers as described herein. For example, the multi-layer material can include two, three, four, five, six, seven, eight, nine, or ten layers. In some embodiments, the multi-layer material can include two or three layers. In some embodiments, the multi-layer material can be a spacer fabric. In some embodiments, collagen can be infused into the armature. In some embodiments, collagen can be disposed on an outermost surface of at least one layer of an armature. In some embodiments, the collagen disposed on an outermost surface of at least one layer of an armature can be a layer of collagen.

[0161] In certain embodiments, the collagen infused composite material comprises an armature and collagen infused into the armature. As described herein, the armature can be infused with collagen to create a collagen-infused composite material, for example an engineered leather fabric, having certain desired characteristics and properties that can be tailored based on the structure of the armature as well as the collagen density within the armature. Exemplary characteristics and properties that can be tailored include, but are not limited to, thickness, tear strength, tensile strength, flexibility, surface roughness, softness, and aesthetic features such as color and or a colored pattern.

[0162] The armature infused with collagen can be a single-layer material or a multi-layer material. The single-layer material can be, for example, a non-woven layer, a woven layer, or a knitted layer as described herein. The multi-layer material can include any number of layers, such as any number of non-woven layers, woven layers, or knitted layers as described herein. For example, the multi-layer material can include two, three, four, five, six, seven, eight, nine, or ten layers. In some embodiments, the multi-layer material can include two or three layers. In some embodiments, the multi-layer material can be a spacer fabric.

[0163] Collagen can be infused into any or all of the layers of an armature to create a collagen-infused composite material. In some embodiments, collagen can be infused into a plurality of layers of an armature. For example, for an armature having three layers, collagen can be infused into at least two of the three layers. In some embodiments,

collagen can be disposed on an outermost surface of at least one layer of a multi-layer armature. For example, a layer of collagen can be disposed on an outermost surface of at least one layer of a multi-layer armature.

[0164] In some embodiments, an un-infused armature, for example armature 110 shown in FIG. 1A, can include a base substrate 120 and one or more non-woven substrates 130. Base substrate 120 includes a topmost surface 122 and a bottommost surface 124 opposite topmost surface 122. Thickness 126 of base substrate 120 is measured between topmost surface 122 and bottommost surface 124. In some embodiments, thickness 126 can be in a range of about 0.5 mm to about 50 mm, including subranges. In some embodiments, thickness 126 can be about 0.5 mm (millimeters), about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 126 can be in a range of about 0.5 mm to about 50 mm, about 1 mm to about 45 mm, about 2 mm to about 40 mm, about 3 mm to about 35 mm, about 4 mm to about 30 mm, about 5 mm to about 25 mm, about 10 mm to about 20 mm, or about 10 mm to about 15 mm. In some embodiments, non-woven substrate 130 can be directly coupled to base substrate 120 to form un-infused armature 110. Un-infused armature 110 can be infused with collagen as described herein.

[0165] Non-woven substrate 130 includes a top surface 132 and a bottom surface 134 opposite top surface 132. A thickness 136 of a non-woven substrate 130 is measured between top surface 132 and bottom surface 134. In some embodiments, thickness 136 can be in a range of about 0.2 mm to about 20 mm, including subranges. In some embodiments, thickness 136 can be about 0.2 mm, about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, or about 20 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 136 can be in a range of about 0.2 mm to about 20 mm, about 0.5 mm to about 15 mm, about 1 mm to about 10 mm, about 2 mm to about 9 mm, about 3 mm to about 8 mm, about 4 mm to about 7 mm, or about 5 mm to about 6 mm.

[0166] In some embodiments, the thickness 136 of non-woven substrate 130 can be selected based on the weight (grams per square meter, gsm) of the non-woven substrate

130 to produce a collagen-infused non-woven substrate having a desired thickness. In general, a higher weight non-woven substrate will collapse (shrink in thickness) less during a collagen infusion process. By tailoring the thickness and/or weight of non-woven substrate 130, a collagen-infused non-woven layer having desired dimensional properties and/or mechanical properties can be produced.

[0167] In some embodiments, collagen-infused composite material 140 can have a thickness 146 in the range of about 0.5 mm to about 50 mm, including subranges. In some embodiments, thickness 146 can be about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 146 can be in a range of about 0.5 mm to about 50 mm, about 1 mm to about 45 mm, about 2 mm to about 40 mm, about 3 mm to about 35 mm, about 4 mm to about 30 mm, about 5 mm to about 25 mm, about 10 mm to about 20 mm, or about 10 mm to about 15 mm. In some embodiments, thickness 146 can be about 0.5 mm to about 10 mm.

[0168] FIG. 1A illustrates a non-woven substrate 130 disposed over topmost surface 122 of base substrate 120. In some embodiments, non-woven substrate 130 can be disposed on topmost surface 122 of base substrate 120. In some embodiments, a second non-woven substrate 170 can be disposed over bottommost surface 124 of base substrate 120. FIG. 1C shows an armature 160 including a first non-woven substrate 130 and a second non-woven substrate 170 according to some embodiments. In some embodiments, second non-woven substrate 170 can be disposed on bottommost surface 124 of base substrate 120. In such embodiments, armature 160 can include a first non-woven substrate 130 disposed on topmost surface 122 of base substrate 120 and a second non-woven substrate 170 disposed on bottommost surface 124 of base substrate 120. A thickness 176 of non-woven substrate 170 is measured between a top surface 172 and a bottom surface 174. In some embodiments, thickness 176 can be equal to thickness 136 of non-woven substrate 130. In some embodiments, thickness 176 can be greater than thickness 136, while in other embodiments, thickness 176 can be less than thickness 136. In some embodiments, thickness 176 can be in a range of about 0.2 mm to about 20 mm, including subranges. In some embodiments, thickness 176 can be about 0.2 mm, about 0.5 mm, about 1 mm,

about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, or about 20 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 176 can be in a range of about 0.2 mm to about 20 mm, about 0.5 mm to about 15 mm, about 1 mm to about 10 mm, about 2 mm to about 9 mm, about 3 mm to about 8 mm, about 4 mm to about 7 mm, or about 5 mm to about 6 mm.

[0169] In some embodiments, the thickness 176 of non-woven substrate 170 can be selected based on the weight (grams per square meter, gsm) of the non-woven substrate 170 to produce a collagen-infused non-woven substrate having a desired thickness. In general, a higher weight non-woven substrate will collapse (shrink in thickness) less during a collagen infusion process. By tailoring the thickness and/or weight of non-woven substrate 170, a collagen-infused non-woven layer having desired dimensional properties and/or mechanical properties can be produced.

[0170] In some embodiments, the first and second non-woven substrates can be the same material. In other embodiments, the first and second non-woven substrates can be different materials. Non-woven substrates 130 and/or 170 can be coupled, directly or indirectly, to base substrate 120 using any of the attachment methods disclosed herein. In some embodiments, non-woven substrates 130 and 170 can be separate substrates of armature 160 disposed over opposing sides of base substrate 120. In some embodiments, non-woven substrates 130 and 170 can be portions of a single substrate wrapped around base substrate 120.

[0171] Collagen can be infused into any or all of non-woven substrate 130, non-woven substrate 170, and base substrate 120 to create a collagen-infused composite material. In some embodiments, for example as shown in FIGs. 1B and 1D, an armature, can be infused with collagen 150 to form a collagen-infused composite material. FIG. 1B shows armature 110 infused with collagen 150 to form a collagen-infused composite material 140. FIG. 1D shows armature 160 infused with collagen 150 to form collagen-infused composite material 180. Collagen 150 can be infused into base substrate 120, first non-woven substrate 130 and/or second non-woven substrate 170 using any of the infusion processes disclosed herein. In some embodiments, non-woven substrate 130 and/or non-woven substrate 170 can be coupled to base substrate 120 via collagen 150 infused into armature 110/160. Collagen 150 infused into the armature can bridge the interface

between non-woven substrate 130 and base substrate 120 and/or the interface between non-woven substrate 170 and base substrate 120, thereby coupling the substrate(s) together. In some embodiments, armatures 110 and 160 can be functionalized prior to collagen infusion according to any of the functionalization processes disclosed herein. In some embodiments, armatures 110 and 160 can be colored as described herein prior to collagen infusion.

[0172] In some embodiments, base substrate 120 can have a first collagen infusion capacity and non-woven substrate 130 can have a second collagen infusion capacity. In some embodiments, collagen 150 can be infused into non-woven substrate 130 at a first collagen density and collagen 150 can be infused into base substrate 120 at a second collagen density less than the first collagen density. In other embodiments, the first collagen density can be less than the second collagen density, and in some embodiments, the first collagen density can be equal to the second collagen density. In some embodiments, the first collagen infusion capacity of base substrate 120 can be less than the second collagen infusion capacity of non-woven substrate 130. In some embodiments, the first collagen infusion capacity of base substrate 120 can be greater than the second collagen infusion capacity of non-woven substrate 130. And, in other embodiments, the first collagen infusion capacity of base substrate 120 can be equal to the second collagen infusion capacity of non-woven substrate 130.

[0173] In some embodiments, second non-woven substrate 170 can have a third collagen infusion capacity, and collagen 150 can be infused into second non-woven substrate 170 at a third collagen density. In some embodiments, the third collagen density can be equal to the first collagen density of base substrate 120. In some embodiments, the third collagen density can be greater than the first collagen density of base substrate 120. And, in other embodiments, the third collagen density can be less than the first collagen density of base substrate 120. In some embodiments, the third collagen infusion capacity of second non-woven substrate 170 can be equal to the second collagen infusion capacity of first non-woven substrate 130. In some embodiments, the third collagen infusion capacity of second non-woven substrate 170 can be greater than, or less than the second collagen infusion capacity of first non-woven substrate 130. In some embodiments, the first collagen infusion capacity of base substrate 120 can be less than the third collagen infusion capacity of non-woven substrate 170. In some embodiments, the first collagen

infusion capacity of base substrate 120 can be greater than the third collagen infusion capacity of non-woven substrate 170. And, in other embodiments, the first collagen infusion capacity of base substrate 120 can be equal to the third collagen infusion capacity of non-woven substrate 170.

[0174] In some embodiments, the first collagen density can be less than or greater than the second collagen density by 5% or more. In some embodiments, the first collagen density can be less than or greater than the third collagen density by 5% or more. In some embodiments, the second collagen density can be less than or greater than the third collagen density by 5% or more.

[0175] In some embodiments, the first collagen infusion capacity can be less than or greater than the second collagen infusion capacity by 5% or more. In some embodiments, the first collagen infusion capacity can be less than or greater than the third collagen infusion capacity by 5% or more. In some embodiments, the second collagen infusion capacity can be less than or greater than the third collagen density by 5% or more.

[0176] In some embodiments, base substrate 120 can have a collagen infusion capacity in the range of about 500% to about 1000%, including subranges. In some embodiments, base substrate 120 can have a collagen infusion capacity of about 500%, about 550%, about 600%, about 650%, about 700%, about 750%, about 800%, about 850%, about 900%, about 950%, or about 1000%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, base substrate 120 can have a collagen infusion capacity in a range of about 500% to about 1000%, about 550% to about 950%, about 600% to about 900%, about 650% to about 850%, about 700% to about 800%, or about 750% to about 800%. In some embodiments, base substrate 120 can have a collagen infusion capacity in the range of about 600% to about 800%.

[0177] In some embodiments, non-woven substrates 130 and 170 can have a collagen infusion capacity in the range of about 800% to about 1400%, including subranges. In some embodiments, non-woven substrates 130 and 170 can have an infusion capacity of about 800%, about 850%, about 900%, about 950%, about 1000%, about 1050%, about 1100%, about 1150%, about 1200%, about 1250%, about 1300%, about 1350%, or about 1400%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, non-woven substrates 130 and 170 can

have a collagen infusion capacity in a range of about 800% to about 1400%, about 850% to about 1350%, about 900% to about 1300%, about 950% to about 1250%, about 1000% to about 1200%, about 1050% to about 1150%, or about 1100% to about 1150%. In some embodiments, non-woven substrates 130 and 170 can have a collagen infusion capacity in the range of 1000% to 1200%.

[0178] In some embodiments, by tailoring the infusion capacity and collagen density in base substrate 120 and non-woven substrates 130 and/or 170, material properties, macroscopic structure, and/or microscopic structure of a collagen-infused composite material can be tailored for specific goals. For example, material properties and surface properties of collagen-infused composite materials 140 and 180 can be tailored to mimic properties of a natural leather.

[0179] In some embodiments, the density of collagen in a collagen-infused composite material can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen in the collagen-infused composite materials can be about 1 mg/cc, about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc, about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, non-woven substrates 130 and 170 can have a collagen infusion capacity in a range of about 800% to about 1400%, about 850% to about 1350%, about 900% to about 1300%, about 950% to about 1250%, about 1000% to about 1200%, about 1050% to about 1150%, or about 1100% to about 1150%. In some embodiments, non-woven substrates 130 and 170 can have a collagen infusion capacity in the range of 1000% to 1200%.

[0180] In some embodiments, the density of collagen in a base substrate, for example spacer fabric 200, can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen in a base substrate can be about 1 mg/cc, about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc,

about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the density of the collagen in a base substrate can be in a range of about 1 mg/cc to about 1,000 mg/cc, about 5 mg/cc to about 900 mg/cc, about 10 mg/cc to about 800 mg/cc, about 20 mg/cc to about 700 mg/cc, about 30 mg/cc to about 600 mg/cc, about 40 mg/cc to about 500 mg/cc, about 50 mg/cc to about 450 mg/cc, about 60 mg/cc to about 400 mg/cc, about 70 mg/cc to about 350 mg/cc, about 80 mg/cc to about 300 mg/cc, about 90 mg/cc to about 250 mg/cc, about 100 mg/cc to about 200 mg/cc, or about 150 mg/cc to about 200 mg/cc.

[0181] In some embodiments, the density of collagen in a non-woven substrate can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen in a non-woven substrate can be about 1 mg/cc, about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc, about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the density of the collagen in a non-woven substrate can be in a range of about 1 mg/cc to about 1,000 mg/cc, about 5 mg/cc to about 900 mg/cc, about 10 mg/cc to about 800 mg/cc, about 20 mg/cc to about 700 mg/cc, about 30 mg/cc to about 600 mg/cc, about 40 mg/cc to about 500 mg/cc, about 50 mg/cc to about 450 mg/cc, about 60 mg/cc to about 400 mg/cc, about 70 mg/cc to about 350 mg/cc, about 80 mg/cc to about 300 mg/cc, about 90 mg/cc to about 250 mg/cc, about 100 mg/cc to about 200 mg/cc, or about 150 mg/cc to about 200 mg/cc. In some embodiments, the density of the collagen in a non-woven substrate can be greater than the density of the collagen in a base substrate.

[0182] In some embodiments, collagen-infused composite materials 140 and 180 can include a top and/or bottom collagen layer as described for collagen-infused composite material 310. The collagen layer(s) can be disposed over non-woven substrates 130 and/or 170. In some embodiments, collagen-infused composite material 180 can have a total thickness 186, measured between exterior surfaces of collagen layers, for example as

discussed herein for collagen-infused composite material 310. In some embodiments, total thickness 186 can be about 0.5 mm to about 50 mm, including subranges. In some embodiments, thickness 186 can be about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 186 can be in a range of about 0.5 mm to about 50 mm, about 1 mm to about 45 mm, about 2 mm to about 40 mm, about 3 mm to about 35 mm, about 4 mm to about 30 mm, about 5 mm to about 25 mm, about 10 mm to about 20 mm, or about 15 mm to about 20 mm. In some embodiments, thickness 186 can be about 0.5 mm to about 10 mm.

[0183] In some embodiments, non-woven substrates 130 and/or 170 can have first and second tear strengths and base substrate 120 can have a third tear strength. In some embodiments, the third tear strength can be greater than the first and/or second tear strengths. In such embodiments, base substrate 120 can be the primary source of strength for an armature, and also a collagen-infused composite material. Other material properties of base substrate 120 and non-woven substrates 130 and/or 170 can be similarly selected to provide desired properties for an armature, and also a collagen-infused composite material.

[0184] In some embodiments, non-woven substrates 130 and/or 170 can be staple non-wovens, melt-blown non-wovens, spunlaid non-wovens, flashspun non-wovens, or a combination thereof. In some embodiments, non-woven substrates 130 and/or 170 can be made by carding, can be air-laid, or can be wet-laid. In some embodiments, the carded, air-laid, or wet-laid substrates can be bonded by, for example, needle-punch, hydroentanglement, lamination, or thermal bonding. In some embodiments, the non-woven substrates 130 and/or 170 can include one or more natural fibers, for example fibers made from cotton, linen, silk, wool, kenaf, flax, cashmere, angora, bamboo, bast, hemp, soya, seacell, milk or milk proteins, spider silk, chitosan, mycelium, cellulose including bacterial cellulose, or wood. Mycelium is the vegetative part of a fungus or fungus-like bacterial colony, composed of a mass of branching, thread-like hyphae. Fungi are composed primarily of a cell wall that is constantly being extended at the apex of the hyphae. Unlike the cell wall of a plant, which is composed primarily of cellulose, or

the structural component of an animal cell, which relies on collagen, the structural oligosaccharides of the cell wall of fungi rely primarily on chitin and beta glucan. Chitin is a strong, hard substance, also found in the exoskeletons of arthropods.

[0185] In some embodiments, non-woven substrates 130 and/or 170 can include one or more synthetic fibers, for example fibers made from polyesters, nylons, aromatic polyamides, polyolefin fibers such as polyethylene, polypropylene. In some embodiments, non-woven substrates 130 and/or 170 can include one or more cellulosic fibers like rayon, lyocell, viscose, Sorbtek®, elastomers such as Lycra®, spandex, or Elastane®, polyester-polyurethane copolymers, aramids. In some embodiments, non-woven substrates 130 and/or 170 can include polymeric fibers with functional particles in the polymer. Exemplary functional particles include ceramic particles mixed in a polymeric resin during an extrusion process for making the polymeric fibers. Such ceramic particles can provide the polymeric fibers with desirable heat dissipation and flame resistance properties. In some embodiments, non-woven substrates 130 and/or 170 can include fibers made of fruit pulp (e.g., grape pulp or apple pulp) or pineapple fibers. In some embodiments, non-woven substrates 130 and/or 170 can include fibers made from recycled materials, for example recycled plastics. In some embodiments, non-woven substrates 130 and/or 170 can include algae fibers. In some embodiments, non-woven substrates 130 and/or 170 can include cork fibers.

[0186] In some embodiments, non-woven substrates 130 and/or 170 can include one or more natural or synthetic materials, wherein the natural material(s) are cellulosic and/or the synthetic material(s) have a chemical structure having a plurality of functional groups (such as hydroxyl groups, amino groups or a combination thereof) suitable for functionalization. In particular embodiments, non-woven substrates 130 and/or 170 are made of cotton fibers, rayon fibers, a blend of PLA (polylactide) fibers and either cotton or rayon fibers, or a blend of PET (polyethylene terephthalate) fibers and cotton fibers. In other particular embodiments, non-woven substrates 130 and/or 170 are made of a blend of 75% cotton fibers and 25% PLA fibers. In other particular embodiments, non-woven substrates 130 and/or 170 are made of a blend of 50% cotton fibers and 50% PET fibers.

[0187] In some embodiments, non-woven substrates 130 and/or 170 can have a weight in the range of about 25 g/m^2 (grams per square meter, gsm) to about 250 g/m^2, including subranges. In some embodiments, non-woven substrates 130 and/or 170 can have a

weight in the range of about 25 g/m^2, about 50 g/m^2, about 75 g/m^2, about 100 g/m^2, about 125 g/m^2, about 150 g/m^2, about 175 g/m^2, about 200 g/m^2, about 225 g/m^2, or about 250 g/m^2, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, non-woven substrate 130 and/or 170 can have a weight in a range of about 25 g/m^2 to about 250 g/m^2, about 50 g/m^2 to about 225 g/m^2, about 75 g/m^2 to about 200 g/m^2, about 100 g/m^2 to about 175 g/m^2, or about 125 g/m^2 to about 150 g/m^2. In some embodiments, non-woven substrate(s) 130 and/or 170 can have a weight of about 50 g/m^2. In some embodiments, non-woven substrates 130 and/or 170 can have a weight of about 100 g/m^2. In some embodiments, non-woven substrates 130 and/or 170 can have a weight of about 200 g/m^2.

[0188] In some embodiments, base substrate 120 can be brushed cotton twill. In some embodiments, base substrate 120 can be a three-dimensional fabric produced by knitting or weaving. In some embodiments, base substrate 120 can be a non-woven material produced by for example, melt blowing, spun bonding, or wet laid process. In some embodiments, the yarns used for preparation of base substrate 120 can be selected from: (i) natural fibers such as cotton, linen, silk, wool, kenaf, or flax, (ii) synthetic fibers such as polyesters, nylons, or aromatic polyamids, (iii) polyolefin fibers such as polyethylene, polypropylene, and the like, and (iv) mixtures thereof. In some embodiments, base substrate 120 can include recombinant proteins fibers, collagen fibers, gelatin fibers, or the like.

[0189] Additional exemplary base substrate 120 materials include woven and nonwoven fibers as well as cotton, wool, cashmere, angora, linen, bamboo, bast, hemp, soya, seacell, fibers produced from milk or milk proteins, silk, spider silk, other peptides or polypeptides including recombinantly produced peptides or polypeptides, chitosan, mycelium, cellulose including bacterial cellulose, wood including wood fibers, rayon, lyocell, vicose, antimicrobial yarn (A.M.Y.), Sorbtek, nylon, polyester, elastomers such as lycra®, spandex or elastane and other polyester-polyurethane copolymers, aramids, carbon including carbon fibers and fullerenes, glass fibers and nonwovens, silicon and silicon-containing fibers, mineral fibers, fibers made of or containing metals or metal alloys, including those comprising iron, steel, lead, gold, silver, platinum, copper, zinc, and titanium. It is also envisioned that combinations of the same can be used or incorporated into an armature.

[0190] In some embodiments, base substrate 120 includes one or more woven fabric layers. In some embodiments, base substrate 120 includes one or more knitted fabric layers. In some embodiments, base substrate 120 can be made of lyocell or modal fibers, for example TENCEL™ fibers. In some embodiments, base substrate 120 can be, or can include a spacer fabric, for example spacer fabric 200, shown in FIG. 2. Spacer fabric 200 includes a first fabric layer 210 and a second fabric layer 220 connected by one or more spacer yarns 230. Spacer yarn(s) 230 are disposed between first fabric layer 210 and second fabric layer 220 and define a distance between an interior surface 214 of first fabric layer 210 and an interior surface 224 of second fabric layer 220. Exterior surface 212 of first fabric layer 210 and exterior surface 222 of second fabric layer 220 can define topmost and bottommost surfaces of spacer fabric 200 (e.g., topmost surface 322 and bottommost surface 324 of spacer fabric 320 shown in FIGs. 3A and 3B).

[0191] First fabric layer 210 and second fabric layer 220 can include one or more layers of fabric material. In some embodiments, first fabric layer 210 and second fabric layer 220 can include one or more textile layers made from staple fibers, filaments, or mixtures thereof. As used herein, "staple fibers" are fibers having a short length, between about 0.2 mm to about 5 cm. Staple fibers can be naturally occurring or can be cut filaments. As used herein, "filaments" are long fibers having a length of 5 cm or more. In some embodiments, first fabric layer 210 and second fabric layer 220 can include one or more layers of a woven material or a knitted material. In some embodiments, exterior surface 212 of first fabric layer 210 can be defined by a woven fabric layer or a knitted fabric layer. In some embodiments, exterior surface 222 of second fabric layer 220 can be defined by a woven fabric layer or a knitted fabric layer. In some embodiments, at least one of the topmost surface or the bottommost surface of spacer fabric 200 can be defined by a woven fabric layer or a knitted fabric layer (e.g., topmost surface 322 and/or bottommost surface 324 of spacer fabric 320 shown in FIG. 3).

[0192] In some embodiments, first fabric layer 210 and second fabric layer 220 can be made from one or more natural fibers, for example fibers made from cotton, linen, silk, wool, kenaf, flax, cashmere, angora, bamboo, bast, hemp, soya, seacell, milk or milk proteins, spider silk, chitosan, mycelium, cellulose including bacterial cellulose, or wood. In some embodiments, first fabric layer 210 and second fabric layer 220 can be made from one or more synthetic fibers, for example fibers made from polyesters, nylons,

aromatic polyamides, polyolefin fibers such as polyethylene, polypropylene, rayon, lyocell, viscose, antimicrobial yarn (A.M.Y.), Sorbtek, nylon, elastomers such as Lycra®, spandex, or Elastane®, polyester-polyurethane copolymers, aramids, carbon including carbon fibers and fullerenes, glass, silicon, minerals, metals or metal alloys including those containing iron, steel, lead, gold, silver, platinum, copper, zinc, and titanium, or mixtures thereof. Spacer yarn(s) 230 can include mono-filament yarn(s) composed of any of the natural or synthetic materials listed above for first fabric layer 210 and second fabric layer 220.

[0193] In some embodiments, first fabric layer 210, second fabric layer 220, and/or spacer yarn(s) can be made of one or more of the natural or synthetic materials described above, wherein the natural material(s) are cellulosic and/or the synthetic material(s) have a chemical structure having a plurality of functional groups (such as hydroxyl groups, amino groups, or a combination thereof) suitable for functionalization.

[0194] FIG. 3A illustrates an un-infused armature 300 configured to be infused with collagen according to some embodiments. Armature 300 can include a spacer fabric 320 having a topmost surface 322 and a bottommost surface 324 opposite topmost surface 322. Spacer fabric 320 corresponds to spacer fabric 200 described above in reference to FIG. 2. Spacer fabric 320 can include a first fabric layer 330, a second fabric layer 332, and one or more spacer yarns 340 connecting first fabric layer 330 to second fabric layer 332.

[0195] Un-infused armature 300 can also include a first non-woven substrate 350 disposed over topmost surface 322 of spacer fabric 320 and a second non-woven substrate 354 disposed over bottommost surface 324 of spacer fabric 320. In this configuration, spacer fabric 320 is disposed between second non-woven substrate 354 and first non-woven substrate 350. In some embodiments, first non-woven substrate 350 can be disposed on topmost surface 322 of spacer fabric 320 and/or second non-woven substrate 354 can be disposed on bottommost surface 324 of spacer fabric 320. In such embodiments, first non-woven substrate 350 and/or second non-woven substrate 354 are in direct contact with spacer fabric 320. In some embodiments, first non-woven substrate 350 and second non-woven substrate 354 can be directly coupled to spacer fabric 320 to form an un-infused armature that is configured to be infused with collagen.

[0196] In some embodiments, first non-woven substrate 350 and/or second non-woven substrate 354 can be mechanically or thermally coupled to spacer fabric 320 by: (i) knitting first non-woven substrate 350 and/or second non-woven substrate 354 to spacer fabric 320, (ii) crocheting first non-woven substrate 350 and/or second non-woven substrate 354 to spacer fabric 320, (iii) stitching first non-woven substrate 350 and/or second non-woven substrate 354 to spacer fabric 320, (iv) felting first non-woven substrate 350 and/or second non-woven substrate 354 to spacer fabric 320, (v) dry laying, (vi) wet laying, (vii) spin bonding, (viii) spin lacing, (ix) melt blowing, (x) spin melting, (xi) hydroentanglement, or (xii) needle-punching to create a plurality of needle-punched entanglements.

[0197] In some embodiments, hydroentanglement can be used to directly couple non-woven fibers of substrates 350 and/or 354 to spacer fabric 320. In some embodiments, the hydroentanglement technique used to directly couple non-woven fibers of substrates 350 and/or 354 to spacer fabric 320 can include spraying water into the non-woven fibers at a jet pressure rate in the range of about 30 Hz to about 45 Hz, including subranges. For example, in some embodiments, water can be sprayed into the non-woven fibers at a jet pressure rate of about 30 Hz, about 35 Hz, about 40 Hz, or about 45 Hz, or in a range having any two of these values as endpoints, inclusive of the endpoints. After hydroentanglement, armature 300 can be dried in an oven at, for example, about 110 °C.

[0198] In some embodiments, a thermal bonding technique can be used to directly couple non-woven fibers of substrate(s) 350 and/or 354 to spacer fabric 320. During thermal bonding, a material of non-woven substrate(s) 350 and/or 354 can be thermally fused to a material of spacer fabric 320. In some embodiments, a polymeric material of non-woven substrate(s) 350 and/or 354 can be thermally fused to a material of spacer fabric 320. In some embodiments, a polymeric material of non-woven substrate(s) 350 and/or 354 can be thermally fused to a polymeric material of spacer fabric 320. In some embodiments, a polymeric material of spacer fabric 320 can be thermally fused to a material of non-woven substrate(s) 350 and/or 354. In some embodiments, the polymeric material of non-woven substrate(s) 350 and/or 354 can be a thermoplastic material. In some embodiments, the polymeric material of spacer fabric 320 can be a thermoplastic material. In some embodiments, thermal bonding can involve heating a polymeric material of spacer fabric 320 and/or a polymeric material of non-woven substrate(s) 350

and/or 354 to a temperature of 400 °F or more. In some embodiments, thermal bonding can involve heating a polymeric material of spacer fabric 320 and/or a polymeric material of non-woven substrate(s) 350 and/or 354 to a temperature of 400 °F or more and applying pressure to press non-woven substrate(s) 350 and/or 354 to spacer fabric 320.

[0199] In some embodiments, needle-punching can be used to directly couple non-woven fibers of substrates 350 and/or 354 to spacer fabric 320. In some embodiments, the needle-punching technique used to directly couple non-woven fibers of substrate(s) 350 and/or 354 to spacer fabric 320 can include a depth of punch of about 5/8''. In some embodiments, the penetrations per square inch (PPSI) for a needle-punching technique used to directly couple non-woven fibers of substrate(s) 350 and/or 354 to spacer fabric 320 can be in a range of about 250 PPSI to about 400 PPSI. In some embodiments, the needle-punching technique used to directly couple non-woven fibers of substrate(s) 350 and/or 354 to spacer fabric 320 can utilize needles with a gauge smaller than 36 gauge, such as 35 gauge, 34 gauge, 33 gauge, 32 gauge, 31 gauge, 30 gauge, 29 gauge, 28 gauge, 27 gauge, 28 gauge, 26 gauge, 25 gauge, 24 gauge, 23 gauge, 22 gauge, 21 gauge, 20 gauge, 19 gauge, 18 gauge, 17 gauge, 16 gauge, 15 gauge, 14 gauge, 13 gauge, 12 gauge, 11 gauge, 10 gauge, 9 gauge, 8 gauge, 7 gauge, 6 gauge, 5 gauge, 4 gauge, 3 gauge, 2 gauge, or 1 gauge. In some embodiments, the needle-punching technique can create a punch pattern in first non-woven substrate 350 and/or second non-woven substrate 354 such that when collagen is infused into armature 300, a surface of collagen-infused non-woven substrate 350 and/or 354 is formed with a non-uniform, rough surface that follows the contour of the punch pattern.

[0200] In some embodiments, first non-woven substrate 350 and/or second non-woven substrate 354 can be directly coupled to spacer fabric 320 by needle-punched entanglements between the non-woven substrate(s) and spacer fabric 320. In some embodiments, first non-woven substrate 350 can be directly coupled to topmost surface 322 of spacer fabric 320 by needle-punched entanglements between first non-woven substrate 350 and spacer fabric 320. In some embodiments, second non-woven substrate 354 can be directly coupled to bottommost surface 324 of spacer fabric 320 by needle-punched entanglements between second non-woven substrate 354 and the spacer fabric 320.

[0201] First non-woven substrate 350 and second non-woven substrate 354 can have a thickness as discussed above for non-woven substrate 130 and non-woven substrate 170. First non-woven substrate 350 and second non-woven substrate 354 can have a weight as discussed above for non-woven substrate 130 and non-woven substrate 170. Also, first non-woven substrate 350 and second non-woven substrate 354 can be made of any of the materials discussed above for non-woven substrate 130 and non-woven substrate 170. In some embodiments, first non-woven substrate 350 and/or second non-woven substrate 354 can be made from one or more natural or synthetic materials, wherein the natural material(s) are cellulosic and/or the synthetic material(s) have a chemical structure having a plurality of functional groups (such as hydroxyl groups, amino groups or a combination thereof) suitable for functionalization.

[0202] In some embodiments, spacer fabric 320 can have a first collagen infusion capacity and non-woven substrates 350 and 354 can have a second and third collagen infusion capacity. In some embodiments, the second and third collagen infusion capacities can be greater than the first collagen infusion capacity. Without wishing to be bound by a particular theory, it is believed that such an increased infusion capacity is a function of the loosely packed fibrous structure of non-woven substrates 350 and 354, which facilitates infusion of collagen 360 between fibers. In some embodiments, the second collagen infusion capacity can be equal to the third collagen infusion capacity. In some embodiments, the second collagen infusion capacity can be greater than, or less than the third collagen infusion capacity.

[0203] Collagen infusion capacity is measured by weighing a material before and after infusion, and comparing the dry weight to the infused weight. For example, a material having a dry weight of 10 g (grams) and an infused weight of 90 g has an 80 g net weight increase, which is 8 times the dry weight; therefore, the infusion capacity of that material is 8, or 800%. In some embodiments, spacer fabric 320 can have a collagen infusion capacity in the range of about 500% to about 1000%, including subranges. In some embodiments, spacer fabric 320 can have a collagen infusion capacity of about 500%, about 550%, about 600%, about 650%, about 700%, about 750%, about 800%, about 850%, about 900%, about 950%, or about 1000%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, spacer fabric 320 can have a collagen infusion capacity in a range of about 500% to about

1000%, about 550% to about 950%, about 600% to about 900%, about 650% to about 850%, about 700% to about 800%, or about 700% to about 750%. In some embodiments, spacer fabric 320 can have a collagen infusion capacity in the range of about 600% to about 800%.

[0204] In some embodiments, non-woven substrates 350 and 354 can have a collagen infusion capacity in the range of about 800% to about 1400%, including subranges. In some embodiments, non-woven substrates 350 and 354 can have a collagen infusion capacity of about 800%, about 850%, about 900%, about 950%, about 1000%, about 1050%, about 1100%, about 1150%, about 1200%, about 1250%, about 1300%, about 1350%, or about 1400%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, non-woven substrates 350 and 354 can have a collagen infusion capacity in a range of about 800% to about 1400%, about 850% to about 1350%, about 900% to about 1300%, about 950% to about 1250%, about 1000% to about 1200%, about 1050% to about 1150%, or about 1050% to about 1100%. In some embodiments, non-woven substrates 350 and 354 can have a collagen infusion capacity in the range of 1000% to 1200%.

[0205] In some embodiments, for example as shown in FIG. 3B, Collagen 360 can be infused into any or all of first non-woven substrate 350, second non-woven substrate 354, and spacer fabric 320 to create a collagen-infused composite material 310. By utilizing non-woven substrates 350/354 disposed over opposing sides of spacer fabric 320 leather-like properties, for example, a leather-like surface texture, can be created on opposing sides of spacer fabric 320.

[0206] In some embodiments, first non-woven substrate 350 and/or second non-woven substrate 354 can be coupled to spacer fabric 320 via collagen 360 infused into armature 300. Collagen 360 infused into armature 300 can bridge the interface between first non-woven substrate 350 and spacer fabric 320 and/or the interface between second non-woven substrate 354 and spacer fabric 320, thereby coupling the substrate(s) and spacer fabric 320 together. For example, in some embodiments, collagen 360, in the form of a hydrogel, can coat surfaces of the substrate(s) and spacer fabric 320 and the interface between the substrate(s) and spacer fabric 320. This coating at the interface can couple the substrate(s) to spacer fabric 320 when the hydrogel dries down.

[0207] In some embodiments, infusion of collagen 360 into armature 300 can result in the formation of a collagen layer defining an exterior surface of collagen-infused composite material 310. In some embodiments, collagen 360 infused into first non-woven substrate 350 can define a collagen layer 370 on a top surface 352 first non-woven substrate 350. Collagen layer 370 can be referred to as a "top collagen layer" of collagen-infused composite material 310. In some embodiments, collagen 360 infused into second non-woven substrate 354 can define a collagen layer 380 on a bottom surface 356 of second non-woven substrate 354. Collagen layer 380 can be referred to as a "bottom collagen layer" of collagen-infused composite material 310.

[0208] Top collagen layer 370 includes an exterior surface 372 and an interior surface 374 opposite exterior surface 372. A thickness 376 of top collagen layer 370 is measured between exterior surface 372 and interior surface 374. For purposes of determining thickness 376 of a collagen layer 370 having a rough surface, the thickness is measured relative to the top of the peaks (e.g., peaks 412). In some embodiments, thickness 376 can be 0.1 mm or more. In some embodiments, thickness 376 can be in the range of about 0.1 mm to about 2 mm, including subranges. In some embodiments, thickness 376 can be about 0.1 mm, about 0.5 mm, about 1 mm, about 1.5 mm, or about 2 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 376 can be in a range of about 0.1 mm to about 2 mm, about 0.5 mm to about 1.5 mm, or about 1 mm to about 1.5 mm.

[0209] In some embodiments, exterior surface 372 of top collagen layer 370 can define a topmost surface of collagen-infused composite material 310 exposed to atmosphere during use. In other words, in some embodiments, no additional layers of material are disposed over exterior surface 372 of top collagen layer 370. In some embodiments, top collagen layer 370 can be continuously distributed over first non-woven substrate 350 such that exterior surface 372 has a continuous surface disposed over first non-woven substrate 350. The continuous exterior surface 372 can extend continuously between perimeter edges of first non-woven substrate 350 or perimeter edges of an area on top surface 352 of first non-woven substrate 350 coated with collagen 360. Formation of a continuous layer of collagen can provide an aesthetic look similar to natural leather by masking underlying layers and substrates of collagen-infused composite material 310.

[0210] Bottom collagen layer 380 includes an exterior surface 382 and an interior surface 384 opposite exterior surface 382. A thickness 386 of bottom collagen layer 380 is measured between exterior surface 382 and interior surface 384. For purposes of determining thickness 386 of a collagen layer 380 having a rough surface, the thickness is measured relative to the top of the peaks (e.g., peaks 412). In some embodiments, thickness 386 can be 0.1 mm or more. In some embodiments, thickness 386 can be in the range of about 0.1 mm to about 2 mm, including subranges. In some embodiments, thickness 386 can be about 0.1 mm, about 0.5 mm, about 1 mm, about 1.5 mm, or about 2 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 386 can be in a range of about 0.1 mm to about 2 mm, about 0.5 mm to about 1.5 mm, or about 1 mm to about 1.5 mm.

[0211] In some embodiments, exterior surface 382 of bottom collagen layer 380 can define a bottommost surface of collagen-infused composite material 310 exposed to atmosphere during use. In other words, in some embodiments, no additional layers of material are disposed over exterior surface 382 of bottom collagen layer 380. In some embodiments, bottom collagen layer 380 can be continuously distributed over second non-woven substrate 354 such that exterior surface 382 has a continuous surface disposed over second non-woven substrate 354. The continuous exterior surface 382 can extend continuously between perimeter edges of second non-woven substrate 354 or perimeter edges of an area on bottom surface 356 of second non-woven substrate 354 coated with collagen 360. Formation of a continuous layer of collagen can provide an aesthetic look similar to natural leather by masking underlying layers and substrates of collagen-infused composite material 310.

[0212] Collagen-infused composite material 310 can have a total overall thickness defined by the sum of the thicknesses for the individual components of collagen-infused composite material 310. In some embodiments, collagen-infused composite material 310 can have an overall thickness 316, measured between exterior surface 372 of collagen layer 370 and exterior surface 382 of collagen layer 380, in the range of about 0.05 mm to about 50 mm, including subranges. In some embodiments, the collagen-infused composite materials can have a thickness 316 of about 0.05 mm, about 0.1 mm, about 0.2 mm, about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 11 mm, about

12 mm, about 13 mm, about 14 mm, about 15 mm, about 16 mm, about 17 mm, about 18 mm, about 19 mm, about 20 mm, about 25 mm, about 30 mm, about 40 mm, or about 50 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, thickness 316 can be in a range of about 0.05 mm to about 50 mm, about 0.1 mm to about 40 mm, about 0.2 mm to about 30 mm, about 0.5 mm to about 25 mm, about 1 mm to about 20 mm, about 2 mm to about 19 mm, about 3 mm to about 18 mm, about 4 mm to about 17 mm, about 5 mm to about 16 mm, about 6 mm to about 15 mm, about 7 mm to about 14 mm, about 8 mm to about 13 mm, about 9 mm to about 12 mm, or about 10 mm to about 11 mm. In some embodiments, collagen-infused composite material 310 can have a thickness 316 greater than 50 mm.

[0213] As shown in FIGs. 4A and 4B, in some embodiments, collagen layer 370 and/or collagen layer 380 of a collagen-infused composite material 400 can have a rough exterior surface 410 having a plurality of peaks 412 and a plurality of valleys 414, as shown in the zoomed-in area 420 of FIG. 4B. Peaks 412 and valleys 414 can create a surface texture similar in appearance and feel to that of a naturel leather (e.g., the grain of pebbled natural leather). In some embodiments, the placement and distribution of peaks 412 and valleys 414 can be defined by a pattern created by a direct or in-direct coupling process used to couple non-woven substrate 350 or 354 to spacer fabric 320. For example, in some embodiments, the placement and distribution of peaks 412 and valleys 414 can be defined by a pattern created by needle-punched entanglements of a non-woven substrate and/or the density and fiber distribution of a non-woven substrate. In some embodiments, peaks 412 and valleys 414 can be created by collagen contracting during drying of collagen-infused composite material 310. During drying, local surface areas can contract more or less based on mechanical reinforcements on armature 300, for example needle-punched entanglements or hydroentanglements. In some embodiments, peaks 412 and valleys 414 can be created be a finishing treatment or an embossing process like in traditional leather manufacturing.

[0214] In some embodiments, collagen-infused composite material 400 can have an increased surface area due to rough exterior surface 410. For example, in some embodiments, peaks 412 and valleys 414 can cause rough exterior surface 410 to have a surface area per square inch of at least about 1% greater than 1 in^2. In other words, in some embodiments, a one square inch sample of collagen-infused composite material

400, including a collagen layer having rough exterior surface 410, can have a surface area that is at least about 1% greater than a one square inch sample of a material having a perfectly smooth surface. In some embodiments, rough exterior surface 410 can have a surface area per square inch of at least about 1% greater than 1 in^2, about 10% greater than 1 in^2, about 20% greater than 1 in^2, about 30% greater than 1 in^2, about 40% greater than 1 in^2, about 50% greater than 1 in^2, about 60% greater than 1 in^2, about 70% greater than 1 in^2, about 80% greater than 1 in^2, about 90% greater than 1 in^2, about 100% greater than 1 in^2, about 150% greater than 1 in^2, about 200% greater than 1 in^2, about 250% greater than 1 in^2, about 300% greater than 1 in^2, about 350% greater than 1 in^2, about 400% greater than 1 in^2, about 450% greater than 1 in^2, or about 500% greater than 1 in^2, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, rough exterior surface 410 can have a surface area per square inch of about 1% greater than 1 in^2 to about 500% greater than 1 in^2, about 10% greater than 1 in^2 to about 450% greater than 1 in^2, about 20% greater than 1 in^2 to about 400% greater than 1 in^2, about 30% greater than 1 in^2 to about 350% greater than 1 in^2, about 40% greater than 1 in^2 to about 300% greater than 1 in^2, about 50% greater than 1 in^2 to about 250% greater than 1 in^2, about 60% greater than 1 in^2 to about 200% greater than 1 in^2, about 70% greater than 1 in^2 to about 150% greater than 1 in^2, about 80% greater than 1 in^2 to about 100% greater than 1 in^2, or about 80% greater than 1 in^2 to about 90% greater than 1 in^2. Unless specified otherwise, a surface area of material disclosed herein is measured using profilometry. For non- transparent materials, optical profilometry is used.

[0215] Any type of collagen, truncated collagen, unmodified or post-translationally modified, or amino acid sequence-modified collagen that can be fibrillated and cross-linked by the methods described herein can be used to produce the collagen-infused composite materials described herein. The degree of fibrillation of the collagen molecules can be determined via x-ray diffraction. This characterization will provide d-spacing values which will correspond to different periodic structures present (e.g., 67 nm spacing vs. amorphous). In some embodiments, the collagen can be substantially homogenous collagen, such as only Type I or Type III collagen or can contain mixtures of two or more different kinds of collagens. In embodiments, the collagen is recombinant collagen.

[0216] For example, a collagen composition can homogenously contain a single type of collagen molecule, for example 100% bovine Type I collagen or 100% Type III bovine

collagen, or can contain a mixture of different kinds of collagen molecules or collagen-like molecules, such as a mixture of bovine Type I and Type III molecules. The collagen mixtures can include amounts of each of the individual collagen components in the range of about 1% to about 99%, including subranges. For example, the amounts of each of the individual collagen components within the collagen mixtures can be about 1%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99%, or within a range having any two of these values as endpoints. For example, in some embodiments, a collagen mixture can contain about 30% Type I collagen and about 70% Type III collagen. Or, in some embodiments, a collagen mixture can contain about 33.3% of Type I collagen, about 33.3% of Type II collagen, and about 33.3% of Type III collagen, where the percentage of collagen is based on the total mass of collagen in the composition or on the molecular percentages of collagen molecules.

[0217] In some embodiments, amino acid residues, such as lysine and proline, in a collagen or collagen-like protein can lack hydroxylation or can have a lesser or greater degree of hydroxylation than a corresponding natural or unmodified collagen or collagen-like protein. In some embodiments, amino acid residues in a collagen or collagen-like protein can lack glycosylation or can have a lesser or greater degree of glycosylation than a corresponding natural or unmodified collagen or collagen-like protein.

[0218] In some embodiments, the collagen solution can be fibrillated into collagen fibrils. As used herein, collagen fibrils refer to nanofibers composed of tropocollagen or tropocollagen-like structures (which have a triple helical structure). In some embodiments, triple helical collagen can be fibrillated to form nanofibrils of collagen. To induce fibrillation, the collagen can be incubated to form the fibrils for a time period in the range of about 1 minute to about 24 hours, including subranges. In some embodiments, the collagen can be incubated for about 1 minute, about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, or about 24 hours, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the collagen can be

incubated for about 1 minute to about 24 hours, about 30 minutes to about 18 hours, about 1 hour to about 12 hours, or about 2 hours to about 6 hours.

[0219] In some embodiments, the collagen fibrils can have an average diameter in the range of about 1 nm (nanometer) to about 1 μm (micron, micrometer), including subranges. In some embodiments, the average diameter of the collagen fibrils can be about 1 nm, about 2 nm, about 3 nm, about 4 nm, about 5 nm, about 10 nm, about 15 nm, about 20 nm, about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 200 nm, about 300 nm, about 400 nm, about 500 nm, about 600 nm, about 700 nm, about 800 nm, about 900 nm, or about 1 μm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the average diameter of the collagen fibrils can be in a range of about 1 nm to about 1 μm, about 2 nm to about 900 nm, about 3 nm to about 800 nm, about 4 nm to about 700 nm, about 5 nm to about 600 nm, about 10 nm to about 500 nm, about 15 nm to about 400 nm, about 20 nm to about 300 nm, about 30 nm to about 200, about 40 nm to about 100 nm, about 50 nm to about 90 nm, about 60 nm to about 80 nm, or about 60 nm to about 70 nm.

[0220] In some embodiments, an average length of the collagen fibrils is in the range of about 100 nm to about 1 mm (millimeter), including subranges. In some embodiments, the average length of the collagen fibrils can be about 100 nm, about 200 nm, about 300 nm, about 400 nm, about 500 nm, about 600 nm, about 700 nm, about 800 nm, about 900 nm, about 1 μm, about 5 μm, about 10 μm, about 20 μm, about 30 μm, about 40 μm, about 50 μm, about 60 μm, about 70 μm, about 80 μm, about 90 μm, about 100 μm, about 200 μm, about 300 μm, about 400 μm, about 500 μm, about 600 μm, about 700 μm, about 800 μm, about 900 μm, or about 1 mm, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the average length of the collagen fibrils can in a range of about 100 nm to about 1 mm, about 200 nm to about 900 μm, about 300 nm to about 800 μm, about 400 nm to about 700 μm, about 500 nm to about 600 μm, about 600 nm to about 500 μm, about 700 nm to about 400 μm, about 800 nm to about 300 μm, about 900 nm to about 200 μm, about 1 μm to about 100 μm, about 5 μm to about 90 μm, about 10 μm to about 80 μm, about 20 μm to about 70 μm, about 30 μm to about 60 μm, or about 40 μm to about 50 μm.

[0221] In some embodiments, the density of the collagen fibrils in a collagen-infused composite material can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen fibrils in the collagen-infused composite materials can be about 1 mg/cc about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc, about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the density of the collagen fibrils in the collagen-infused composite materials can be in a range of about 1 mg/cc to about 1,000 mg/cc, about 5 mg/cc to about 900 mg/cc, about 10 mg/cc to about 800 mg/cc, about 20 mg/cc to about 700 mg/cc, about 30 mg/cc to about 600 mg/cc, about 40 mg/cc to about 500 mg/cc, about 50 mg/cc to about 450 mg/cc, about 60 mg/cc to about 400 mg/cc, about 70 mg/cc to about 350 mg/cc, about 80 mg/cc to about 300 mg/cc, about 90 mg/cc to about 250 mg/cc, about 100 mg/cc to about 200 mg/cc, or about 150 mg/cc to about 200 mg/cc.

[0222] In some embodiments, the density of the collagen fibrils in a base substrate, for example spacer fabric 200, can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen fibrils in a base substrate can be about 1 mg/cc, about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc, about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the density of the fibrils in a base substrate can be in a range of about 1 mg/cc to about 1,000 mg/cc, about 5 mg/cc to about 900 mg/cc, about 10 mg/cc to about 800 mg/cc, about 20 mg/cc to about 700 mg/cc, about 30 mg/cc to about 600 mg/cc, about 40 mg/cc to about 500 mg/cc, about 50 mg/cc to about 450 mg/cc, about 60 mg/cc to about 400 mg/cc, about 70 mg/cc to about 350

mg/cc, about 80 mg/cc to about 300 mg/cc, about 90 mg/cc to about 250 mg/cc, about 100 mg/cc to about 200 mg/cc, or about 150 mg/cc to about 200 mg/cc.

[0223] In some embodiments, the density of the collagen fibrils in a non-woven substrate can be in the range of about 1 mg/cc to about 1,000 mg/cc, including subranges. In some embodiments, the density of the collagen fibrils in a non-woven substrate can be about 5 mg/cc, about 10 mg/cc, about 20 mg/cc, about 30 mg/cc, about 40 mg/cc, about 50 mg/cc, about 60 mg/cc, about 70 mg/cc, about 80 mg/cc, about 90 mg/cc, about 100 mg/cc, about 150 mg/cc, about 200 mg/cc, about 250 mg/cc, about 300 mg/cc, about 350 mg/cc, about 400 mg/cc, about 450 mg/cc, about 500 mg/cc, about 600 mg/cc, about 700 mg/cc, about 800 mg/cc, about 900 mg/cc, or about 1,000 mg/cc, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the density of the collagen fibrils in a non-woven substrate can be in a range of about 1 mg/cc to about 1,000 mg/cc, about 5 mg/cc to about 900 mg/cc, about 10 mg/cc to about 800 mg/cc, about 20 mg/cc to about 700 mg/cc, about 30 mg/cc to about 600 mg/cc, about 40 mg/cc to about 500 mg/cc, about 50 mg/cc to about 450 mg/cc, about 60 mg/cc to about 400 mg/cc, about 70 mg/cc to about 350 mg/cc, about 80 mg/cc to about 300 mg/cc, about 90 mg/cc to about 250 mg/cc, about 100 mg/cc to about 200 mg/cc, or about 150 mg/cc to about 200 mg/cc. In some embodiments, the density of the collagen fibrils in a non-woven substrate can be greater than the density of the collagen fibrils in a base substrate.

[0224] In some embodiments, the collagen fibrils can exhibit a unimodal, bimodal, trimiodal, or multimodal distribution. For example, a collagen-infused composite material can be composed of two different fibril preparations, each having a different range of fibril diameters arranged around one of two different modes. Such collagen mixtures can be selected to impart additive, synergistic, or a balance of physical properties to the collagen-infused composite materials.

[0225] In some embodiments, the collagen fibrils form networks. For example, individual collagen fibrils can associate to exhibit a banded pattern. These banded fibrils can then associate into larger aggregates of fibrils. However, in some embodiments, the fibrillated collagen can lack a higher order structure. For example, the collagen fibrils can be unbundled and provide a strong and uniform non-anisotropic structure to collagen-infused composite materials. In other embodiments, the collagen fibrils can be bundled or aligned

into higher order structures. For example, the collagen fibrils can have an orientation index in the range of 0 to about 1, including subranges. In some embodiments, the orientation index of the collagen fibrils can be 0, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, or about 1, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the orientation index of the collagen fibrils can be in a range of 0 to about 1, about 0.1 to about 0.9, about 0.2 to about 0.8, about 0.3 to about 0.7, about 0.4 to about 0.6, or about 0.4 to about 0.5. An orientation index of 0 describes collagen fibrils that are perpendicular to other fibrils, and an orientation index of 1.0 describes collagen fibrils that are completely aligned.

[0226] In some embodiments, fibrils can align with other fibrils over 50, 100, 200, 300, 400, 500 μm or more of their lengths or can exhibit little, or no alignment. In some embodiments, some collagen fibrils can be bundled or aligned into higher order structures.

[0227] In some embodiments, methods disclosed herein make it possible to produce a collagen-infused composite material including collagen fibrils differing in diameter from those produced by an animal expressing the same type of collagen. The characteristics of natural collagens, such as fibril diameter and degree of crosslinking between fibrils are affected by genetic and environmental factors such as the species or breed of the animal and by the condition of the animal , for example the amount of fat, type of feed (e.g. grain, grass), and level of exercise.

[0228] In some embodiments, a collagen-infused composite material can be fibrillated and processed to contain collagen fibrils that resemble or mimic the properties of collagen fibrils produced by particular species or breeds of animals or by animals raised under particular conditions.

[0229] Alternatively, in some embodiments, fibrillation and processing conditions can be selected to provide collagen fibrils distinct from those found in nature, such as by decreasing or increasing the fibril diameter, degree of alignment, or degree of crosslinking compared to fibrils in natural leather.

[0230] In some embodiments, a cross-linked network of collagen, sometimes called a hydrogel, can be formed as collagen is fibrillated, or it can form a network after fibrillation. In some embodiments, the process of fibrillating collagen can form a gel-like

network. In some embodiments, once formed, a fibrillated collagen network can be further stabilized by incorporating molecules with di-, tri-, or multifunctional reactive groups that include chromium, amines, carboxylic acids, sulfates, sulfites, sulfonates, aldehydes, hydrazides, sulfhydryls, diazarines, aryl-, azides, acrylates, epoxides, or phenols.

[0231] In some embodiments, once the collagen fibrils, sometimes characterized as a hydrogel, have formed or during formation, they can be cross-linked. For example, the fibrillated collagen hydrogel can be treated with compounds containing chromium or at least one aldehyde group, or vegetable tannins prior to gel formation, during gel formation, or after gel formation, to further stabilize the fibrillated collagen hydrogel. For example, collagen fibrils pre-treated with acrylic polymer followed by treatment with a vegetable tannin (e.g., Acacia Mollissima) can exhibit increased hydrothermal stability. In some embodiments, glyceraldehyde can be used as a cross-linking agent to increase the thermal stability, proteolytic resistance, and mechanical characteristics (e.g. Young's modulus, tensile stress) of a fibrillated collagen hydrogel.

[0232] Depending on the temperature and volume of starting material, the fibrillation and hydrogel formation can occur somewhat quickly after induction and be largely complete after an hour and a half, as shown by the absorbance values leveling off after 70 minute's time passing. An increase in storage modulus (or viscoelastic qualities of the material) of the fibrillated collagen hydrogel after induction from around 1 Pa (for the solution of collagen) to approximately 400 Pa for the fibrillated collagen hydrogel can occur.

[0233] Animal skin typically includes fibrils that are ordered into higher-order structures, including the presence of banding (having regular lacunar regions) and formation of multiple fibrils into aligned fibers, which can then bundled into collagen bundles. In certain embodiments, and in contrast, the collagen hydrogels and therefore the collagen-infused composite materials described herein can have a primarily disorganized collagen fibril structure throughout the entire thickness (in some cases entire volume) of the material. For example, the collagen structure of the composite materials including a collagen hydrogel can be primarily unbundled and un-oriented along any particular axis. In some variations, the collagen fibrils can be unbanded (e.g., greater than 10% unbanded, greater than 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or unbanded throughout the volume).In some embodiments, the orientation of the collagen fibrils within the volume

(or throughout the volume) can be un-oriented or randomly oriented, and this lack of orientation can be the same throughout the volume, rather than changing through the volume thickness as in natural leather. In some cases, the orientation of the collagen fibrils in natural leather can change from bundles of collagen fibrils that are vertically oriented to bundles that are horizontally oriented over the thickness of the leather. Any of the properties that are the same at any level thickness of the hydrogel and therefore a resulting leather-leather material can be referred to herein as "uniformly" the same throughout the thickness.

[0234] In some embodiments, collagen-infused composite materials can have a uniform distribution of fibrils throughout the thickness of a hydrogel.

[0235] In some embodiments, a fibrillated collagen network can be polymerized with other agents (e.g. polymers that are capable of polymerizing or other suitable fibers), which could be used to further stabilize the matrix and provide the desired end structure. Hydrogels based upon acrylamides, acrylic acids, and their salts can be prepared using inverse suspension polymerization. In some embodiments, hydrogels described herein can be prepared from polar monomers. In some embodiments, the hydrogels used can be natural polymer hydrogels, synthetic polymer hydrogels, or a combination of the two. In some embodiments, the hydrogels used can be obtained using graft polymerization, crosslinking polymerization, networks formed of water-soluble polymers, radiation crosslinking, and so on. In some embodiments, a small amount of crosslinking agent can be added to the hydrogel composition to enhance polymerization.

[0236] In some embodiments, collagen or collagen-like proteins can be polymerized into dimers, trimers, and higher order oligomers prior to fibrillation, and/or chemically modifying the collagen or collagen-like proteins to promote crosslinking between the collagen or collagen-like proteins.

[0237] In some embodiments, collagen or collagen-like proteins can be functionalized with one or a combination of chromium, an amine, carboxylic acid, a sulfate, a sulfite, a sulfonate, an aldehyde, a hydrazide, a sulfhydryl, a diazirine, an aryl, an azide, an acrylate, an epoxide, or a phenol group.

[0238] In some embodiments, a coloring agent can be incorporated into a collagen-infused composite material. In some embodiments, the coloring agent can be incorporated into the collagen before infusion into an armature. In some embodiments, the coloring

agent can be incorporated into the collagen after infusion into an armature. In some embodiments, the coloring agent can be incorporated into an armature before infusion of collagen. In some embodiments, the coloring agent can be incorporated into an armature before functionalization of the armature. In some embodiments, the coloring agent can be incorporated into an armature after functionalization of the armature.

[0239] In some embodiments, a first coloring agent can be incorporated into the collagen and a second coloring agent can be incorporated into the armature, depending on the desired aesthetic of the collagen-infused composite material. In some embodiments the coloring agent can be a dye, for example an acid dye, a fiber reactive dye, a direct dye, a direct dye, a sulfur dye, a basic dye, or a reactive dye. In some embodiments, the coloring agent can be pigment, for example a lake pigment. In some embodiments, a coloring agent can be incorporated into a spacer fabric. In some embodiments, a first coloring agent can be incorporated into the collagen and a second coloring agent can be incorporated into the spacer fabric, depending on the desired aesthetic of the collagen-infused composite material.

[0240] A fiber reactive dye includes chromophores that contain pendant groups capable of forming covalent bonds with nucleophilic sites in fibrous, cellulosic substrates in the presence of an alkaline pH and raised temperature. These dyes can achieve high wash fastness and a wide range of brilliant shades. Exemplary fiber reactive dyes, include but are not limited to, sulphatoethylsulphone (Remazol), vinylsulphone, and acrylamido dyes. These dyes can dye protein fibers such as silk, wool and nylon by reacting with fiber nucleophiles via a Michael addition. Direct dyes are anionic dyes capable of dying cellulosic or protein fibers. In the presence of an electrolyte such as sodium chloride or sodium sulfate, near boiling point, these dyes can have an affinity to cellulose. Exemplary direct dyes include, but are not limited to, azo, stilbene, phthalocyanine, and dioxazine.

[0241] In some embodiments, a dye can be incorporated into an armature before infusion of collagen. In some embodiments, a dye can be incorporated into an armature before functionalization of the armature. In some embodiments, a dye can be incorporated into an armature after functionalization of the armature. The weight percent of dye used to dye an armature can be used to control the depth of color of an armature post-dyeing. In some embodiments, about 0.01 wt.% to about 10 wt.% dye, based on armature weight, can be used. A dye can be applied by submerging an armature in a bath including dye, water, and

a salt. The dye can be pre-dissolved in water before adding it to the bath. In some embodiments, the bath can be stirred using, for example, a stir bar, while the armature is submerged in the bath. In some embodiments, after stirring, the temperature of the bath can be increased. In some embodiments, sodium carbonate can be added to the bath to increase the temperature of the bath. The increased temperature of the bath can be tailored to control the depth of color of an armature post-dyeing. In some embodiments, for example for a reactive dye, the temperature can be about 30 °C to about 40 °C. In some embodiments, for example for a direct dye, the temperature can be about 75 °C to about 100 °C. After raising the temperature, the bath can be mixed again. After sufficient time, the armature is taken out of the bath, is washed, allowed to cool, and dried. In some embodiments, the armature can be dried at room temperature. In some embodiments, the armature can be dried at an elevated temperature in an oven, for example at 75 °C.

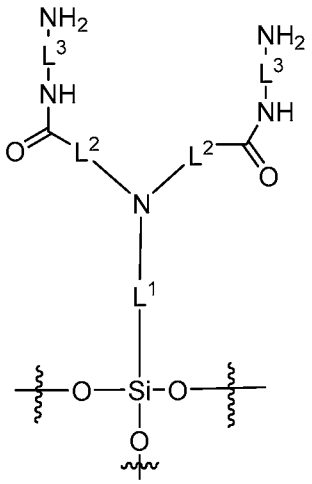
[0242] In some embodiments, a coloring agent can be used to achieve a uniform color distribution on a collagen-infused armature. In some embodiments, a coloring agent can be used to achieve a complex color distribution on a collagen-infused armature. For example, in some embodiments, the coloring agent can create a first color on peaks 412 of rough exterior surface 410 of a collagen layer and a second color in valleys 414 of rough exterior surface 410. The color on peaks 412 can be darker or lighter than the color in valleys 414. This can create a non-uniform color distribution that can resemble the color distribution of dye in dyed natural leather, for example, a dyed crocodile skin leather. The color of different areas (e.g., peaks and valleys) on a collagen-infused armature can be characterized by a three-coordinate color space, for example the CIELab space. In this system, each color is characterized by the lightness value (L*), a chroma value (a*), and a hue value (b*). Through use of a spectrophotometer, these three values can be measured and differences in color between different areas on a collagen-infused armature can be characterized. CIELab colors can be measured using ISO/CIE 11664-4:2019 ("Colorimetry – Part 4: CIE 1976 L*a*b Colour Space").

[0243] In some embodiments, the color on a peak 412, measured with a spectrophotometer, can have one or more of a lightness value, a chroma value, or a hue value that is at least about 10% higher or at least about 10% lower than the lightness value, chroma value, and hue value the color in a valley 414. In some embodiments, the color on a peak 412 can have a lightness value of at least about 10% higher or at least

about 10% lower than the lightness value of the color in a valley 414. In some embodiments, the color on a peak 412 can have a chroma value of at least about 10% higher or at least about 10% lower than the chroma value of the color in a valley 414. In some embodiments, the color on a peak 412 can have a hue value of at least about 10% higher or at least about 10% lower than the hue value of the color in a valley 414.

[0244] In some embodiments, armature 300 can be functionalized prior to infusion of collagen 360.

[0245] In some embodiments, an armature described herein is functionalized with one or more reactive groups, such as one or more primary amino groups, suitable for crosslinking with dyes, with the collagen present in a collagen-infused armature, or a combination thereof (a "functionalized armature"). In certain embodiments, the functionalized armature is functionalized with a plurality of functional groups according to Formula I:



Formula I

[0246] wherein

[0247] each L^1 is $-(CR^4R^5)_m\text{-}CH_2-$;

[0248] each L^2 is $-(CR^6R^7)\text{-}(CR^8R^9)-$;

[0249] each L^3 is $-(CR^{10}R^{11})_n-$;

[0250] each R^4 and R^5 are, independently selected from the group consisting of hydrogen, $C_{1\text{-}10}$-alkly, $C_{1\text{-}10}$-alkoxy, $C_{2\text{-}10}$-alkenyl, $C_{2\text{-}10}$-alkynyl, $C_{3\text{-}10}$-cyclic alkyl, $C_{3\text{-}10}$-cyclic alkenyl, $C_{3\text{-}10}$-heterocycle, $C_{4\text{-}10}$-aryl, and $C_{4\text{-}10}$-heteroaryl;

[0251] each R^6, R^7, and R^8 are independently selected from the group consisting of H, C_{1-10}-alkyl, C_{2-10}-alkenyl, C_{2-10}-alkynyl, C_{3-10}-cyclic alkyl, C_{3-10}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, and C_{4-10}-heteroaryl;

[0252] each R^9 is H;

[0253] each R^{10} and R^{11} are independently, H, halogen, an C_{1-10}-alkly, C_{1-10}-alkoxy, C_{2-10}-alkenyl, C_{2-10}-alkynyl, C_{3-10}-cyclic alkyl, C_{3-10}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, or C_{4-10}-heteroaryl;

[0254] m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and

[0255] n is 1, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0256] In some embodiments, the armature is functionalized with a plurality of functional groups according Formula I, wherein

[0257] each of L^1, L^2, and L^3 are defined as described above; further wherein

[0258] each R^4 and R^5 are independently selected from the group consisting of H, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl;

[0259] each R^6, R^7, and R^8 are independently selected from the group consisting of H, C_{1-4}-alkly, C_{2-4}-alkenyl, C_{2-4}-alkynyl, C_{5-7}-cyclic alkyl, and C_{5-7}-cyclic alkenyl;

[0260] each R^9 is H;

[0261] each R^{10} and R^{11} are independently selected from the group consisting of H, halogen, C_{1-4}-alkly, C_{1-4}-alkoxy, C_{2-4}-alkenyl, C_{2-4}-alkynyl, C_{5-7}-cyclic alkyl, and C_{5-7}-cyclic alkenyl;

[0262] m is 2, 3, 4, 5, or 6; and

[0263] n is 1, 2, 3, 4, or 5.

[0264] In yet another embodiment, the armature is functionalized with a plurality of functional groups according to Formula I, wherein

[0265] each of L^1, L^2, and L^3 are defined as described above;

[0266] each R^4 and R^5 are H;

[0267] each R^6 and R^7 are H or methyl;

[0268] each R^8 is H or methyl;

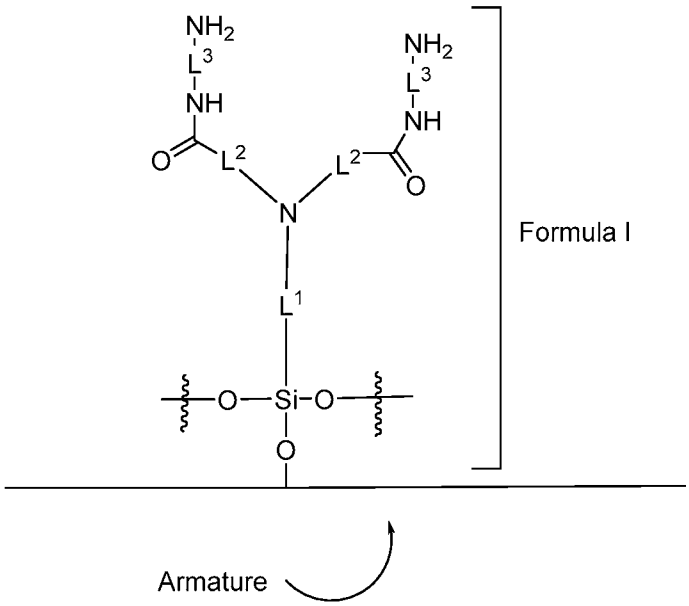
[0269] each R^9 is H;

[0270] each R^{10} and R^{11} are H;

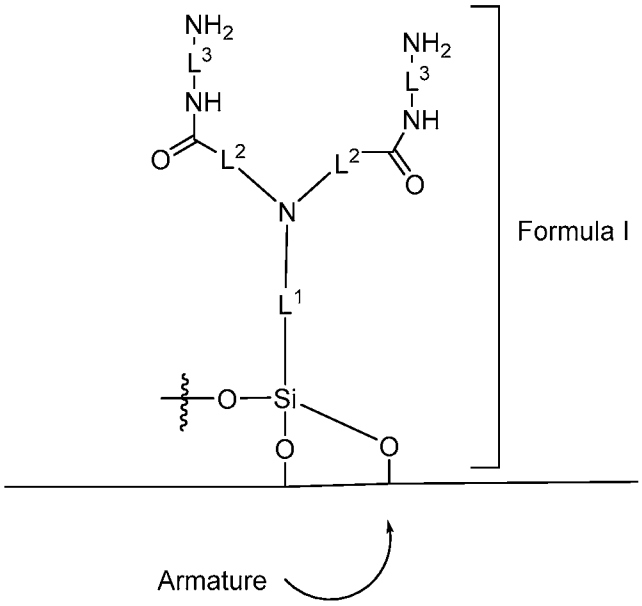
[0271] m is 2; and

[0272] n is 2.

[0273] Without wishing to be bound by a particular theory, it is believed that the plurality of functional groups of Formula I can be bound to the armature in a variety of configurations. For example, in certain configurations, the silicon atom in Formula I can be bound to the armature via a single Si-O bond, as shown below.

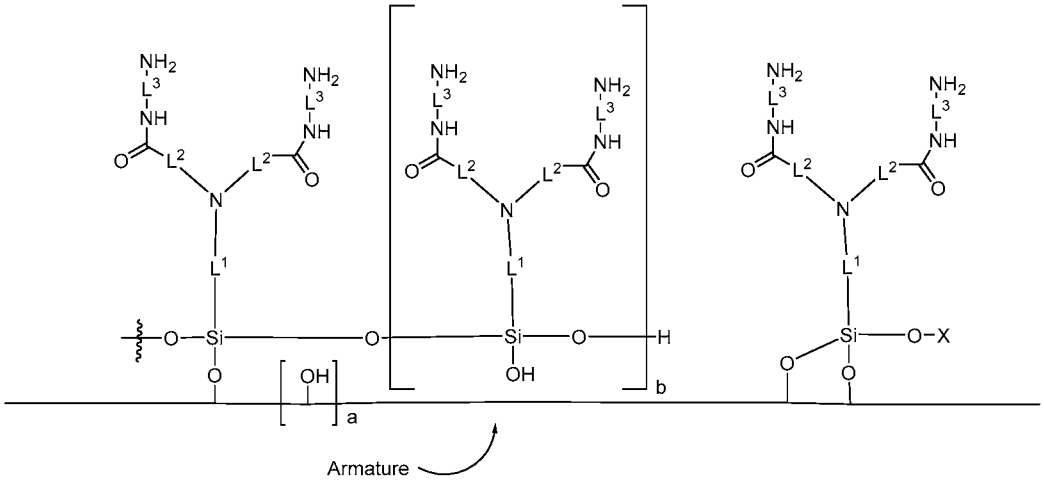


[0274] Alternatively, the silicon atom in Formula I can be bound to the armature via two Si-O bonds as shown below.



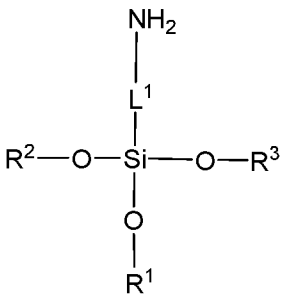
[0275] When a functional group of Formula I is bound to the functionalized armature via a single Si-O bond, Formula I can optionally be bound to one or more other molecules of Formula I through Si-O-Si linkages. In this configuration, certain groups according to Formula I may only be bound to other molecules of Formula I and may not be bound to

the armature at all. An exemplary configuration showing compounds of Formula I bound to the armature via single Si-O bonds and two Si-O bonds is shown below in Configuration I. Configuration I is shown for purposes of explanation only and does not represent the precise configuration of functional groups on the armature. In Configuration I, "a" is an integer from 0 to 4, "b" is an integer from 1 to 4, and X is H or another compound of Formula I that is optionally bound to the armature and/or another compound of Formula I.



Configuration I

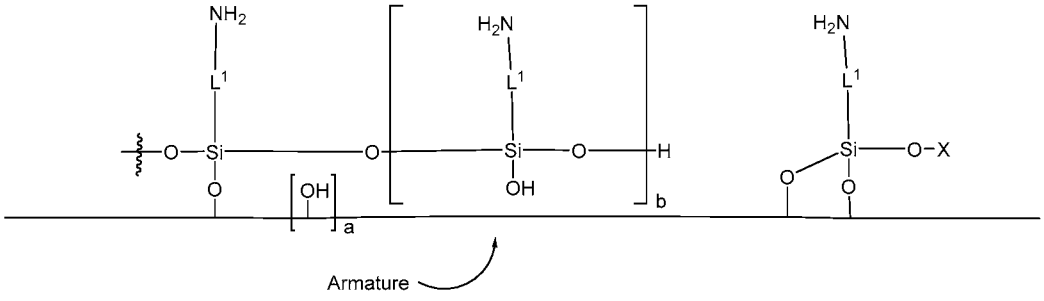
[0276] Typically, the functionalized armature can be prepared by reacting the armature with a silanization reagent having a structure according to Formula II in a silanization reaction,



Formula II

wherein each of R^1, R^2, and R^3 are independently selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, -CH=CH_2, -CH=$CHCH_3$, -$CH(CH_3)$=CH_2, -CH=$CHCH_2CH_3$, -CH=$C(CH_3)_2$, -$CH(CH_3)$=$CH(CH_3)$, and -CH_2CH=$CHCH_3$; and L^1 has the definition noted above; to provide a silanized armature that, after dehydration and condensation, has an approximate

configuration according to Configuration II wherein a, b, and X are as described above. As with Configuration I, Configuration II is shown for purposes of example only and is not intended to describe the exact configuration resulting from the reaction of a compound of Formula II with the armature.



Configuration II

[0277] In some embodiments of the silanization reaction, R^1, R^2, and R^3 in Formula II are the same and are selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, -CH=CH_2, -CH=$CHCH_3$, -$CH(CH_3)$=CH_2, -CH=$CHCH_2CH_3$, -CH=$C(CH_3)_2$, -$CH(CH_3)$=$CH(CH_3)$, and -CH_2CH=$CHCH_3$.

[0278] In some embodiments of the silanization reaction, R^1, R^2, and R^3 in Formula II are the same and are methyl or ethyl.

[0279] In some embodiments of the silanization reaction, R^1, R^2, and R^3 in Formula II are the same and are either methyl or ethyl and L^1 is $–(CR^4R^5)_m$-CH_2– wherein m is 1, 2, 3, 4, 5, or 6 and each R^4 and R^5 are independently selected from the group consisting of hydrogen, $C_{1\text{-}10}$-alkly, $C_{1\text{-}10}$-alkoxy, $C_{2\text{-}10}$-alkenyl, $C_{2\text{-}10}$-alkynyl, $C_{3\text{-}10}$-cyclic alkyl, $C_{3\text{-}10}$-cyclic alkenyl, $C_{3\text{-}10}$-heterocycle, $C_{4\text{-}10}$-aryl, and $C_{4\text{-}10}$-heteroaryl.

[0280] In some embodiments of the silanization reaction, R^1, R^2, and R^3 in Formula II are the same and are either methyl or ethyl and L^1 is $–(CR^4R^5)_m$-CH_2–; wherein m is 2, 3, 4, 5, or 6 and each R^4 and R^5 are independently selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[0281] In some embodiments of the silanization reaction, R^1, R^2, and R^3 in Formula II are methyl, L^1 is $–(CR^4R^5)_m$-CH_2–, m is 2, and each of R^4 and R^5 are hydrogen. In this embodiment of the silanization reaction, the compound of Formula II is known to those of ordinary skill in the art as (3-aminopropyl)trimethyoxysilane or APTMS (CAS No. 13822-56-5). APTMS is commercially available from sources including, but not limited to, Millipore Sigma.

[0282] In some embodiments of the silanization reaction, each of R^1, R^2, and R^3 in Formula II are ethyl, L^1 is $-(CR^4R^5)_m-CH_2-$, m is 2, and each of R^4 and R^5 are hydrogen. In this embodiment of the silanization reaction, the compound of Formula II is known to those of ordinary skill in the art as (3-aminopropyl)triethoxysilane or APTES (CAS No. 919-30-2). APTES is commercially available from sources including, but not limited to, Millipore Sigma.

[0283] In some embodiments, the silanization reaction can be performed by suspending the armature in a suitable solvent having an appropriate pH and adding the silanization reagent (i.e. the compound of Formula II) to the solvent/armature combination. In some embodiments, the suitable solvent can be any solvent that dissolves the silanization reagent. In certain embodiments, the solvent can be water, methanol, ethanol, propanol, isopropanol, or a combination thereof. In particular embodiments, the solvent can be water.

[0284] In some embodiments, the solvent can have a pH ranging from about 7 to about 14, from about 8 to about 13, from about 9 to about 12, or from about 10 to about 11. In one embodiment, the pH can be about 7, about 8, about 9, about 10, about 11, about 12, about 13, or about 14. The desired pH can be obtained by adding a sufficient amount of an appropriate base (organic or inorganic) to obtain the desired pH, provided that the base does not interfere with the reaction between the armature and the compound of Formula II. In some embodiments, the desired pH can be obtained by adding one or more organic bases including, but not limited to, trimethylamine (TEA), N,N-diisopropylethylamine (DIPEA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), pyridine, 2,6-lutidine, imidazole, 1-methylimidazole (NMI), 1,4-diazabicyclo[2.2.2]octane (DABCO), 4-dimethylaminopyridine (DMAP), or a combination thereof. In certain embodiments, the desired pH can be obtained by adding one or more inorganic bases including, but not limited to, NaOH, KOH, $Ca(OH)_2$, LiOH, or a combination thereof to the solvent.

[0285] In some embodiments, the silanization reagent, i.e. the compound of Formula II, can be added to the solvent/armature combination as a weight percentage of the weight of the dry armature. In some embodiments, the silanization reagent can be added in an amount equivalent to about 0.1 wt.% to about 50 wt.% of the weight of the dry armature. In other embodiments, the silanization reagent can be added in an amount equivalent to about 0.5 wt.% to about 40 wt.% of the weight of the dry armature, about 1 wt.% to about

30 wt.% of the weight of the dry armature, about 1.5 wt.% to about 20 wt.% of the weight of the dry armature, about 2 wt.% to about 10 wt.% of the weight of the dry armature, about 2.5 wt.% to about 5 wt.% of the weight of the dry armature, or about 3 wt.% to about 4 wt.% of the weight of the dry armature. In particular embodiments, the silanization reagent can be added to the solvent/armature combination in an amount equivalent to about 3 wt.% to about 4 wt.% of the weight of the dry armature

[0286] In some embodiments, silanization reaction proceeds for a reaction time of at least about 4 hours. In some embodiments, the reaction time of the silanization reaction is about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, or about 24 hours. In particular embodiments, the reaction time of the silanization reaction is about 24 hours.

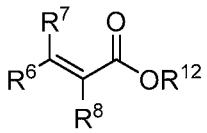
[0287] In some embodiments, the silanization reaction takes place at a temperature in the range of about 0 ºC to about 150 °C, about 10 °C to about 140 °C, about 20 °C to about 130 °C, about 20 °C to about 120 °C, about 20 °C to about 110 °C, or about 20 °C to about 100°C. In particular embodiments, the silanization reaction takes place at room temperature (about 23 °C).

[0288] In certain embodiments, after a certain amount of time and/or after the silanization reagent has been sufficiently consumed as measured by TLC, HPLC, or other appropriate methodology, the silanized armature can be washed with a wash solvent, as needed, to remove impurities, excess reagent, and/or to neutralize the pH within the silanized armature. In some embodiments, the wash solvent can be any solvent that can solubilize the unreacted silanization reagents and be miscible with the reaction solvent of choice. In some embodiments, the wash solvent can be water, methanol, ethanol, propanol, isopropanol, acetone, or a combination thereof. In particular embodiments, the wash solvent can be water. In some embodiments, the water can be distilled water.

[0289] In certain embodiments, the silanization reaction further comprises heating the washed silanized armature in an oven (such as a vacuum oven or equivalent device) for a period of time sufficient to remove any residual wash solvent in the silanized armature and to induce dehydration and condensation to form a dry silanized armature. In some embodiments, the heating process can take place a temperature ranging from about 50 °C

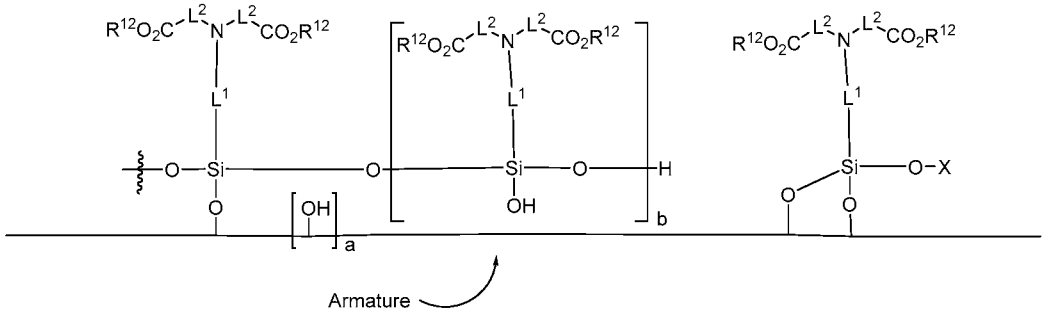
to about 150 °C, from about 60 °C to about 140 °C, from about 70 °C to about 130 °C, from about 80 °C to about 120 °C, from about 90 °C to about 110 °C, from about 95 °C to about 105 °C, or from about 99 °C to about 101 °C. In some embodiments, the period of time can be from about 1 to about 10 hours, from about 2 to about 9 hours, from about 3 to about 8 hours, from about 4 to about 7 hours, from about 4.5 to about 6 hours, or from about 5 to about 5.5 hours. In some embodiments, the drying process can take at a pressure of 1 atmosphere. In other embodiments the pressure can be reduced to aid in the drying process.

[0290] In some embodiments, the dry silanized armature can then undergo a Michael addition reaction with an α,β-unsaturated carboxylic acid or ester according to Formula IV:



Formula IV

wherein each of R^6, R^7, and R^8 is defined as described above; and wherein R^{12} is selected from the group consisting of hydrogen, C_{1-4}-alkly, C_{2-4}-alkenyl, C_{2-4}-alkynyl, C_{5-7}-cyclic alkyl, C_{5-7}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, and C_{4-10}-heteroaryl, to provide a carboxylic acid or ester functionalized armature having an approximate configuration according to Configuration III:



Configuration III

wherein each of L^1, L^2, R^2, R^3, and R^{12} is defined as described above. As noted above, the configure shown in Configuration III is exemplary and does not describe the exact configuration of functional groups on the armature.

[0291] In some embodiments, each R^2 and R^3 are independently selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, -CH=CH_2,

-CH=CHCH$_3$, -CH(CH$_3$)=CH$_2$, -CH=CHCH$_2$CH$_3$, -CH=C(CH$_3$)$_2$, -CH(CH$_3$)=CH(CH$_3$), and -CH$_2$CH=CHCH$_3$;

[0292] each R^4 and R^5 in L^1 are independently selected from the group consisting of H, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl;

[0293] m is 1, 2, 3, or 4;

[0294] R^6, R^7, and R^8 in L^2 are each independently selected from the group consisting of H, C_{1-10}-alkly, C_{2-10}-alkenyl, C_{2-10}-alkynyl, C_{3-10}-cyclic alkyl, C_{3-10}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, and C_{4-10}-heteroaryl;

[0295] R^9 is H; and

[0296] R^{12} is selected from the group consisting of hydrogen, C_{1-4}-alkly, C_{2-4}-alkenyl, C_{2-4}-alkynyl, C_{5-7}-cyclic alkyl, C_{5-7}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, and C_{4-10}-heteroaryl.

[0297] In particular embodiments, R^2 and R^3 are both methyl or both ethyl;

[0298] each R^4 and R^5 in L^1 are H;

[0299] m is 2; and

[0300] R^6, R^7, R^8, and R^9 in L^2 are each H or R^6, R^7, and R^9 are H and R^8 is methyl; and

[0301] R^{12} is H or methyl.

[0302] In some embodiments, the compound of Formula IV used in the Michael addition reaction has a structure wherein

[0303] each R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, C_{1-4}-alkly, C_{2-4}-alkenyl, C_{2-4}-alkynyl, C_{5-7}-cyclic alkyl, or C_{5-7}-cyclic alkenyl; and

[0304] R^{12} is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[0305] In some embodiments, the compound of Formula IV in the Michael addition reaction is acrylic acid.

[0306] In other embodiments, the compound of Formula IV in the Michael addition reaction is methacrylic acid.

[0307] In a further embodiment, the compound of Formula IV in the Michael addition reaction is methyl methacrylate.

[0308] In some embodiments, the carboxylic acid or ester functionalized armature can be prepared by suspending the silanized armature in a solvent and adding the α,β-unsaturated

carboxylic acid or ester according to Formula IV to the suspension. In some embodiments, the suitable solvent can be any solvent that dissolves the α,β-unsaturated carboxylic acid or ester. In certain embodiments, the solvent can be methanol, ethanol, propanol, isopropanol, acetone, tetrahydrofuran, or a combination thereof.

[0309] In some embodiments, the compound of Formula IV can be added to the suspended silanized armature in an amount equivalent to about 50 wt.% to about 200 wt.% of the weight of the silanized armature, about 60 wt.% to about 190 wt.% of the weight of the silanized armature, about 70 wt.% to about 180 wt.% of the weight of the silanized armature, about 80 wt.% to about 170 wt.% of the weight of the silanized armature, about 90 wt.% to about 160 wt.% of the weight of the silanized armature, about 100 wt.% to about 150 wt.% of the weight of the silanized armature, about 100 wt.% to about 140 wt.% of the weight of the silanized armature, about 100 wt.% to about 130 wt.% of the weight of the silanized armature, about 100 wt.% to about 120 wt.% of the weight of the silanized armature, or about 100 wt.% to about 110 wt.% of the weight of the silanized armature. In particular embodiments, the compound of Formula IV can be added to the suspended silanized armature in an amount equivalent to about 100 wt.% to about 120 wt.% of the weight of the silanized armature.

[0310] In certain embodiments, the Michael addition reaction is conducted in the dark.

[0311] In certain embodiments, the Michael addition reaction between the silanized armature and the compound of Formula IV can be conducted over about 12 to about 48 hours. In particular embodiments, the Michael addition reaction between the silanized armature and the compound of Formula IV can be conducted over about 12 to about 24 hours. In still another embodiment, the Michael addition reaction between the silanized armature and the compound of Formula IV can be conducted over about 48 hours.

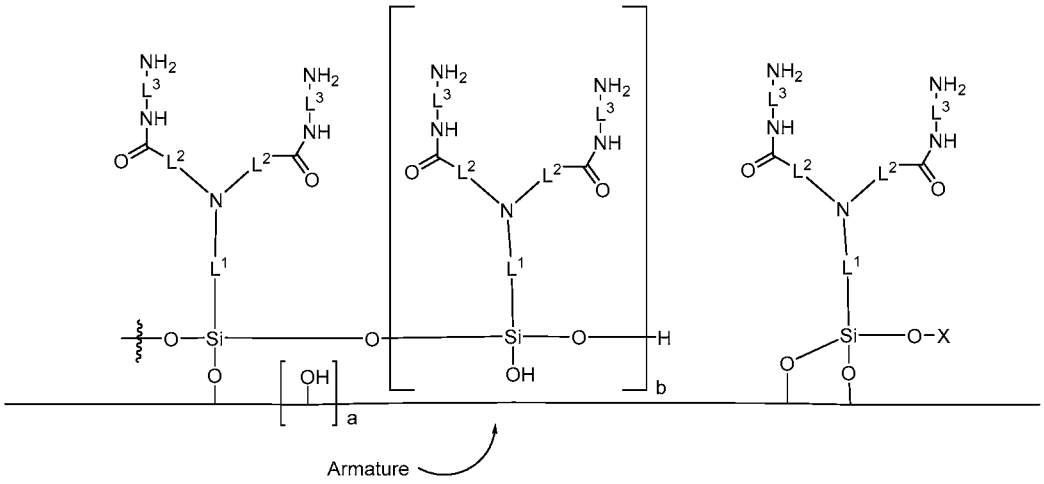
[0312] In certain embodiments, the Michael addition reaction can be conducted at a temperature ranging from about 0 ºC to about 100 °C, from about 0 °C to about 80 °C, from about 0 °C to about 60 °C, from about 0 °C to about 40 °C, or from about 0 °C to about 30 °C. In some embodiments, the Michael addition reaction can be conducted at a temperature ranging from about 15 °C to about 30 °C. In some embodiments, the Michael addition reaction can be conducted at a temperature ranging from about 20 °C to about 25 °C. In still other embodiments, the Michael addition reaction can be conducted at a temperature ranging from about 20 °C to about 25 °C for about 24 to about 48 hours, and

in particular embodiments, about 48 hours. The Michael addition reaction temperature can be selected based on the solvent used. For example, a temperature of about 100 °C can be used when water is the solvent. As another example, a temperature of about 65 °C can be used when methanol is the solvent.

[0313] Upon completion of the reaction as determined by time, TLC, HPLC, or other analytical method, the carboxylic acid or ester functionalized armature can be washed with any compatible wash solvent such as, but not limited to, water, methanol, ethanol, propanol, isopropanol, and combinations thereof. A solvent is "compatible" when it will dissolve excess reagent but not otherwise dissolve or swell the carboxylic acid or ester functionalized armature. It is within the skill of the ordinarily skilled artisan to select a compatible solvent. In particular embodiments, the wash solvent can be distilled water. In some embodiments, the carboxylic acid or ester functionalized armature can undergo a second wash with a second wash solvent selected from the group consisting of water, methanol, ethanol, propanol, isopropanol, and combinations thereof. In particular embodiments, the second wash solvent can be methanol.

[0314] In certain embodiments, the carboxylic acid or ester functionalized armature can subject to a drying process in an oven (such as a vacuum oven or equivalent device) for a period of time sufficient to remove any residual solvent in the carboxylic acid or ester functionalized armature to provide a dry carboxylic acid or ester functionalized armature. In some embodiments, the drying process can take place a temperature ranging from about 20 °C to about 150 °C, about 30 °C to about 140 °C, about 40 °C to about 130 °C, about 50 °C to about 120 °C, about 60 °C to about 110 °C, about 70 °C to about 100 °C, or about 80 °C to about 90 °C. In some embodiments, the drying process can take place at a temperature of about 65 °C. In some embodiments, the period of time can be from about 1 to about 10 hours, from about 2 to about 9 hours, from about 3 to about 8 hours, from about 4 to about 7 hours, from about 4.5 to about 6 hours, or from about 5 to about 5.5 hours. In some embodiments, the drying process can take at a pressure of 1 atmosphere. In other embodiments the pressure can be reduced to aid in the drying process.

[0315] In certain embodiments, the carboxylic acid functionalized armature can react with a diamine having the formula: $NH_2(CR^{10}R^{11})_nNH_2$ in an amidation reaction, wherein R^{10}, R^{11}, and n are defined as described above, to provide the functionalized armature having the general structure previously shown:



[0316] In some embodiments of the amidation reaction, the diamine used to prepare the functionalized armature can have a structure wherein each R^{10} and R^{11} are independently H, C_{1-10}-alkly, C_{1-10}-alkoxy, C_{2-10}-alkenyl, C_{2-10}-alkynyl, C_{3-10}-cyclic alkyl, C_{3-10}-cyclic alkenyl, C_{3-10}-heterocycle, C_{4-10}-aryl, and C_{4-10}-heteroaryl; and

[0317] n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0318] In some embodiments of the amidation reaction, the diamine has a structure wherein n is 1, 2, 3, or 4, and each R^{10} and R^{11} are independently selected from the group consisting of H, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[0319] In some embodiments of the amidation reaction, the diamine has a structure wherein n is 2 and each R^{10} and R^{11} are H.

[0320] In some embodiments of the amidation reaction, the functionalized armature can be prepared by suspending the carboxylic acid functionalized armature in an amidation solvent and adding the diamine to the suspension. In certain embodiments, the amidation solvent can be water, methanol, ethanol, propanol, isopropanol, or a combination thereof.

[0321] In some embodiments, the diamine can be added to the suspended carboxylic acid functionalized armature in the amidation solvent in an amount equivalent to about the mass of the of the carboxylic acid functionalized armature. In some embodiments, the diamine can be added to the suspended carboxylic acid functionalized armature in the amidation solvent in an amount in the range of about 80% to about 120% the mass of the carboxylic acid functionalized armature, including subsrange. For example, the diamine can be added to the suspended carboxylic acid functionalized armature in the amidation solvent in an amount of about 80%, about 90%, about 100%, about 110%, or about 120%

of the mass of the carboxylic acid functionalized armature, or within a range having any two of these values as endpoints, inclusive of the endpoints.

[0322] In certain embodiments, the carboxylation and amidation reactions can be performed iteratively. That is, following the amidation reaction described above, a second Michael addition reaction can be performed using the reagents and conditions described herein for the Michael addition reaction. The second Michael addition reaction can be followed by a second amidation reaction. This sequence of reactions can be repeated as often as desired to obtain functionalized armatures having different properties and reactivities.

[0323] The collagen-infused composite materials described herein can have physical and mechanical properties similar to those of natural leather. For example, the collagen-infused composite materials can have similar thickness, tear strength, tensile strength, flexibility, and softness values as those of natural leather.

[0324] In some embodiments, a base substrate (e.g., spacer fabric 320) can provide a collagen-infused composite material with mechanical properties similar to those of natural leather. For example, properties of exemplary samples of base substrates are summarized in Tables 1 and 2, below. Sample No. 1 was a spacer fabric made with TENCEL™ fabric layers connected by cotton spacer yarns. Sample No. 2 was a spacer fabric made with TENCEL™ fabric layers connected by TENCEL™ spacer yarns. Sample No. 3 was a spacer fabric made with TENCEL™ fabric layers connected by TENCEL™ spacer yarns. Sample No. 4 was a spacer fabric made with fabric layers composed of TENCEL™ yarn connected by cotton and polyacrylate monofilament as spacer yarns.

Sample No.	Thickness (mm)	Area Density (GSM)	Strength (MPa)		Strain (%)	
			Course Direction	Wale Direction	Course Direction	Wale Direction
1	1.18	460	14.7	12.9	153.5	305.9
2	1.33	516	27.2	9.8	133.6	127.7
3	1.90	825	31.4	0.4	58	211.8
4	1.22	437	14.8	8.8	228	171.5

Table 1

Sample No.	Compressive Modulus under Compression (kPa)	Compressive Modulus under Recovery (kPa)	Thermal Conductivity under Compression (W/m•K)	Thermal Conductivity under Recovery (W/m•K)
1	41.96	79.31	0.067	0.065
2	29.83	86.88	0.078	0.069
3	18.27	34.63	0.116	0.091
4	31.54	48.58	0.063	0.059

Table 2

[0325] Table 3, below, shows properties of various samples of commercially available leathers, having about the same thickness as the base substrates shown in Tables 1 and 2. The data in Tables 1–3 show that the base substrates have mechanical properties that are nearly equivalent to, or better than, those of natural leather.

Natural Leather Sample ID	Compressive Modulus under Compression (kPa)	Compressive Modulus under Recovery (kPa)	Thermal Conductivity under Compression (W/m•K)	Thermal Conductivity under Recovery (W/m•K)
A	178.1415	356.9804	0.0859	0.0801
B	123.1538	486.7098	0.0814	0.0780
C	76.0693	201.7745	0.1361	0.1044
D	227.3206	625.1523	0.0954	0.0916

Table 3

[0326] In some embodiments, the base substrate (e.g., spacer fabric 320) can provide a non-collagen-infused armature with strength. For example, a base substrate can have a first tear strength and non-woven substrate(s) can have a second tear strength. Tear strength, or tear resistance, is a measure of how well a material can withstand the effects of tearing. Tear resistance can be measured by a variety of methods, for example the method provided by ASTM D 412 or the method provided by ISO 3377 (also called the "Bauman tear"). The method provided by ASTM D 624 can also be used to measure the resistance to the formation of a tear and the resistance to the expansion of a tear. Regardless of the method used, first, a cut is made in the material sample tested to induce a tear. Then, the sample is held between two grips and a uniform pulling force is applied until sample tears in two. Tear resistance is then calculated by dividing the force applied by the thickness of the material. Unless specified otherwise, a tear strength value reported herein is measured by ISO 3377.

[0327] In some embodiments, the tear strength of a base substrate (e.g., spacer fabric 320) can be greater than the tear strength of non-woven substrate(s) (e.g., first non-woven substrate 350 and/or second non-woven substrate 354). In some embodiments, the tear strength of a base substrate can be in the range of about 20 N (newtons) to about 500 N, including subranges. In some embodiments, the tear strength of a base substrate can be about 20 N, about 50 N, about 100 N, about 150 N, about 200 N, about 250 N, about 300 N, about 350 N, about 400 N, about 450 N, or about 500 N, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the tear strength of a base substrate can be in a range of about 20 N to about 500 N, about 50 N to about 450 N, about 100 N to about 400 N, about 150 N to about 350 N, about 200 N to about 300 N, or about 250 N to about 300 N. In embodiments including a base substrate with directional mechanical properties, the tear strength in either the wale direction and/or the course direction can be in the range of about 20 N to about 500 N, including subranges.

[0328] The tear strength of a non-woven substrate can be in the range of about 20 N to about 500 N, including subranges. In some embodiments, the tear strength of a non-woven substrate can be about 20 N, about 50 N, about 100 N, about 150 N, about 200 N, about 250 N, about 300 N, about 350 N, about 400 N, about 450 N, or about 500 N, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the tear strength of a non-woven substrate can be in a range of about 20 N to about 500 N, about 50 N to about 450 N, about 100 N to about 400 N, about 150 N to about 350 N, about 200 N to about 300 N, or about 250 N to about 300 N.

[0329] In some embodiments, the collagen-infused composite materials described herein can have a tear strength that is at least about 1% greater than that of a natural leather of the same thickness. In some embodiments, the collagen-infused composite material can have a tear strength that is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 100%, about 150%, or about 200% greater than that of natural leather of the same thickness. For example, in some embodiments, the collagen-infused composite material can have a tear strength in a range of about 1% to about 200%, about 2% to about 150%, about 3% to about 100%,

about 4% to about 50%, about 5% to about 45%, about 6% to about 40%, about 7% to about 35%, about 8% to about 30%, about 9% to about 25%, about 10% to about 20%, or about 10% to about 15% greater than that of natural leather of the same thickness.

[0330] In some embodiments, the collagen-infused composite material can have a tear strength in the range of about 20 N to about 500 N, including subranges. In some embodiments, the tear strength of the collagen-infused composite material can be about 20 N, about 30 N, about 40 N, about 50 N, about 60 N, about 70 N, about 80 N, about 90 N, about 100 N, about 125 N, about 150 N, about 175 N, about 200 N, about 225 N, about 250 N, about 275 N, about 300 N, about 325 N, about 350 N, about 375 N, about 400 N, about 425 N, about 450 N, about 475 N, or about 500 N, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the tear strength of the collagen-infused composite material can be in a range of about 20 N to about 500 N, about 30 N to about 475 N, about 40 N to about 450 N, about 50 N to about 425 N, about 60 N to about 400 N, about 70 N to about 375 N, about 80 N to about 350 N, about 90 N to about 325 N, about 100 N to about 300 N, about 125 N to about 275 N, about 150 N to about 250 N, about 175 N to about 225 N, or about 175 N to about 200 N.

[0331] Tensile strength, or ultimate tensile strength (UTS), is the capacity of a material to withstand loads in tension without failing. Unless specified otherwise, a tensile strength value disclosed herein is measured according the method provided by ASTM D 412. In some embodiments, the collagen-infused composite materials described herein can have a tensile strength in the range of about 1 kPa (kilopascal) to about 100 MPa (megapascals), including subranges. In some embodiments, the collagen-infused composite materials can have a tensile strength of about 1 kPa, about 50 kPa, about 100 kPa, about 200 kPa, about 300 kPa, about 400 kPa, about 500 kPa, about 600 kPa, about 700 kPa, about 800 kPa, about 900 kPa, about 1 MPa, about 5 MPa, about 10 MPa, about 20 MPa, about 30 MPa, about 40 MPa, about 50 MPa, about 60 MPa, about 70 MPa, about 80 MPa, about 90 MPa, or about 100 MPa, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the collagen-infused composite materials can have a tensile strength in a range of about 1 kPa to about 100 MPa, about 50 kPA to about 90 MPa, about 100 kPa to about 80 MPa, about 200 kPa to about 70 MPa, about 300 kPa to about 60 MPa, about 400 kPa to about 50 MPa, about

500 kPa to about 40 MPa, about 600 kPa to about 30 MPa, about 700 kPa to about 20 MPa, about 800 kPa to about 10 MPa, about 900 kPa to about 5 MPa, or about 900 kPa to about 1 MPa.

[0332] Elastic modulus (also known as Young's modulus) is a value that measures a material's resistance to being deformed elastically (i.e., non-permanently) when a force is applied to it. The elastic modulus of a material is defined as the slope of its stress–strain curve in the elastic deformation region. A stiffer material will have a higher elastic modulus. In some embodiments, the elastic modulus can be measured using a texture analyzer. In some embodiments, the collagen-infused composite materials can have an elastic modulus of at least 100 kPa. For example, the collagen-infused composite materials can have an elastic modulus in a range from about 100 kPa to about 1,000 MPa, including subsranges. For example, in some embodiments, the collagen-infused composite materials can have an elastic modulus in a range of about 100 kPa to about 1,000 MPa, about 200 kPa to about 900 MPa, about 300 kPa to about 800 MPa, about 400 kPa to about 700 MPa, about 500 kPA to about 600 MPa, about 600 kPa to about 500 MPa, about 700 kPa to about 400 MPa, about 800 kPa to about 300 MPa, about 900 kPa to about 200 MPa, about 1 MPa to about 100 MPa, about 2 MPa to about 50 MPa, about 3 MPa to about 10 MPa, about 4 MPa to about 9 MPa, about 5 MPa to about 8 MPa, or about 6 MPa to about 7 MPa.

[0333] In some embodiments, a collagen-infused composite material can be capable of elongating up to 300% from its relaxed state length. For example, a collagen-infused composite material can be capable of elongating in a range of about 1% to about 300%, about 2% to about 250%, about 3% to about 200%, about 4% to about 150%, about 5% to about 100%, about 6% to about 90%, about 7% to about 80%, about 8% to about 70%, about 9% to about 60%, about 10% to about 50%, about 20% to about 40%, or about 30% to about 40% greater than its relaxed state length.

[0334] Softness, also referred to as "hand feel" of a material can be determined by ISO 17235. In some embodiments, an exterior surface of a collagen-infused composite material described herein can have a softness in a range of about 2 mm to about 12 mm, including subranges. In some embodiments, an exterior surface can have a softness of about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 11 mm, or about 12 mm, or within a range having

any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, an exterior surface can have a softness in a range of about 2 mm to about 12 mm, about 3 mm to about 11 mm, about 4 mm to about 10 mm, about 5 mm to about 9 mm, about 6 mm to about 8 mm, or about 6 mm to about 7 mm. Unless specified otherwise, a softness value disclosed herein is determined by ISO 17235.

[0335] Flexibility, or strain, of a material can be determined by measuring its elongation at failure when a tensile force is applied, for example using the equation: $\frac{\Delta L}{L}$, where ΔL is the change in length of the material after the tensile force is applied, and L is the original length of the material. Flexibility can also be measured according to the method provided by ASTM D 412. In some embodiments, the collagen-infused composite materials described herein can have a flexibility in the range of about 1% to about 30%, including subranges. In some embodiments, the collagen-infused composite materials can have a flexibility of about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, or about 30%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the collagen-infused composite materials can have a flexibility in a range of about 1% to about 30%, about 5% to about 25%, about 10% to about 20%, or about 10% to about 15%. In some embodiments, the collagen-infused composite materials can have a flexibility greater than 30%. Unless specified otherwise, a flexibility value disclosed herein is measured by ASTM D 412.

[0336] In some embodiments, the collagen-infused composite materials described herein can be subjected to the same, or similar finishing treatments as those used to treat natural leather. The treatment process for natural leather typically has three steps: preparation of the hide, tanning, and retanning. Tanning can be performed in any number of well-understood ways, including by contacting the collagen-infused composite materials with a vegetable tanning agent, blocked isocyanate compounds, chromium compound, aldehyde, syntan, natural resin, tanning natural oil, or modified oil. Blocked isocyanate compounds can include X-tan. Vegetable tannins can include pyrogallol- or pyrocatechin-based tannins, such as valonea, mimosa, ten, tara, oak, pinewood, sumach, quebracho, and chestnut tannins. Chromium tanning agents can include chromium salts such as chromium sulfate. Aldehyde tanning agents can include glutaraldehyde and oxazolidine compounds. Syntans can include aromatic polymers, polyacrylates, polymethacrylates, copolymers of

maleic anhydride and styrene, condensation products of formaldehyde with melamine or dicyandiamide, lignins, and natural flours.

[0337] To tan a composite material, the material's pH can be adjusted, for example lowered to a pH in the range of about 2.5 to about 3.0 in the presence of 10% salts (for example sodium chloride, sodium sulfate, or sodium salts), to allow for penetration of the tanning agent. Following penetration, the pH of the composite material can be adjusted again, for example raised to a pH in the range of about 3.5 to about 4.0, to fix the tanning agent. In some embodiments, a composite material can be soaked in a bath including 2 wt.% (based on the weight of collagen infused into the composite material) of chromium (III) sulfate and the pH can be adjusted as necessary for penetration and fixation. For example, for a 10 gram composite material with 10% mass of collagen, 0.02 gram of chrome chromium (III) sulfate powder can be dissolved in enough water to cover the composite material in a container (the amount of water will depend on container dimensions). The composite material can then be added to the container and the container can be agitated, for example on an orbital shaker at 50 rpm. The agitation can be performed at a pH of about 2.8 to about 3.2 and for a time sufficient to allow penetration of the chromium (III) sulfate into the composite material. After penetration, the pH of the bath can be increased and fixation of the chromium (III) sulfate can be performed at a pH between about 3.8 and about 4.2. The duration of the fixation step can be selected to achieve a desired color for the composite material.

[0338] In some embodiments, the collagen-infused composite material can be tanned by crosslinking infused collagen fibrils. Crosslinking reactions can stabilize the collagen structure and, in some cases, can form a network between collagen molecules. Any suitable crosslinking agent known in the art can be used to crosslink the collagen fibrils, for example, mineral salts such as those based on chromium, formaldehyde, hexamethylene diisocyanate, glutaraldehyde, polyepoxy compounds, gamma irradiation, and ultraviolet irradiation with riboflavin. Other suitable crosslinkers can include isocyantes, carbodiimide, poly(aldehyde), poly(azyridine), mineral salts, poly(epoxies), enzymes, thiirane, phenolics, novolac, resole as well as other compounds that have chemistries that react with amino acid side chains such as lysine, arginine, aspartic acid, glutamic acid, hydroxylproline, or hydroxylysine.

[0339] In some embodiments, the collagen can be chemically modified to promote chemical and/or physical crosslinking between the collagen fibrils. Chemical crosslinking is possible due to reactive groups such as lysine, glutamic acid, and hydroxyl groups on the collagen molecule that project from collagen's rod-like fibril structure. Crosslinking that involves these reactive groups prevents the collagen molecules from sliding past each other under stress, thereby increasing the mechanical strength of the collagen fibrils. Chemical crosslinking reactions can include, for example, reactions with the ε-amino group of lysine or reaction with carboxyl groups of the collagen molecule. In some embodiments, enzymes such as transglutaminase can also be used to generate crosslinks between glutamic acid and lysine to form a stable γ-glutamyl-lysine crosslink. Inducing crosslinking between functional groups of neighboring collagen molecules is known in the art.

[0340] In some embodiments, the collagen can be crosslinked or lubricated during fibrillation. In some embodiments, the collagen can be crosslinked or lubricated after fibrillation. For example, collagen fibrils can be treated with compounds containing chromium, at least one aldehyde group, or vegetable tannins prior to network formation, during network formation, or during network gel formation.

[0341] In some embodiments, up to about 20 wt.% of a crosslinking agent, based on total weight of a collagen solution can be used to crosslink collagen during fibrillation. In some embodiments, about 1 wt.%, about 2 wt.%, about 3 wt.%, about 4 wt.%, about 5 wt.%, about 6 wt.%, about 7 wt.%, about 8 wt.%, about 9 wt.%, about 10 wt.%, about 15 wt.%, or about 20 wt.%, or an amount of crosslinking agent within a range having any two of these values as endpoints, inclusive of the endpoints, can be used. For example, in some embodiments, about 1 wt.%, to about 20 wt.%, about 2 wt.% to about 15 wt.%, about 3 wt.% to about 10 wt.%, about 4 wt.% to about 9 wt.%, about 5 wt.% to about 8 wt.%, or about 6 wt.% to about 7 wt.% of a crosslinking agent, based on total weight of a collagen solution, can be used. In some embodiments, the crosslinking agent can include tanning agents used for conventional leather. In some embodiments, the crosslinking agents can be covalently bound to the collagen fibrils. In some embodiments, the crosslinking agents can be non-covalently associated with the collagen fibrils.

[0342] In some embodiments, after tanning, the composite material can be retanned. Retanning refers to post-tanning treatments. Such treatments can include tanning a second

time, wetting, sammying, dehydrating, neutralization, adding a coloring agent such as a dye, fat liquoring, fixation of unbound chemicals, setting, conditioning, softening, and/or buffing.

[0343] In some embodiments, lubricants used during fat liquoring include fats, biological, mineral or synthetic oils, cod oil, sulfonated oil, polymers, organofunctional siloxanes, or other hydrophobic compounds or agents used for fat liquoring conventional leather, or mixtures thereof. Other lubricants can include surfactants, anionic surfactants, cationic surfactants, cationic polymeric surfactants, anionic polymeric surfactants, amphiphilic polymers, fatty acids, modified fatty acids, nonionic hydrophilic polymers, nonionic hydrophobic polymers, poly acrylic acids, poly methacrylic, acrylics, natural rubbers, synthetic rubbers, resins, amphiphilic anionic polymer and copolymers, amphiphilic cationic polymer and copolymers and mixtures thereof as well as emulsions or suspensions of these in water, alcohol, ketones, and other solvents. Lubricants can be incorporated in any amount that facilitates movement of the collagen fibrils, or that confers leather-like properties such as flexibility, decrease in brittleness, durability, or water resistance. In some embodiments, the amount of lubricant applied to a collagen-infused composite material can be in the range of about 0.1 wt.% to about 60 wt.% of the collagen-infused composite material. In some embodiments, the amount of lubricant applied can be about 0.1 wt.%, about 1 wt.%, about 5 wt.%, about 10 wt.%, about 15 wt.%, about 20 wt.%, about 25 wt.%, about 30 wt.%, about 35 wt.%, about 40 wt.%, about 45 wt.%, about 50 wt.%, about 55 wt.%, or about 60 wt.%, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the amount of lubricant applied can be in a range of about 0.1 wt.% to about 60 wt.%, about 1 wt.% to about 55 wt.%, about 5 wt.% to about 50 wt.%, about 10 wt.% to about 45 wt.%, about 15 wt.% to about 40 wt.%, about 20 wt.% to about 35 wt.%, or about 25 wt.% to about 30 wt%.

[0344] In some embodiments, during dehydration, water can be removed by filtration, evaporation, freeze-drying, solvent exchange, vacuum drying, convection drying, heating, irradiating or microwaving, or by other known methods for removing water. In some embodiments, the chemical crosslinking of the collagen can remove bound water from collagen by consuming hydrophilic amino acid residues such as lysine, arginine, and hydroxylysine. In some embodiments, the water content of a collagen-infused composite

material after dehydration is no more than about 60 wt.%, for example, no more than about 5 wt.%, about 10 wt.%, about 15 wt.%, about 20 wt.%, about 30 wt.%, about 35 wt.%, about 40 wt.%, about 50 wt.%, or about 60 wt.% of the collagen-infused composite material. Unless specified otherwise, water content is measured by equilibration at 65% relative humidity at 25 ℃ and at 1 atm.

[0345] A tanned composite material can be mechanically or chemically finished. For example, mechanical finishing can include polishing the composite material to yield a shiny surface; ironing and plating the composite material to achieve a flat, smooth surface; embossing the composite material to create a three-dimensional print or pattern on the material's surface; or tumbling the composite material to provide a more evident grain and smooth surface. Chemical finishing can involve the application of a film, a natural or synthetic coating, or other treatment. Chemical treatments can be applied, for example, by spraying, curtain coating, or roller coating.

[0346] The collagen-infused composite materials described herein can be used as a replacement for natural leather in a variety of applications. For example, the collagen-infused composite materials can be used in footwear, garments, gloves, furniture, vehicle upholstery, and other good and products, such as overcoats, coats, jackets, shirts, trousers, pants, shorts, swimwear, undergarments, uniforms, emblems or letters, costumes, ties, skirts, dresses, blouses, leggings, gloves, mittens, shoes, shoe components such as sole, quarter, tongue, cuff, welt, and counter, dress shoes, athletic shoes, running shoes, casual shoes, athletic, running or casual shoe components such as toe cap, toe box, outsole, midsole, upper, laces, eyelets, collar, lining, Achilles notch, heel, and counter, fashion or women's shoes and their shoe components such as upper, outer sole, toe spring, toe box, decoration, vamp, lining, sock, insole, platform, counter, and heel or high heel, boots, sandals, buttons, sandals, hats, masks, headgear, headbands, head wraps, and belts; jewelry such as bracelets, watch bands, and necklaces; gloves, umbrellas, walking sticks, wallets, mobile phone or wearable computer coverings, purses, backpacks, suitcases, handbags, folios, folders, boxes, and other personal objects; athletic, sports, hunting or recreational gear such as harnesses, bridles, reins, bits, leashes, mitts, tennis rackets, golf clubs, polo, hockey, or lacrosse gear, chessboards and game boards, medicine balls, kick balls, baseballs, and other kinds of balls, and toys; book bindings, book covers, picture frames or artwork; furniture and home, office or other interior or exterior furnishings

including chairs, sofas, doors, seats, ottomans, room dividers, coasters, mouse pads, desk blotters, or other pads, tables, beds, floor, wall or ceiling coverings, flooring, automobile, boat, aircraft and other vehicular products including seats, headrests, upholstery, paneling, steering wheel, joystick or control coverings and other wraps or coverings.

[0347] FIG. 5 illustrates a method 500 for manufacturing a collagen-infused composite material, for example collagen-infused composite material 310, according to some embodiments. Unless stated otherwise, the steps of method 500 need not be performed in the order set forth herein. Additionally, unless specified otherwise, the steps of method 500 need not performed sequentially. The steps can be performed concurrently or simultaneously.

[0348] In step 502, an armature can be functionalized as described herein. In some embodiments, the entirety of the armature can be functionalized in step 502. In some embodiments, a portion, for example one or more layers or substrates of the armature, can be functionalized in step 502. In some embodiments, method 500 does not include step 502.

[0349] In step 504, the armature can be colored as described herein. In some embodiments, the entirety of the armature can be colored in step 504. In some embodiments, a portion, for example one or more layers or substrates of the armature, can be colored in step 504. In some embodiments, method 500 does not include step 504.

[0350] In step 506, a collagen solution is prepared. In some embodiments, the collagen solution can be a collagen suspension. In some embodiments, the collagen solution can be fibrillated prior to infusion into an armature in step 506.

[0351] In step 508, the collagen solution is infused into the armature as described herein. For example, the collagen solution can be infused into the armature by soaking the armature in the collagen solution. In some embodiments, the armature soaked in the in the collagen solution can be exposed to vacuum to facilitate infusion of the collagen solution into the armature.

[0352] To estimate the amount of collagen to use during infusion step 508, the infusion capacity of the armature can be measured by weighing the dry armature and comparing the dry weight of the armature to a wet weight of the armature upon water infusion. The infusion capacities of different armatures can be estimated based on known equivalences. In some embodiments, a solution including collagen in an amount that is about 1 to about

3 times the armature infusion capacity can be prepared. For example, the amount of collagen in a solution can be about 1 to about 2.7 times the armature infusion capacity, about 1.8 to about 2.5 times the armature infusion capacity, or about 1.9 to about 2.2 times the armature infusion capacity.

[0353] In some embodiments, the amount of collagen in a solution can be about 1.5 to about 3 times the armature infusion capacity.

[0354] In some embodiments, infusion of the collagen solution into the armature in step 508 can be achieved by submerging the armature in a bath including the collagen solution and soaking the armature in the bath. In some embodiments, the armature can be submerged in the collagen solution at a temperature below about 15 °C, for example below about 14 °C, below about 13 °C, below about 12 °C, below about 11 °C, below about 10 °C, below about 9 °C, below about 8 °C, below about 7 °C, below about 6 °C, below about 5 °C, below about 4 °C, below about 3 °C, below about 2 °C, or below about 1 °C. The lower limit of the temperature is limited only by the need to maintain the collagen in solution. The lower limit of the temperature can be about 0.5 °C.

[0355] In some embodiments, infusion of the collagen solution into the armature in step 508 can be achieved by filtering the collagen solution through the armature. In some embodiments, the armature can be submerged into a collagen solution, and then exposed to vacuum. The collagen solution containing the submerged armature can be exposed to vacuum at a pressure in the range of about 20 inches Hg to about 726 inches Hg, including subranges. In some embodiments, the negative pressure of the vacuum can be about 20 inches Hg, about 50 inches Hg, about 100 inches Hg, about 150 inches Hg, about 200 inches Hg, about 250 inches Hg, about 300 inches Hg, about 350 inches Hg, about 400 inches Hg, about 450 inches Hg, about 500 inches Hg, about 550 inches Hg, about 600 inches Hg, about 650 inches Hg, about 700 inches Hg, or about 726 inches Hg, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the negative pressure of the vacuum can be in a range of about 20 inches Hg to about 726 inches Hg, about 50 inches Hg to about 700 inches Hg, about 100 inches Hg to about 650 inches Hg, about 150 inches Hg to about 600 inches Hg, about 200 inches Hg to about 550 inches Hg, about 250 inches Hg to about 450 inches Hg, about 300 inches Hg to about 400 inches Hg, or about 300 inches Hg to about 350 inches Hg. In some embodiments, the collagen solution can be exposed

to the vacuum at least one time, at least twice, at least 3 times, or at least 4 times. The upper limit to the number of times the vacuum is applied is limited only by that which does not adversely impact the structure or integrity of the armature and the collagen.

[0356] In step 510, the infused collagen can be fibrillated. Fibrillating the collagen in step 510 forms a collagen hydrogel on and within the armature. In some embodiments, to promote fibrillation of the collagen in step 510, the pH of the collagen solution can be raised by adding a buffer or adjusting a salt concentration of the solution. In some embodiments, the pH can be raised at a temperature below about 10 °C, for example at a temperature in a range of about 0.5 °C to about 10 °C. In some embodiments, fibrillation can be facilitated by including a nucleating agent. Salts used for fibrillation can include phosphate salts and chloride salts, such as Na_3PO_4, K_3PO_4, KCl, and NaCl. Additional exemplary salts include any conjugate salt of an acid such as a sulfate, a phosphate, a chloride, an acetate, a nitrate and a citrate. The salt concentration during fibrillation can be in the range of about 10 mM to about 2M, including subranges. In some embodiments, the salt concentration can be about 10 mM, about 50 mM, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, about 1 M, about 1.5 M, or about 2 M, or within a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the salt concentration can be in a range of about 10 mM to about 2 M, about 50 mM to about 1.5 M, about 100 mM to about 1 M, about 200 mM to about 900 mM, about 300 mM to about 800 mM, about 400 mM to about 700 mM, or about 500 mM to about 600 mM. The acids, salt concentration, salt type, pH, temperature, and collagen concentration for a fibrillation step affects how fast fibrils are formed.

[0357] In some embodiments, inducing fibrillation can include adding a nucleating agent. In some embodiments, the nucleating agent can include a branched collagen microgel, a collagen micro or nanoparticle, metallic particles, or a naturally or synthetically derived fiber. In some embodiments, the nucleating agent can have a concentration between about 1 mM to about 100 mM.

[0358] Is some embodiments, the pH of the collagen solution can be adjusted to a pH in a range of about 6 to about 10. In some embodiments, the pH of the collagen solution can be adjusted to a pH in a range of about 7 to about 8.5. In some embodiments, the pH of the collagen solution can be adjusted to a pH in a range of about 7.2 to about 7.5. In some

embodiments, the pH of the collagen solution can be adjusted to a pH of about 6.5, about 7.0, or greater. In some embodiments, the pH can be adjusted to a range of about 6.8 to about 7.6, a range of about 7.0 to about 7.4, or a range of about 7.1 to about 7.3. In some embodiments, the salt concentration and pH can be simultaneously adjusted to induce or promote fibrillation. In some embodiments, the temperature is about 10 °C or below while adjusting the pH and/or adding the salt solution. In certain embodiments, the temperature is below about 10 °C, about 9 °C, about 8 °C, about 7 °C, about 6 °C, about 5 °C, about 4 °C , about 3 °C, about 2 °C, about 1 °C, or about 0 °C while adjusting the pH and/or adding the salt solution. In some embodiments, after adjusting the pH of the collagen solution to within an appropriate range, fibrillation can be conducted at a temperature in a range of between about 10 °C and about 40 °C, between about 15 °C and about 37 °C, between about 15 °C and about 25 °C, between about 20 °C and about 25 °C, or between about 15 °C and about 20 °C. In certain embodiments, the temperature is about 10 °C, about 11 °C , about 12 °C, about 13 °C, about 14 °C, about 15 °C, about 16 °C, about 17 °C, about 18 °C, about 19 °C, about 20 °C, about 21 °C , about 22 °C, about 23 °C, about 24 °C, about 25 °C, about 26 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 31 °C, about 32 °C, about 33 °C, about 34 °C, about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, or about 40 °C during fibrillation.

[0359] Collagen fibrillation in step 510 can be a function of temperature, concentration, pH and time. Fibrillation time can require a few minutes to a few days depending on the chosen conditions. In some embodiments, fibrillation can be conducted in about 1 hour, about 2 hours, about 5 hours, or about 24 hours.

[0360] Following collagen fibrillation of the infused armature, the amount of collagen incorporated therein can range from about 10% to about 300%, about 50% to about 100%, about 30% to about 250%, about 50% to about 200%, about 75% to about 150%, or about 100% to about 125%, based on the weight of the armature before infusion and fibrillation.

[0361] Fibrillation of infused collagen in step 510 can form an exterior collagen layer on an armature (e.g., collagen layer 370 and/or collagen layer 380). After fibrillation, the collagen-infused armature can be partially dried in step 512.

[0362] In some embodiments, after fibrillation, chemical cross-linkers can be added to stabilize the fibrils. Exemplary cross-linkers include, but are not limited to, aldehydes,

chromium, blocked isocyanates such as x-tan, syntans, natural vegetable tanning agents, or a mixture of these. In some embodiments, if desired, an armature already containing collagen, can be infused with more collagen or other proteins, followed again by crosslinking for stabilization. In some embodiments, a stabilizing cross-linker can have a concentration in a range of about 1nM to about 100 nM.

[0363] In some embodiments, a color can be added to the infused collagen in step 514. The color can be added using a coloring agent as described herein. In some embodiments, a coloring agent can be added to the collagen solution prior to infusion into the armature in step 508. In some embodiments, a coloring agent can be added any time before drying a collagen-infused armature. In some embodiments, method 500 does not include step 514.

[0364] In step 516, the collagen-infused armature can be tanned as described herein. In some embodiments, step 516 can include one or more re-tanning steps. In some embodiments, method 500 does not include step 516.

[0365] In step 518, the collagen-infused armature can be dried. In some embodiments, the collagen-infused armature can be dried in an oven at a temperature in the range of about 30 ºC to about 100 ºC, including subranges. In some embodiments, the collagen-infused armature can be dried in an oven at a temperature of about 30 ºC, about 35 ºC, about 40 ºC, about 45 ºC, about 50 ºC, about 55 ºC, about 60 ºC, about 65ºC, about 70 ºC, about 75 ºC, about 80 ºC, about 85 ºC, about 90 ºC, about 95 ºC, or about 100 ºC, or in a range having any two of these values as endpoints, inclusive of the endpoints. For example, in some embodiments, the collagen-infused armature can be dried at a temperature in a range of about 30 ºC to about 100 ºC, about 35 ºC to about 95 ºC, about 40 ºC to about 90 ºC, about 45 ºC to about 85 ºC, about 50 ºC to about 80 ºC, about 55 ºC to about 75 ºC, about 60 ºC to about 70 ºC, or about 60 ºC to about 65 ºC. In some embodiments, the collagen-infused armature can be hung to air dry. In some embodiments, the collagen-infused armature can be dried at room temperature (23°C). If desired, the dried collagen-infused composite material can be subjected to any of the tanning processes described herein.

[0366] In some embodiments, the resulting collagen-infused composite material can contain fillers, density modifiers such as hollow microspheres, dyes, pigments, oils, preservatives, stabilizers, and/or other minor components. In some embodiments, finishes

known in the art can be applied by roller coating or by spraying, or another process known in the art. The finish can be a single coat or multiple coats. For example, a base coat can be based on polyurethane or other chemistry and can optionally contain pigments, agents to affect gloss, while adhesive and top coats can be added if desired to adjust color and provide abrasion resistance. In some embodiments, a collagen-infused composite material can be subjected to staking before final drying to adjust hand feel before or after finishing.

[0367] Exemplary filler materials include nanoparticles, microparticles, or various polymers such as syntans commonly used in the tanning industry. In some embodiments, a filler material can be part of a final dehydrated composite material. In some embodiments, a filler material can be sacrificial. For example, the filler material can be degraded or dissolved away leaving open space for a more porous fibril network. In some embodiments, a filler material can serve to control the organization of a dehydrated fibril network by keeping fibrils spaced apart during dehydration. The shape and dimension of a filler material can be used to control the orientation of the dehydrated fibril network.

[0368] In some embodiments, a secondary material can be incorporated into the final collagen-infused composite material product. In some embodiments, the secondary component can be incorporated into the collagen-infused composite material. In some embodiments, the collagen-infused composite material can be at least partially incorporated into a secondary material, or coated on, layered on, or laminated to a secondary material. For example, a collagen-infused composite material can encapsulate a secondary material, a secondary material can be coated on one side with a collagen-infused composite material, a secondary material coated on both external sides with a collagen-infused composite material, or one or more layers of a secondary material can be laminated to one or more layers of a collagen-infused composite material.

[0369] In some embodiments, a filler material or secondary component can include one or more of polymeric microsphere(s), bead(s), fiber(s), wire(s), or organic salt(s). Other additional materials can also be embedded or otherwise incorporated into a collagen-infused composite material described herein. Exemplary additional materials include other peptides or polypeptides including recombinantly-produced peptides or polypeptides, chitosan, mycelium, cellulose including bacterial cellulose, wood including wood fibers, aramids, carbon including carbon fibers and fullerenes, glass including glass

fibers and nonwovens, silicon and silicon-containing compounds, minerals, including mineral particles and mineral fibers, and metals or metal alloys, including those comprising iron, steel, lead, gold, silver, platinum, copper, zinc and titanium, which can be in the form of particles, fibers, wires or other forms suitable for incorporating into a composite material. In some embodiments, a filler material can include an electrically conductive material, magnetic material, fluorescent material, bioluminescent material, phosphorescent material or other photoluminescent material, or combinations thereof. Mixtures or blends of these components can be embedded or incorporated into a collagen-infused composite material, for example, to modify the chemical and physical properties disclosed herein.

[0370] In some embodiments, materials such as lubricants, humectants, dyes, and other treating agents can be uniformly distributed through a collagen-infused composite material using a method as described herein. This is an advantage compared to conventional leather tanning and fat liquoring, which due to its structural heterogeneity often makes uniform treatment impossible. Further, as chemical agents can be incorporated before network formation, the amount of treatment chemicals can be reduced because there is reduced chemical loss by not having to penetrate a collagen network from a float containing the treatment chemicals. Unlike high temperatures often used to treat natural leather, a collagen-infused composite material described herein can be heated at ambient temperature or, in some embodiments, at a temperature no greater than 37 °C during processing before evaporating away the solvent to form a fibrillated collagen material. Alternatively, in some embodiments, collagen fibrils can be cross-linked and lubricated in suspension before forming a network between fibrils during dehydration or through the addition of a binding agent to the suspension or to the dehydrated material.

[0371] As previously mentioned, the density and to some extent, the pattern of collagen fibril formation can be controlled by adjusting the pH of a collagen solution during fibrillation induction along with the concentration of fibrils during dehydration. In some embodiments, although the overall size of the fibrils can be similar the natural bovine corium, the arrangement of these fibrils can be quite different. Such ultrastructural differences between the collagen fibrils within a fibrillated collagen hydrogel and natural tissue such as bovine corium (and resulting leather made therefrom) can result in a

collagen-infused composite material that is as soft or softer than natural leather, more pliable than natural leather, and/or that has an appearance similar to natural leather.

[0372] In some embodiments, collagen can be infused to form a collagen-infused composite material and a secondary material can be laminated to one side of the composite material using an adhesive, or the like. Suitable adhesives include, but are not limited to, hot melt adhesives, emulsion polymer adhesives, and the like. A collagen-infused composite material can be coated with adhesive by known techniques such as slot die casting, kiss coating, and the like. In some embodiments, the secondary material can be applied to the collagen-infused composite material and passed through rollers under heat to laminate the materials.

[0373] In some embodiments, a secondary material can be dispersed throughout a collagen-infused composite material to create a composite structure. In some embodiments, the density of the secondary material can range from about 1μg/mL to about 500 mg/mL. In some embodiments, the ratio of fibrillated collagen to secondary material can range from about 1:100 to about 100:1. In some embodiments, the ratio of dried collagen to secondary material in a collagen-infused composite material can range from about 1:100 to about 100:1.

[0374] In some embodiments, the secondary material can be a photoluminescent material, for example, a photoluminescent fabric, nonwoven, felt, carbon fiber, or three-dimensional object. In some embodiments, a collagen solution can be poured over one side of a secondary material, the secondary material can be flipped over, and collagen solution can be poured over the other side of the secondary material.

[0375] In some embodiments, after or during dehydration, a fibrillated collagen material can be treated with lubricants and/or oils to impart greater flexibility and suppleness to the fibrillated collagen material. In some embodiments, using a combination of oil and solvent can allow the oil to better penetrate the fibrillated collagen network compared to using oil by itself. Oil by itself can penetrate the exposed surfaces but may not readily infiltrate the entire thickness of the fibrillated collagen material in a reasonable amount of time. Once the oil/solvent composition has penetrated the entire thickness of the material, the solvent can then be removed. Suitable oils and lubricants can include but are not limited to castor oil, pine oil, lanolin, mink oil, neatsfoot oil, fish oil, shea butter, aloe, and so forth.

[0376] In some embodiments, lubricating the dehydrated a cross-linked fibrillated collagen network or hydrogel to form a leather-like material can result in a material having properties that are similar, or better, than the properties of natural leather. Solutions that include a combination of oils and organic solvent can increase the mass and the softness (inversely proportional to the slope of the stress-strain curve) of a dehydrated fibrillated collagen material. The combination of oils and organic solvents can penetrate the dehydrated fibrillated collagen material, and once penetrated through; the oils can remain distributed throughout the material, while the organic solvents are able to evaporate away. In some embodiments, the use of oils alone may not be as effective in penetrating entirely through the dehydrated fibrillated collagen material.

[0377] In some embodiments, a fibrillated collagen material can be treated similarly to natural leather derived from animal hide or skin, and be re-tanned, dyed, and/or finished. Additional processing steps can include crosslinking, re-tanning, and/or surface coating. Crosslinking and re-tanning can include sub-processes such as wetting back (re-hydrating semi-processed material), sammying (45-55% water is squeezed from the material), splitting (material is split into one or more layers), shaving (material is thinned), neutralization (pH of material is adjusted to, for example, between about 4.5 and about 6.5), dyeing (material is colored), fat liquoring (fats, oils, waxes are fixed to the material), filling (dense/heavy chemicals to make the material harder and heavier), stuffing (fats, oils, waxes added), fixation (unbound chemicals are bonded/trapped and removed), setting (grain flatness are imparted and excess water removed), drying (material is dried to desired moisture levels, for example, about 10-25%), conditioning (moisture is added to a material to, for example, about a 18-28% level), softening (physical softening of a material by separating the fibers), or buffing (abrading surface of a material to reduce nap and grain defects). Surface coating can include any one or combination of the following steps: oiling (material coated with raw oil or oils), buffing, spraying, roller coating, curtain coating, polishing, plating, embossing, ironing, or glazing.

[0378] Unlike animal hides, where the hide has to be trimmed to obtain the desired thickness or dimensions, collagen-infused composite materials described herein can be fabricated with a wide range of thicknesses as well as the desired dimensions for a particular product.

[0379] The production of collagen-infused composite materials described herein can generate relatively less waste by bypassing the step of removing excess proteins, fats, and hair necessary for treating natural animal hide in the leather production process. This can result in less environmental impact.

[0380] The embodiments discussed herein will be further clarified in the following examples. It should be understood that these examples are not limiting to the embodiments described above.

EXAMPLE 1: Armature Functionalization

[0381] The functionalization of armatures, such as cellulose-based armatures, with free hydroxyl groups, was performed by the following process. First, an un-infused armature (brushed cotton twill, Whaleys Bradford Ltd Fabrics) was placed in a water bath at pH 10 (adjusted with 1N sodium hydroxide) with 2 wt% of (3-aminopropyl)-trimethoxysilane (APTMS) based on the weight of the armature. The armature was left in the bath overnight, at room temperature, shaking on an orbital shaker at 70 rpm. The armature was then washed with distilled water and placed in an oven at 100°C for at least 3 hours, depending on the size of the armature, to generate a first amine functional armature.

[0382] The amine-functionalized armature was then placed in a methanol bath at room temperature in a closed, opaque container and set to shake on an orbital shaker at 60 rpm for 48 hours. The armature was then removed from the bath, washed in water, and placed in an oven at 65 °C until dry to generate a carboxy-functionalized armature ready for subsequent infusion or functionalization. The methanol bath included 4 molar equivalents of methacrylic acid based on the number of primary amine groups present on the functionalized armature. The amount of methacrylic was determined by weighing the amine functionalized armature and calculating the approximate molar quantity primary amine groups available for reaction. This calculation was performed by assuming each monomeric glucose unit in the cellulose based armature reacted with one molecule of APTMS, calculating the molecular weight of each APTMS functionalized monomer after hydrolysis and dehydration (263.33 g/mol for the fabric in the APTMS functionalization step after functionalization and drying), and further assuming that armature had a molecular weight equal to the weight of the functionalized monomer. Based on these assumptions and calculations, a 10 g sample of amine functionalized armature was

assumed to have 37.98 mmol of primary amine available for further reaction (10 g / 263.33 g/mol) such that 151.92 mmol of methacrylic acid was added.

[0383] The carboxy-functionalized armature was placed into a methanol bath at room temperature in a closed container containing ethylene diamine. Ethylene diamine was added to the bath in an amount determined by calculating the new molecular weight of the monomeric units in the armature (435.45 g/mol) and determining the number of carboxy groups . The ethylene diamine was added to the methanol bath in a 1:4 mole ratio of armature monomer to diamine (22.96 mmol for a 10 g sample of carboxy-functionalized armature). The ethylene diamine was added to the methanol bath and allowed to disperse before the carboxy-functionalized armature was submerged. The container was then sealed and set to shake on an orbital shaker at room temperature for 48 hours. Upon completion of the reaction time, the newly amine-functionalized armature was washed in excess water and dried in an oven at 65 °C to generate a second amine functionalized armature.

EXAMPLE 2: Collagen Solution Preparation and Infusion

[0384] A collagen solution was prepared by dissolving Bovine Type I collagen in 0.01 N hydrochloric acid (pH ~2.2) to a concentration of 1 gram per liter overnight (~16 hours). The collagen solution was then placed in a 4 °C refrigerator for storage. While the collagen solution cooled, an armature was prepared for infusion. The armature used in this example was a brushed cotton twill (10g) that was washed in 0.1% SYNTHRAPOL® detergent and tap water at 98 °C for 5 minutes, rinsed with fresh tap water 3 times, and dried in the oven at 100 °C. The armature infusion capacity was measured by first weighing the dry armature (10 g), and then comparing the dry weight to the weight of the armature after water uptake (80 g, total weight with the armature was 90 g). Therefore, the infusion capacity (IC) of the brushed cotton twill was 800% (weight of dry armature divided by the weight of water uptake). The infusion capacity factor (ICF) used for this example was 2. The amount of collagen solution used in the infusion process was determined by multiplying IC by ICF by the weight of the armature, which was 160 mL (8 times 2 times 10). The collagen solution was measured out and placed over ice. Phosphate buffered saline (10X) was adjusted to pH 11.5 using 10 N sodium hydroxide, then 1 part of the phosphate buffered saline (10x) was mixed with 9 parts of the collagen

solution to generate a mixed solution. The mixed solution was then stirred at 450 rpm for 2 minutes. The temperature and pH of the mixed solution were checked to ensure the solution was below 10 °C and pH was 7.2 ± 0.2.

[0385] The armature was then submerged in the mixed solution and underwent 2 vacuum-purge cycles (20 inHg) for 2 to 5 minutes per cycle. The cycles were turning the pump off, flipping the armature and applying vacuum again to generate an infused armature. The infused armature was removed from the mixed solution, placed in plastic wrap and the collagen was allowed to fibrillate at room temperature (about 23 °C) overnight. At this point, the fibrillated armature could be tanned and/or retanned utilizing traditional leather processes as collagen fibrils are physically entangled throughout the armature. When the collagen was fibrillated, it formed a visible white "film" on exterior surfaces of the armature. Additionally, after stabilization of the collagen, there was a measurable difference in the weight of the infused armature as compared to the un-infused armature. This collagen preparation and infusion process can be used on both functionalized and un-functionalized armatures. This collagen preparation and infusion process can also be used to infuse collagen into other armatures as described herein, for example the armature of Example 5.

EXAMPLE 3: Tanning (and optionally coloring) an Infused Armature

[0386] The infused armature from Example 2 was tanned in X-tan (from Lanxess). The X-tan solution was prepared at 5 wt.% of tanning agent, based on the mass of collagen infused into the armature, and dissolved in a phosphate buffered saline solution with a pH adjusted to 7.5 +- 0.1 using 1 N sodium hydroxide to generate a float. The infused armature was placed flat in a container. The X-tan solution was then added to the container. The volume of float was adjusted to be around 2x the wet sample mass. The container was heated to 33 °C and shaken on an orbital shaker at 60 rpm. The pH was measured and adjusted to 7.5 using 1 N sodium hydroxide every hour. After 6 hours, the infused armature was taken out of the X-tan solution, drained, weighed and stored wet for further processing to generate a tanned collagen-infused composite material. If the infused armature has not been colored up to this point and it is desired to be colored, it can be colored during the last hour of the tanning process using the following steps. First, add 0.1 wt.% to 10 wt.% of a fiber reactive dye or an acid dye, based on weight of the

infused armature before tanning, to the float. The amount of dye used can be selected based on the shade of color desired. Second, adjust the pH of the tanning solution to about 7.5, if not at about 7.5 after the dye addition.

EXAMPLE 4: Retanning (an optionally coloring) an Infused Armature

[0387] The tanned infused armature from Example 3 (not colored) was retanned in a bath with syntans, such as Tanigan HS (from Lanxess). A syntan solution was prepared by dissolving 50 wt.% of syntan, based on the mass of collagen infused into the armature, in distilled water with enough volume to submerge the armature in a container while flat. The pH of the syntan solution was adjusted to 5-6 using 1 N formic acid. The container was then placed in a water bath at 32-34 °C for 2-3 hours and shaken on an orbital shaker at 40 rpm. The pH of the syntan solution was monitored and adjusted to 5-6 every hour. After 2-3 hours, the pH of the syntan solution was lowered gradually to 3.8 using 1 N formic acid and left for 1hour shaking at 32-34°C to generate a retanned infused material. If the infused armature has not been colored up to this point and it is desired to be colored, it can be colored during the last hour of the tanning process using the following steps. First, lower the pH of the syntan solution to about 3.8. Second, add 0.1 wt.% to 10 wt.% of a fiber reactive dye or an acid dye, based on weight of the infused armature before tanning, to the solution. The amount of dye used can be selected based on the shade of color desired. Third, allow the armature to shake in the bath for an hour.

[0388] The retanned (not colored) infused material was then put in a bath with a fat liquor (Truposol Ben, from Trumpler). A fat liquor solution was prepared by dissolving a 50% offer of fat liquor in collagen in distilled water, with enough volume to submerge the retanned infused armature, while flat. The pH was adjusted to be between 4-5 using 1N formic acid. The container was placed in a water bath at 30 °C for 1 hour and shaken on an orbital shaker at 40 rpm. The pH of the solution was then lowered gradually to 3.5 using 1 N formic acid and left for 1 hour shaking at 32-35 °C. The retanned infused material armature was then removed from the container, covered, and left overnight. The infused armature was hung to air dry and staked before it was fully dry (at approximately 20% moisture) to generate a fat liquored infused material.

EXAMPLE 5: Armature Formation

[0389] A multi-layer armature was made according to the following example. A 12/1 S Tencel yarn was used on a Shima Seiki SWG041 N1 machine with a knitting gauge of 15 and a 4/4 tuck stitch to generate a spacer fabric. Cellulosic fibers (25 mm in length and 1.5 D in diameter) were carded to generate a nonwoven substrate (200 gsm and 15 mm thickness). The nonwoven substrate was needled-punched (draft ration 1, speed 100 rpm, needle depth 5/8", and 50 PPSI). The needle-punched nonwoven substrate was placed on top of the spacer fabric and needle-punched again (draft ration 1.02, speed 500 RPM, needle depth 5/8", 400 PPSI) to directly couple the non-woven substrate to the spacer fabric, thereby creating a multi-layer armature.

EXAMPLE 6: Un-infused Armature Coloring

[0390] The cellulosic armature of Example 5 was colored using a dye according to the following exemplary processes. Other cellulosic armatures can also be colored according to these processes. First, two armatures were washed in 0.12 wt.% SYNTHRAPOL® textile detergent, based on weight of the armature. The armatures were submerged in the textile detergent by one inch at 98 °C for 5 minutes. Then the armatures were rinsed with fresh tap water 3 times each and dried in the oven at 100 °C. One of the armatures was then dyed using a fiber reactive dye and the other armature was dyed using a direct dye.

[0391] For the armature dyed with the fiber reactive dye, the dye was pre-dissolved in 1 mL (milliliter) of water then transferred to a vessel with enough DI water to submerge the armature by one inch. Depending on the desired depth of color of the armature post-dyeing, 0.01 wt.% to 10 wt.% of the fiber reactive die, based on the dry weight of the armature, is used. Then, 200 wt.% of salt, based on the dry weight of armature, was pre-dissolved and added to the vessel with the dye and the water to form a dye bath. The armature was then placed in the bath such that it was submerged by about 1 inch and the bath was mixed at a low rpm using a stir bar for 45 minutes. After 45 minutes, 1 oz of sodium carbonate per pound of the armature was added to bring the heat of the dye bath up to between 31 °C and 40.5 °C. The bath was then mixed again at low rpm using a stir bar for 60 minutes. After stirring, the armature was taken out of the dye bath and washed with 0.12 wt.% SYNTHRAPOL® textile detergent mixed with cool tap water, based on

weight of armature, until the water ran clear. After washing, the armature was dried at 75 °C.

[0392] For the armature dyed with a direct dye, the dye was pre-dissolved in 1 mL of water and then transferred to a vessel with enough DI water to submerge armature by one inch. Depending on the desired depth of color of the armature post-dyeing, 0.01 wt.% to 10 wt% of the direct die, based on the dry weight of the armature, is used. Then, 50 wt.% of salt, based on the dry weight of armature, was pre-dissolved and added to the vessel with the dye and the water to from a dye bath. The armature was then placed in the dye bath and the bath temperature was brought up to between 79 °C and 93 °C. The bath was then mixed at a low rpm using a stir bar for 30-60 minutes. The mixing time can be selected based on the desired depth of color of the armature post-dyeing. After mixing, the armature was taken out of the dye bath and washed with 0.12 wt.% SYNTHRAPOL® textile detergent mixed with cool tap water, based on weight of armature, until the water ran clear. After washing, the armature was dried at 75 °C.

[0393] While various embodiments have been described herein, they have been presented by way of example, and not limitation. It should be apparent that adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It therefore will be apparent to one skilled in the art that various changes in form and detail can be made to the embodiments disclosed herein without departing from the spirit and scope of the present disclosure. The elements of the embodiments presented herein are not necessarily mutually exclusive, but can be interchanged to meet various situations as would be appreciated by one of skill in the art.

[0394] Embodiments of the present disclosure are described in detail herein with reference to embodiments thereof as illustrated in the accompanying drawings, in which like reference numerals are used to indicate identical or functionally similar elements. References to “one embodiment,” “an embodiment,” “some embodiments,” “in certain embodiments,” etc., indicate that the embodiment described can include a particular feature, structure, or characteristic, but every embodiment can not necessarily include the particular feature, structure, or characteristic. Moreover, such phrases are not necessarily referring to the same embodiment. Further, when a particular feature, structure, or characteristic is described in connection with an embodiment, it is submitted that it is

within the knowledge of one skilled in the art to affect such feature, structure, or characteristic in connection with other embodiments whether or not explicitly described.

[0395] The examples are illustrative, but not limiting, of the present disclosure. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in the field, and which would be apparent to those skilled in the art, are within the spirit and scope of the disclosure.

[0396] It is to be understood that the phraseology or terminology used herein is for the purpose of description and not of limitation. The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A collagen-infused composite material, comprising:

an armature, the armature comprising:

a spacer fabric comprising a topmost surface and a bottommost surface opposite the topmost surface,

a first non-woven substrate disposed over the topmost surface of the spacer fabric, and

a second non-woven substrate disposed over the bottommost surface of the spacer fabric such that the spacer fabric is disposed between the second non-woven substrate and the first non-woven substrate; and

collagen infused into the spacer fabric, the first non-woven substrate, and the second non-woven substrate.

2. The composite material of claim 1, wherein the first non-woven substrate is disposed on the topmost surface of the spacer fabric.

3. The composite material of claim 1 or claim 2, wherein the second non-woven substrate is disposed on the bottommost surface of the spacer fabric.

4. The composite material of any of claims 1–3, wherein the collagen couples the first non-woven substrate to the spacer fabric and couples the second non-woven substrate to the spacer fabric.

5. The composite material of any of claims 1–4, wherein the first non-woven substrate is directly coupled to the spacer fabric.

6. The composite material of claim 5, wherein the first non-woven substrate is directly coupled to the spacer fabric by a plurality of needle-punched entanglements between the first non-woven substrate and the spacer fabric.

7. The composite material of any of claims 1–6, wherein the collagen infused into the first non-woven substrate defines a collagen layer on a top surface of the first non-woven substrate, the collagen layer being continuously distributed over the top surface of the first non-woven substrate.

8. The composite material of claim 7, wherein the collagen layer on the top surface of the first non-woven substrate comprises a rough top surface, and wherein the rough top surface has a surface area per square inch of at least 10% greater than 1 in^2.

9. The composite material of claim 8, further comprising a coloring agent, wherein the rough top surface comprises a plurality of peaks and a plurality of valleys, and wherein a color of the peaks is different than a color of the valleys.

10. The composite material of claim 9, wherein the color of the peaks is darker than the color of the valleys.

11. The composite material of claim of claim 9, wherein the coloring agent comprises a dye.

12. The composite material of claim 11, wherein the dye is a fiber reactive dye or an acid dye.

13. The composite material of any of claims 1–12, wherein at least one of the topmost surface of the spacer fabric or the bottommost surface of the spacer fabric is defined by a woven fabric layer or a knitted fabric layer.

14. The composite material of any of claims 1–13, wherein the armature is functionalized.

15. The composite material of any of claims 1–8, 13, or 14, further comprising a coloring agent.

16. The composite material of claim 15, wherein the coloring agent comprises a dye.

17. The composite material of claim 16, wherein the dye is a fiber reactive dye or an acid dye.

18. A collagen-infused composite material, comprising:

an armature comprising:

a base substrate having a first collagen infusion capacity and a first tear strength, the base substrate comprising a topmost surface and a bottommost surface opposite the topmost surface, and

a non-woven substrate having a second collagen infusion capacity and a second tear strength, the non-woven substrate disposed over the topmost surface of the base substrate; and

collagen infused into the base substrate and the non-woven substrate,

wherein the collagen is infused into the non-woven substrate at a first collagen density, and

wherein the collagen is infused into the base substrate at a second collagen density less than the first collagen density.

19. The composite material of claim 18, wherein the first collagen infusion capacity is less than the second collagen infusion capacity.

20. The composite material of claim 18 or claim 19, wherein the base substrate comprises at least one of: a woven fabric layer or a knitted fabric layer.

21. The composite material of any of claims 18–20, wherein the first tear strength is greater than the second tear strength.

22. A method for manufacturing a composite material, the method comprising:

preparing a collagen solution;

infusing the collagen solution into an armature to form a collagen-infused armature, the armature comprising a first non-woven substrate, a second non-woven substrate, and a spacer fabric, wherein the first non-woven substrate is directly coupled to

a topmost surface of the spacer fabric and wherein the second non-woven substrate is directly coupled to a bottommost surface of the spacer fabric;

fibrillating the infused collagen solution; and

drying the collagen-infused armature.

23. The method of claim 22, wherein the first non-woven substrate is directly coupled to the topmost surface of the spacer fabric by a plurality of needle-punched entanglements between the first non-woven substrate and the spacer fabric, and wherein the second non-woven substrate is directly coupled to the bottommost surface of the spacer fabric by a plurality of needle-punched entanglements between the second non-woven substrate and the spacer fabric.

24. The method of claim 22 or claim 23, further comprising adding a coloring agent before drying the collagen-infused armature.

25. The method of claim 24, wherein the collagen-infused armature comprises a rough top surface defined by a collagen layer, the rough top surface comprises a plurality of peaks and a plurality of valleys, and a color of the coloring agent on the peaks is different than a color of the coloring agent in the valleys.

26. The method of claim 25, wherein the coloring agent comprises a dye.

27. The method of any of claims 22–26, wherein infusing the collagen solution into the armature comprises filtering the collagen solution through the armature.

28. The method of any of claims 22–27, wherein infusing the collagen solution into the armature comprises soaking the armature in a bath comprising the collagen solution.

29. The method of any of claims 22–28, further comprising functionalizing the armature before infusing the collagen solution into the armature.

30. The method of any of claims 22–29, further comprising tanning the collagen-infused armature.

31. The method of claim 30, wherein tanning the collagen-infused armature is performed before drying the collagen infused armature.

32. The method of claim 30 or claim 31, further comprising retanning the tanned collagen-infused armature.

33. The method of claim 32, wherein retanning the tanned collagen-infused armature is performed before drying the collagen infused armature.

34. The method of any of claims 30–33, wherein the armature is functionalized such that tanning the collagen infused armature results in the formation of chemical crosslinks between the collagen molecules and the functionalized armature.

35. A composite material comprising collagen and an armature having a thickness of about 0.5 mm to about 50 mm.

36. The composite material of claim 35, wherein the thickness is about 0.5 mm, about 1 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm.

37. The composite material of claim 35 or claim 36, wherein the collagen is recombinant collagen.

38. The composite material of any of claims 35–37, wherein the armature comprises a cellulosic material.

39. The composite material of any of claims 35–38, wherein the armature comprises multiple layers.

40. The composite material of claim 39, wherein the armature comprises two, three, four, five, six, seven, eight, nine or ten layers.

41. The composite material of claim 40, wherein the armature comprises two or three layers.

42. The composite material of claim 41, wherein the armature comprises three layers and wherein the collagen is infused into at least two of the three layers and wherein collagen is disposed on at least an outermost surface of at least one of the three layers.

43. The composite material of any of claims 35–42, wherein the collagen and the armature are tanned or dyed.

44. The composite material of any of claims 35–43, wherein the armature comprises one or more materials selected from the group consisting of cotton, linen, silk, wool, kenaf, flax, cashmere, angora, bamboo, bast, hemp, soya, seacell, milk or milk proteins, spider silk, chitosan, mycelium, cellulose including bacterial cellulose, wood, polyester, nylon, an aromatic polyamide, a polyethylene, a polypropylene, a polypropylene/polyethylene copolymer, rayon, lyocell, viscose, antimicrobial yarn (A.M.Y.), Sorbtek, nylon, Lycra®, spandex, Elastane®, polyester-polyurethane copolymers, aramids, carbon fibers, fullerenes, glass, silicon, minerals, metals, and combinations thereof.

45. The composite of claim 44, wherein the metals are metal alloys selected from the group consisting of iron, steel, lead alloys, gold alloys, silver alloys, platinum alloys, copper alloys, zinc alloys, and titanium alloys.

46. The composite of any of claims 35–45, wherein the armature is functionalized.

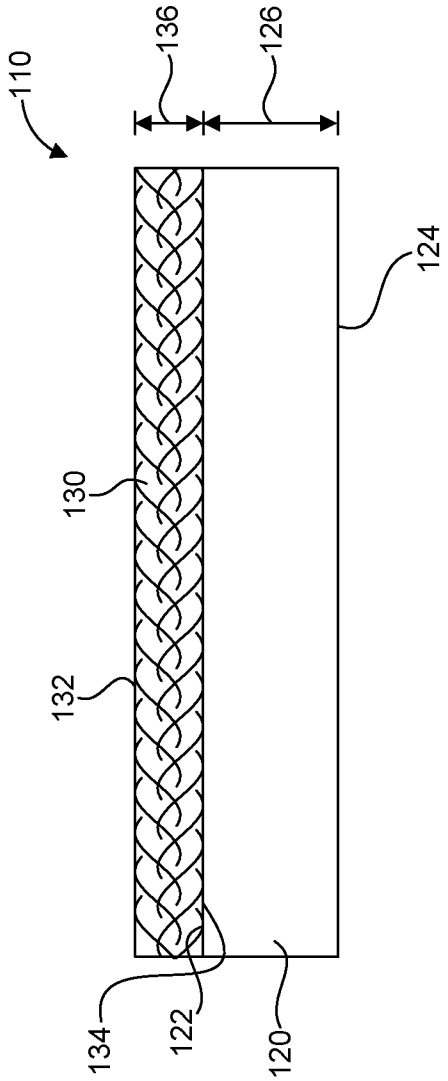


FIG. 1A

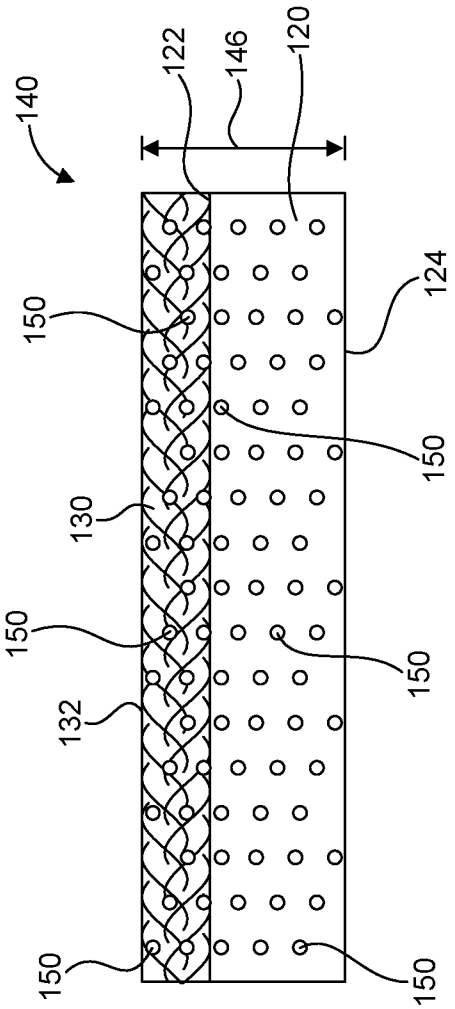


FIG. 1B

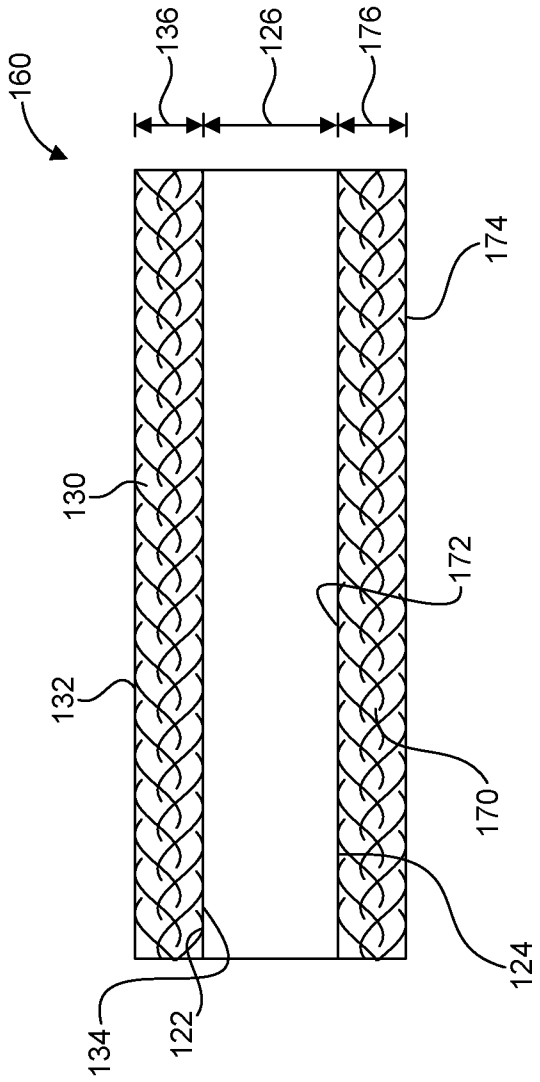


FIG. 1C

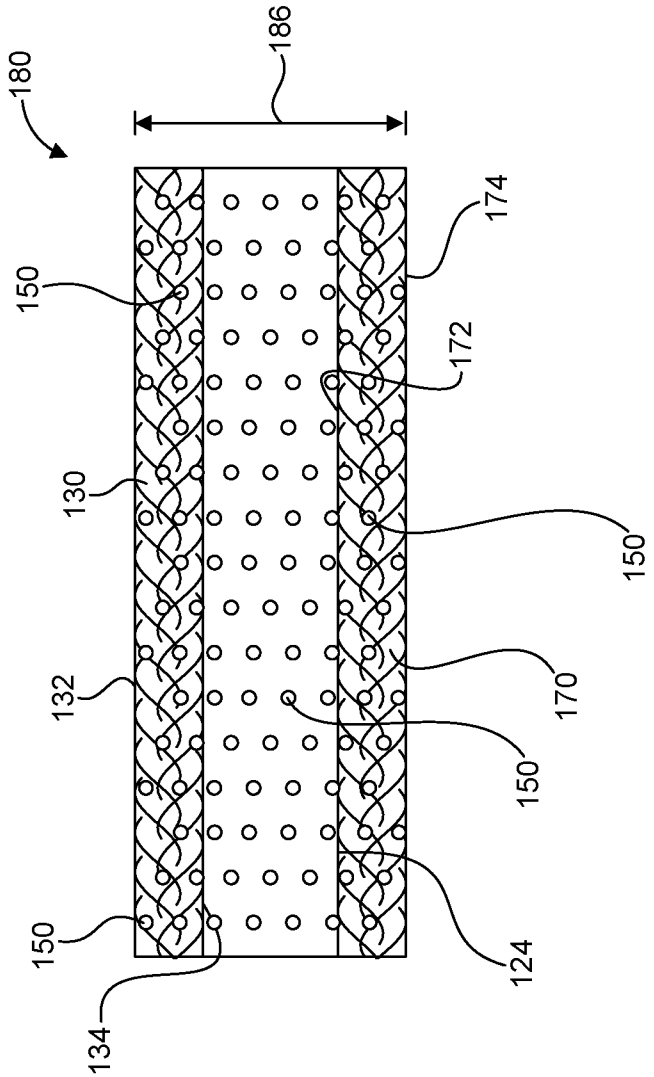


FIG. 1D

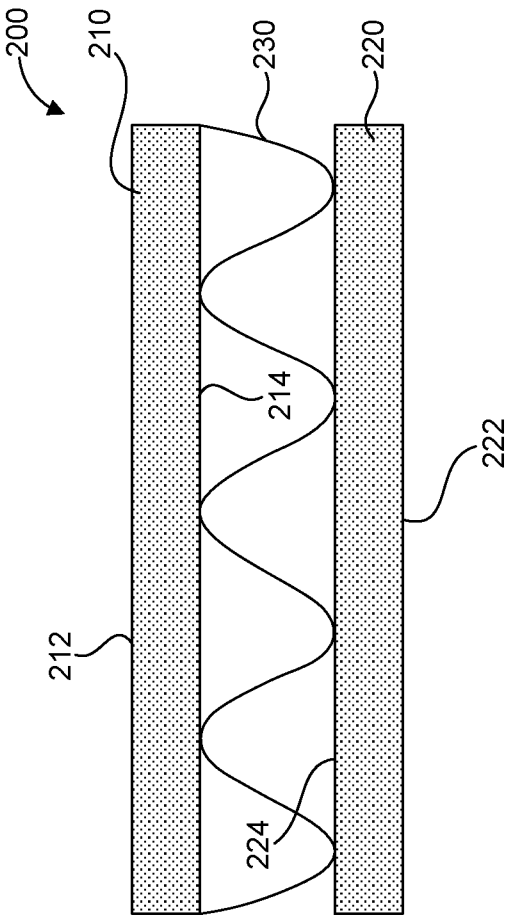


FIG. 2

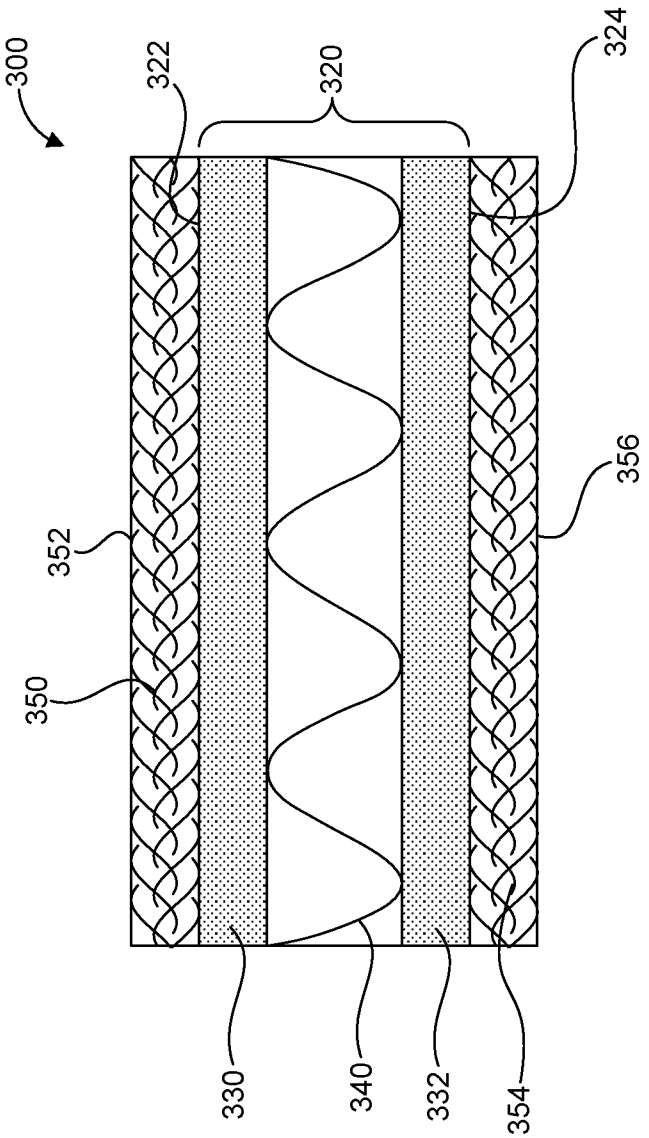


FIG. 3A

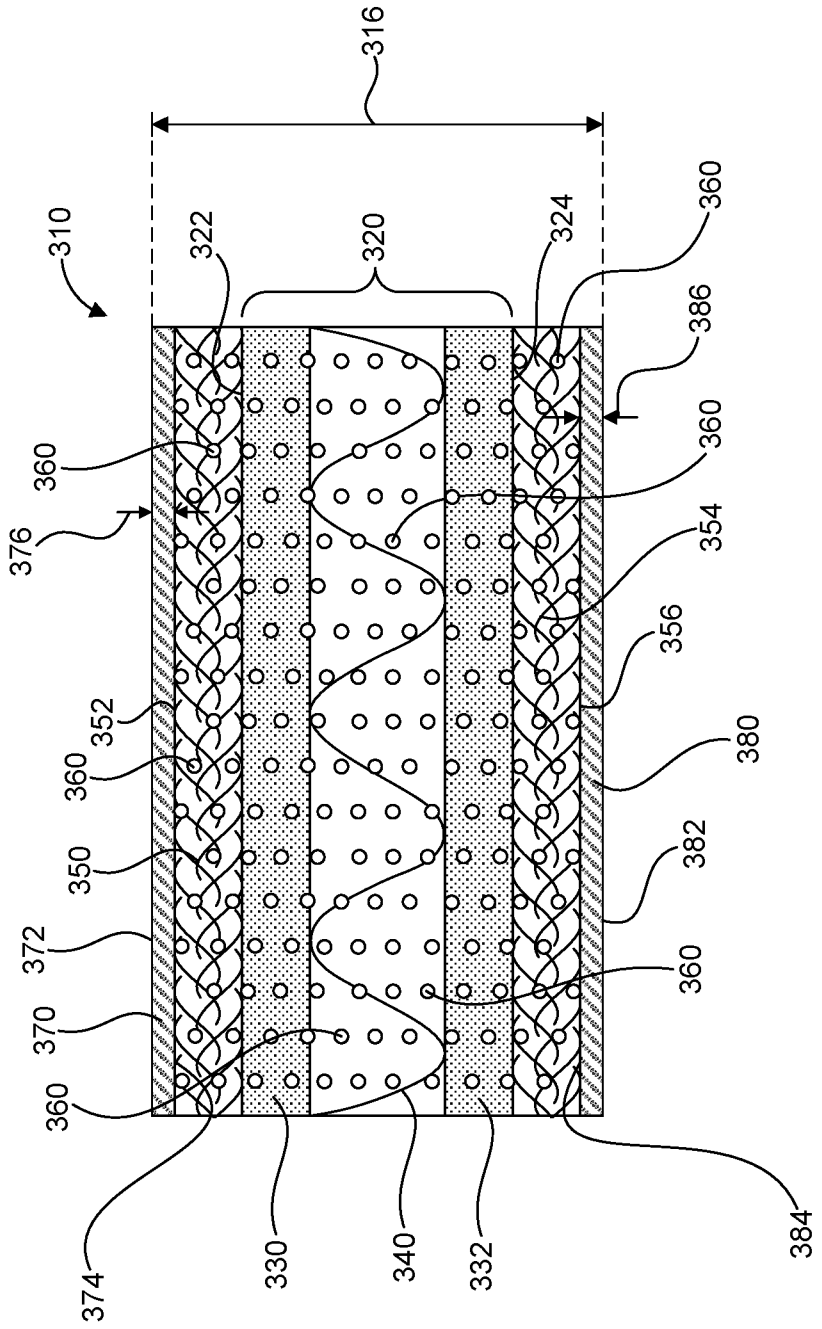

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FIG. 3B

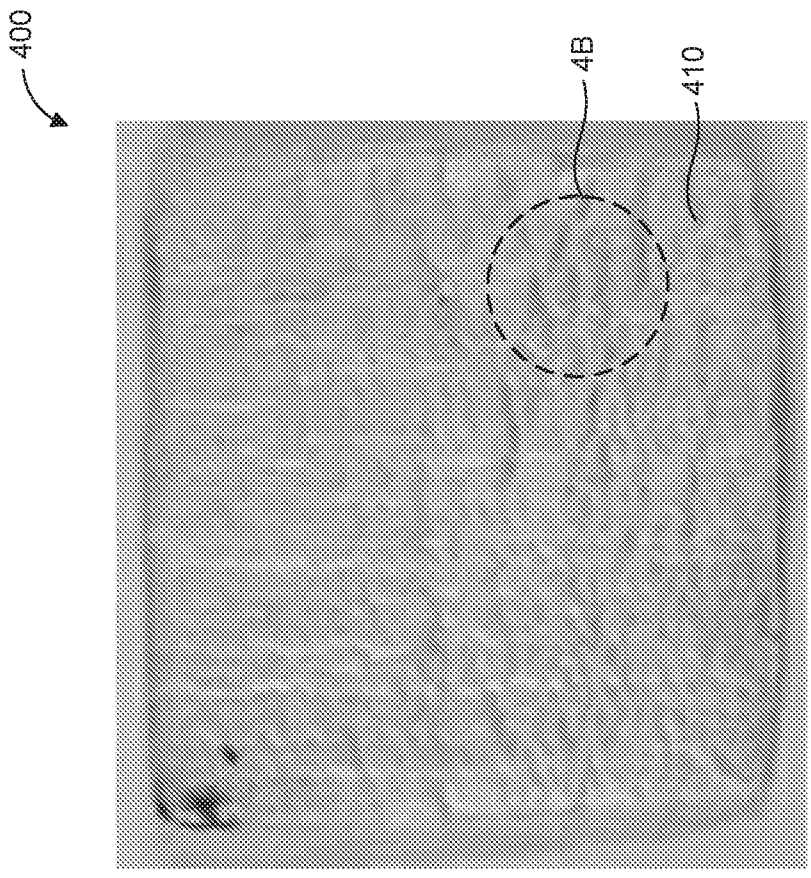

FIG. 4A

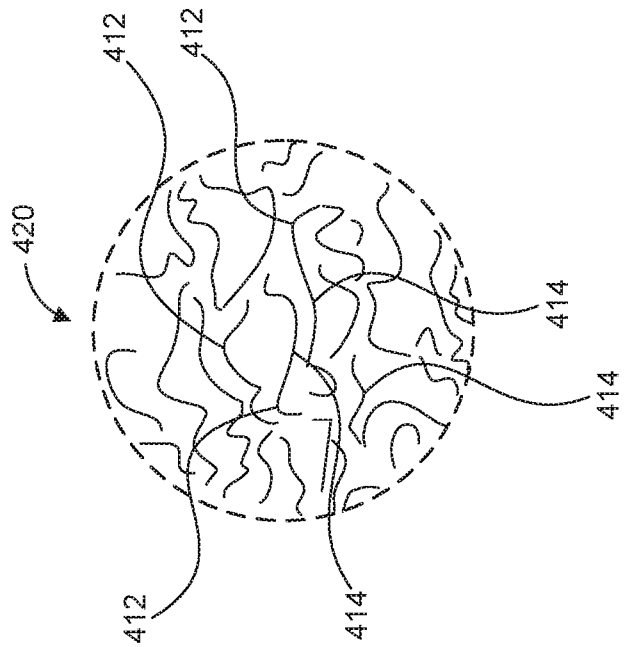

FIG. 4B

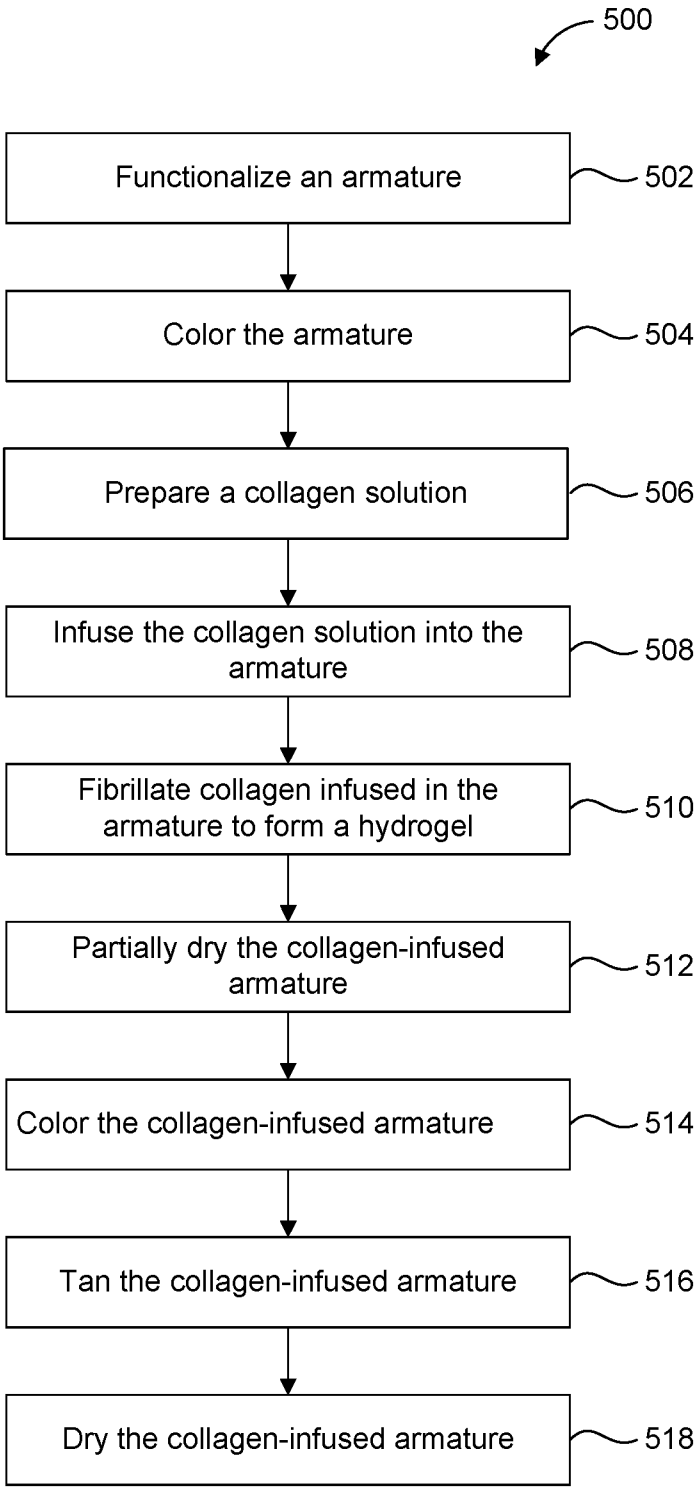
500
Functionalize an armature
502
Color the armature
504
Prepare a collagen solution
506
Infuse the collagen solution into the armature
508
Fibrillate collagen infused in the armature to form a hydrogel
510
Partially dry the collagen-infused armature
512
Color the collagen-infused armature
514
Tan the collagen-infused armature
516
Dry the collagen-infused armature
518

FIG. 5

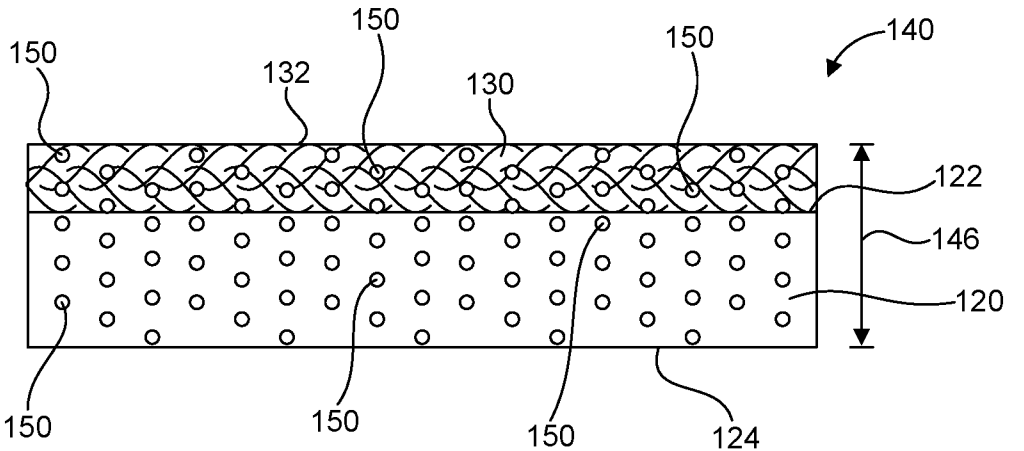

FIG. 1B