molecules. Hydrogels may be formed. Piluso et al. teach that propiolic acid may be conjugated by means of a carbodiimide activating agent and subsequently a gel may be formed via a bivalent linker. Reactive linkers can thus be avoided. However, this process is rather complex and requires several laborious steps and toxic agents. CN-A 111440340 describes a rather complex multi-step process for obtaining a silk fibroin-sodium hyaluronate cross-linked network. The silk fibroin is partly digested by contacting it with a tyrosinase and hydrogen peroxide. Hyaluronate is reacted with a derivative of ethanesulfonic acid, N-hydroxysuccinimide and a carbodiimide activator. In a third step, the two already reacted solutions are combined with each other. Such step is comparably laborious. Furthermore, undesirable reactive ingredients such as N-hydroxysuccinimide are used and can have detrimental effects when residuals remain in the obtained hydrogels.

WO 2019/175036 provides a porous biomaterial for tissue regeneration. This application teaches reacting fibroin moieties with formyl hyaluronic acid. Thereby, a direct linkage between the fibroin moieties and hyaluronic acid is obtained without a linker moiety introduced in between. A disadvantage of this method is that the rather reactive formyl hyaluronic acid is used and that imine groups are formed which are rarely found in nature. The reaction is taught including a reaction using a freeze-drying and drying step.

In view of above, there is still an unmet need for an efficient method for preparing a bio-based material that requires only low procedural efforts, that avoids xenobiotic linker moieties introduced into the hydrogel and minimizes the residuals of toxic reagents. It is further desired to obtain amide bonds and to avoid the need of the intermediate formation of formyl groups. Particularly desirable is a method that provides injectable hydrogel (e.g., usable as super-volumizer) for facial and body re-shaping.

Surprisingly, it has been found that a bio-based material having beneficial properties can be obtained from a method comprising the reaction of a fibroin moiety comprising primary amino residues with polysaccharide moieties comprising carboxylic acid residues or salt thereof with an activating agent forming an activation, thereby forming amide bonds.

The present disclosure relates to a method for preparing a cross-linked material, said method comprising:

(i) contacting the following components with each other:
  (A) one or more fibroin moieties comprising primary amino residues or salts thereof,
  (B) one or more polysaccharide moieties comprising carboxylic acid residues or salts thereof,
  (C) one or more activating agents that effect reaction of carboxylic acid residues with amino residues thereby forming amide bonds, and
  (D) one or more solvents; and
(ii) allowing a reaction of at least some of the carboxylic acid residues with at least some of the primary amino residues to form amide bonds conjugating the one or more fibroin moieties covalently with the one or more polysaccharide moieties; and
(iii) optionally purifying the cross-linked material obtained from step (ii).

In one aspect, the present disclosure relates a method for preparing a cross-linked material, said method comprising:
(i) contacting the following components with each other:
  (A) one or more fibroin moieties comprising primary amino residues or salts thereof,
  (B) one or more polysaccharide moieties comprising carboxylic acid residues or salts thereof,
  (C) one or more triazine-based activating agents that effect reaction of carboxylic acid residues with amino residues thereby forming amide bonds, and
  (D) one or more solvents; and
(ii) allowing a reaction of at least some of the carboxylic acid residues with at least some of the primary amino residues to form amide bonds conjugating the one or more fibroin moieties covalently with the one or more polysaccharide moieties; and
(iii) optionally purifying the cross-linked material obtained from step (ii).

In another aspect, the present disclosure relates to a cross-linked material obtainable from a method as described herein, wherein said cross-linked material is further characterized in that it does not comprise:
(a) imide groups;
(b) imine groups;
(c) epoxy groups; and
(d) xenobiotic linker moieties.

In another aspect, the present disclosure relates to a cross-linked material comprising or consisting of

(A) one or more fibroin moieties having a weight average molecular weight of at least 5 kDa, and

(B) one or more hyaluronic acid moieties having a weight average molecular weight of at least 50 kDa,

wherein the one or more fibroin moieties are covalently conjugated with the with the one or more hyaluronic acid moieties via amide bonds without an interconnecting linker structure,

wherein said cross-linked material is further characterized in that it does not comprise:

(a) imide groups;

(b) imine groups;

(c) epoxy groups; and

(d) xenobiotic linker moieties.

It has been found that such cross-linked material bears unexpectedly beneficial properties. It has been found that cross-linked material obtainable from the method of the present disclosure has a long-lasting stability. Viscosity is widely maintained over weeks, even when incubated at elevated temperatures. Likewise, also enzymatic degradability is desirably diminished. It was surprisingly found that xenobiotic linker structures as commonly used in the prior rat could be avoided.

The cross-linked material of the present disclosure may essentially consist of amino acid moieties and polysaccharide moieties, which can also found in nature. In aqueous environment, the obtained cross-linked material may form a hydrogel.

It may also be used to mimic an extracellular matrix and may, thus, induce cell proliferation and/or cellular migration. It may be well used as a filler (e.g., dermal filer) which may be populated by cells. The cross-linked material of the present disclosure may have good shear-thinning properties. Preferably, it has thixotropic properties. Thus, it is preferably less viscous when stressed. Accordingly, it may be injected very well, while still being rather viscous in its target area (when e.g., administered in a subcutaneous area). Comparably low extrusion forces are required. Gels of high viscosity and low extrusion force are obtainable. It may optionally serve as a super-volumizer.

In another aspect, the present disclosure relates to an injectable composition comprising a cross-linked material as described herein and a liquid or viscous carrier and optionally further components, preferably wherein the cross-linked material is a hydrogel and/or a super-volumizer.

In another aspect, the present disclosure relates to use of an injectable composition as described herein for cosmetic applications comprising facial and body re-shaping and rejuvenation, preferably including filling of wrinkles, improving facial lines, breast reconstruction or augmentation, rejuvenation of the skin, buttocks augmentation, remodeling of cheekbones, soft-tissue augmentation, filling facial wrinkles, improving glabellar lines, improving nasolabial folds, improving marionette lines, improving buccal commissures, improving peri-lip wrinkles, improving crow's feet, improving subdermal support of the brows, malar and buccal fat pads, improving tear troughs, improving nose appearance, augmentation of lips, augmentation of cheeks, augmentation of peroral region, augmentation of infraorbital region, resolving facial asymmetries, improving jawlines, augmentation of chin, or a combination of two or more thereof.

The process of the present disclosure can be conducted without undue burden with comparably low efforts. No lubrication phase is found.

Claims

1. A method for preparing a cross-linked material, said method comprising:
   (i) contacting the following components with each other:
      (A) one or more fibroin moieties comprising primary amino residues or salts thereof,
      (B) one or more polysaccharide moieties comprising carboxylic acid residues or salts thereof,
      (C) one or more triazine-based activating agents that effect reaction of carboxylic acid residues with amino residues thereby forming amide bonds, and
      (D) one or more solvents; and
   (ii) allowing a reaction of at least some of the carboxylic acid residues with at least some of the primary amino residues to form amide bonds conjugating the one or more fibroin moieties covalently with the one or more polysaccharide moieties; and
   (iii) optionally purifying the cross-linked material obtained from step (ii).

2. The method of claim 1, wherein the one or more fibroin moieties have a weight average molecular weight of at least 5 kDa, in the range of 5 to 1000 kDa, in the range of 5 to 400 kDa, in the range of 10 to 400 kDa, or in the range of 100 to 150 kDa, preferably wherein the one or more fibroin moieties are silk fibroin moieties, more preferably silk fibroin moieties having at least having at least 80% sequence homology to a natural insect or spider silk fibroin moiety.

3. The method of any of claims 1 or 2, wherein the one or more polysaccharide moieties comprise or consist of hyaluronic acid, heparosan, chondroitin sulfate, and carboxymethyl cellulose.

4. The method of any of claims 1 to 3, wherein the one or more polysaccharide moieties comprise or consist of one or more hyaluronic acid moieties.

5. The method of any of claims 1 to 4, wherein the one or more polysaccharide moieties have a weight average molecular weight of at least 50 kDa, in particular in the range of 50 to 4000 kDa.

6. The method of any of claims 1 to 5, wherein the mass ratio between the one or more fibroin moieties (A) and the one or more polysaccharide moieties (B) A : B is in the range of 5:1 to 1:20, preferably in the range of 1:1 to 1:10, in particular in the range of 1:1 to 1:5.

7. The method of any of claims 1 to 6, wherein the one or more triazine-based activating agents are selected from the group consisting of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium, a salt thereof, and/or 2-chloro-4,6,-dimethoxy-1,3,5-triazine, and combinations thereof.

8. The method of any of claims 1 to 7, wherein the triazine-based activating agent is 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium or a salt thereof, in particular 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride.

9. The method of any of claims 1 to 8, wherein the method is further characterized in that:
   (a) it comprises a step (iii) of purifying the cross-linked material by filtration, washing and/or dialysis, in particular crossflow filtration, diafiltration and/or dead-end filtration;
   (b) steps (i) and (ii) are conducted in a single batch; and/or
   (c) steps (i) and (ii) and optional step (iii) are conducted at a temperature in the range of from 5 to 90°C, in particular 18 to 30°C.

10. The method of any of claims 1 to 9, wherein the method comprises:
   (i) contacting the following components with each other:
      (A) one or more silk fibroin moieties having a weight average molecular weight of at least 5 kDa that comprises primary amino residues or salts thereof,
      (B) one or more hyaluronic acid moieties having a weight average molecular weight of at least 50 kDa that comprises carboxylic acid residues or salts thereof,
      (C) one or more triazine-based activating agents that promote effect thereby forming amide bonds, in particular wherein the

activating agent is 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium or a salt thereof; and

(D) one or more solvents; and

(ii) allowing a reaction of at least some of the carboxylic acid residues with at least some of the primary amino residues to form amide bonds conjugating the one or more silk fibroin moieties covalently with the one or more hyaluronic acid moieties; and

(iii) optionally purifying the cross-linked material obtained from step (ii).

11. A cross-linked material obtainable from a method of any of claims 1 to 10, wherein said cross-linked material is further characterized in that it does not comprise:

(a) imide groups;

(b) imine groups;

(c) epoxy groups; and

(d) xenobiotic linker moieties.

12. A cross-linked material comprising or consisting of

(A) one or more fibroin moieties having a weight average molecular weight of at least 5 kDa, and

(B) one or more hyaluronic acid moieties having a weight average molecular weight of at least 50 kDa,

wherein the one or more fibroin moieties are covalently conjugated with the with the one or more hyaluronic acid moieties via amide bonds without an interconnecting linker structure,

wherein said cross-linked material is further characterized in that it does not comprise:

(a) imide groups;

(b) imine groups;

(c) epoxy groups; and

(d) xenobiotic linker moieties.

13. An injectable composition comprising a cross-linked material of claim 10 or 11 and a liquid or viscous carrier and optionally further components, preferably wherein the cross-linked material is a hydrogel and/or a super-volumizer.

14. Use of an injectable composition of claim 13 for cosmetic applications comprising facial and body re-shaping and rejuvenation, preferably including filling of wrinkles, improving facial lines, breast reconstruction or augmentation, rejuvenation of the skin, buttocks augmentation, remodeling of cheekbones, soft-tissue augmentation, filling facial wrinkles, improving glabellar lines, improving nasolabial folds, improving marionette lines, improving buccal commissures, improving peri-lip wrinkles, improving crow's feet, improving subdermal support of the brows, malar and buccal fat pads, improving tear troughs, improving nose appearance, augmentation of lips, augmentation of cheeks, augmentation of peroral region, augmentation of infraorbital region, resolving facial asymmetries, improving jawlines, augmentation of chin, or a combination of two or more thereof.