OBJECT OF THE INVENTION

[0016] In view of the above, the object of the present invention is to provide an *in situ* cross-linkable composition, which can be easily extruded through fine needles and after administration forms *in situ* a cross-linked hydrogel with desired properties (e.g., in terms of mechanical, chemical, rheological, biological and immunological properties) for augmenting, filling or replacing soft tissues in various cosmetic and therapeutic applications.

SUMMARY

[0017] The above object is solved by the provision of two functionalized polysaccharide derivatives, which spontaneously form covalent cross-links following injection in the body of a patient (i.e. under *in vivo* conditions). The thus formed *in situ* cross-linked polysaccharide network in the form of a hydrogel acts as a soft tissue filler, e.g. a dermal filler. The *in situ* cross-linkable polysaccharide composition of the present invention is advantageous in that the two functionalized polysaccharide derivatives can be co-injected in liquid form, thereby enabling co-injection with low extrusion forces even through fine needles. Desirably, the *in situ* gel formation does not generate any harmful by-products. The only by-product is water that is readily absorbed by the formed hydrogel and/or the surrounding tissues. In addition, the *in situ* formed hydrogel has the desired properties in terms of tissue integration, skin improvement, tissue shaping capacity and volumizing ability.

[0018] In one aspect, the present invention refers to the use of a first polysaccharide derivative and a second polysaccharide derivative for the in situ formation of a cross-linked hydrogel in cosmetic applications, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second polysaccharide derivatives are sterilized, and wherein the nucleophilic group and the electrophilic group form a covalent linkage in situ following co-injection of the first and second polysaccharide derivatives to a target site in the body of a patient, resulting in the formation of a cross-linked hydrogel at the target site, wherein the first polysaccharide derivative is a hydrazide functionalized first hyaluronic acid (HA) derivative and the second polysaccharide derivative is an aldehyde functionalized

second hyaluronic acid (HA) derivative. In one example, there is disclosed the use of a first polysaccharide derivative and a second polysaccharide derivative for the *in situ* formation of a cross-linked hydrogel in cosmetic applications, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second polysaccharide derivatives are sterilized, and wherein the nucleophilic group and the electrophilic group form a covalent linkage *in situ* following co-injection of the first and second polysaccharide derivatives to a target site in the body of a patient, resulting in the formation of a cross-linked hydrogel at the target site.

[0019] In another aspect, the present invention refers to the use of a first polysaccharide derivative and a second polysaccharide derivative as defined herein for the *in situ* formation of a cross-linked hydrogel in therapeutic applications in the manufacture of a medicament, for treating stress urinary incontinence, vaginal dryness, and vesico-ureteral reflux. In one example, there is disclosed the use of a first polysaccharide derivativeand a second polysaccharide derivative, as defined in the present invention for the *in situ* formation of a cross-linked hydrogel in therapeutic applications in the manufacture of a medicament, for treating stress urinary incontinence, vaginal dryness, vesico-ureteral reflux, vocal fold insufficiency, and vocal fold medialization.

[0020] In a further example, there is disclosed a combination of a first hyaluronic acid (HA) derivative and a second hyaluronic acid (HA) derivative as defined herein, preferably in the form of a first precursor solution that comprises the first hyaluronic acid (HA) derivative and a second precursor solution that comprises the second hyaluronic acid (HA) derivative.

[0021] In another aspect, the present invention refers to a multi-barrel syringe system prefilled with at least (a) a first precursor solution as defined herein in one barrel and (b) a second precursor solution as defined herein in another barrel. In a yet further example, there is disclosed a multi-barrel syringe system prefilled with at least (a) a first precursor solution, comprising a first polysaccharide derivative as defined herein, in one barrel and (b) a second precursor solution, comprising a second polysaccharide derivative as defined herein, in another barrel.

[0022] In still another example, there is disclosed a kit for the *in situ* formation of a cross-linked polysaccharide hydrogel, comprising (i) a first container comprising a first precursor solution of a first polysaccharide derivative as defined herein and (ii) a second container comprising a second precursor solution of a second polysaccharide derivative as defined herein and, optionally, (iii) instructions for use.

[0023] In yet another aspect, the present invention provides a method for the in situ formation of a cross-linked hydrogel in cosmetic applications, comprising the steps of (a) providing a first precursor solution of a first polysaccharide derivative and, separately thereof, a second precursor solution of a second polysaccharide derivative, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second precursor solutions are sterilized, wherein the first polysaccharide derivative is a hydrazide functionalized first hyaluronic acid (HA) derivative and the second polysaccharide derivative is an aldehyde functionalized second hyaluronic acid (HA) derivative, (b) mixing the first precursor solution and the second precursor solution into a in situ cross-linkable mixed solution, and (c) injecting the mixed solution to a target site in the body of a patient, wherein the nucleophilic group of the first polysaccharide derivative and the electrophilic group of the second polysaccharide derivative form a covalent linkage in situ to result in the formation of a cross-linked hydrogel at the target site. In another example, there is disclosed a method for the *in situ* formation of a cross-linked polysaccharide hydrogel in cosmetic applications, comprising the steps of

(a) providing a first precursor solution of a first polysaccharide derivative and, separately thereof, a second precursor solution of a second polysaccharide derivative, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second precursor solutions are sterilized,

(b) mixing the first precursor solution and the second precursor solution into a *in situ* cross-linkable mixed solution, and

(c) injecting the mixed solution to a target site in the body of a patient, wherein the nucleophilic group of the first polysaccharide derivative and the electrophilic group of the second polysaccharide derivative form a covalent linkage *in situ* to result in the formation of a cross-linked hydrogel at the target site.

[0023a] In still another example, there is disclosed a method for the *in situ* formation of a cross-linked hydrogel in therapeutic applications, comprising the steps of:

a) providing a first precursor solution of a first polysaccharide derivative and, separately thereof, a second precursor solution of a second polysaccharide

derivative, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second precursor solutions are sterilized,

b) mixing the first precursor solution and the second precursor solution

into a *in situ* cross-linkable mixed solution,

wherein the mixed solution is to be administered to a patient, wherein the nucleophilic group of the first polysaccharide derivative and the electrophilic group of the second polysaccharide derivative form a covalent linkage *in situ* to result in the formation of a cross-linked hydrogel.

[0024] Particular embodiments of the present invention are set forth in the appended claims.

CLAIMS

1. Use of a first polysaccharide derivative and a second polysaccharide derivative for the *in situ* formation of a cross-linked hydrogel in cosmetic applications, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second polysaccharide derivatives are sterilized, and wherein the nucleophilic group and the electrophilic group form a covalent linkage *in situ* following co-injection of the first and second polysaccharide derivatives to a target site in the body of a patient, resulting in the formation of a cross-linked hydrogel at the target site,
   wherein the first polysaccharide derivative is a hydrazide functionalized first hyaluronic acid (HA) derivative and the second polysaccharide derivative is an aldehyde functionalized second hyaluronic acid (HA) derivative.

2. The use of claim 1, wherein the *in situ* formation of a covalent linkage occurs spontaneously upon co-injection and/or wherein the *in situ* formation of a covalent linkage results in the release of no other by-product than water.

3. The use of claim 1 or 2, wherein the first polysaccharide derivative is present in the form of a first sterile precursor solution and the second polysaccharide derivative is present in the form of a second sterile precursor solution.

4. The use of claim 3, wherein the first and second sterile precursor solutions each have a complex viscosity of 0.001 Pa·s to 5.0 Pa·s, as determined by oscillatory rheological measurements at 1 Hz and 25°C, or have each an injection force of from 0.01 N to 15 N, as measured through a 30G needle at an extrusion rate of 0.21 mm/sec using a 1.0 ml glass syringe, or both.

5. The use of claim 3 or 4, wherein the first precursor solution and the second precursor solution are mixed during co-injection but prior to entering the body of the patient to form a liquid *in situ* cross-linkable composition, and wherein said liquid *in situ* cross-linkable composition preferably has a complex viscosity of 0.1 Pa·s to 100 Pa·s, as determined by oscillatory rheological measurements at 1 Hz and 25°C, or has an

injection force of 0.01 N to 20 N, as measured through a 30G needle at an extrusion rate of 0.21 mm/sec using a 1.0 ml glass syringe, or both.

6. The use of any one of claims 3 to 5, wherein the concentration of the first polysaccharide derivative present in the first precursor solution is from 0.1 wt.% to 5.0 wt.%, and the concentration of the second polysaccharide derivative present in the second precursor solution is from 0.1 wt.% to 5.0 wt.%, and/or wherein the weight ratio of the first polysaccharide derivative to the second polysaccharide derivative comprised in the liquid *in situ* cross-linkable composition that is injected to a target site in the body of a patient is from 15:85 to 85:15.

7. The use of any one of claims 1 to 6, wherein the aldehyde moiety is introduced into the polysaccharide without breaking the cyclic saccharide rings of the polysaccharide backbone.

8. The use of any one of claims 1 to 7, wherein the first and second polysaccharide derivatives are independently selected from the group consisting of carboxylated cellulose and carboxylated cellulose derivatives, carboxymethyldextran, carboxymethylstarch, hyaluronic acid, alginate, heparosan, heparin, heparin sulfate, pectin, chondroitin sulfate, dermatan sulfate, cellulose, chitosan or chitin.

9. The use of any one of claims 1 to 8, wherein the first HA derivative is functionalized with a hydrazide moiety at a carboxyl group of a saccharide unit of HA, or the second HA derivative is preferably functionalized with an aldehyde moiety made by conversion of a $-CH_2OH$ group into a -CHO group, or both.

10. The use of any one of claims 1 to 9, wherein the *in situ* cross-linkable composition is used in the treatment of wrinkles and lines of the skin, glabellar lines, nasolabial folds, chin folds, marionette lines, jawlines, buccal commissures, perioral wrinkles, crow's feet, cutaneous depressions, scars, temples, subdermal support of the brows, malar and buccal fat pads, tear troughs, nose, lips, cheeks, chin, perioral region, infraorbital region, and facial asymmetries.

11. Use of a first polysaccharide derivative and a second polysaccharide derivative as defined in any one of claims 1 to 9 for the *in situ* formation of a cross-linked hydrogel in therapeutic applications in the manufacture of a medicament, for treating stress urinary incontinence, vaginal dryness, and vesico-ureteral reflux.

12. A multi-barrel syringe system prefilled with at least (a) a first precursor solution as defined in any one of claims 3 to 9 in one barrel and (b) a second precursor solution as defined in any one of claims 3 to 9 in another barrel.

13. A method for the *in situ* formation of a cross-linked hydrogel in cosmetic applications, comprising the steps of:

(a) providing a first precursor solution of a first polysaccharide derivative and, separately thereof, a second precursor solution of a second polysaccharide derivative, wherein the first polysaccharide derivative is functionalized with a nucleophilic group and the second polysaccharide derivative is functionalized with an electrophilic group, and both the first and second precursor solutions are sterilized,

wherein the first polysaccharide derivative is a hydrazide functionalized first hyaluronic acid (HA) derivative and the second polysaccharide derivative is an aldehyde functionalized second hyaluronic acid (HA) derivative,

(b) mixing the first precursor solution and the second precursor solution into a *in situ* cross-linkable mixed solution, and

(c) injecting the mixed solution to a target site in the body of a patient, wherein the nucleophilic group of the first polysaccharide derivative and the electrophilic group of the second polysaccharide derivative form a covalent linkage *in situ* to result in the formation of a cross-linked hydrogel at the target site.