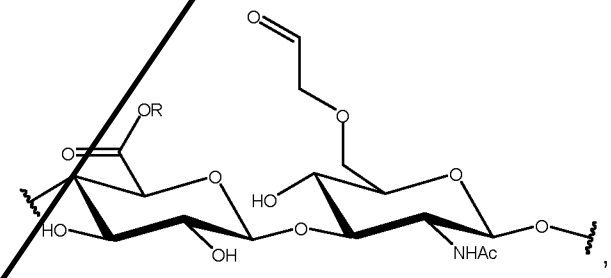
CLAIMS

1. A modified hyaluronic acid derivative, wherein the -CH_2-OH group of at least one N-acetyl-D-glucosamine unit is modified into an aldehyde group having the structure -CH_2-O-CH_2-CHO.

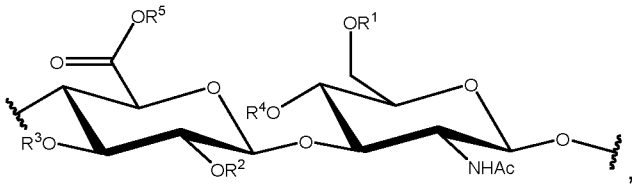
2. The modified hyaluronic acid derivative of claim 1, wherein the degree of modification is 1.0% to 20.0%, preferably 1.0% to 15.0%, more preferably 1.0% to 10.0%, more preferably 1.5% to 10.0%, more preferably 1.5% to 8.0%, more preferably 1.8% to 7.0%, more preferably 2.0% to 6.9%, the degree of modification being defined as the number of -CH_2-O-CH_2-CHO groups divided by the total number of N-acetyl-D-glucosamine units present in the modified hyaluronic acid derivative, and/or wherein the modified hyaluronic acid derivative has a weight average molecular weight of 0.1 to 2.5 MDa, preferably 0.2 to 1.5 MDa, more preferably 0.4 to 1.3 MDa, even more preferably 0.6 to 1.1 MDa.

3. The modified hyaluronic acid derivative according to claim 1 or 2, comprising at least one disaccharide unit of the following structure



wherein R is selected from hydrogen, an alkali metal ion, preferably Na, and an alkaline earth metal ion, and

optionally further comprising at least one disaccharide unit of the following structure



wherein R^1, R^2, R^3 and R^4 are independently selected from H and $-CH_2-CHO$ and R^5 is selected from hydrogen, an alkali metal ion, preferably Na, an alkaline earth metal ion, and $-CH_2-CHO$, provided that at least one of R^1, R^2, R^3, R^4 and R^5 is $-CH_2-CHO$ and, if R^1 is $-CH_2-CHO$, at least one of R^2, R^3, R^4 and R^5 is $-CH_2-CHO$.

4. A method for preparing the modified hyaluronic acid derivative of any one of claims 1 to 3, comprising the following steps:

a) providing a glycerol-modified hyaluronic acid, which is characterized in that the $-CH_2-OH$ group of at least one N-acetyl-D-glucosamine unit is modified into a moiety of the following formula $-CH_2-O-CH_2-CHOH-CH_2OH$;

b) dissolving the glycerol-modified hyaluronic acid in an aqueous medium to obtain solubilized glycerol-modified hyaluronic acid;

c) reacting said solubilized glycerol-modified hyaluronic acid with an oxidizing agent, preferably a periodate, more preferably sodium periodate, to convert at least a part of said $-CH_2-O-CH_2-CHOH-CH_2OH$ groups into aldehyde groups having the formula $-CH_2-O-CH_2-CHO$, thereby obtaining an aldehyde-modified hyaluronic acid derivative.

5. The method of claim 4, further comprising one or more of the following steps:

d) optionally stopping the reaction of step c), preferably by adding a vicinal diol (1,2-diol), more preferably ethylene glycol;

e) optionally purifying the modified hyaluronic acid derivative, preferably by precipitating the modified hyaluronic acid derivative in an organic solvent, preferably ethanol, isopropanol or a mixture thereof, re-dissolving the precipitate in saline and re-precipitating the modified hyaluronic acid derivative in said organic solvent;

f) optionally drying the modified hyaluronic acid derivative obtained in step e).

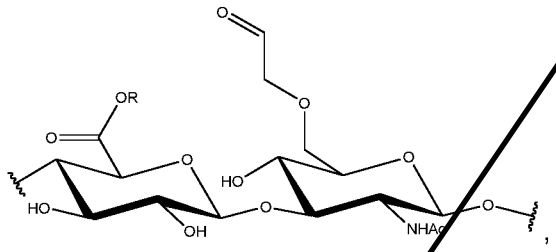
6. The method of claim 4 or 5, wherein the glycerol-modified hyaluronic acid has a weight average molecular weight of 0.1 to 5.0 MDa, preferably 1.0 to 3.0, more preferably 1.0 to 2.0 MDa, even more preferably 1.1 to 1.9, even more preferably 1.2 to 1.8 MDa, even more preferably 1.3 to 1.7 MDa, even more preferably 1.4 to 1.6 MDa and/or a degree of modification of 5 to 25%, preferably 10 to 20%, the degree of modification being defined as the number of $-CH_2-O-CH_2-CHOH-CH_2OH$ groups divided by the total number of N-acetyl-D-glucosamine units present in the glycerol-modified hyaluronic acid
and/or wherein
(i) step c) is performed at a temperature of 4 to 35°C, preferably 15 to 35°C, more preferably 20 to 30°C, even more preferably 20 to 25°C, even more preferably about 21 to 23°C, even more preferably about 22°C; and/or
(ii) step c) is performed for a duration of 5 to 120 minutes, preferably 5 to 65 minutes, more preferably 10 to 60 minutes, even more preferably 10 to 50 minutes, even more preferably 10 to 40 minutes, even more preferably 10 to 30 minutes, even more preferably 10 to 20 minutes; and/or
(iii) the oxidizing agent is present in an amount of 0.01 to 0.5 molar equivalents, preferably 0.01 to 0.3 molar equivalents, more preferably 0.04 to 0.3 molar equivalents, even more preferably 0.04 to 0.1, based on the molar amount of disaccharide repeating units of the glycerol-modified hyaluronic acid; and/or
(iv) said glycerol-modified hyaluronic acid is present in an amount of 2 to 50 g/L, preferably 2 to 40 g/L, more preferably 3 to 38 g/L, more preferably 4 to 36 g/L.

7. A modified hyaluronic acid derivative obtained by the method of any one of claims 4 to 6, wherein the modified hyaluronic acid derivative preferably
   i) has a degree of modification of 1.0% to 20.0%, preferably 1.0% to 15.0%, more preferably 1.0% to 10.0%, more preferably 1.5% to 10.0%, more preferably 1.5% to 8.0%, more preferably 1.8% to 7.0%, more preferably 2.0% to 6.9%, the degree of modification being defined as the number of $-CH_2-O-$

CH_2-CHO groups divided by the total number of N-acetyl-D-glucosamine units present in the modified hyaluronic acid derivative, and/or

ii) has a weight average molecular weight of 0.1 to 2.5 MDa, preferably 0.2 to 1.5 MDa, more preferably 0.4 to 1.3 MDa, even more preferably 0.6 to 1.1 MDa, and/or

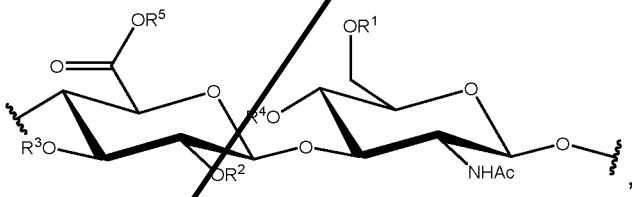
iii) comprises at least one disaccharide unit of the following structure



,

wherein R is selected from H, an alkali metal ion, preferably Na, and an alkaline earth metal ion, and

optionally further comprises at least one disaccharide unit of the following structure



,

wherein R^1, R^2, R^3 and R^4 are independently selected from H and -CH_2-CHO and R^5 is selected from hydrogen, an alkali metal ion, preferably Na, an alkaline earth metal salt, and -CH_2-CHO, provided that at least one of R^1, R^2, R^3, R^4 and R^5 is -CH_2-CHO and, if R^1 is -CH_2-CHO, at least one of R^2, R^3, R^4 and R^5 is -CH_2-CHO.

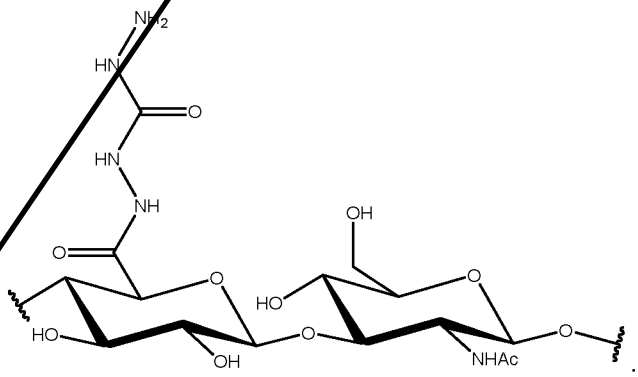
8. Use of the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 for the *in situ* formation of a crosslinked hydrogel in aesthetic applications, preferably for treating wrinkles and lines of the skin, including glabellar lines, nasolabial folds, chin folds, marionette lines, jawlines, buccal commissures, perioral wrinkles and 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.

9. Use of the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 for the formation of a pre-formed crosslinked hydrogel.

10. A modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 for use in the *in situ* formation of a crosslinked hydrogel for therapeutic applications, preferably for treating stress urinary incontinence, vaginal dryness, vesico-ureteral reflux, vocal fold insufficiency, and vocal fold medialization.

11. The use of any one of claims 8-9 or the modified hyaluronic acid derivative for use according to claim 10, wherein the modified hyaluronic acid derivative is used together with a second polysaccharide derivative, which comprises one or more nucleophilic functional groups capable of forming a covalent bond with one or more aldehyde groups of the modified hyaluronic acid derivative, wherein the second polysaccharide is preferably a hyaluronic acid derivative and said nucleophilic functional group is preferably a hydrazide functional group, and wherein the second polysaccharide is more preferably a hyaluronic acid derivative comprising at least one disaccharide unit having the following structure:

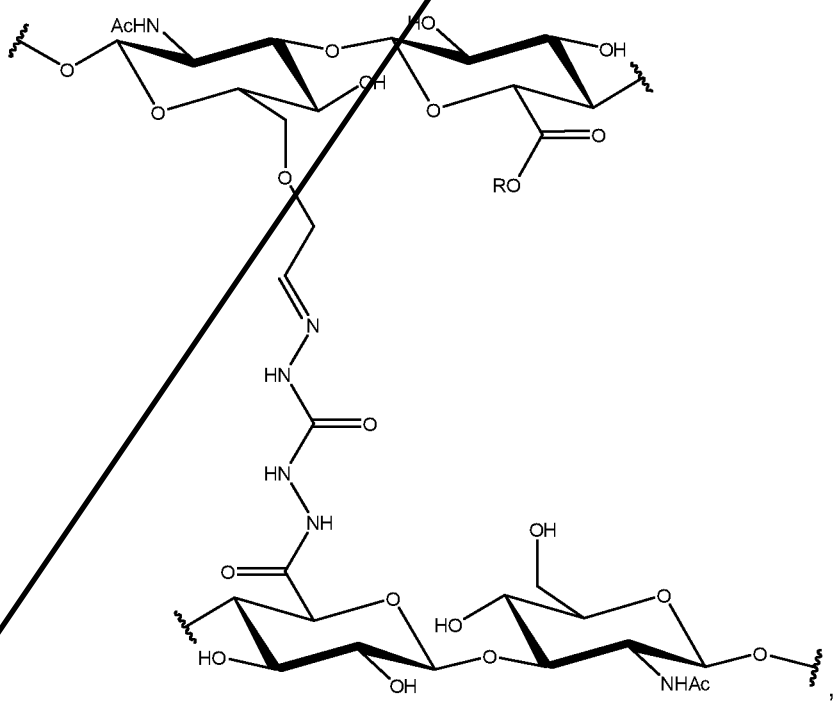


12. The use of claim 11 or the modified hyaluronic acid derivative for use according to claim 11, wherein

(i) the aldehyde group of the modified HA derivative and the nucleophilic functional group of the second polysaccharide derivative spontaneously form a covalent bond upon co-injecting the modified hyaluronic acid derivative and the second polysaccharide derivative to a target site in the body of a subject, thereby forming a crosslinked hydrogel at the target site, or

(ii) the aldehyde group of the modified HA derivative and the nucleophilic functional group of the second polysaccharide derivative spontaneously form a covalent bond upon contacting the modified hyaluronic acid derivative and the second polysaccharide derivative, wherein preferably the modified HA derivative and the second polysaccharide derivative are present in a buffer medium, preferably a physiological buffer medium.

13. A crosslinked hydrogel, comprising the following structural unit:

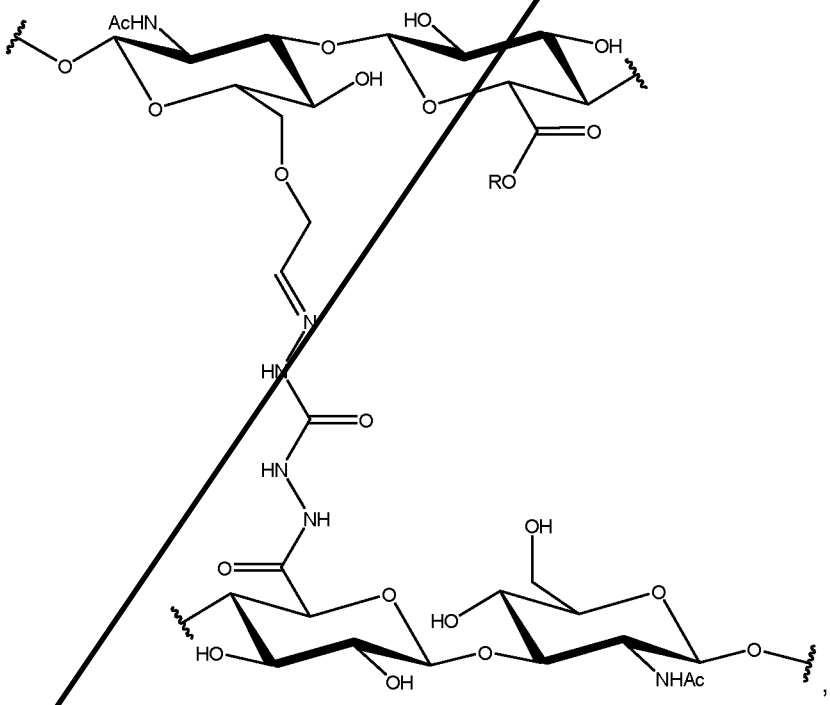


wherein "Ac" denotes -C(O)CH_3 and R is selected from hydrogen, an alkali metal ion, preferably Na, and an alkaline earth metal ion.

14. The crosslinked hydrogel according to claim 13, wherein the crosslinked hydrogel is a pre-formed crosslinked hydrogel.

15. The crosslinked hydrogel according to claim 14, wherein the crosslinked hydrogel further comprises an uncrosslinked polysaccharide, preferably hyaluronic acid, and/or wherein the crosslinked hydrogel further comprises an anesthetic agent, preferably lidocaine.

16. A crosslinked hydrogel obtained by contacting the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 and a second polysaccharide derivative as defined in claim 11, wherein the crosslinked hydrogel preferably comprises the following structural unit:



wherein "Ac" denotes $-C(O)CH_3$ and R is selected from hydrogen, an alkali metal ion, preferably Na, and an alkaline earth metal ion.

17. The crosslinked hydrogel according to claim 16, wherein said contacting is performed *in situ*.

18. The crosslinked hydrogel according to claim 16, wherein said contacting is performed *in vitro*, wherein said contacting is preferably performed in a buffer, preferably a physiological buffer.

19. A method, preferably an aesthetic method, of preparing a crosslinked hydrogel, the method comprising the following steps:
    a) providing a first precursor solution comprising the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 and, separately thereof, a second precursor solution comprising the second polysaccharide derivative as defined in claim 11;
    b) mixing the first precursor solution and the second precursor solution into an *in situ* crosslinkable mixture; and
    c) injecting the *in situ* crosslinkable mixture to a target site in the body of a patient to form a crosslinked gel at the target site.

20. The method of claim 19, wherein in step a) the first precursor solution and the second precursor solution are present in different barrels of a multi-barrel syringe and mixing of step b) occurs during extrusion from said multi-barrel syringe.

21. A method of preparing a crosslinked hydrogel, the method comprising the following steps:
    a) providing a buffer, preferably a physiological buffer;
    b) adding the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 and adding the second polysaccharide derivative as defined in claim 11 to the buffer,
    and optionally adding uncrosslinked polysaccharide, preferably hyaluronic acid, to the buffer;
    c) crosslinking the modified hyaluronic acid derivative and the second polysaccharide derivative in the buffer to thereby prepare a crosslinked hydrogel;

d) optionally adding uncrosslinked polysaccharide to the crosslinked hydrogel obtained in step c);

e) optionally sieving and degassing the hydrogel obtained in step c) or d);

f) optionally filling the hydrogel obtained in step c), d), or e) in a container, preferably a syringe, more preferably a ready-to-use syringe;

g) optionally sterilizing the container comprising the gel obtained in step f).

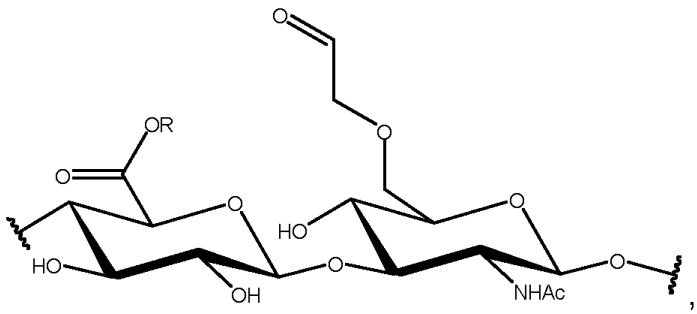
22. A kit for the *in situ* formation of a crosslinked hydrogel, comprising (i) a first container containing a first precursor solution comprising the modified hyaluronic acid derivative of any one of claims 1 to 3 and 7 and (ii) a second container containing a second precursor solution comprising the second polysaccharide derivative as defined in claim 11, and optionally, (iii) instructions for use.

CLAIMS

1. A modified hyaluronic acid derivative, in which the -CH_2-OH group of at least one N-acetyl-D-glucosamine unit is modified into an aldehyde group having the structure - CH_2-O-CH_2-CHO, in which the degree of modification is 1.0% to 20.0%, the degree of modification being defined as the number of -CH_2-O-CH_2-CHO groups divided by the total number of N-acetyl-D-glucosamine units present in the modified hyaluronic acid derivative.

2. A modified hyaluronic acid derivative according to Claim 1, in which the degree of modification is 1.0% to 15.0%, preferably 1.0% to 10.0%, more preferably 1.5% to 10.0%, more preferably 1.5% to 8.0%, more preferably 1.8% to 7.0%, more preferably 2.0% to 6.9%, and/or in which the modified hyaluronic acid derivative has a weight average molecular weight of 0.1 to 2.5 MDa, preferably 0.2 to 1.5 MDa, more preferably 0.4 to 1.3 MDa, even more preferably 0.6 to 1.1 MDa.

3. A modified hyaluronic acid derivative according to Claim 1 or 2, comprising at least one disaccharide unit of the following structure



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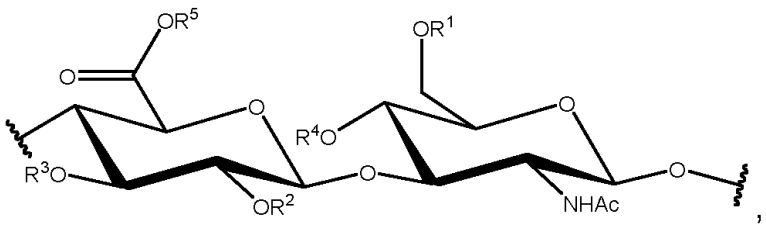
in which R is selected from hydrogen, an alkali metal ion, preferably Na, and an alkaline earth metal ion, and

optionally further comprising at least one disaccharide unit of the following structure



in which R^1, R^2, R^3 and R^4 are independently selected from H and -CH_2-CHO and R^5 is selected from hydrogen, an alkali metal ion, preferably Na, an alkaline earth metal ion, and -CH_2-CHO, provided that at least one of R^1, R^2, R^3, R^4 and R^5 is -CH_2-CHO and, if R^1 is -CH_2-CHO, at least one of R^2, R^3, R^4 and R^5 is -CH_2-CHO.

4. A method for preparing the modified hyaluronic acid derivative according to Claims 1, 2 or 3, comprising the following steps:

(a) providing a glycerol-modified hyaluronic acid, which is characterized in that the -CH_2-OH group of at least one N-acetyl-D-glucosamine unit is modified into a moiety of the following formula -CH_2-O-CH_2-CHOH-CH_2OH;

(b) dissolving the glycerol-modified hyaluronic acid in an aqueous medium to obtain solubilized glycerol-modified hyaluronic acid;

(c) reacting the solubilized glycerol-modified hyaluronic acid with an oxidizing agent, preferably a periodate, more preferably sodium periodate, to convert at least a part of the -CH_2-O-CH_2-CHOH-CH_2OH groups into aldehyde groups having the formula -CH_2-O-CH_2-CHO, thereby obtaining an aldehyde-modified hyaluronic acid derivative, wherein step (c) is performed for a duration of 5 to 65 minutes.

5. A method according to Claim 4, further comprising one or more of the following steps:

(d) optionally stopping the reaction of step (c), preferably by adding a vicinal diol (1,2-diol), more preferably ethylene glycol;

(e) optionally purifying the modified hyaluronic acid derivative, preferably by precipitating the modified hyaluronic acid derivative in an organic solvent, preferably ethanol, isopropanol or a mixture thereof, re-dissolving the precipitate in saline and re-precipitating the modified hyaluronic acid derivative in the organic solvent;

(f) optionally drying the modified hyaluronic acid derivative obtained in step (e).

6. A method according to Claim 4 or 5, in which the glycerol-modified hyaluronic acid has a weight average molecular weight of 0.1 to 5.0 MDa, preferably 1.0 to 3.0, more preferably 1.0 to 2.0 MDa, even more preferably 1.1 to 1.9, even more preferably 1.2 to 1.8 MDa, even more preferably 1.3 to 1.7 MDa, even more preferably 1.4 to 1.6 MDa and/or a degree of modification of 5 to 25%, preferably 10 to 20%, the degree of modification being defined as the number of -CH_2-O-CH_2-CHOH-CH_2OH groups divided by the total number of N-acetyl-D-glucosamine units present in the glycerol-modified hyaluronic acid

and/or in which

(i) step (c) is performed at a temperature of 4 to 35°C, preferably 15 to 35°C, more preferably 20 to 30°C, even more preferably 20 to 25°C, even more preferably about 21 to 23°C, even more preferably about 22°C; and/or

(ii) step (c) is performed for a duration of 10 to 60 minutes, preferably 10 to 50 minutes, more preferably 10 to 40 minutes, even more preferably 10 to 30 minutes, even more preferably 10 to 20 minutes; and/or

(iii) the oxidizing agent is present in an amount of 0.01 to 0.5 molar equivalents, preferably 0.01 to 0.3 molar equivalents, more preferably 0.04 to 0.3 molar equivalents, even more preferably 0.04 to 0.1, based on the molar amount of disaccharide repeating units of the glycerol-modified hyaluronic acid; and/or

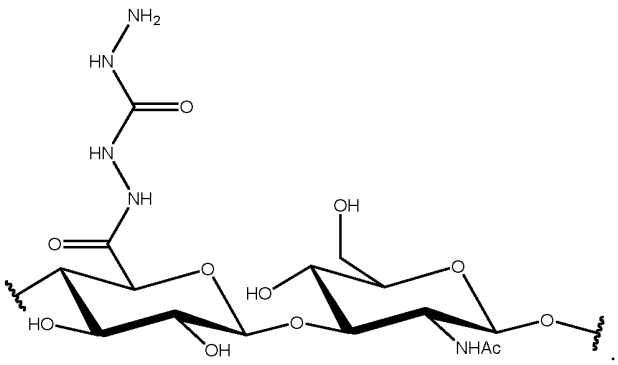
(iv) the glycerol-modified hyaluronic acid is present in an amount of 2 to 50 g/L, preferably 2 to 40 g/L, more preferably 3 to 38 g/L, more preferably 4 to 36 g/L.

7. Use of a modified hyaluronic acid derivative according to any of Claims 1 to 3 for the *in situ* formation of a crosslinked hydrogel in aesthetic applications, preferably for treating wrinkles and lines of the skin, including glabellar lines, nasolabial folds, chin folds, marionette lines, jawlines, buccal commissures, perioral wrinkles and 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.

8. Use of a modified hyaluronic acid derivative according to any of Claims 1 to 3 for the formation of a pre-formed crosslinked hydrogel.

9. A modified hyaluronic acid derivative according to any of Claims 1 to 3 for use in the *in situ* formation of a crosslinked hydrogel for therapeutic applications, preferably for treating stress urinary incontinence, vaginal dryness, vesico-ureteral reflux, vocal fold insufficiency, and vocal fold medialization.

10. Use according to Claim 7 or 8 or a modified hyaluronic acid derivative for use according to Claim 9, in which the modified hyaluronic acid derivative is used together with a second polysaccharide derivative, which comprises one or more nucleophilic functional groups capable of forming a covalent bond with one or more aldehyde groups of the modified hyaluronic acid derivative, in which the second polysaccharide is preferably a hyaluronic acid derivative and the nucleophilic functional group is preferably a hydrazide functional group, and in which the second polysaccharide is more preferably a hyaluronic acid derivative comprising at least one disaccharide unit having the following structure:



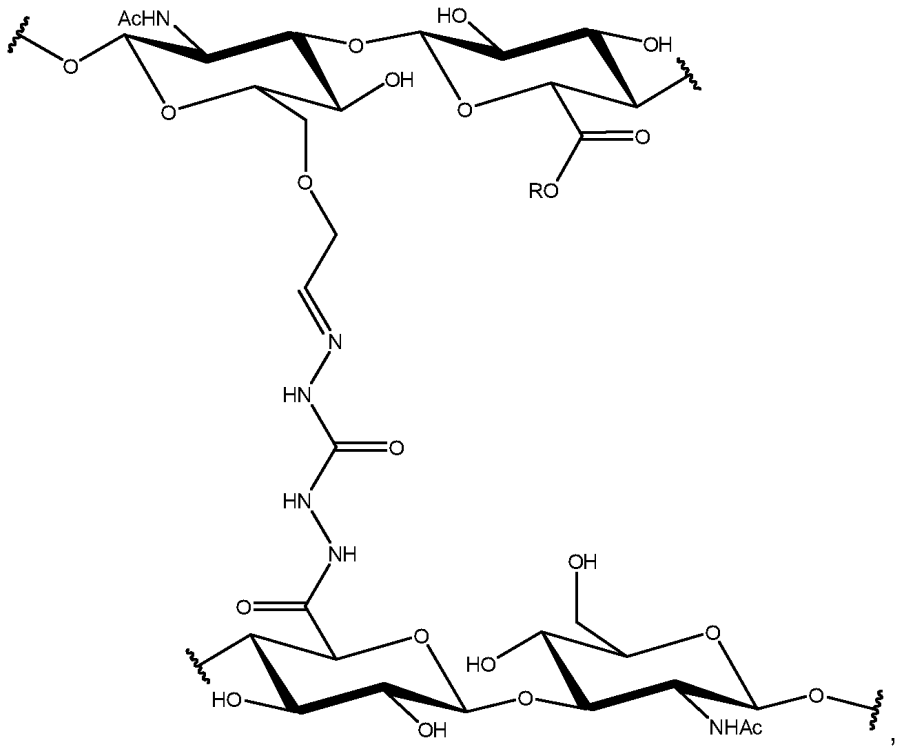
11. Use according to Claim 10 or a modified hyaluronic acid derivative for use according to Claim 10, in which

(i) the aldehyde group of the modified HA derivative and the nucleophilic functional group of the second polysaccharide derivative spontaneously form a covalent bond upon co-injecting the modified hyaluronic acid derivative and the second polysaccharide derivative to a target site in the body of a subject, thereby forming a crosslinked hydrogel at the target site, or

(ii) the aldehyde group of the modified HA derivative and the nucleophilic functional group of the second polysaccharide derivative spontaneously form a covalent bond upon contacting the modified hyaluronic acid derivative and the second polysaccharide

derivative, in which preferably the modified HA derivative and the second polysaccharide derivative are present in a buffer medium, preferably a physiological buffer medium.

12. A crosslinked hydrogel, comprising the following structural unit:



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in which "Ac" denotes -C(O)CH_3 and R is selected from hydrogen, an alkali metal ion, preferably Na, and an alkaline earth metal ion.

13. A crosslinked hydrogel according to Claim 12, in which the crosslinked hydrogel is a pre-formed crosslinked hydrogel.

14. A crosslinked hydrogel according to Claim 13, in which the crosslinked hydrogel further comprises an uncrosslinked polysaccharide, preferably hyaluronic acid, and/or in which the crosslinked hydrogel further comprises an anaesthetic agent, preferably lidocaine.

15. A method, preferably an aesthetic method, of preparing a crosslinked hydrogel, the method comprising the following steps:

(a) providing a first precursor solution comprising the modified hyaluronic acid derivative according to any of Claims 1 to 3 and 7 and, separately thereof, a second precursor solution comprising the second polysaccharide derivative as defined in Claim 10;

(b) mixing the first precursor solution and the second precursor solution into an *in situ* crosslinkable mixture; and

(c) injecting the *in situ* crosslinkable mixture to a target site in the body of a patient to form a crosslinked gel at the target site.

16. A method according to Claim 15, in which in step (a) the first precursor solution and the second precursor solution are present in different barrels of a multi-barrel syringe and mixing of step (b) occurs during extrusion from the multi-barrel syringe.

17. A method of preparing a crosslinked hydrogel, the method comprising the following steps:

(a) providing a buffer, preferably a physiological buffer;

(b) adding a modified hyaluronic acid derivative according to any of Claims 1 to 3 and adding a second polysaccharide derivative as defined in Claim 10 to the buffer, and optionally adding uncrosslinked polysaccharide, preferably hyaluronic acid, to the buffer;

(c) crosslinking the modified hyaluronic acid derivative and the second polysaccharide derivative in the buffer to thereby prepare a crosslinked hydrogel;

(d) optionally adding uncrosslinked polysaccharide to the crosslinked hydrogel obtained in step (c);

(e) optionally sieving and degassing the hydrogel obtained in step (c) or (d);

(f) optionally filling the hydrogel obtained in step (c), (d), or (e) in a container, preferably a syringe, more preferably a ready-to-use syringe;

(g) optionally sterilizing the container comprising the gel obtained in step (f).

18. A kit for the *in situ* formation of a crosslinked hydrogel, comprising (i) a first container containing a first precursor solution comprising a modified hyaluronic acid derivative

according to any of Claims 1 to 3 and (ii) a second container containing a second precursor solution comprising the second polysaccharide derivative as defined in Claim 10, and optionally, (iii) instructions for use.