

WHAT IS CLAIMED:

1. A method of preparing a hydrogel comprising crosslinked glycosaminoglycan (GAG) molecules, comprising crosslinking a GAG having a molecular weight of at least 1.5 MDa with a crosslinker, wherein the concentration of GAG is between 2% to 10% (w/w) and the molar ratio of the crosslinker to GAG is less than or equal to 2%, to obtain a glycosaminoglycan hydrogel crosslinked by amide bonds.
2. The method of preparing a hydrogel according to claim 1, wherein
if the GAG concentration is between 1% to 4.5% (w/w), then the concentration of crosslinker is between 0.8 to 2 mol% per GAG disaccharide; and
if the GAG concentration is between 4.6% to 5.9% (w/w), then the concentration of crosslinker is between 0.5 to 0.8 mol% per GAG disaccharide; and
if the GAG concentration is between 6% to 12% (w/w), then the concentration of crosslinker is between 0.3 to 0.5 mol% per GAG disaccharide.
3. The method according to claim 1 or 2, wherein the crosslinker is a di- or multinucleophile functional crosslinker.
4. The method according to claim 3, wherein the di- or multinucleophile functional crosslinker is an aliphatic or aromatic diamino derivative, a peptide, or a peptide sequence.
5. The method according to claim 4, wherein the di- or multinucleophile functional crosslinker comprises a spacer group selected from the group consisting of di-, tri-, tetra-, and oligosaccharides.
6. The method according to claim 5, wherein the di- or multinucleophile functional crosslinker is diaminotrehalose (DATH).
7. The method according to any one of claims 3-6, wherein the crosslinking comprises:
(1) providing a solution of glycosaminoglycan (GAG) molecules;

(2) activating carboxyl groups on the glycosaminoglycan molecules with a coupling agent to form activated glycosaminoglycan molecules;

(3) crosslinking the activated glycosaminoglycan (GAG) molecules via their activated carboxyl groups using a di- or multinucleophile functional crosslinker to obtain a glycosaminoglycan hydrogel crosslinked by amide bonds.

8. The method according to claim 7, wherein the coupling agent is a triazine-based coupling agent, such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM).

9. The method according to any one of claims 1-8, wherein the crosslinking is performed at a pH of 5.0 – 9.0, preferably at a pH of 6.0 – 8.0.

10. The method according to any one of claims 1-9, further comprising formulating the crosslinked hydrogel to a final glycosaminoglycan (GAG) concentration of 10 - 45 mg/mL.

11. The method according to any one of claims 1-10, further comprising sterilizing the crosslinked hydrogel.

12. The method according to any one of claims 1-11, wherein the glycosaminoglycan (GAG) is hyaluronic acid (HA).

13. The method according to any one of claims 1-12, wherein the GAG has a molecular weight of 1.5 -10 MDa, preferably 1.5 – 3.5 MDa.

14. The method according to any one of claims 1-13, wherein the concentration of GAG is between 3-5 % (w/w).

15. The method according to any one of claims 1-14, wherein the molar ratio of crosslinker to GAG is between 0.9-1.1%.

16. The method according to any one of claims 1-15, wherein the GAG is hyaluronic acid (HA) having a molecular weight of 1.5 – 3.5 MDa and the crosslinker is diaminotrehalose

(DATH), and further wherein the concentration of HA is between 3-5 % (w/w) and the molar ratio of DATH to HA is between 0.9-1.1%.

17. The hydrogel product obtained by the method according to any one of claims 1-16.
18. A hydrogel product comprising glycosaminoglycan (GAG) molecules as the swellable polymer, wherein the glycosaminoglycan molecules are crosslinked by amide bonds and wherein the apparent molecular weight (M_{wapp}) of the crosslinked glycosaminoglycan molecules is above 1.0 MDa; and wherein the thermostability of the swellable polymer (NormGelC) is above 80% after 24 hours.
19. The hydrogel product according to claim 18, wherein the glycosaminoglycan molecules are covalently crosslinked via crosslinks comprising a spacer group selected from the group consisting of di-, tri-, tetra-, and oligosaccharides.
20. The hydrogel product according to claim 19, wherein the spacer group is trehalose.
21. The hydrogel product according to any one of claims 18-20, wherein the glycosaminoglycan (GAG) is hyaluronic acid (HA).
22. The hydrogel product according to any one of claims 18-21, wherein the hydrogel product is sterilized.
23. A method of cosmetically treating skin, which comprises administering to the skin a hydrogel product according to any one of claims 18-22.