Claims

1. A composition for a soft polysaccharide hydrogel capable of conversion to a hard polysaccharide hydrogel, the soft polysaccharide hydrogel comprising:
one or more water soluble high acyl gellan gum polymers;
one or more water soluble low acyl gellan gum polymers; and
one or more water soluble chemically modified gellan gum polymers or one or more peptide modified gellan gum polymers, wherein the soft polysaccharide hydrogel exhibits a homogeneous matrix structure and the hard polysaccharide hydrogel exhibits an aggregated matrix network structure.

2. The composition according to claim 1, wherein the soft polysaccharide hydrogel is suitable for injection uses.

3. The composition according to claim 2, wherein the soft polysaccharide hydrogel exhibits shear thinning and self-healing rheological properties, by allowing the soft polysaccharide hydrogel to be converted into a liquid state by a shearing force, or to recover its hydrogel state once the shearing force is ceased.

4. The composition according to claim 3, wherein the shearing force is exerted by pipetting, syringe injection, or pump perfusion or a combination thereof.

5. The composition according to claim 1, wherein the hard polysaccharide hydrogel exhibits 3-D gel structures with rheological properties such that when the hard gel is broken by pipetting or shearing, the hard gel breaks into smaller gel particles, and has an affinity for one or more bioactive molecules.

6. The composition according to claim 1, wherein the hard polysaccharide hydrogel has a storage modulus value of greater than about 10 Pa.

7. The composition according to claim 1, wherein the hard polysaccharide hydrogel maintains its gel formation at a temperature equal to or below about 80° C., but is capable of being broken into smaller gel particles when disturbed with an external force.

8. The composition according to claim 1, wherein the one or more chemically modified gellan gum polymers are selected from the group consisting of:
a) organic molecules selected from the group consisting of: polymers of natural or synthetic origin, chemically modified or co-polymers, hyaluronate, chitosan, collagen, polyethyleneglycol anticoagulants, contrasting agents, chemotherapeutic agents, and signaling pathway molecules; and
b) inorganic molecules selected from the group consisting of: bioactive glass, hydroxyapatite, calcium phosphate and iron.

9. The composition according to claim 5, wherein the one or more bioactive molecules are added into the gellan gum solution before heating are selected from the group consisting of: cells, peptides, proteins, lipids, polysaccharides, growth factors, growth hormone, antibodies, enzymes, cell receptors, cell ligands, antibiotics, anti-microbial, anti-fungi, antimycotics, and functional peptide molecules with an NH_2, a COOH and an $CONH_2$ group comprising: a tripeptide Arg-Gly-Asp (RGD) motif, a Ile-Lys-Val-Ala-Val (IKVAV) peptide, an Arg-Glu-Asp-Val (REDV) peptide, a laminin sequence Tyr-Ile-Gly-Ser-Arg (YIGSR), and poly-Lysine.

10. The composition according to claim 1, wherein the soft polysaccharide hydrogel is converted into the hard polysaccharide hydrogel by submerging the soft polysaccharide hydrogel in an aqueous solution of extra phosphate buffer, cell culture media, or ionic solutions, or a combination thereof.

11. The composition according to claim 1, wherein one or more bioactive molecules are in contact with, adhered to, or embedded in the soft and hard polysaccharide hydrogels while maintaining their bioactivities.

12. The composition according to claim 1, wherein each of the one or more bioactive molecules are suspended in or entrapped in the soft and hard polysaccharide hydrogels while maintaining their bioactivities.

13. The composition according to claim 1, wherein a solution of gellan gum modified by peptides is formed by adding peptide into the gellan gum solution as a mixture and then heating at a temperature of about 100° C. or above and a pressure of about 1 to about 40 psi, and for a time period of about 3 to about 30 min.

14. The composition according to claim 1, wherein the composition comprises from about 0.001% to about 20% of the one or more high acyl gellan gum polymers, about 0.001% to about 20% of the one or more low acyl gellan gum polymers, about 0.001% to about 20% of the one or more modified gellan gum polymers, and further comprises from about 0.00001% to about 30% of the one or more bioactive molecules.

15. The composition of according to claim 14, wherein the composition has a storage modulus value of about 10 Pa.

16. The composition according to claim 1, wherein the composition comprises from about 0.01% to about 10% of the one or more high acyl gellan gum polymers, about 0.01% to about 10% of the one or more low acyl gellan gum polymers, about 0.01% to about 10% of the one or more modified gellan gum polymers, and further comprises from about 0.001% to about 20% of the one or more bioactive molecules.

17. The composition of according to claim 16, wherein the composition has a storage modulus value of about 10 Pa to about 20000 Pa.

18. A method for forming a polysaccharide hydrogel according to claim 1, the method comprising the steps of:
dissolving water-soluble gellan gum polymers in a water based solvent with a solid content higher than 0.001% w/v at a temperature ranging from about 4° C. to about 99° C.;
heating the solution to a temperature of about 100° C. or higher and at a pressure of about 1 psi or higher for 3 minutes or longer; and reticulation at a temperature ranging from about 4° C. to about 60° C. by directly mixing the solution with phosphate buffer (PBS), cell culture media or ionic solutions to trigger the polysaccharide hydrogel formation, wherein a storage modulus (G′) of the polysaccharide hydrogel increases upon mixing and surpasses about 10 Pa within 30 minutes such that the system sustains bioactive molecules suspended within its hydrogel matrix for 3D growth; and
adding chemicals or bioactive molecules such that the chemicals or bioactive molecules are in contact with, adhered to, suspended, embedded or entrapped in the polysaccharide hydrogel formed.

19. The method according to claim 18, wherein the water based solvent comprises water, phosphate buffer solution (PBS), saline solution, and cell culture medium.

20. The method according to claim 18, the solid content is used in amounts ranging from about 0.001% (w/v) to 10% (w/v).

21. The method according to claim 18, wherein a soft hydrogel is formed when the solution and a trigger solution of an ionic concentration of about 0.01% (w/v) or higher are mixed with a ratio range from about 100:1 to about 1:1.

22. The method according to claim 18, wherein a hard hydrogel is formed when the solution and a trigger solution of a high ionic concentration of about 0.01% (w/v) are mixed with a ratio range from about 1:1 to about 1:100.

23. The method according to claim 18, wherein, a hard hydrogel is formed when the solution and a trigger solution of a normal ionic concentration of about 0.01% (w/v) are mixed with a ratio range from about 1:1 to about 1:20.

24. The method according to claim 18, wherein the solution is heated to a temperature ranging from about 100° C. to about 132° C. and preferably from about 100° C. to about 121° C. before reticulation.

25. The method according to claim 18, wherein the solution is heated under a pressure ranging from about 1 psi to about 25 psi before reticulation.

26. The method according to claim 18, wherein the solution is heated under a pressure ranging from about 1 psi to about 15 psi before reticulation.

27. The method according to claim 18, wherein the solution is heated for from about 1 minute to about 30 minutes before reticulation.

28. The method according to claim 18, wherein the solution is heated for from about 5 minutes to about 20 minutes before reticulation.

29. The composition according to claim 10, wherein the soft polysaccharide hydrogel formed is converted into the hard polysaccharide hydrogel by submersion in an aqueous solution of cell culture media or ionic solution.

30. The hard polysaccharide hydrogel formed and interconverted by the method according to claim 10, wherein one or more bioactive molecules can be added to the hard polysaccharide hydrogel before or after the hard polysaccharide hydrogel formation, or before or after the exchange of hydrogel and the surrounding media, by being mixed with the gellan gum solution or trigger solutions.

31. A method of of a polysaccharide hydrogel, which is derived from a composition according to claim 1, as a versatile platform for drug discovery and biomedical applications, comprising cell viability assay, live/dead assay, high-throughput screening, fluorescent staining and imaging, histological analysis, and 3D bio-printing.

32. The method according to claim 31, wherein living cells are grown on the top of, or embedded, encapsulated in the hydrogel and are harvested out of hydrogel system by breaking the hydrogel and dissolving the hydrogel with DI water or low ionic concentration solution.

33. The method according to claim 31, wherein a two-step procedure is provided for in vitro 3D cell culture in the hydrogel, comprising:
1) living cells being mixed with gellan gum solution or trigger solution such as cell culture media before the hydrogel formation, wherein the cells are homogeneously suspended in the soft hydrogel and ready to transfer to an individual cell culture plate or different container by pipetting or injection; and
2) once the soft gel is placed in the final container, where the extra trigger solutions are added on the top or surround the soft gel and convert it into the hard hydrogel, wherein the 3D matrix structure is further stabilized and nutrition or other biomolecules are added into the hydrogel system and exchange between the hydrogel and surrounding media.

34. The method according to claim 31 for 2D hydrogel coating cell culture study, wherein the soft hydrogel is added directly to the surface of the culture plate to coat the cell culture plate, and then living cells can be added on the top of hydrogel and grow in 2D and some cells can penetrate into hydrogel from the top and grow as 3D structure.

35. The method of claim 18 further comprising the step of: adding sodium citrate to the water-soluble gellan gum polymers forming a gellan gum polymer solution; and adjusting the pH of the gellan gum polymer solution to a neutral pH.

36. The method of claim 21 wherein the soft hydrogel comprises a fiber structure suitable for injection.

37. The method of claim 22 wherein the hard hydrogel comprises an agglomeration structure.

38. The composition according to claim 12, wherein each of the one or more biomolecules are attached to, suspended in or entrapped in the aggregated matrix network structure of the hard polysaccharide hydrogel.

39. The composition according to claim 12, wherein each of the one or more biomolecules are attached to, suspended in or entrapped in the homogeneous matrix structure of the soft polysaccharide hydrogel.

40. The composition according to claim 1, wherein cells are added to a surface or suspended in or entrapped in the soft or hard polysaccharide hydrogel.