

Claims

1. A microstructure suitable for encapsulating biologically active materials, wherein the microstructure comprises a negatively charged polymer.
- 5 2. A macrostructure suitable for encapsulating biologically active materials, wherein the macrostructure comprises a negatively charged polymer.
3. The structure of claim 1 or 2, wherein the negatively charged polymer is capable of emulating glycosaminoglycans.
- 10 4. The structure of any one of the preceding claims, wherein the structure further comprises a biocompatible, biodegradable polymer and at least one structural component capable of emulating extracellular matrix (ECM).
- 15 5. The structure of claim 4, wherein the biocompatible, biodegradable polymer, the at least one structural component and the negatively charged polymer are natural, semi-synthetic or fully synthetic.
- 20 6. The structure of any one of claims 4 to 5, wherein the biocompatible, biodegradable polymer is capable of providing mechanical stability.
- 25 7. The structure of any one of claims 4 to 6, wherein the biocompatible, biodegradable polymer is selected from a group consisting of polysaccharide, polyester, protein, polyhydroxybutyrate, poly glycolic acids, polyglycolide, polylactide, chitosan, poly-(D,L)-lactic acid, ethylhydroxyethyl cellulose, polycaprolactone, polycaprolactone diol, polylysine, polyglycolic acid, polybenzyl-L-glutamic acid, polymaleic acid, hydrogels, polyethylene glycol (PEG), their derivatives and combinations thereof.
- 30 8. The structure of claim 7, wherein the polysaccharide is an $\alpha(1\rightarrow4)$ linked polysaccharide.
9. The structure of claim 8, wherein the polysaccharide is agarose or alginate.
- 35 10. The structure of any one of claims 4 to 9, wherein the at least one structural component is selected from a group consisting of proteins, non-proteins, synthetic materials emulating structural components and mixtures thereof.
- 40 11. The structure of claim 10, wherein the proteins are selected from a group consisting of serum-derived proteins, extracellular matrix proteins, synthetically modified structural proteins, aggrecan, biglycan, collagens, gelatine, decorin, elastin, fibrinogen, fibrin, fibulins, fibrillins, tenascins, fibronectin, heparan sulphate proteoglycans, keratins, laminins, vimentin, vitronectin and combinations thereof.
12. The structure of claim 11, wherein the protein is collagen and/or fibrinogen.

- 5 13. The structure of any one of claims 4 to 12, wherein the negatively charged polymer is selected from a group consisting of glycosaminoglycan-like (GAG) polysaccharides, non-glycosaminoglycan (non-GAG) polysaccharides, non-polysaccharides, amino acids, poly amino acids and macromolecules.
- 10 14. The structure of claim 13, wherein the macromolecules are selected from the group consisting of oligomers and peptides with a molecular mass of less than 5 kDa, oligosaccharides, di-, tri- and tetrasaccharides, tetra-, penta-, hexa-, hepta-, octa- to icosopolymers and polymers greater in monomer units, low molecular weight heparin fractions, peptides, sulfonated carbohydrates, carbohydrate phosphates, nucleic acids, charged semi-synthetic or synthetic oligomers, peptide hormones, growth factors, physiological proteins, glycosaminoglycan (GAG), structural proteins and combinations thereof.
- 15 15. The structure of any one of claims 1 to 14, wherein the negatively charged polymer is dextran sulfate, cellulose sulphate, pullulan sulphate, chondroitin sulfate, dermatan sulfate, heparan sulphate, keratan sulfate, and hyaluronan.
- 20 16. The structure of claim 15, wherein the negatively charged polymer is not an $\alpha(1\rightarrow4)$ linked polysaccharide and/or is an $\alpha(1\rightarrow6)$ linked polysaccharide.
- 25 17. The structure of claim 16, wherein the negatively charged polymer is dextran sulphate.
18. The structure of claim 1, wherein the microstructure is a microcapsule, film, patch, bead, capsule, hydrogel, gel, microbead, injectable liquid, and moulded microstructure.
- 30 19. The structure of claim 18, wherein the structure is a microcapsule having a diameter of between 1 to 2000 μm .
20. The structure of claim 2, wherein the structure is a macrocapsule having a diameter of between 2 to 20 mm.
- 35 21. The structure of any one of claims 1 to 20, wherein the structure contains between 0.1 to 25% w/v of the biocompatible, biodegradable polymer.
22. The structure of claim 21, wherein the structure contains between 0.3% to 0.4% w/v of the biocompatible, biodegradable polymer.
- 40 23. The structure of any one of claims 1 to 22, wherein the structure contains between 1 to 8000 μg of the negatively charged polymer per ml.
24. The structure of any one of claims 1 to 23, wherein the structure contains between 0.01 to 99% w/v of at least one structural component capable of emulating extracellular matrix (ECM).
- 45 25. The structure of any one of claims 1 to 24, wherein the biologically active material is selected from a group consisting of liver-derived cells, pancreas-derived cells, umbilical cord-derived

cells, umbilical cord blood derived cells, brain-derived cells, spleen-derived cells, bone marrow derived cells, adipose derived cells, cells derived from IPS technology, cells derived from embryonic stem cells, genetically engineered cells, pluripotent cells, multipotent cells, neural cells, astrocytes, hepatocytes, fibroblasts, mesenchymal cells, pericytes, cardiomyocytes, cardiomyocyte progenitor cells, hematopoietic cells, endothelial cells, endothelial progenitors, smooth muscle cells, keratinocytes, stem cells and progenitors cells, cell mixtures, medicaments, pharmaceutical compositions, growth factors, differentiation factors, transcription factors, nucleic acids, amino acids, proteins, protein fragments and combinations thereof.

26. The structure of any one of claims 1 to 25, wherein the biologically active material is an adherent or non-adherent cell.

27. The structure of any one of claims 1 to 26, wherein the biologically active material is conjugated to the biocompatible, biodegradable polymer for slow release and/or time release.

28. The structure of claim 27, wherein the biologically active material is a stem cell selected from the group consisting of mesenchymal stem cells, neural stem cells, hematopoietic stem cells, endothelial progenitor cells and adipose-derived stem cells.

29. The structure of claim 1, wherein the structure is a microcapsule comprising agarose, collagen, fibrinogen and dextran sulfate.

30. The structure of claim 2, wherein the structure is a macrocapsule comprising agarose, collagen, fibrinogen and dextran sulfate.

31. Use of the structure for encapsulating biologically active materials of any one of claims 1 to 30 for the regeneration of organs and tissue.

32. The use of claim 31, wherein the organs and tissue are selected from a group consisting of liver, bone, heart, pancreas, lung, skeletal muscle, skin, cartilage, tendon, ligaments, adipose tissue, tissue of the lymphatic circulatory system, tissue of the vascular system, neural cells, periphery neurons and tissue of the central nervous system.

33. The use of claims 31 to 32, wherein the biologically active material comprises stem cells.

34. Use of structure of any one of claims 1 to 30 in the manufacture of a medicament for the treatment of an ischemic disease, muscular dystrophy and diseases arising from genetic deficiencies.

35. The use of claim 34, wherein the ischemic disease is selected from the group consisting of biliary ischemia, bone-related ischemia, cerebral ischemia, colonic ischemia, coronary ischemia, foot-related ischemia, hepatic ischemia, mesenteric ischemia, myocardial ischemia, optical nerve ischemia, retinal ischemia and spinal ischemia.

36. The use of claim 34 or 35, wherein the ischemic disease is myocardial infarction.

37. A method of manufacturing structures according to any one of claims 1 to 30, wherein the method comprises emulsifying methods, polymerization methods, forming methods, moulding methods, casting methods and coating methods.
38. A method of manufacturing structures according to any one of claims 1 to 30, wherein the method comprises
- Mixing a biologically active material with a biocompatible, biodegradable polymer, at least one structural component capable of emulating ECM and a negatively charged polymer, resulting in a biomaterial mix;
 - Adding the biomaterial mix to an emulsifier to form an emulsion;
 - Isolating the formed structures from the emulsion; and optionally
 - Incubating the formed structures with a polymerization agent.
39. A method of manufacturing microcapsules/macrocapsules according to any one of claims 1 to 30 using an emulsion based method, wherein the method comprises:
- Mixing an aqueous phase, wherein the aqueous phase comprises a solution of a biologically active material and/or cellular cargo with an oil or non-aqueous phase containing an emulsifier;
 - Forming microcapsules/macrocapsules by shaking, membrane emulsification, droplet generator, microfluidics or any other method that is essentially emulsion based or forming the aqueous phase containing biomaterial components and cellular cargo into droplets that subsequently solidify in liquid phase, air phase or at the solid/air or solid liquid interface;
 - Generating desired shapes and sizes using an extrusion or mould-based method;
 - Allowing the aqueous phase containing biologically active material to solidify prior to a process that leads to smaller units;
 - Isolating the formed microcapsules or macrocapsules from the emulsion; and optionally
 - Incubating the formed microcapsules/macrocapsules with a polymerization agent.
40. The method of claims 37 to 39, wherein the emulsifier is span80 and/or polymerisation agent is thrombin.

Claims

1. A structure suitable for encapsulating biologically active materials, wherein the structure comprises a mixture comprising a negatively charged polymer, a biocompatible, biodegradable polymer, at least one structural component, wherein the at least one structural component is capable of emulating extracellular matrix (ECM); wherein the biocompatible, biodegradable polymer is selected from a group consisting of polysaccharide, polyester, polyhydroxybutyrate, poly glycolic acids, polyglycolide, polylactide, chitosan, poly-(D,L)-lactic acid, ethylhydroxyethyl cellulose, polycaprolactone, polycaprolactone diol, polylysine, polyglycolic acid, polybenzyl-L-glutamic acid, polymaleic acid, hydrogels, polyethylene glycol (PEG) and their derivatives.
2. The structure of claim 1, wherein the structure is a microstructure or a macrostructure.
3. The structure of claim 1, wherein the negatively charged polymer is capable of emulating glycosaminoglycans.
4. The structure of claim 1, wherein the biocompatible, biodegradable polymer, the at least one structural component and the negatively charged polymer are natural, semi-synthetic or fully synthetic.
5. The structure of any one of claims 1 to 4, wherein the biocompatible, biodegradable polymer is capable of providing mechanical stability.
6. The structure of claim 5, wherein the polysaccharide is an $\alpha(1 \rightarrow 4)$ linked polysaccharide.
7. The structure of claim 6, wherein the polysaccharide is agarose or alginate.
8. The structure of any one of claims 1 to 7, wherein the at least one structural component is selected from a group consisting of proteins, non-proteins, synthetic materials emulating structural components and mixtures thereof.
9. The structure of claim 8, wherein the proteins are selected from a group consisting of serum-derived proteins, extracellular matrix proteins, synthetically modified structural proteins, aggrecan, biglycan, collagens, gelatine, decorin, elastin, fibrinogen, fibrin, fibulins, fibrillins, tenascins, fibronectin, heparan sulphate proteoglycans, keratins, laminins, vimentin, vitronectin and combinations thereof.
10. The structure of claim 9, wherein the protein is collagen and/or fibrinogen.
11. The structure of any one of claims 1 to 10, wherein the negatively charged polymer is selected from a group consisting of glycosaminoglycan-like (GAG) polysaccharides, non-glycosaminoglycan (non-GAG) polysaccharides, non-polysaccharides, amino acids, poly amino acids and macromolecules.
12. The structure of claim 11, wherein the macromolecules are selected from the group consisting of oligomers and peptides with a molecular mass of less than 5 kDa, oligosaccharides, di-, tri- and tetrasaccharides, tetra-, penta-, hexa-, hepta-, octa- to icoso-polymers and polymers greater in monomer units, low molecular weight heparin fractions, peptides, sulfonated carbohydrates, carbohydrate phosphates, nucleic acids, charged semi-synthetic or synthetic oligomers, peptide hormones, growth factors, physiological proteins, glycosaminoglycan (GAG), structural proteins and combinations thereof.

13. The structure of any one of claims 1 to 12, wherein the negatively charged polymer is dextran sulfate, cellulose sulphate, pullulan sulphate, chondroitin sulfate, dermatan sulfate, heparan sulphate, keratan sulfate, and hyaluronan.
- 5 14. The structure of claim 13, wherein the negatively charged polymer is not an $\alpha(1\rightarrow4)$ linked polysaccharide and/or is an $\alpha(1\rightarrow6)$ linked polysaccharide.
15. The structure of claim 14, wherein the negatively charged polymer is dextran sulphate.
- 10 16. The structure of claim 2, wherein the microstructure is a microcapsule, film, patch, bead, capsule, hydrogel, gel, microbead, injectable liquid, and moulded microstructure .
17. The structure of claim 16, wherein the structure is a microcapsule having a diameter of between 1 to 2000 μm .
- 15 18. The structure of claim 2, wherein the structure is a macrocapsule having a diameter of between 2 to 20 mm.
- 20 19. The structure of any one of claims 1 to 18, wherein the structure contains between 1 to 8000 μg of the negatively charged polymer per ml.
- 25 20. The structure of any one of claims 1 to 19, wherein the structure contains between 0.01 to 99% w/v of at least one structural component capable of emulating extracellular matrix (ECM).
- 30 21. The structure of any one of claims 1 to 20, wherein the biologically active material is selected from a group consisting of liver-derived cells, pancreas-derived cells, umbilical cord-derived cells, umbilical cord blood derived cells, brain-derived cells, spleen-derived cells, bone marrow derived cells, adipose derived cells, cells derived from IPS technology, cells derived from embryonic stem cells, genetically engineered cells, pluripotent cells, multipotent cells, neural cells, astrocytes, hepatocytes, fibroblasts, mesenchymal cells, pericytes, cardiomyocytes, cardiomyocyte progenitor cells, hematopoietic cells, endothelial cells, endothelial progenitors, smooth muscle cells, keratinocytes, stem cells and progenitors cells, cell mixtures, medicaments, pharmaceutical compositions, growth factors, differentiation factors, transcription factors, nucleic acids, amino acids, proteins, protein fragments and combinations thereof.
- 35 22. The structure of any one of claims 1 to 21, wherein the biologically active material is an adherent or non-adherent cell.
- 40 23. The structure of any one of claims 1 to 22, wherein the biologically active material is conjugated to the biocompatible, biodegradable polymer for slow release and/or time release.
- 45 24. The structure of claim 23, wherein the biologically active material is a stem cell selected from the group consisting of mesenchymal stem cells, neural stem cells, hematopoietic stem cells, endothelial progenitor cells and adipose-derived stem cells.
- 50 25. The structure of claim 2, wherein the structure is a microcapsule comprising agarose, collagen, fibrinogen and dextran sulfate.
26. The structure of claim 2, wherein the structure is a macrocapsule comprising agarose, collagen, fibrinogen and dextran sulfate.

27. The structure of any one of claims 1 to 26, wherein the structure is suitable for encapsulating biologically active materials, wherein the structure comprises a mixture comprising a negatively charged polymer, a biocompatible, biodegradable polymer, at least one structural component, wherein the at least one structural component is capable of emulating extracellular matrix (ECM); wherein the negatively charged polymer is dextran sulfate, wherein the biocompatible, biodegradable polymer is agarose, wherein the structure comprises two structural components, wherein the structural components are collagen and fibrinogen.
28. Use of the structure for encapsulating biologically active materials of any one of claims 1 to 27 for the regeneration of organs and tissue.
29. The use of claim 28, wherein the organs and tissue are selected from a group consisting of liver, bone, heart, pancreas, liver, lung, skeletal muscle, skin, cartilage, tendon, ligaments, adipose tissue, tissue of the lymphatic circulatory system, tissue of the vascular system, neural cells, periphery neurons and tissue of the central nervous system.
30. The use of claims 28 to 29, wherein the biologically active material comprises stem cells.
31. Use of structure of any one of claims 1 to 27 in the manufacture of a medicament for the treatment of an ischemic disease, muscular dystrophy and diseases arising from genetic deficiencies.
32. The use of claim 31, wherein the ischemic disease is selected from the group consisting of biliary ischemia, bone-related ischemia, cerebral ischemia, colonic ischemia, coronary ischemia, foot-related ischemia, hepatic ischemia, mesenteric ischemia, myocardial ischemia, optical nerve ischemia, retinal ischemia and spinal ischemia.
33. The use of claim 31 or 32, wherein the ischemic disease is myocardial infarction.
34. A method of manufacturing structures according to any one of claims 1 to 27, wherein the method comprises emulsifying methods, polymerization methods, forming methods, moulding methods, casting methods and coating methods.
35. A method of manufacturing structures according to any one of claims 1 to 27, wherein the method comprises
 - i. Mixing a biologically active material with a biocompatible, biodegradable polymer, at least one structural component capable of emulating ECM and a negatively charged polymer, resulting in a biomaterial mix;
 - ii. Adding the biomaterial mix to an emulsifier to form an emulsion;
 - iii. Isolating the formed structures from the emulsion; and optionally
 - iv. Incubating the formed structures with a polymerization agent.
36. A method of manufacturing microcapsules/macrocapsules according to any one of claims 1 to 27 using an emulsion based method, wherein the method comprises:
 - i. Mixing an aqueous phase wherein the aqueous phase comprises a solution of a biologically active material and/or cellular cargo with an oil or non-aqueous phase containing a emulsifier;
 - ii. Forming microcapsules/macrocapsules by shaking, membrane emulsification, droplet generator, microfluidics or any other method that is essentially emulsion based or forming the aqueous phase containing biomaterial components and cellular cargo into droplets that subsequently solidify in liquid phase, air phase or at the solid/air or solid liquid interface;

- iii. Generating desired shapes and sizes using an extrusion or mould-based method;
 - iv. Allowing the aqueous phase containing biologically active material to solidify prior to a process that leads to smaller units;
 - 5 v. Isolating the formed microcapsules or macrocapsules from the emulsion; and optionally
 - vi. Incubating the formed microcapsules/macrocapsules with a polymerization agent.
- 10 37. The method of claims 34 to 36, wherein the emulsifier is span80 and/or polymerisation agent is thrombin.