

## MATERIAL AND STRUCTURES FOR CELL DELIVERY

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10201402976V, filed 5 June 2014, the contents of it being hereby incorporated by reference in its entirety for all purposes.

### FIELD OF THE INVENTION

[0002] The present invention relates to biochemistry. In particular, the present invention relates to micro- or macro-structures made of polymers, methods of using such structures, uses of such structures and methods of manufacturing thereof.

### BACKGROUND OF THE INVENTION

[0003] Delivery of adult stem cells, such as mesenchymal stem cells, has been investigated extensively for tissue regeneration and repair. However, administration of stem cells into tissue, such as the myocardium has resulted in a less optimal outcome. Although literature on delivery of stem cells in animal studies has reported improvement in functional recovery, the translation to the clinics has been more challenging. Whilst clinical studies have reported some convincing data, no significant improvement has been shown following stem cells treatment. Hence, there is a need to provide an alternative delivery system of stem cells.

[0004] As practiced with other tissue engineering applications, the issue of cell delivery into tissue can be aided with the use of a suitable encapsulation biomaterial. In comparison to other moldable hydrogels such as fibrinogen, the microcapsules show superior mechanical stability, allowing for a better protection of injected cells, and hence, better long-term retention of cells within the host tissue.

[0005] Historically, microcapsules were employed to deliver allogeneic or xenogeneic cells. Designed to be non-degradable and semipermeable, these microcapsules were meant to protect encapsulated cells from the host immune system. Unfortunately, alginate is known to be immunogenic due to presence of protein impurities in the polysaccharide hydrogel, and has been shown to induce a foreign body response (FBR), which can result in collagenous encapsulation and therefore isolation of encapsulated cells from the surrounding tissues. This immunogenic response is not restricted to alginates alone. In general, microcapsules for such applications fail in the long-term, due to rejection.

[0006] Nevertheless, most microcapsules for tissue engineering applications are derived using such materials. Therefore many are non-degradable, do not allow histointegration of delivered cells and induce a FBR. Often the interior does not provide an appropriate microenvironment to support

long-term cell survival of delivered cells. Moreover, whilst staining for live cells over a short time is deemed sufficient to prove proper cell support, a clear decrease in cell density would invariably be observed. Thus, there is a need to provide for an alternative structure suitable for encapsulating biologically active materials.

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### SUMMARY OF THE INVENTION

[0007] In one aspect, there is provided a microstructure suitable for encapsulating biologically active materials. The microstructure comprises a negatively charged polymer.

10 [0008] In one aspect, there is provided a macrostructure suitable for encapsulating biologically active materials. The macrostructure comprises a negatively charged polymer.

[0009] In another aspect, there is provided the use of a microstructure/macrostructure as described herein for the regeneration of organs and tissue.

15 [0010] In yet another aspect, there is provided the use of a structure as described herein in the manufacture of a medicament for the treatment of an ischemic disease, muscular dystrophy and diseases arising from genetic deficiencies.

[0011] In yet another aspect, there is provided a method of manufacturing the structure as described herein. The method comprises emulsifying methods, polymerization methods, forming methods, moulding methods, casting methods and coating methods.

20 [0012] In yet another aspect, there is provided another method of manufacturing the structures as described herein. This other method comprises the steps of: (i) mixing the 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 said biomaterial mix to an emulsifier to form an emulsion; (iii) isolating the formed structures from the emulsion; and optionally (iv) incubating the formed microstructures with a polymerization agent.

25 [0013] In yet another aspect, there is provided a method of manufacturing microcapsules/macrocapsules as described herein using an emulsion based method. This method comprises the steps of (i) mixing an aqueous phase, wherein the aqueous phase comprises a solution of 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  
30 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; (v)

isolating the formed microcapsules/macrocapsules from the emulsion; and optionally (vi) incubating the formed microcapsules/macrocapsules with a polymerization agent.

### BRIEF DESCRIPTION OF THE DRAWINGS

- 5 [0014] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:
- [0015] **Fig. 1** shows the extent of cellular support provided by the various microcapsules. In particular, live-dead cell staining of encapsulated cells is shown in a) and c), which show encapsulation at day 1 and day 3 post-encapsulation, respectively. Quantification of live-to-dead cell staining area ratio at the respective days are shown as bar graphs in b) and d), which show results from day 1 and day 3 post-encapsulation, respectively. Aga: short abbreviation for agarose. \* $p < 0.05$  indicates significant difference from all other conditions. Thus, Fig. 1 shows addition of extracellular matrix (ECM) proteins and/or dextran sulfate (DxS) within microcapsules support cell survival.
- 10 [0016] **Fig. 2** shows results on the studies of the fibrillogenesis caused by dextran sulfate (DxS). In particular, a) shows a line graph of the kinetics of collagen fibrillogenesis in the presence of various concentrations of dextran sulfated (DxS), as followed by turbidimetry; b) shows a bar graph showing the nucleation rate and elongation rate of collagen as calculated from time-resolved spectrophotometry data; c) shows images of collagen hydrogels assembly in the presence of dextran sulfate (DxS) as taken by 3D confocal reflexion microscopy. Scale bar is 50  $\mu\text{m}$ ; d) shows a graph of the kinetics of collagen fibrillogenesis in the presence of agarose and dextran sulfate (DxS) at concentrations as present in microcapsule material. Thus, Fig. 2 shows the presence of dextran sulfate (DxS) influences the architecture of gels.
- 20 [0017] **Fig. 3** shows the enhancement of cell-microenvironment interactions by dextran sulfate (DxS) and collagen I. In particular, a) shows a phase contrast and fluorescent microscopy of microcapsules containing FITC-labelled dextran sulfate (DxS). FITC-labelled dextran sulfate (DxS) appears as bright spots in black background; and b) shows phase contrast and fluorescent microscopy of microcapsules containing phalloidin-stained fibroblasts. Stained cells appear as bright spots in black background. Thus, Fig. 3 shows interactions of dextran sulfate (DxS) with collagen I within the microcapsules resulting in a stronger cell spreading.
- 25 [0018] **Fig. 4** shows the investigation into the properties and cell functionalities within microcapsules. In particular, a) shows the result of cell counting kit-8 (CCK-8) colorimetric assay (a metabolic assay) looking into the long-term cell survival and cell proliferation of mesenchymal stem cells over three weeks of culture. Fig. 4a shows mesenchymal stem cells can proliferate and have long-term survival in the Col-Fb-DxS100 microcapsules (i.e. microcapsules composed of collagen I, fibrin and 100  $\mu\text{g/ml}$  dextran sulfate); b) shows phase contrast pictures at various magnifications of
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microcapsules showing out-migrating cells; c) shows microcapsules size distribution as diameter measurements. Fig. 4 shows the various microcapsules size and pore-size of microcapsules that are composed of collagen I, fibrin and 100 µg/ml dextran sulfate (i.e. Col-Fb-DxS100).

[0019] **Fig. 5** shows the investigation of the functionality of cells encapsulated in microcapsules.

5 In particular, a) shows the result of an enzyme-linked immunosorbent assay (ELISA) study on the amount of vascular endothelial growth factor (VEGF) in the culture medium of encapsulated mesenchymal stem cells, which were either non-treated or treated with 10 µM ciclopirox olamine (CPX); b) shows the microscope images of differentiation of out-migrating mesenchymal stem cells into adipocytes and osteoblasts; c) confocal microscopy images of encapsulated mesenchymal stem  
10 cells four days in culture in the presence of ascorbic acid. A strong staining (bright spots) for collagen I confirmed its presence post encapsulation; however fluorescence decreased in the presence of encapsulated MSCs, indicating degradation by cells. In sections through microcapsules and MSCs, a granular staining for collagen I in close proximity to the cytoskeleton was observed, suggesting newly synthesized collagen I (far left panel). Collagen IV was observed at a similar manner close to the  
15 cytoskeleton, although it appeared more frequently (center panel). A similar pattern was observed for heparin sulfate proteoglycans (HSPGs; far right panel). Thus, Fig. 5 shows encapsulation of mesenchymal stem cells (MSCs) in the microcapsule as described in the Example section allows the mesenchymal stem cells to remain functional and are capable of modifying their microenvironment within the microcapsules.

20 [0020] **Fig. 6** shows studies on the *in vivo* functionality of microcapsules Col-Fb-DxS100. In particular, a) shows histological hematoxylin and eosin stain (H&E) staining of microcapsules immediately after injection into rat thigh muscle; b) shows phase contrast and fluorescent microscopy images of TRITC-labelled agarose containing microcapsules as well as H&E staining four weeks post injection. Fluorescent positive cells are seen as bright spots in panels labelled TRITC-agarose or  
25 darkened area in phase-contrast panels (bottom three panels). Fig. 6 shows Col-Fb-DxS100 microcapsules exhibit sufficient mechanical stability for intramuscular injection; c) shows immunohistochemistry images of rat calf muscles injected with TRITC-labelled agarose containing microcapsules. Grey spots: DAPI (nuclei staining), Bright spots: immunostaining against CD11b, iNOS, CD206 and collagen I (Col I), collagen III (Col III) and rat endothelial cell antigen 1 (Reca-1).  
30 White arrows: phagocytized agarose. Immunohistochemistry demonstrated that many of the infiltrated cells expressed CD11b, confirming their monocytic origin (first four images labelled “CD11b”). Of note, a specifically strong CD11b staining was found in close proximity to biomaterial, indicating that macrophages directly attach and surround to the biomaterial. Higher exposure times identified small traces of TRITC-labeling in close proximity to CD11b positive cells around the main biomaterial  
35 location (white arrows), indicating that biomaterial was phagocytized and transported out by

macrophages. Very little staining for iNOS, an M1 marker, was observed indicating that microcapsules did not polarize the endogenous macrophages into a chronic pro-inflammatory state (panels labeled "iNOS"). On the other hand, cells positive for the mannose receptor (CD206; an M2 marker; stained cells seen as bright spots) were prominent around the biomaterial, indicating an anti-inflammatory and 'wound-healing' state around the biomaterial (far right panels). In accordance with this finding, collagen type I staining was strongly displayed in spaces between muscle fibers (far left, top row of bottom column labelled "Col I"), but was weaker around the microcapsule fragments (far left, bottom row of bottom column labelled "Col I"). Loosely distributed, thin collagen I fibers with a random distribution indicated that no fibrotic response was induced, which normally can be identified by a dense parallel alignment of collagen fibers. A similar loose and random distribution of fibers was observed for collagen type III around the biomaterial (middle column labelled "Col III"). In general high collagen III content in ECM is a signature of granulation tissue, further indicating the 'wound-healing' state around the biomaterial. This observation was further supported by cells stained positive for rat endothelial cell antigen 1 (Reca-1) around the biomaterial (far right column labelled "Reca-1"), indicating the presence of infiltrated endothelial cells and blood vessels. Therefore, Fig. 6 shows Col-Fb-DxS100 microcapsules degraded slowly *in vivo*, and does not induce a fibrotic foreign body response but rather induced a 'healing' environment around the implant.

[0021] **Fig. 7** shows the results of *in vivo* transplantation test. Fig. 7 shows a magnetic resonance imaging (MRI) image of T1-weighted short-axis analysis (T1W1, "spin-lattice" relaxation time, a basic pulse sequence in MRI) of rat left ventricle after implantation of microcapsules. For images showing signal from encapsulated MSCs, the same section through the same rat left ventricle is shown over 6 weeks. Arrows pointing to black void indicate ultrasmall super-paramagnetic iron oxide (USPIO) signal from labelled rat bone-marrow derived-mesenchymal stem cells (bmMSCs). Thus, Fig. 7 shows Col-Fb-DxS100 microcapsules enable retention and long-term tracking of MSCs in the myocardium.

[0022] **Fig. 8** shows a net negatively charged polycarbohydrate, such as sulfated dextran (branched glycan) polymer.

#### DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0023] One of the methods known in the art of transplantation includes the use of a suitable encapsulation biomaterial. However, encapsulation technologies known in the art can often trigger host immune response or unable to provide suitable microenvironment for the encapsulated cells to thrive. Thus, there is a need to provide an alternative encapsulation biomaterial.

[0024] Thus, in one aspect, there is provided a microstructure suitable for encapsulating biologically active materials. The microstructure comprises a negatively charged polymer.

[0025] As used herein, the term “microstructure” refers to a construct, structure or arrangement of the present disclosure that may have a diameter of about 1 $\mu$ m to about 2mm. In one example, the microstructure may be a microcapsule, film, patch, bead, capsule, hydrogel, gel, microbead, injectable liquid, and moulded microstructure.

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[0026] In one example, the microstructure may be a microcapsule that may have a diameter of between about 1  $\mu$ m to about 2000  $\mu$ m, for example, the microcapsule may be between about 1  $\mu$ m to about 50  $\mu$ m, or between about 50  $\mu$ m to about 100  $\mu$ m, or between about 100  $\mu$ m to about 200  $\mu$ m, or between about 200  $\mu$ m to about 400  $\mu$ m, or between about 400  $\mu$ m to about 800  $\mu$ m, or between about 800  $\mu$ m to about 1000  $\mu$ m, or between about 1000  $\mu$ m to about 1500  $\mu$ m, or between about 1500  $\mu$ m to about 2000  $\mu$ m. The microcapsule as exemplified in the Example section, in Fig. 4c, may have a diameter of about 60  $\mu$ m to about 80  $\mu$ m, or about 80  $\mu$ m to about 100  $\mu$ m, or about 100  $\mu$ m to about 120  $\mu$ m, or about 120  $\mu$ m to about 140  $\mu$ m, or about 140  $\mu$ m to about 160  $\mu$ m. In one example, the microcapsule may have a diameter of about 100  $\mu$ m.

[0027] It is envisaged that the structure as described herein may be provided in large scale that is not bound by the actual dimension of the structure. Hence, in another aspect, there is provided a macrostructure suitable for encapsulating biologically active materials. The macrostructure comprises a negatively charged polymer.

[0028] As used herein, the term “macrostructure” refers to a construct, structure or arrangement of the present disclosure that may have a diameter of about 2mm to about 100mm. In one example, the macrostructure may be a macrocapsule, film, patch, bead, capsule, hydrogel, gel, beads such as macrobead, injectable liquid and moulded microstructure. In one example, the macrostructure may be a macrocapsule that may have a diameter of between about 2 mm to about 20mm, or between about 20 mm to 1000 mm. In one example, the macrocapsule may have between about 2 mm to about 4mm, or between about 4 mm to about 8 mm, or between about 8 mm to about 12 mm, or between about 12 mm to about 16 mm, or between about 16 mm to about 20 mm or between about 20 mm to about 100 mm or between about 100 mm to about 1000 mm.

[0029] As used herein, the term “encapsulating” refers to a process for coating biologically active materials or placing the biologically active materials into an encasement (casing or capsules) so that the coating (encasement, casing or capsule) will encase the biologically active materials, at least to some extent, in such a manner that the biologically active materials does not have direct cellular contact with the cellular environment the microstructure has been placed into. For example, the structure would prevent the encapsulated biologically active material from having direct cellular contacts with the cells of the tissue in which the encapsulated biologically active material have been placed (grafted or injected) into. In one example, the encapsulation does not prevent extracellular

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fluid to come in contact with the biologically active material. Hence, the encapsulated biologically active material is still in fluid contact with the extracellular fluid.

[0030] As used herein, the phrase “negatively charged polymer” refers to a polymer that has a negative charge when located in an aqueous solution or an aqueous system such as blood or an extracellular fluid. As used herein, the term “polymer” refers to a material that is a homopolymer, copolymer, terpolymer or the like. In one example, the negatively charged polymer may be capable of emulating glycosaminoglycan. That is, the negatively charged polymer may be mimicking the properties of glycosaminoglycan in an extracellular matrix. It was found that having negatively charged polymer in a microstructure/macrostructure is beneficial for the stability of the biologically active materials as negatively charged polymer, such as glycosaminoglycan (GAG), can interact with extracellular matrix proteins or cell receptors, sequester and present growth factors, and actively take part in cell-microenvironment signaling. In one example, the negatively charged polymer may not be capable of providing mechanical stability to the structure. In one example, the negatively charged polymer may not have gelatinous properties. That is, in one example, the negatively charged polymer may not be able to form gelatinous structure. In one example, negatively charged polymer binds to extracellular matrix proteins such as collagen I and affects how these proteins are presented to the cells. For example, less collagen I fibrillogenesis occurs in the presence of negatively charged polymers (see Fig. 2). Although various glycosaminoglycan (GAG) possess specialized functions, they are all net-negatively charged polycarbohydrates with a protein core. Thus, in one example, the negatively charged polymer may include, but is not limited to, glycosaminoglycan-like (GAG) polysaccharides, non-glycosaminoglycan (non-GAG) polysaccharides, non-polysaccharides, amino acids, poly amino acids, macromolecules (such as glycosaminoglycan) and the like.

[0031] As used herein, the term “macromolecule” refers to a molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass. The lower size limit for a macromolecule can be further defined as to have sufficient monomeric units to interact or bind with at least one component of the material by hydrogen bonding, electrostatic or steric interaction. For example, the minimum size of heparan sulfate fragments to mediate interaction of vascular endothelial growth factor (VEGF) with its receptor is about an octa-saccharide for stable interaction, which is larger than the oligomere size to provide stable electrostatic interaction –tri or tetra saccharide). The upper size limit of a macromolecule can be derived from functional parameters – solubility in aqueous phase and viscosity. Typically, macromolecules are smaller than 1000 kDa. In exceptional cases, mainly synthetic polymers, the molecular weight may reach a maximum of 2500 kDa. In one example, macromolecules with a molecular mass greater than 5000 kDa may not be suitable for use in the structure as described herein.

[0032] As used herein, the term “poly amino acids” refers to proteins generally with a molecular mass between 5 kDa and 500 kDa, with or without a negative charge. These could be further defined as natural proteins and peptides, synthetically modified natural proteins and peptides or fully synthetic proteins, peptides, and poly amino acids whose isoelectric point indicates a negative net-charge at a pH range suitable for the intended application. Examples of negatively charged poly amino acids are poly glutamic acids, poly aspartic acids and poly amino acids that have a significant proportion of these amino acids to render the net charge negative at pH range between 3-9. Negatively charged macromolecules, polymers and macromolecules may also fall under this definition of poly amino acids, resulting in that at least a fraction of the monomeric component is derived from synthetic materials (e.g. non-amino acids). Accordingly, in one example, the negatively charged polymer may be a partially synthetic or fully synthetic polymer.

[0033] In one example, the macromolecules may include, but are not limited to, 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 (GAGs), structural proteins and combinations thereof. As used herein the term “peptide hormone” refers to proteins with endocrine functions, for example insulin.

[0034] In one example, the macromolecule may have negatively charged side groups. In one example, the negatively charged side groups may be selected from a group consisting of phosphate, carboxylate, sulfate and derivatives thereof. For example, the macromolecules may include, but are not limited to, polystyrene sulphate, vinyl sulphate and the like.

[0035] In one example, the glycosaminoglycan-like (GAG) polysaccharides may include, but are not limited to, chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, dextran sulfate, hyaluronan and the like.

[0036] In one example, the non-glycosaminoglycan (non-GAG) polysaccharides may include, but are not limited to, dextran sulfate, cellulose sulphate, pullulan sulphate and the like.

[0037] In one example, the non-polysaccharides may include, but are not limited to, polystyrene sulfate, polyvinyl sulfate, polyvinyl phosphate, negatively charged polyaminoacids (e.g. polyglutamic acid), derivatives and the like. In one example, the negatively charged polymer may be a natural, semi-synthetic or fully synthetic polymer.

[0038] In one example, the negatively charged polymer may include, but is not limited to, dextran sulfate, cellulose sulphate, pullulan sulphate, chondroitin sulfate, dermatan sulfate, heparin sulphate and the like. In one example, the negatively charged polymer may not have a linear backbone of an

$\alpha(1\rightarrow4)$  linked repeating units (polysaccharide) and/or may have a linear backbone of an  $\alpha(1\rightarrow6)$  linked repeating units (such as polysaccharide). The negatively charged polymer as exemplified in the Example section of the present disclosure is sulfated dextran (DxS) or dextran sulfate polymer, which is a negatively charged polycarbohydrate. Thus, in one example, the negatively charged polymer may be dextran sulfate, which is a sulfated dextran polymer.

[0039] As illustrated in the Example section of the present disclosure, the structure as described herein may contain between about 1  $\mu\text{g}$  to about 8000  $\mu\text{g}$  of the negatively charged polymer per ml of structure. That is, the structure as described herein may have between about 1 to about 500  $\mu\text{g}$ , or between about 1 to about 1000  $\mu\text{g}$ , or between about 1000 to about 5000  $\mu\text{g}$ , or between about 5000 to about 10000  $\mu\text{g}$ , or between about 250 to about 750  $\mu\text{g}$ , or between about 2000 to about 4000  $\mu\text{g}$ , or between about 4000 to about 8000  $\mu\text{g}$  of the negatively charged polymer per ml of structure.

[0040] As used herein, the phrase "biologically active material" refers to an agent, substance or cell, which when provided into a subject, is capable of modulating the subject's physiology. In one example, the "biologically active material" may be an agent, substance or cell intended for use in the treatment or prevention of disease in a subject, such as a mammal, such as human, dog, cat, rodent, horse, non-human primates, and the like. In one example, biologically active material may include, but is not limited to, liver-derived cells, pancreas-derived cells (such as pancreatic islets), 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.

[0041] In one example, the biologically active material may be an adherent or non-adherent cell. In yet another example, the biologically active material may be a stem cell, including but not limited to, mesenchymal stem cells, neural stem cells, hematopoietic stem cells, endothelial progenitor cells and adipose-derived stem cells. For example, as illustrated in the Example section, the biologically active material is a mesenchymal stem cell. In this example, the encapsulated mesenchymal stem cell may be provided into a subject requiring heart tissue regeneration. In another example, the biologically active material may be a pancreatic islet. In this example, the encapsulated islet cells may be provided into a subject having diabetes such as Type 1 or Type 2 diabetes.

[0042] As illustrated in the Example section, the structure of the present disclosure may further comprise supplementation with components of extracellular matrix. In one example, the structure as

described herein may further comprise a biocompatible, biodegradable polymer and at least one structural component capable of emulating extracellular matrix (ECM). As used herein, the term “biocompatible” refers to the property of being biologically compatible with living tissue of a host organism by not producing a toxic, injurious, foreign body response or immunological response. In one example, the term “biocompatible” may also refer to a material which performs its desired function when introduced into an organism without inducing significant inflammatory response, immunogenicity, or cytotoxicity to native cells, tissues or organs.

[0043] As used herein, the phrase “foreign body response” refers to an immunological response of a biological tissue to the presence of any foreign material in the tissue, which can include protein adsorption, chronic inflammation, multinucleated foreign body giant cells and fibrosis.

[0044] As used herein, the term “biodegradable polymer” refers to a polymer or polymers which degrade *in vivo*, and wherein erosion of the polymer or polymers over time occur concurrent with or subsequent to release of the biologically active material. Thus, in one example, the slow degradation of the biocompatible, biodegradable polymer results in the slow release and/or time release of the biologically active material that is encapsulated in the microstructure (such as for example conjugated into the biocompatible, biodegradable polymer). In one example, the biologically active material may be conjugated to the biocompatible, biodegradable polymer for slow release and/or time release. However, it is understood that the biodegradable polymer as described herein may not be degraded by proteolytic degradation that may occur in view of the host immune system’s reaction to the microstructure. Thus, in one example, the biocompatible, biodegradable polymer may not be proteolytically degradable or essentially not proteolytically degradable. In one example, the biocompatible, biodegradable polymer may be capable of providing mechanical stability (for example through gelation of the biocompatible, biodegradable polymer). In one example, the biocompatible, biodegradable polymer may be soluble in aqueous solution. In one example, the biocompatible, biodegradable polymer, may be natural, semi-synthetic or fully synthetic.

[0045] To achieve the desired properties, the biocompatible, biodegradable polymer may include, but is not limited to, a polysaccharide, polyester, protein, polyhydroxybutyrate, poly glycolic acids, polyglycolide, polylactide, chitosan, hyaluronic acid, 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. In one example, the biocompatible, biodegradable polymer may have a linear backbone of an  $\alpha(1\rightarrow4)$  linked linear repeating units (polysaccharide). In one example, the biocompatible, biodegradable polymer may be a polysaccharide such as agarose or alginate.

[0046] As used herein, the term “alginate” refers to a class of linear polysaccharide copolymers formed from 1-4-glycosidically linked  $\beta$ -D-mannuronate (M) and its C-5 epimer  $\alpha$ -L-guluronate (G).

Alginates are naturally occurring biopolymers produced by a variety of organisms, including marine brown algae and at least two genera of bacteria (*Pseudomonas* and *Azotobacte*). Typically, commercial alginates are isolated from marine algae, including *Macrocystis pyrifera*, *Ascoph lllum nodosum*, and various types of *Laminaria*.

5 [0047] "Agarose", as used herein, refers to a biomaterial that is not degradable enzymatically within tissue, but has been shown to be phagocytized by macrophages. In contrast to other enzymatically degradable materials, it remains longer within the host tissue, thus allowing for a slow and gradual release of encapsulated biologically active material into the surrounding tissues of transplant (or graft) recipient. Similarly to alginate, agarose is a polysaccharide derived from seaweed,  
10 but is neutral in charge. It has previously been reported that neutrally charged polymers induce lower foreign body response (FBR) than charged ones. Nevertheless, agarose has been reported to induce a foreign body response at higher concentrations; therefore it was found that a specific range of percentages (w/v of total microstructure) of biocompatible, biodegradable polymer, such as agarose, is beneficial in avoiding a foreign body response (FBR). As agarose was found to be insufficiently  
15 immunoprotective and allows extrusion of cells, it was previously dismissed for uses in allogeneic or xenogeneic cell transplantation. However, despite common general knowledge in the art, the inventors of the present disclosure surprisingly found these properties enables agarose to be a suitable candidate for delivery of a biologically active material into a subject.

[0048] To achieve the balance between biodegradability whilst still proteolytically non-degradable, and do not cause the initiation of the foreign body response, the inventors of the present  
20 disclosure found a specific range of percentages of biocompatible, biodegradable polymer (w/v of total structure) is ideal in providing such properties. In one example, the structure as described herein may contain between about 0.1 to about 0.5% w/v (of the total w/v of the structure) of the biocompatible, biodegradable polymer. In one example, the structure as described herein may contain  
25 between about 0.1 to about 0.5%, or between about 0.5 to about 1%, or between about 0.25 to about 0.6%, or between about 0.3 to about 0.5%, or between about 1 to about 2%, or between about 1.5 to about 2.5%, or between about 2.5 to about 5%, or between about 5 to about 10%, or between about 10 to about 15%, or between about 15 to about 20%, or between about 20 to about 25%, or between about 7 to about 11%, or between about 13 to about 17%, or between about 17 to about 23% w/v of  
30 the biocompatible, biodegradable polymer. In one example, the structure may contain between about 0.3% to about 0.4% w/v of the biocompatible, biodegradable polymer.

[0049] It was further found that low percentages (w/v of structure) of the biocompatible, biodegradable polymer, such as agarose, do not allow for optimal mechanical stability for intramuscular injection. Therefore, to provide for optimum mechanical stability, the inventors of the  
35 present disclosure added various structural components, such as collagen I and fibrinogen, which

polymerize to form a gel, which subsequently increases the stability of the structure. In addition, these structural components also provide a matrix for the encapsulated biologically active material, such as cells, allowing cellular attachment. Anchorage and interaction of cells with the surrounding microenvironment play a role in providing a proper arrangement of the cytoskeleton, affecting cell signaling, metabolism and ensuring proper cell function. Fibrin is a pro-angiogenic matrix component during wound healing and provides further wound-healing signals to encapsulated cells. Thus, in one example, the structure as described herein further comprises at least one structural component.

[0050] As used herein, the phrase “structural component” refers to scaffolding polymers or “polymer scaffold”, which refers to material that mimics, resembles or simulates the structure and function of the extracellular matrix. The term “extracellular matrix” refers to the extracellular part of cellular structure (for example, organisms, tissues, biofilms) that typically provides structural and biological support to the surrounding cells. In one example, the structural component may be natural, semi-synthetic or fully synthetic. In one example, the structure as described herein may have at least one structural component. In another example, the structure as described herein may have at least two, at least three, at least four, at least five, at least six, at least seven, at least eight or more structural components. In one example, the structural components may include, but are not limited to, proteins, non-proteins, synthetic materials emulating structural components and mixtures thereof. In one example, the structural component may be a non-protein, which may be synthetic materials containing amino acid sequences allowing cell interaction. In one example, the structural component may be proteins including, but are not limited to, 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. In one example, the collagens that may include, but are not limited to, collagen I, collagen II, collagen III, collagen IV, collagen V, and the like. In one example, the structural component may be a protein, which may be collagen and/or fibrinogen or derivatives thereof. As illustrated in the Example section, the structure may comprise at least two structural components namely collagen I and fibrinogen or derivatives thereof. Thus, in one example, the structure may have at least two structural components such as collagen and fibrinogen.

[0051] In one example, the structure may contain between about 0.01 to about 40% w/v, or about 40% (w/v) to about 99% (w/v) of at least one structural component capable of emulating extracellular matrix. That is, the structure may contain between about 0.01 to about 1%, or between about 1 to about 5%, or between about 5 to about 15%, or between about 15 to about 30%, or between about 30 to about 50%, or between about 0.05 to about 2%, or between about 2 to about 10 %, or between about 10 to about 20%, or between about 20 to about 40% w/v, or between about 40 to about 50% w/v, or between about 50 to about 60% w/v, or between about 60 to about 70% w/v, or between about

70 to about 80% w/v, or between about 80 to about 90% w/v, or between about 90 to about 99% w/v of at least one structural component capable of emulating extracellular matrix (ECM).

[0052] As would be understood by the skilled person in the art, the components of the structure as illustrated in the Example section may be substituted with suitable materials as exemplified above.

5 Thus, in one example, the components of the structure as described herein may be substituted with a naturally occurring polymer known in the art, synthetic materials known in the art or a semi-synthetic material known in the art. Thus, in one example, the biocompatible, biodegradable polymer, the at least one structural component and the negatively charged polymer of the structure as described herein may be natural, semi-synthetic or fully synthetic.

10 [0053] As illustrated in the Example section of the present disclosure, the structure may be a microcapsule comprising agarose and at least two structural components capable of emulating ECM and a negatively charged polymer. In one example, the structure may be a microcapsule/macrocapsule comprising agarose, collagen, at least one further structural component capable of emulating ECM and a negatively charged polymer. In another example, the structure may be a microcapsule and/or  
15 macrocapsule comprising agarose, collagen, fibrinogen and a negatively charged polymer. In another example, the structure may be a microcapsule/macrocapsule comprising or consisting of agarose, collagen, fibrinogen and dextran sulfate.

[0054] In one example, the microcapsules/macrocapsules as described herein, may comprise of a composition of the following materials, with the biocompatible, biodegradable polymer being agarose  
20 or a material with similar physical characteristics; structural components, including proteins such as serum-derived proteins, which may be selected from extracellular matrix proteins, or derivatives thereof, such as gelatin, or synthetic materials emulating structural proteins, as well as all synthetically modified structural proteins (such as alkylated collagen and the like), which would include collagen and fibrinogen; biocompatible materials or polymers which may be highly charged  
25 for the purpose to complex (i.e. aggregate/coagulate, and the like) with structural proteins or other microcapsule components. In one example, one purpose of the components of the microstructure/macrosstructure as described herein is to emulate glycosaminoglycan and extracellular matrix structures, which the inventors of the present disclosure found to be surprisingly beneficial in providing optimal cell microenvironment that leads to growth and cell viability even six weeks post  
30 transplantation (see Fig. 7). In one example, the components of the microstructure/macrosstructure as described herein has a role in retaining small molecular weight components like growth factors (VEGF) and present them to the encapsulated cells or to achieve a steady release of these small molecules components. For example, heparin or heparin sulphate mediates interaction of VEGF with its receptors.

[0055] In another aspect, there is provided a structure as described herein for encapsulating biologically active material. In this aspect, the structure as described herein may comprise a biocompatible, biodegradable polymer that is alginate or agarose or combinations thereof; a structural protein that is collagen I or hydrolysed collagen or combinations thereof; another structural protein that is fibrinogen; and a negatively charged polymer that is dextran sulfate.

[0056] Fig. 7 of the present disclosure illustrates the growth and viability of mesenchymal stem cells in rat hearts even after six weeks post-transplantation. Thus, another aspect of the present disclosure is the use of a structure as described herein for the regeneration of organs and tissue. In one example, the organs and tissue may include, but are not limited to, 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, tissue of the central nervous system, and the like. In one example, the biologically active material may comprise stem cells. In one example, as exemplified in the Example section, the biologically active material may comprise mesenchymal stem cells.

[0057] With observation of viability and growth of transplanted cells, the present disclosure also provides the use of a structure as described herein in the manufacture of a medicament for the treatment of an ischemic disease, muscular dystrophy and diseases arising from genetic deficiencies. In one example, the ischemic disease may include, but is not limited to, 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, spinal ischemia and the like. In one example, the ischemic disease may be myocardial infarction.

[0058] As would be understood by the skilled person in the art, the form of the biologically active material and the delivery route of the structure would define the physical requirements of the structure, and hence influence the form of the structure. Additionally, it is also understood by the skilled person in the art that the structure may be prepared in liquid form prior to transplantation for ease of injection. Upon injection into the body, the liquid structure may subsequently solidify within the host subject. Optionally,  $\text{CaCl}_2$  dihydrate may be used to prevent the clotting of fibrinogen during the formation of structures such as microcapsules and/or macrocapsules. Furthermore, although a number of multivalent cations can have the same effect, the skilled person in the art would understand suitable cations would be non-toxic. For example, strontium is one example of non-toxic suitable cations. The anion or hydrate water may or may not be crucial to facilitate the process (various anions or hydrates are possible).

[0059] In another aspect, there is provided a method of manufacturing the structures as described herein, wherein the method comprises emulsifying methods, polymerization methods, forming methods, moulding methods, casting methods and coating methods. As used herein, the term

“moulding” implies the use of an actual mould, whereas forming implies forming a structure without the use of a mould. In one example, the polymerization and forming methods may refer to methods that polymerize units of pre-formed microstructures. As would be understood by the skilled person in the art, a polymerization itself does not indicate the formation of structures but rather the build-up of a macromolecule in some way. Another term known in the field of micro-fabrication is curing, for example the process to solidify silicon polymers into patches, implants, microfluidic devices are known as curing silicon (e.g. PDMS), that may also involve a curing agent that induces the process in some cases. In one example, methods used to form units of desired structures by division from raw material, pre- or post-solidification may also be utilised.

5 [0060] In another aspect, there is provided a method of manufacturing the structures as described herein, wherein the method may comprise the steps of (a) 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; (b) adding said biomaterial mix to an emulsifier to form an emulsion; (c) isolating the formed structures from the emulsion; and  
15 optionally (d) incubating the formed structures with a polymerization agent.

[0061] In yet another aspect, there is provided a method of manufacturing microcapsules and/or macrostructure as described herein, using an emulsion based method. In this aspect, the method may comprise the steps of (a) mixing an aqueous phase, wherein the aqueous phase comprises a solution of biologically active material and/or cellular cargo with an oil or non-aqueous phase containing a  
20 emulsifier; (b) forming microcapsules and/or macrocapsules, microstructures and/or macrostructures or beads 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; (c) generating desired shapes and sizes using an extrusion or mould-based  
25 method; (d) allowing the aqueous phase containing biologically active material to solidify prior to a process that leads to smaller units; (e) isolating the formed microcapsules and/or macrocapsules from the emulsion; and optionally (f) incubating the formed microcapsules/macrocapsules with a polymerization agent.

[0062] In one example, the emulsifier may be SPAN<sup>®</sup>80 (IUPAC name is: [2-(3,4-dihydroxyoxolan-2-yl)-2-hydroxyethyl]octadec-9-enoate). In one example, the polymerization agent  
30 may be thrombin.

[0063] As used herein, the term “about”, in the context of a diameter of a microstructure, means +/- 5% of the stated value, +/- 4% of the stated value, +/- 3% of the stated value, +/- 2% of the stated value, +/- 1% of the stated value, or +/- 0.5% of the stated value.

[0064] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0065] The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0066] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

## EXPERIMENTAL SECTION

[0067] **Materials & Methods**

[0068] **Cell culture:**

[0069] Human bone marrow MSCs (ATCC), rat bone marrow MSCs (isolated from rat femur) and IMR-90 fibroblasts (ATCC) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C and 5% CO<sub>2</sub> in a humidified incubator.

[0070] **Encapsulation of stromal cells:**

[0071] Low temperature gelling agarose (Sigma) was dissolved and autoclaved in PBS at a stock concentration of 2% (w/v). Dextran sulfate (DxS) with a molecular weight of 500kDa was prepared as a stock solution of 10mg/ml in deionized water. A concentrated CaCl<sub>2</sub> dihydrate solution (2M) was prepared in deionized water. Collagen I (bovine skin, Advanced Biomatrix) was neutralized using 80% collagen stock solution 10% 10xPBS and 10% 0.1M NaOH. Final neutralized collagen stock had a concentration of 2.48 mg/ml. Fibrinogen stock was prepared fresh every time at a concentration of

40mg/ml in PBS. Thrombin aliquots were stored at a concentration of 2 mg/ml at -20°C. MSCs or fibroblasts were encapsulated at a cell concentration of  $0.5-1 \times 10^6$  cells in 100  $\mu$ l biomaterial. For this purpose, cells were harvested and the exact number of cells per sample was collected in a pellet. All components of the biomaterial mixture were brought to and kept at 37°C just before mixing. PBS or collagen I and/or fibrinogen were warmed to 37°C first, and then pre-warmed agarose was added at a final concentration of 0.4% (w/V). Next D<sub>x</sub>S and CaCl<sub>2</sub> were added and the biomaterial composition was gently mixed (Exact biomaterial composition details can be found in table 1). CaCl<sub>2</sub> increased cell viability during encapsulation and allowed isolation of fibrinogen-containing microcapsules, which otherwise strongly aggregated during the first purification step of the microcapsules. After biomaterial mixtures were prepared, they were mixed immediately with the cell pellet. The cell-biomaterial suspension was added into 1ml of pre-warmed soya bean oil with 0.5% (w/v) Span® 8 (Sigma Aldrich, USA). An emulsification was formed by manually shaking. Whole vial containing the emulsification was placed on ice for 10 min to allow solidification of agarose. The emulsion was layered on top of 1 ml of HBSS and centrifuged at 200g for 10 minutes. Solidified agarose acted as a scaffold for pre-mature microcapsules. The oil phase was removed and the microcapsules pellet gently collected and placed into fresh HBSS. Was no fibrinogen present in the microcapsule material, encapsulated cells are washed twice with HBSS. Fibrinogen was one of the components of the microcapsule material, 100  $\mu$ l of cell-biomaterial suspension were added to 1 ml of HBSS and incubated with 10  $\mu$ l thrombin at room temperature. Thrombin incubation took place on a horizontal shaker (400 rpm) for 30 minutes. Afterwards, encapsulated cells were collected and washed twice with full DMEM media. All encapsulated cells were plated into adherent surface plates over night to allow attachment of cells, which were not permanently trapped within the microcapsules. On the next day all microcapsules were transferred into non-adherent plates.

[0072] Table 1. Composition of one example of a microcapsule material

| V=100 $\mu$ l        | PBS/Col I/Fibrin                        | Ag         | D <sub>x</sub> S (10 mg/ml) | CaCl <sub>2</sub> |
|----------------------|-----------------------------------------|------------|-----------------------------|-------------------|
| PBS                  | 80 $\mu$ l                              | 20 $\mu$ l | 0.1-10 $\mu$ l              | 2 $\mu$ l         |
| Collagen I (2mg/ml)  | 80 $\mu$ l                              | 20 $\mu$ l | 0.1-10 $\mu$ l              | 2 $\mu$ l         |
| Fibrinogen (20mg/ml) | 40 $\mu$ l PBS/ 40 $\mu$ l Fibrinogen   | 20 $\mu$ l | 0.1-10 $\mu$ l              | 2 $\mu$ l         |
| Fibrinogen (20mg/ml) | 40 $\mu$ l Col I/ 40 $\mu$ l Fibrinogen | 20 $\mu$ l | 0.1-10 $\mu$ l              | 2 $\mu$ l         |
| Collagen I (1mg/ml)  | Fibrinogen                              |            |                             |                   |

25 [0073] **Live-dead cell staining**

[0074] Live dead cell staining was performed accordingly to manufacturer's instruction (Life technology, L3224). Microcapsules were collected and washed with PBS. They were incubated in 200  $\mu$ l staining solution consisting of PBS with Calcein AM (1:1600 dilution) and ETDH-1 (1:400 dilution) for 45 minutes. Microcapsules were pelleted and 15  $\mu$ l of pellet was pipetted onto a glass slide, covered with a cover slip and sealed with nail polish. Samples were imaged immediately

afterwards using the Nikon upright Ni-E microscope. Staining areas were quantified using ImageJ software.

[0075] **Immunostaining/ phalloidin staining and microscopy**

[0076] Microcapsules were collected, washed twice with PBS and fixed with 4% paraformaldehyde for 10 minutes with 3 subsequent PBS washes. For DxS tracking FITC-conjugated DxS was used and no further staining was necessary for imaging. The images were taken with the Nikon N-STORM/TIRF microscope at 40x magnification and an exposure time of 10 seconds. Samples without added DxS did not give any signal. For phalloidin staining alone, microcapsules were dried on a glass slide. Immunostaining took place in suspension. For phalloidin and immunostaining, fixed microcapsules were blocked for 1 hour with 3%BSA and incubated for 1.5 hours with the primary antibody. After 3 washes with PBS, samples were incubated with FITC-conjugated phalloidin and/or fluorescent-labelled secondary antibodies for 30 minutes. For ECM imaging, stained microcapsules were embedded in a fibrin gel (5mg/ml) for confocal microscopy (Olympus Multi View 1200 System). Stained cryosections were mounted and imaged using an epifluorescence microscope (Olympus, IX71). Phase contrast pictures of live samples for microcapsule diameter quantifications were taken with a Nikon TS100-F(1) microscope. Quantifications were performed using ImageJ software.

[0077] **Collagen fibrillogenesis measurements**

[0078] All described material mixtures were prepared as described in the protocol for encapsulation of stromal cells excluding cells and  $\text{CaCl}_2$ . They were added into a 96 wells-plate and absorbance was measured immediately at 37°C using a plate reader over a duration of 2 hours.

[0079] **VEGF ELISA**

[0080] Encapsulated mesenchymal stem cells (MSCs) were cultured either alone or in the presence of 10  $\mu\text{M}$  CPX (ciclopirox olamine) for 24 hours. Supernatants of encapsulated cells were analyzed for secreted VEGF using the DuoSet ELISA kits (R&D System) following the manufacturer's instructions. Results were normalized to cell numbers of originally encapsulated MSCs.

[0081] **Differentiation into adipocytes and osteoblasts**

[0082] Encapsulated cells were seeded into adherent surface 24 well-plate wells and allowed to migrate out of capsules for 1 week. Osteogenic differentiation took place directly after that. For adipogenic differentiation, cells were cultured until confluence before differentiation. For osteoblast differentiation, media composed of HG DMEM containing 10% FBS and 1% P/S with dexamethasone ( $1 \times 10^{-7}$  M), ascorbic acid at 100  $\mu\text{M}$  and  $\beta$ -glycerophosphate at 10 mM, was prepared freshly every time. Media was changed every 3 to 4 days for 4 weeks. Cells were washed 2 times with PBS, fixed using 4% formaldehyde and washed 2 more times with PBS. Deposited

hydroxyapatite was stained using 40 mM alizarin red. Wells were washed 3 times with water and air-dried. For adipocyte differentiation, induction media was composed of HG DMEM containing 10% FBS and 1% P/S with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1 $\mu$ M dexamethasone, 0.2 mM indomethacin and 10  $\mu$ g/ml insulin and the maintenance media of HG DMEM 10% FBS and 1% P/S and 10  $\mu$ g/ml insulin. Cells were induced for 4 days and then kept in maintenance media for 3 days. After 3-4 cycles of induction cells were washed 2 times with PBS, fixed using 4% formaldehyde and washed 2 more times with PBS. Lipid droplets were stained with lipophilic Nile red fluorescent dye (0.05 mg/ml in water) and DAPI (0.5  $\mu$ g/ml) for 1 hour. Wells were washed 3 times with PBS and stored in PBS. Cells were viewed with an epifluorescence microscope (Olympus, IX71).

10 [0083] **CCK-8 proliferation assay**

[0084] Cell counting Kit-8 (CCK-8) assay (Sigma Aldrich, USA) was performed according to manufacturer's instructions. Encapsulated cells were cultured in non-adherent plates and were transferred into adherent surface plates one day before CCK-8 assay was performed to isolate single cells. On the day of the assay encapsulated cells were re-suspended in fresh media and 150  $\mu$ l of cell suspension were mixed with 15  $\mu$ l of CCK-8 solution in a 96 wells-plate. Incubation took place for 4 hours at 37°C and 5% CO<sub>2</sub> in a humidified incubator. Cell suspensions were collected and centrifuged and 100  $\mu$ l of the supernatant were placed into a new 96 well plate. Absorbance readings were taken at 450 nm and 650 nm for reference. Results are plotted as a fold-change from absorbance reading on day 1.

20 [0085] **FBR *in vivo***

[0086] All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC, A\*STAR, Singapore). Mechanical stability and host body response towards the material was evaluated by injecting empty microcapsules into the leg muscle (thigh and calf) of Wistar rats. The rats were euthanized immediately post injection or after 4 weeks, respectively. Tissues were isolated and fixed with paraformaldehyde for 2-3 hours. After overnight incubation in 20% sucrose, tissues were embedded in OCT and cryosectioned. Cryosections were then immunostained.

[0087] **Cardiac surgery and MRI**

[0088] Male Wistar rats (~250 g) were anaesthetized with 2% isoflurane-oxygen (intubated and ventilated using a small animal ventilation apparatus (VetTech)). A thoracotomy was performed at the fourth intercostal space, and a 7/0 premilene suture was tied around the left anterior descending coronary artery approximately 3 mm from its origin. The ischemic area was identified visually on the basis of blanching. Immediately after coronary artery occlusion, 1 $\times$ 10<sup>6</sup> labelled cells (suspended in 100  $\mu$ l of PBS) were implanted into both the lesioned and peri-infarcted areas (three sites of injection;

50 µl into infarcted area and 25 µl into two peri-infarcted areas) with a 27-gauge needle. Animals received analgesics and antibiotics for three days post-surgery.

[0089] Post-implantation stem cell localization and heart function were assessed in anaesthetized rats using high-resolution MRI acquired on a 7T horizontal-bore animal scanner (Cliniscan, Bruker) 2 days post-implantation and then on week 1, 2, 4 and 6 using a cardiac array coil.

[0090] A stack of contiguous 2 mm short-axis ECG-gated gradient echo cine-MRIs were acquired to cover the entire left ventricle using the following parameters: TR=7.1 ms, TE=1.43 ms, FOV=60×60 mm, matrix 256×256, flip angle 25°, slice thickness 2 mm, 6 averages, 24 frames per cycle.

## [0091] Results

### [0092] Supplementation of microcapsules with ECM proteins and DxS supports MSC survival.

[0093] Microcapsules were generated by suspending biomaterial-cell mixtures in a water-in-oil emulsion, using soybean oil containing 0.5% SPAN<sup>®</sup> 80. Agarose concentration as low as 0.4% was sufficient to generate stable microcapsules. Therefore all biomaterial compositions containing dextran sulfate (DxS), collagen I and/or fibrinogen were mixed with 0.4% agarose. Microcapsules containing fibrinogen were incubated additionally with thrombin to yield fibrin. Live-dead cell staining at early time-points were used to evaluate the ability of the material to support mesenchymal stem cells survival (Fig. 1).

[0094] The effects were quantified by calculating the ratio of live to dead cell staining area. It is apparent even at day one that the addition of collagen I to the encapsulation media significantly increased the live-to-dead cell staining ratio (Fig. 1a,b). Interestingly, the addition of dextran sulfate (DxS) to agarose alone decreased the ratio in a dose-dependent manner (Fig. 1,b). Substitution of collagen I with fibrin, without DxS or at lower DxS concentrations, resulted in strong aggregation of capsules during washing. At higher DxS concentration, instable capsules were formed, releasing many cell clusters during handling (Fig. 1a, arrows). However, remaining capsules showed good cell survival, higher than compared to any of the collagen I - agarose capsules (Fig. 1a,b). Similarly, to fibrin-agarose microcapsules, a combination of collagen I and fibrin did not result in stable capsules at low DxS concentrations. However, at higher concentrations of DxS, stable microcapsules were formed with a peak live-to-dead cell staining area ratio obtained with 100 µg/ml DxS (Fig. 1a,b).

[0095] Following three days of cultivation, microcapsules lacking ECM proteins and/or containing high concentrations of DxS (1000 µg/ml) resulted in low cell densities (Fig. 1c). However, capsules containing collagen I or collagen I mixed with fibrin resulted in a similar density of cells (Fig. 1c). It was also observed that the live-to-dead cell ratios were only comparable between

conditions of a similar cell density. Low densities of cells (due to early cell death) resulted in lower number of dead cells (red staining), therefore high values for live-to-dead cell ratios. Therefore, the present disclosure compared microcapsules containing collagen I and DxS (concentrations 0-100 µg/ml) or collagen I mixed with fibrin and 100 µg/ml DxS. Cell survival in capsules increased in a dose-dependent manner with the DxS concentration (Fig. 1d). Highest cell survival rates were found in microcapsules composed of a mixture of collagen I, fibrin and 100 µg/ml DxS (Fig. 1d).

[0096] **DxS modulates collagen I fibrillogenesis**

[0097] The effect of dextran sulfate on collagen I fibrillogenesis was assessed by time-resolved spectrophotometry. Upon aggregation of collagen monomers into fibers, collagen solutions absorb light at 313 nm due to the ability of tubular structures (fibers) to diffract near-UV light. Absorbance at 313 nm increases over time in a sigmoidal fashion, from which the rates of collagen nucleation and elongation can be derived. During nucleation, collagen I monomers assemble to form nuclei, which are further elongated by additional monomers to form collagen fibers. The presence of DxS, from 0 to 20 µg/ml, delayed the increase of absorbance from the baseline and reduced the rate of absorbance increase (Fig. 2a). No significant increase in absorbance was observed for DxS concentrations 50 and 100 µg/ml. Calculated nucleation and elongation rates of collagen, calculated from sigmoidal curves, both decreased in the presence of DxS in a dose-dependent manner (Fig. 2b). Rates for DxS concentrations 50 and 100 µg/ml could not be calculated due to any change in absorbance. Resulting collagen hydrogels were imaged in 3D via confocal reflection microscopy according to methods known in the art, for example Jawerth LM, Munster S, Vader DA, Fabry B, Weitz DA. A blind spot in confocal reflection microscopy: the dependence of fiber brightness on fiber orientation in imaging biopolymer networks. Biophys J 2010; 98(3): L1-3. The presence of DxS markedly influenced the architecture of gels, reducing the amount of visible fibers for DxS concentrations between 0 and 10 µg/ml (Fig. 2c). No structure was visible for DxS concentrations of 20 µg/ml and above. Agarose affected collagen fibrillogenesis only in the absence of DxS as assessed by turbidimetry. Agarose (0.4%) induced an early nucleation in the absence of DxS, but had no significant effect in the presence of 100 µg/ml DxS (Fig 2d).

[0098] **DxS aggregates with collagen I and enhances cell-microenvironment interactions.**

[0099] FITC-conjugated DxS at the optimal concentration of 100 µg/ml was tracked in microcapsules, with and without supplementation of collagen I (Fig. 3a). DxS could only be tracked in agarose-collagen I capsules, whereas in agarose capsules alone fluorescent signal was below the detection limit (Fig. 3a). Fluorescent signal from DxS co-localized with collagen fibrils within the microcapsules, indicating DxS-collagen I interactions (Fig 3a, magnified). Overall this indicates that DxS aggregated with collagen monomers and inhibited fiber elongation.

[00100] Aggregation of collagen I monomers by DxS will lead to a different presentation of binding sites to the cells. Phalloidin staining of the actin cytoskeleton confirmed encapsulated cells form extrusion in the presence of collagen I, which are further enhanced by DxS supplementation (Fig. 3b). Therefore co-localization of collagen and DxS, and aggregation of collagen I monomers by  
 5 DxS enhances cell-ECM interactions.

[00101] **Microcapsules composed of collagen I, fibrin and 100 µg/ml DxS (Col-Fb-DxS100) demonstrate optimal characteristics and functionality**

[00102] Live-dead cell staining allows the monitoring of cell survival over only a short period of time. In order to monitor cell numbers over a longer period, proliferation assays are necessary. Human  
 10 MSC survival and proliferation over three weeks were tested with a metabolic assay (CCK-8) under selected conditions. The measured enzymatic activity of dehydrogenases is considered to be directly proportional to cell numbers in this assay. Starting at day 2 post encapsulation, cell numbers in microcapsules containing only agarose remained constant over 3 weeks (Fig. 4a), indicating that majority of cell death was occurring shortly post encapsulation (Fig. 1 a,c). MSCs in collagen I –  
 15 agarose microcapsules with or without DxS displayed no significant increase in cell number (Fig. 4a). Therefore, the presence of collagen I and DxS seemed only to affect cell survival in the initial post encapsulation phase (Fig. 1). Interestingly, microcapsules consisting of agarose supplemented with collagen I, fibrin and 100 µg/ml DxS (Col-Fb-DxS100) seem not only to be superior to other conditions shortly after cell encapsulation (Fig. 1), but also supported cell proliferation and survival in  
 20 long-term culture (Fig. 4a). As a result it was decided to investigate this biomaterial composition further.

[00103] As Col-Fb-DxS100 microcapsules showed the best support of cell survival, microcapsule properties and cell functionality within capsules were further investigated. Microcapsules had an average diameter of  $106 \mu\text{m} \pm 26 \mu\text{m}$  (average  $\pm$  SD) (Fig 4 b, and c). When cells in microcapsules  
 25 were cultured on adherent tissue culture plates, cells were able to migrate out of capsules, indicating the presence of large pore sizes allowing the exit of mesenchymal stem cells (Fig. 4b). Interestingly attached microcapsules could be washed off easily from the plates, leaving cells and cell debris behind, indicating that microcapsules did not absorb on to the surface, but were only partially attached via outwardly migrated cells.

[00104] The efficacy of MSC on tissue repair is currently thought to be via a dual mechanism, namely the ability of MSCs to differentiate into other cell types into functional tissue, and via the secretion of paracrine factors, to support the host tissue. Therefore functionality of MSCs post encapsulation was evaluated (Fig. 5).

[00105] Encapsulated human MSCs were shown to secrete VEGF, an important growth factor for  
 35 angiogenesis and therefore tissue repair (Fig. 5a). Hypoxic conditions were emulated using ciclopirox

olamine (CPX), a prolyl hydroxylase inhibitor. Emulating hypoxic conditions, CPX administration stabilizes the transcription factor HIF-1 $\alpha$ , which then activates various down-stream angiogenic processes. Therefore, incubation with CPX resulted in a significant (24%) increase in VEGF secretion, indicating that encapsulated MSCs secrete paracrine factors and respond to external/environmental stimuli (Fig. 5a). Furthermore, migrated MSCs were subjected to standard differentiation protocols into adipocytes and osteoblasts (Fig. 5b). Successful differentiation was confirmed by both Nile red staining of accumulated fat droplets in adipocytes, and alizarin red staining of hydroxyapatite deposited in osteoblast cultures (Fig. 5b).

[00106] In order to study cell-microenvironment interactions, encapsulated cells were stained with phalloidin (green fluorescent – seen as bright spots) and dapi (blue nucleus- seen as dull spots). ECM proteins (collagen I, collagen IV, fibronectin and heparin sulfate proteoglycans (HSPG)) were immunostained (red fluorescent) (Fig. 5c). A strong staining for collagen I was confirmed within microcapsules, however the fluorescent intensity decreased in the presence of encapsulated MSCs, indicating degradation by cells (Fig. 5c). Higher magnification of sections through capsules and cells confirmed newly synthesized collagen I in close proximity to the cytoskeleton. Collagen IV was observed at a similar location close to the cytoskeleton, although it appeared more frequent. A similar pattern was observed for HSPGs (Fig. 5c).

[00107] **Microcapsules composed of collagen I, fibrin and 100  $\mu$ g/ml DxS (Col-Fb-DxS100) do not induce a fibrotic body response and are biodegradable *in vivo***

[00108] *In vitro* results of Col-Fb-DxS100 microcapsules were promising and therefore they were tested for their functionality *in vivo*. Microcapsules were injected into rat thigh muscle using a 27 gauge needle and animals were euthanized immediately. Cryosections through the muscle confirmed intact capsules between muscle fibers (Fig. 6a). Therefore microcapsules exhibit sufficient mechanical stability for intramuscular injection.

[00109] To study the foreign body response, microcapsules were injected into rat calf muscles, and animals euthanized four weeks later. Upon initiation of a foreign body response, a fibrotic capsules should form around the implant after this period of time. Microcapsules were generated using TRITC-conjugated agarose to allow easier tracking of capsules. Strong signal from TRITC could be detected in small areas in the muscle (Fig. 6b). However, no spherical shape was observed, indicating that microcapsules disintegrated over time. Hematoxylin and Eosin (H&E) staining confirmed an infiltration of cells (Fig. 6b).

[00110] Immunohistochemistry identified that many of the infiltrated cells expressed CD11b, confirming their monocytic origin (Fig. 6c). Of note, a specifically strong CD11b staining was found in close proximity to biomaterial, indicating that macrophages directly attach and surround to the biomaterial (Figure 6c, CD11b). Higher exposure times identified small traces of TRITC-labeling in

close proximity to CD11b positive cells around the main biomaterial location (6c, white arrows), indicating that biomaterial was phagocytized and transported out by macrophages. Very little staining for the inflammatory marker iNOS, an M1 marker, was observed indicating that microcapsules did not polarize the endogenous macrophages into a chronic proinflammatory state (Fig. 6c). On the other hand, cells positive for the mannose receptor (CD206) a M2 marker were prominent around the biomaterial, indicating an anti-inflammatory and 'wound-healing' state around the biomaterial (Fig. 6c). In accordance with this finding, collagen I staining appeared only in the spaces in between the muscle fibers (as expected), but was absent around the microcapsule fragments, indicating that no fibrotic response was induced (Fig 6c), which normally can be identified by a dense parallel alignment of collagen fibers. A similar loose and random distribution of fibers was observed for collagen type III around the biomaterial. In general high collagen III content in ECM is a signature of granulation tissue, further indicating the 'wound-healing' state around the biomaterial. This observation was further supported by cells stained positive for rat endothelial cell antigen 1 (Reca-1) around the biomaterial (Figure 6c), indicating the presence of infiltrated endothelial cells and blood vessels. Therefore Col-Fb-DxS100 microcapsules degraded slowly *in vivo*, did not induce a fibrotic foreign body response but rather induced a healing environment around the implant.

**[00111] USPIO-labelled rat bmMSCs encapsulated in collagen I, fibrin and 100 µg/ml DxS (Col-Fb-DxS100) microcapsules can be tracked long-term by MRI, when injected into the myocardium.**

[00112] As Col-Fb-DxS100 microcapsules showed a reasonable response *in vivo*, they were tested for the suitability to deliver MSCs into the myocardium. MSCs were isolated from wistar rat femur bone marrow, expanded and labelled with TAT peptide derivatized ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles coated with dextran (IODEX-TAT-FITC). 10 weeks old wistar rats underwent a thoracotomy at the fourth intercostal space with subsequent left anterior descending coronary artery (LAD) ligation.

[00113] Labelled cells were injected either as a cell suspension or encapsulated into the infarcted areas, as well as the peri-infarct area. Rats underwent T1-weighted multislice cine-imaging of the short axis of the left ventricle. MSCs, injected as single cells, were either not detected at all or only at an early time-point post-surgery (Fig. 7a, first image, arrow). In contrast, encapsulated MSCs could be tracked for the whole duration of the study, although signal intensity decreased overtime (Fig. 7, arrows in remaining images). Thereafter, animals were euthanized and heart tissues were harvested for further analysis. Prussian blue staining (Fig. 7b, blue staining) enabled visualization of iron-oxide nanoparticles in H&E stained sections. Areas with visualized nanoparticles were observed in both conditions: injected MSC suspensions and encapsulated MSCs (Fig. 7b). In both conditions, prussian blue staining appeared always in areas of connective tissue, indicative of remodeled tissue due to the

infarct. However, cardiac sections with injected encapsulated cells showed larger patches of staining in general. As no FITC signal was detectable in the tissue sections, an anti-FITC antibody (Fig. 7c, red) was used to visualize the IODEX nanoparticles. Sections were co-stained against CD11b to ensure that nanoparticles were still within the transplanted rat MSCs and not taken up by macrophages (Fig. 7c). Staining for nanoparticles co-localized or overlaid with oval-shaped intact nuclei in both conditions, indicating integrated stromal cells (Fig. 7c). An overlapping with CD11b staining was very rare (MSCs suspension, white arrow), but confirmed that dead transplanted MSCs were scavenged by macrophages. Interestingly, in cardiac sections with injected encapsulated MSCs some of the larger patches of stained nanoparticles were surrounded or in proximity to macrophages (CD11b staining). The CD11b staining was often not in direct contact with staining for nanoparticles and showed a pattern comparable to the one observed around the biomaterial composition in the host response study (Fig. 6c). Therefore CD11b staining was an indirect indication of the retention of microcapsule. Of note, a fraction of transplanted rat MSCs had migrated out of the microcapsules and integrated into the host tissue, whereas some of the transplanted MSCs were still found within the biomaterial (Fig. 6c). Therefore Col-Fb-DxS100 capsules were proven suitable to deliver therapeutic cells into the myocardium.

[00114] The optimized microcapsule biomaterial for cell encapsulation is a finely tuned composite of agarose, collagen I, fibrin and DxS. All components play a role for the performance of the microcapsule. Although materials such as agarose (or substituted by alginate) in combination with collagen, fibrin and fibronectin have been investigated before, the resulting microcapsule biomaterials had limited performance. Most encapsulated cells (MSCs or fibroblast) had a decreased viability over time; cell-material interaction, cell functionality post encapsulation, and *in vivo* applications were often not examined. Furthermore, this proves that the supplementation of microcapsules with extracellular matrix (ECM) proteins alone is not sufficient for the optimal performance of cells and biomaterial. In addition to fibrous and non-fibrous proteins, important components of the extracellular matrix (ECM) are glycosaminoglycans (GAGs). As all GAGs are negatively charged polycarbohydrates, the present disclosure used dextran sulfate (DxS), an off-the-shelf sulfated glucose polymer as a substitute for GAGs. Dextran and DxS are degraded into glucose and non-toxic. Both are approved for medical applications. Interestingly (as it is a surprising and unexpected finding), DxS plays a role for production of microcapsules containing fibrin, as the absence of DxS leads to aggregation of fibrin-containing microcapsules during purification from the oil phase. Moreover, DxS has additional functionality, in that it interacts with the supplemented proteins, enhancing cell-microenvironment interaction with downstream effects on cell survival. Nevertheless, it is the combination of all the components, which allows for the optimal functionality of the biomaterial. Collagen I and fibrin provide mechanical stability and anchorage for the cells, and are both approved

for biomedical application. Moreover, even the refined soybean oil, which was used in processing the microcapsules, is FDA-approved as a lipid injectable emulsion for intravenous feeding. Span®80 and agarose are food additives and generally recognized as safe (GRAS) by FDA. By selecting approved or safe materials for the biomaterial composition, a translation into a clinical setting greatly is simplified.

[00115] Once the microenvironment within the microcapsules is established, it was surprisingly found that MSCs show not only increased survival, but also proliferation rates within the microcapsules. MSCs perform all functions that are reported to contribute to tissue repair when transplanted, as they are able to differentiate into other lineages and secrete paracrine factors.

[00116] On translation into an *in vivo* setting, the microcapsule composition exhibited sufficient mechanical stability for intramuscular injection and a reasonable body response with phagocytizing macrophages, but without a fibrotic capsule formation. It is notable that the body response was studied in the absence of MSCs, which possess inherent immunosuppressive properties. It was demonstrated previously that the strong foreign body response towards alginate-PLL microcapsules could be alleviated by encapsulated MSCs.

[00117] Microcapsules degrade slowly *in vivo*, therefore allowing a slow and gradual release of cells into the host tissue. Moreover, when injected into the myocardium of immune-competent rats, MR signal from encapsulated MSCs could be tracked for the duration of the study. In contrast, injections of single cell suspension resulted in no or a weak signal at early time-points post surgery.

Therefore Col-Fb-DxS100 microcapsules proved to be suitable for stem cell delivery into the myocardium. Furthermore, stem cell delivery in Col-Fb-DxS100 microcapsules seems to overcome current shortcoming of limited cell retention in cell-based therapies of heart diseases and therefore could improve the therapeutic effect of MSCs.

**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.

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.
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. 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.
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.
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.
19. The structure of claim 18, wherein the structure is a microcapsule having a diameter of between 1 to 2000  $\mu\text{m}$ .
20. The structure of claim 2, wherein the structure is a macrocapsule having a diameter of between 2 to 20 mm.
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.
23. The structure of any one of claims 1 to 22, wherein the structure contains between 1 to 8000  $\mu\text{g}$  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).
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, 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.
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
- 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.
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:
- 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 an 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;
  - v. Isolating the formed microcapsules or macrocapsules from the emulsion; and optionally
  - vi. 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.