CLAIMS

1. A method of manufacturing hydrogel microparticles comprising one or more species of living cells attached thereon and/or encapsulated therein, the method comprising:-
    a) dissolving a hydrogel-forming agent in an aqueous medium to form a solution, wherein the hydrogel-forming agent comprises a physically cross-linkable polymer;
    b) suspending one or more species of living cells in the solution to form a cell suspension;
    c) dispersing the cell suspension into an organic oil to form a microemulsion; and
    d) subjecting the microemulsion to conditions that allow the hydrogel-forming agent to form hydrogel microparticles comprising one or more species of living cells attached thereon and/or encapsulated therein.

2. The method according to claim 1, wherein the physically cross-linkable polymer is selected from the group consisting of gelatin, alginate, pectin, furcellaran, carageenan, chitosan, derivatives thereof, copolymers thereof, and mixtures thereof.

3. The method according to claim 1 or 2, wherein the hydrogel-forming agent comprises or consists essentially of gelatin.

4. The method according to any one of claims 1 to 3, wherein the amount of hydrogel-forming agent in the solution is between about 1 % (w/v) to about 10 % (w/v), wherein the amount of hydrogel-forming agent in the solution is preferably about 5 % (w/v).

5. The method according to any one of claims 1 to 4, (i) wherein the living cells are eukaryotic cells or prokaryotic cells or archaea, and/or (ii) wherein the living cells comprise or consist essentially of chondrocytes.

6. The method according to claim 5, wherein the eukaryotic cells are non-anchorage dependent cells, wherein the non-anchorage dependent cells are preferably selected

from the group consisting of chondrocytes, embryonic stem cells, adult stem cells, endodermal lineage cells, and carcinoma cells used for regenerative medicine.

7. The method according to claim 5, wherein the eukaryotic cells are anchorage dependent cells, wherein the anchorage dependent cells are preferably selected from the group consisting of osteogenic cells, fibroblasts, epidermal cells, adipocytes, neural cells, endothelial cells, epithelial cells, keratinocytes, hepatocytes, myocytes, cells from joint ligaments, and cells from the nucleus pulposis.

8. The method according to any one of claims 1 to 7,
(i) wherein the organic oil is selected from the group consisting of soya oil, corn oil, sunflower oil, rapeseed oil, cotton seed oil, peanut oil, olive oil, sesame seed oil, rice germ oil, fish oil, whale oil, palm oil, coconut oil, hemp oil, canola oil, wheat germ oil, safflower oil, linseed oil, tung oil, castor oil, and mixtures thereof; and/or
(ii) wherein the organic oil comprises or consists essentially of soya oil; and/or
(iii) wherein dissolving the hydrogel-forming agent in the aqueous medium is carried out at a temperature in the range from about 20 °C to about 45 °C, wherein dissolving the hydrogel-forming agent in the aqueous medium is preferably carried out at a temperature of about 37 °C.

9. The method according to any one of claims 1 to 8, (i) wherein dispersing the cell suspension into the organic oil is carried out under continuous stirring; and/or (ii) wherein subjecting the microemulsion to conditions that allow the hydrogel-forming agent to form hydrogel microparticles comprises cooling the microemulsion at a temperature in the range from about 0 °C to about 10 °C.

10. The method according to any one of claims 1 to 9, further comprising extracting the organic oil after the hydrogel microparticles are formed and at least one centrifugation and at least one washing step after extracting the organic oil.

11. A composition comprising a mixture of a degradable hydrogel and at least one hydrogel microparticle comprising one or more species of living cells attached

thereon and/or encapsulated therein, wherein the at least one hydrogel microparticle is obtainable by the method of any one of claims 1 to 10.

12. The composition of claim 11, wherein at least one of the degradable hydrogel and the at least one hydrogel microparticle comprises a porogen agent that effects degradation of the hydrogel microparticle.

13. The composition according to claim 12,

(i) wherein the porogen agent further comprises an enzyme selected from the group consisting of a protease, a glycosidase, an actinase, a caseinase, a chondroitinase, a dermatanase, an elastase, a gelatinase, a heparanase, a hyaluronidase, a keratinase, a lipase, a metalloproteinase, a staphylokinase, a streptokinase, chymotrypsin, endopeptidase V8, trypsin, thermolysin, pepsin, plasmin, and combinations thereof; and/or

(ii) wherein the degradable hydrogel is selected from the group consisting of hydrogels made from natural polymers, synthetic hydrogels, and their combination thereof, wherein (a) the natural polymer is preferably selected from the group consisting of polysaccharides, glycosaminoglycans, and proteins, wherein the polysaccharide is preferably selected from the group consisting of alginate, agarose, chitosan, dextran, starch, and gellan gum; (b) the synthetic hydrogel is preferably selected from the group consisting of hydrogels made from a hydrophilic monomer, hydrogels made from a hydrophilic polymer, hydrogels made from a hydrophilic copolymer, and combinations thereof, wherein the hydrophilic monomer is preferably selected from the group consisting of 2-hydroxyethyl methacrylate, 2-hydroxyethyl acrylate, 2-hydroxyethyl methacrylamide, 2-hydroxyethyl acrylamide, N-2-hydroxyethyl vinyl carbamate, 2-hydroxyethyl vinyl carbonate, 2-hydroxypropyl methacrylate, hydroxyhexyl methacrylate, hydroxyoctyl methacrylate, acrylic acid, methacrylic acid, itaconic acid, fumaric acid, crotonic acid, maleic acid, monomethyl maleate ester, monoethyl maleate ester, monomethyl fumarate ester, monoethyl fumarate ester, (meth)acrylamide, crotonic amide, cinnamic amide, maleic diamide, fumaric diamide, methanethiole, ethanethiol, 1-propanethiol, butanethiol, tert-butyl mercaptan, pentanethiols, p-styrenesulfonic acid, vinylsulfonic acid, p-a-

methylstyrenesulfonic acid, isoprene sulfonide and salts thereof, wherein the hydrophilic polymer is preferably selected from group consisting of polymers and oligomers of glycolide, lactide, polylactic acid, polyesters of a-hydroxy acids, including lactic acid and glycolic acid, such as the poly(a-hydroxy) acids including polyglycolic acid, poly-DL-lactic, poly-L-lactic acid, and terpolymers of DL-lactide and glycolide, e-caprolactone and e-caprolactone copolymerized with polyesters, polylactones and polycaprolactones including poly(e-caprolactone), poly(8-valerolactone) and poly (gamma-butyrolactone); polyanhydrides, polyorthoesters, other hydroxy acids, polydioxanone, collagen-hydroxyethylmethacrylate (HEMA), poly(hydroxylethyl methacrylate) (PHEMA), and other biologically degradable polymers that are non-toxic or are present as metabolites in the body; and/or

(iii) wherein the degradable hydrogel comprises alginate; and/or

(iv) wherein the hydrogel microparticle comprises a degradable material which degrades at body temperature; and/or

(v) wherein the hydrogel microparticle comprises surface cross-linked gelatin; and/or

(vi) wherein the hydrogel microparticle comprises gelatin which is cross-linked with a cross-linking agent, wherein the cross-linking agent is preferably selected from the group consisting of genipin, formaldehyde, glutaraldehyde, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), N-hydroxysulfosuccinimide (Sulfo-NHS), and mixtures thereof; and/or

(vii) wherein the hydrogel microparticle comprises gelatin which is cross-linked in a 0.25 wt% genipin solution; and/or

(viii) wherein the hydrogel microparticle has a maximal dimension of between about 50 μm to about 200 μm; and/or

(ix) wherein the composition is an injectable composition; and/or

(x) wherein the hydrogel microparticle has a surface which provides additional reactive groups which allow binding of further molecules; and/or

(xi) wherein the degradable hydrogel and/or the hydrogel microparticle further comprises at least one kind of biologically active molecule, wherein the biologically active molecule is preferably selected from the group consisting of growth factor, cytokine, antibiotic, anti-inflammatory agent, analgesic, and a cell attachment molecule.

14. A method of manufacturing a scaffold for tissue engineering, the method comprising:

a) providing a composition comprising a mixture of a degradable hydrogel and at least one hydrogel microparticle comprising one or more species of living cells attached thereon and/or encapsulated therein, wherein the at least one hydrogel microparticle is obtainable by the method of any one of claims 1 to 10;

b) incubating the composition under conditions which allow proliferation of the one or more species of living cells and degradation of the at least one hydrogel microparticle in the degradable hydrogel to allow the one or more species of living cells to proliferate and to allow the at least one hydrogel microparticle to degrade; and

c) degrading the degradable hydrogel of the incubated mixture to obtain a scaffold.

15. The method according to claim 14, wherein

(i) at least one of the hydrogel microparticle and the degradable hydrogel comprises a porogen agent that effects degradation of the hydrogel microparticle, (a) wherein the porogen agent comprises or consists essentially of an enzyme, wherein the enzyme is preferably selected from the group consisting of a collagenase, a protease, a glycosidase, an actinase, a caseinase, a chondroitinase, a dermatanase, an elastase, a gelatinase, a heparanase, a hyaluronidase, a keratinase, a lipase, a metalloproteinase, a staphylokinase, a streptokinase, chymotrypsin, endopeptidase V8, trypsin, thermolysin, pepsin, plasmin, and combinations thereof; and/or (b) wherein the porogen agent comprises or consists essentially of collagenase; and/or

(ii) the degradable hydrogel comprises one or more species of living cells, wherein the one or more species of living cells comprised in the degradable hydrogel and/or the hydrogel microparticle are preferably non-anchorage dependent cells selected from the group consisting of chondrocytes, embryonic stem cells, adult stem cells, endodermal lineage cells, and carcinoma cells used for regenerative medicine.