

Claims

1. A biofunctional three-dimensional hydrogel suitable for the growth and the expansion of freshly isolated or frozen intestinal cells and the formation of intestinal organoids therefrom, wherein said hydrogel is the reaction product of
 - at least one precursor molecule, which is a multi-arm PEG containing ethylenically unsaturated groups selected from the group consisting of vinylsulfone and acrylate moieties,
 - a crosslinker molecule containing at least two, preferably two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction, and
 - at least one biofunctional ligand selected from the group consisting of natural laminins, for example laminin-111, recombinant laminin-isoforms, preferably laminin-511, and biofunctional fragments thereof,characterized in that the crosslinker molecule comprises at least one RGD motif.
2. The hydrogel according to claim 1, wherein said multi-arm PEG is selected from the group consisting of a 4-arm and a 8-arm PEG.
3. The hydrogel according to claim 1 or 2, wherein said hydrogel has a shear modulus in the range of 50 to 1000 Pa.
4. The hydrogel according to any of the preceding claims, wherein said hydrogel is
 - i) the reaction product of a 8-arm PEG vinylsulfone cross-linked with a peptide comprising at least two thiol groups and at least two RGD motifs, or

- ii) comprises the reaction product of a 8-arm PEG vinyl-sulfone crosslinked with a peptide comprising at least two thiol groups and at least two RGD motifs and/or the reaction product of a 8-arm PEG acrylate crosslinked with a peptide comprising at least two thiol groups and at least two RGD motifs.
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5. The hydrogel according to any of the preceding claims, wherein the shear modulus of said hydrogel degrades over time.
 6. The hydrogel according to any of the preceding claims, wherein said hydrogel is insensitive to enzymatic degradation.
 7. The hydrogel according to any of the preceding claims, wherein said crosslinker molecule is a peptide comprising at least two RGD motifs and at least two cysteine moieties.
 8. The hydrogel according to claim 7, wherein said crosslinker molecule is Ac-GCREGRGDSPGGRGDSPGERCG-NH₂.
 9. Kit of parts for the growth and expansion of freshly isolated or frozen intestinal cells and the formation of intestinal organoids therefrom, comprising
 - at least one precursor molecule, which is a multi-arm PEG containing ethylenically unsaturated groups selected from the group consisting of vinylsulfone and acrylate moieties,
 - a crosslinker molecule containing at least two, preferably two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction, wherein the crosslinker molecule comprises at least one RGD motif, and

- at least one biofunctional ligand selected from the group consisting of natural laminins, for example laminin-111, recombinant laminin isoforms, preferably laminin-511, and biofunctional fragments thereof,
 - 5 - and a culture medium, wherein said culture medium comprises R-spondin 1, preferably R-spondin 1 conditioned medium, and Wnt3a, preferably Wnt3a conditioned medium.
- 10 10. Kit of parts according to claim 9, wherein said crosslinker molecule is a peptide comprising at least two RGD motifs and at least two cysteine moieties.
11. Kit of parts according to any of claims 9 to 10, wherein
15 said precursor molecule is an 8-arm PEG vinylsulfone or a combination of an 8-arm PEG vinylsulfone and an 8-arm PEG acrylate.
12. A method of preparing a three-dimensional hydrogel according
20 to any of claims 1 to 8, the method comprising the steps of
a1) dispensing one or more different hydrogel precursor molecules, which is a multi-arm PEG containing ethylenically unsaturated groups selected from the group consisting
25 of vinylsulfone and acrylate moieties, onto a substrate or into discrete volumes of a substrate, preferably a multi-well plate, and adding to said hydrogel precursor molecules
- one or more crosslinker molecules containing at least two, preferably two nucleophilic groups capable of
30 reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction, wherein said crosslinker molecule comprises at least one RGD motif, and

- at least one biofunctional ligand selected from the group consisting of natural laminins, for example laminin-111, recombinant laminin isoforms, preferably laminin-511, and biofunctional fragments thereof; or
- 5 a2) dispensing a resuspended unreacted powder, preferably a lyophilized unreacted powder, onto a substrate or into discrete volumes of a substrate, preferably a multi-well plate, wherein said unreacted powder comprises
 - one or more different hydrogel
 - 10 precursor molecules, which is a multi-arm PEG containing ethylenically unsaturated groups selected from the group consisting of vinylsulfone and acrylate moieties
 - one or more crosslinker molecules
 - 15 containing at least two, preferably two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction, wherein said crosslinker molecule comprises at least one RGD motif, and
 - 20 - at least one biofunctional ligand selected from the group consisting of natural laminins, for example laminin-111, recombinant laminin isoforms, preferably laminin-511, and biofunctional fragments thereof;
- 25 b) adding cells, preferably freshly isolated or frozen intestinal cells from a biopsy of a human, onto the substrate or into said discrete volumes of the substrate or into the hydrogel precursor formulations of a1) or a2) before addition onto the substrate or into said discrete volumes of the
- 30 substrate; and
- c) crosslinking said hydrogel precursor molecules and said crosslinker molecules to form a hydrogel.

13. A method for the growth and expansion of freshly isolated or frozen intestinal cells, comprising the steps of:
- a) de novo formation of organoids from freshly isolated or frozen human intestinal cells, using a kit of parts as described herein, by incubating the hydrogel of said kit with said freshly isolated or frozen intestinal cells in the presence of the culture medium of said kit,
 - b) growing, optionally passaging, and expanding of cells from the intestinal organoids from step a) using a kit of parts according to any of claims 9 to 11,
 - c) optionally differentiation of the organoids from step a), in the presence of a modified culture medium inducing cell differentiation,
- characterized that in said method only fully synthetic or fully defined semi-synthetic hydrogels are used.
14. The method according to claim 13, wherein in said method only fully defined semi-synthetic hydrogels are used which are self-degradable.
15. The method according to claim 13, wherein in said method only fully synthetic hydrogels are used which are self-degradable.