In particular, the present disclosure is related to a biofunctional three-dimensional hydrogel suitable for the growth and 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 and preferably it is not known to be enzymatically degradable.

In an aspect, there is provided 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 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, recombinant laminin-isoforms, and biofunctional fragments thereof,

characterized in that the crosslinker molecule comprises at least one RGD motif.

In another aspect, there is provided a 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 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, recombinant laminin isoforms, and biofunctional fragments thereof,
- and a culture medium, wherein said culture medium comprises R-spondin 1, preferably R-spondin 1 conditioned medium, and Wnt3a, preferably Wnt3a conditioned medium.

In another aspect, there is provided a method of preparing a three-dimensional hydrogel as defined above, 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 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 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
- at least one biofunctional ligand selected from the group consisting of natural laminins, recombinant laminin isoforms, and biofunctional fragments thereof; or

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 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 containing at least 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
- at least one biofunctional ligand selected from the group consisting of natural laminins, recombinant laminin isoforms, and biofunctional fragments thereof;

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 substrate; and

c) crosslinking said hydrogel precursor molecules and said crosslinker molecules to form a hydrogel.

In another aspect, there is provided 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 as defined above,

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.

Compared to the prior-art hydrogels from Gjorevski 2016 & 2017, WO 2017/036533 A1 and WO 2017/037295 Al, the hydrogel of the present disclosure is less expensive. Since it does not require the presence of enzymes and activating factors for crosslinking, the hydrogel of the present disclosure can be easily upscaled for commercial purposes. And this is also because gel precursors described above in this disclosure (i.e. multi-arm PEG

containing ethylenically unsaturated groups, crosslinker molecule containing two nucleophilic groups, and the at least one biofunctional ligand) do not require addtional steps for the preparation of the gel precursor as it is required in the prior art gels from Gjorevski 2016 & 2017, WO 2017/036533 A1 and WO 2017/037295 A1.

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 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, recombinant laminin-isoforms, and biofunctional fragments thereof,

   characterized in that the crosslinker molecule comprises at least one RGD motif.

2. The hydrogel according to claim 1, wherein the crosslinker molecule contains two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction.

3. The hydrogel according to claim 1, wherein the natural laminin is laminin-111 or the recombinant laminin-isoform is laminin-511.

4. The hydrogel according to any one of claims 1 to 3, wherein said multi-arm PEG is selected from the group consisting of a 4-arm and a 8-arm PEG.

5. The hydrogel according to any one of claims 1 to 4, wherein said hydrogel has a shear modulus in the range of 50 to 1000 Pa.

6. The hydrogel according to any of the preceding claims, wherein said hydrogel is
   i) the reaction product of a 8-arm PEG vinylsulfone crosslinked 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 vinylsulfone 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.

7. The hydrogel according to any of the preceding claims, wherein the shear modulus of said hydrogel degrades over time.

8. The hydrogel according to any of the preceding claims, wherein said hydrogel is insensitive to enzymatic degradation.

9. 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.

10. The hydrogel according to claim 9, wherein said crosslinker molecule is Ac-GCREGRGDSPGGRGDSPGERCG-NH_2.

11. 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 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, recombinant laminin isoforms, and biofunctional fragments thereof,
    - and a culture medium, wherein said culture medium comprises R-spondin 1, preferably R-spondin 1 conditioned medium, and Wnt3a, preferably Wnt3a conditioned medium.

12. Kit of parts according to claim 11, wherein the crosslinker molecule contains two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction.

13. Kit of parts according to claim 11, wherein the natural laminins is laminin-111 or the recombinant laminin-isoform is laminin-511.

14. Kit of parts according to any one of claims 11 to 13, wherein said crosslinker molecule is a peptide comprising at least two RGD motifs and at least two cysteine moieties.

15. Kit of parts according to any one of claims 11 to 14, wherein said precursor molecule is an 8-arm PEG vinylsulfone or a combination of an 8-arm PEG vinylsulfone and an 8-arm PEG acrylate.

16. A method of preparing a three-dimensional hydrogel according to any of claims 1 to 10, 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 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 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
- at least one biofunctional ligand selected from the group consisting of natural laminins, recombinant laminin isoforms, and biofunctional fragments thereof; or

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 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 containing at least 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
- at least one biofunctional ligand selected from the group consisting of natural laminins, recombinant laminin isoforms, and biofunctional fragments thereof;

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 substrate; and

c) crosslinking said hydrogel precursor molecules and said crosslinker molecules to form a hydrogel.

17. The method according to claim 16, wherein the crosslinker molecule contains two nucleophilic groups capable of reacting with said ethylenically unsaturated groups of said multi-arm PEG in a Michael addition reaction.

18. The method according to claim 16, wherein the natural laminin is laminin-111 or the recombinant laminin-isoform is laminin-511.

19. 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 according to claims 11 to 15, 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.

20. The method according to claim 19, wherein in said method only fully defined semi-synthetic hydrogels are used which are self-degradable.

21. The method according to claim 19, wherein in said method only fully synthetic hydrogels are used which are self-degradable.