appendiceal cancer patients were cultured in hyaluronic acid/collagen-based hydrogels to develop a platform for drug response prediction. However, like Matrigel also Collagen is a naturally-derived matrix and suffers from similar problems.

So far, there has not been a report of a successful expansion of freshly isolated or frozen human cells from biopsies or tissue resections (i.e. cells which have been obtained directly from a human and which have not been pre-cultured or pre-established in another system) and subsequent formation of organoids therefrom in a fully defined and/or a fully synthetic hydrogel matrix that is not a naturally-derived matrix such as Matrigel or collagen. Despite the clear need for such an approach, as stated in the above discussed prior art, until now the gold standard still is the use of Matrigel for at least the first step of expansion of the cells. This is proof for the difficulties involved in making a semi-synthetic or fully synthetic three-dimensional hydrogel system work.

It was the problem underlying the present invention to provide a method for expansion of freshly isolated or frozen human cells from biopsies and subsequent formation of intestinal organoids therefrom, wherein said method completely avoids the use of a naturally-derived matrix such as Matrigel and provides intestinal organoids suitable for clinical applications and generated in a commercially feasible manner, i.e. cost-effective, reliable, reproducible, automatizable and up-scalable.

According to the present disclosure, said problem is solved by a hydrogel, a kit of parts and a method as defined in the independent claims.

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, 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.

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, 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,
- 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 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 Al.

Especially, it is very suitable for manufacturing upscale and automatization of their use, i.e. for the application of the method using pipetting robots etc. The hydrogel of the present disclosure is very reliably reproducible. Furthermore, in contrast to the enzymatically cross-linked gel of Gjorevski et al., the hydrogel of the present disclosure is compatible with commercially available culture media, e.g. IntestiCult™ Organoid Growth Medium from STEMCELL Technologies, i.e. it does not disintegrate prematurely.

As compared to the prior-art hydrogels from Cruz-Acuna 2017 & 2018 and WO 2018/165565 A1, the hydrogel of the present disclosure is based on a different chemistry, more suitable for automatization, i.e. for the application of the method using pipetting robots etc., and can be up-scaled more easily. This is because, during the manufacturing process all gel precursor materials of the present disclosure (i.e. multi-arm PEG containing ethylenically unsaturated groups, crosslinker molecule containing two nucleophilic groups, and the at least one biofunctional ligand) can be premixed and lyophilized without reacting prematurely, which is not possible with the system described by Cruz-Acuna et al., as their precursors would react under those conditions.

An important aspect of the hydrogel of the present disclosure is the fact that the at least one RGD motif is provided within the crosslinker molecule, as compared to the prior art hydrogels where the RGD motif is provided in a ligand that is attached (in a “dangling” manner) to the crosslinked hydrogel. The approach of the present disclosure allows the increase of the amount of RGD motifs in the hydrogel to a considerable extent. It has been surprisingly found that this results in a significant improve-

ment of the hydrogel characteristics for the purposes of the present disclosure.

According to the present disclosure, it has been surprisingly found that the hydrogels discussed herein are suitable for de novo formation of organoids from freshly isolated or frozen intestinal cells (single cells and/or clusters) from a human biopsy or tissue resection, the growth, passaging and expansion of these cells and optionally subsequent differentiation of organoids therefrom, when used in combination with a suitable culture medium. Thus, according to the present disclosure, the necessity of having to use a naturally-derived matrix such as Matrigel is completely avoided.

According to the present invention, the term “freshly isolated or frozen human cells from biopsies or tissue resections” refers to cells which have been obtained directly from a human by any of the mentioned procedures and which have not been pre-cultured or pre-established in another system before being used in a method of forming organoids. Typically, such fresh cells are collected and used in the method of the present invention immediately or within a period of up to 3 to 4 days. If the cells are not used immediately after collection, they may be frozen for storage purposes, under conventionally used conditions. The collected cells may be single and/or “clusters” of cells, including dissociated cells, crypts and pieces of tissue. According to a preferred embodiment of the present invention, epithelial cells are used.

According to the present invention, the term “de novo formation of organoids” refers to freshly isolated or frozen human cells (e.g. human biopsy or tissue resection) that have been grown ex-vivo (i.e. outside the original organism) for the first time.