

Summary ~~of the invention~~

The present ~~invention~~-disclosure expands on the above discussed prior art by providing a cell growth kit that comprises extra-
5 cellular matrix conditions that are specifically preselected for a certain disease or healthy tissue and thus allows a more accurate and efficient prediction of an outcome of a drug therapy for said specific disease or toxicity for said specific healthy tissue. In the above prior art, this potential of three-dimen-
10 sional fully defined (including fully synthetic) hydrogels has not been recognized.

Based upon previously conducted experiments and/or knowledge, it is possible to estimate suitable conditions for growth and sub-
15 sequent testing of specific tissue types, such as cancer cells or normal/healthy cells. However, while this may address certain characteristics of the respective tissue type, it is still not sufficient to address the multiple phenotypic subpopulations of a tissue type, that in e.g. the case of cancer cells vary in
20 their ability to initiate metastases and in their sensitivity to anticancer therapy. Accordingly, even performing an assay with a specific tissue type under a single ex vivo culture condition that has been previously established as being suitable for the growth of said specific tissue type does not provide the desired
25 optimal assistance and improvement of the treatment of a patient having a certain disease.

The present ~~invention~~-disclosure provides an array of extracellular matrix (ex vivo culture) conditions that are based on a
30 preselection of extracellular matrix conditions that have been established as being suitable for a certain tissue type, but provides variations of said preselected extracellular matrix conditions. With this approach, a significantly more focused assay can be con-

ducted. Whereas in a conventional assay with non-preselected extracellular matrix conditions (e.g. conventional screening of extracellular matrix conditions) a certain number of extracellular matrix conditions employed in said assay will not be suitable, in the method of the present invention-disclosure employing preselected extracellular matrix conditions all extracellular matrix conditions are principally suitable for the intended purpose, and it is possible in a significantly more focused way to identify optimal extracellular matrix conditions for a specific phenotypic subpopulation of a certain tissue type of a patient to be treated. Thus, the present invention-disclosure provides an improvement with respect to personalized medicine.

Thus, the first aspect of the present invention-disclosure is related to a method to be performed with one tissue type, optionally in combination with other cells such as stromal cells or immune cells, comprising the steps of:

- a) providing a fully defined hydrogel matrix array with discrete volumes by crosslinking, onto a substrate or into discrete volumes of a substrate, preferably a multi-well plate, different combinations of one or more different hydrogel precursor molecules, optionally in the presence of one or more biologically active molecules, optionally at least one crosslinking agent and cells of the tissue type to be tested, so as to create fully defined three-dimensional extracellular matrix conditions that differ from each other in their biological, biophysical and/or biochemical characteristics;
- b) allowing said cells to grow and expand in said discrete volumes of said hydrogel matrix array in the presence of one or more different culture media;
- c) performing an operation with the cells grown in said discrete volumes of said hydrogel matrix array;

wherein a specific combination of hydrogel features has been pre-selected for the said one tissue type to be tested.

According to the present ~~invention~~disclosure, in step b) of the
5 above method the cells are grown and expanded until a sufficient amount of cells is reached. If a sufficient amount of cells is reached, the desired operation (e.g. drug testing or the creation/establishment of a cell repository/biobank) can be performed in step c). Preferably, step b) (cell expansion) is per-
10 formed manually, wherein increasing the cell number after each passage is important. However, it is also possible to perform step b) automatically and/or in a miniaturized manner.

Said method can be a combinatorial method, i.e. a method where a
15 plurality of combinations of ex vivo conditions (extracellular matrix conditions, etc.) and drugs are examined simultaneously.

In one ~~example~~embodiment, said operation to be performed with the cells grown in said discrete volumes of said hydrogel matrix array may be the addition of one or more drugs to said discrete
20 volumes of said hydrogel matrix array. According to said ~~exam-~~pleembodiment, the method is a drug screening test, in order to identify one or more drugs that are suitable for treating a condition associated with cells from the tested tissue type. This
25 can be used in the field of personalized medicine.

If according to a preferred ~~example~~embodiment of the present ~~in-~~vention-disclosure said tissue type is derived from a specific patient, e.g. freshly isolated or frozen cells from a biopsy or
30 resection of said patient, said drug screening test is an improvement as to precision and/or personalized medicine, since it helps identifying precisely the most suitable treatment for said certain patient.

According to one ~~exampleembodiment~~ of the present ~~disclosurein-~~
~~vention~~, said tissue type with which the method is performed may
comprise both cancer cells as well as other cell types, includ-
ing stromal cells, for example cancer associated fibroblasts
5 (CAF), or immune cells.

According to a preferred ~~exampleembodiment~~ of the present ~~dis-~~
~~closureinvention~~, the tissue type is lung cancer, preferably
non-small cell lung cancer overexpressing c-Met, and the hydro-
10 gel matrix is preselected as being a non self-degradable PEG hy-
drogel, wherein the crosslinking agent and said optional bioac-
tive agent do not comprise any RGD motif. Preferably, the cul-
ture medium to be used in said ~~exampleembodiment~~ comprises FBS
(serum) or Wnt agonist such as R-spondin.

15 According to another preferred ~~exampleembodiment~~ of the present
~~disclosureinvention~~, the tissue type is pancreatic ductal adeno-
carcinoma (PDAC) cells, and the hydrogel matrix is preselected
as being a non self-degradable PEG hydrogel having a stiffness
20 in the range of 50 to 3000 Pa, preferably 50 to 2000 Pa and most
preferably 50 to 1000 Pa, wherein at least one of the crosslink-
ing agent and/or said optional bioactive agent comprise a RGD
motif. Preferably, the culture medium to be used in said ~~exam-~~
~~pleembodiment~~ comprises Wnt agonists such as R-spondin and Wnt
25 3a.

According to another preferred ~~exampleembodiment~~ of the present
~~disclosureinvention~~, the tissue type is colorectal cancer (CRC)
cells, and the hydrogel matrix is preselected as being PEG hy-
30 drogel having at least an initial stiffness in the range of 50
to 2000 Pa, and optionally furthermore comprising one or more
biologically active molecules comprising laminin, preferably
laminin-111 or laminin-511, and especially preferable natural
mouse laminin-111 or recombinant human laminin-511, wherein at
35 least one of the

crosslinking agent and/or said optional bioactive agent comprise a RGD motif. Preferably, the culture medium to be used in said exampleembodiment comprises Wnt agonists such as R-spondin and Wnt 3a.

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According to another preferred exampleembodiment of the present disclosureinvention, the tissue type is breast cancer cells, and the hydrogel matrix is preselected as being preferably an enzymatic-degradable PEG hydrogel, wherein at least one of the crosslinking agent preferably comprises an enzymatically degradable motif, preferably a MMP-sensitive motif and said hydrogel optionally furthermore comprises one or more biologically active molecules comprising laminin, preferably laminin-111, and especially preferable natural mouse laminin-111. Preferably, the culture medium to be used in said exampleembodiment comprises FBS (serum) or Wnt agonist such as R-spondin.

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According to another preferred exampleembodiment of the present disclosureinvention, the tissue type is cancer cells that grow ex vivo more slowly than their healthy/normal counterparts (e.g. epithelial and/or stromal cells), preferably prostate cancer cells, and the hydrogel matrix is preselected as being a PEG hydrogel, preferably having a stiffness in the range of 50 to 2000 Pa, wherein said crosslinking agent and said optional bioactive agent do not comprise any RGD motif.

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According to another preferred exampleembodiment of the present disclosureinvention, the tissue type is cancer cells, preferably pancreatic ductal adenocarcinoma (PDAC) cells, in combination with stromal cells, preferably fibroblasts, and the hydrogel matrix is preselected as being a PEG hydrogel having a stiffness preferably in the range of 50 to 3000 Pa, preferably 50 to 2000 Pa and most preferably 50 to 1000 Pa, wherein at least one of the crosslinking agents comprises an enzymatically degradable motif, prefera-

bly a MMP-sensitive motif, and wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif. Preferably, the culture medium to be used in said exampleembodiment comprises Wnt agonists such as R-spondin and Wnt 3a, and more preferably also FBS (fetal bovine serum).

In another exampleembodiment, said operation to be performed with the cells grown in said discrete volumes of said hydrogel matrix array may be drug screening/testing on healthy organoid cells, in particular in the field of precision medicine. According to a preferred exampleembodiment, healthy/normal organoids (e.g. colon or intestinal organoids, normal/healthy prostate cells or healthy cells of other organs) may be used as control conditions and/or as cytotoxic assay (e.g. to test the toxicity of a drug) in drug tests with diseased cells of the same organ. For example, healthy/normal colon or intestinal organoids can be used in drug tests as control conditions where drugs are tested on, e.g. cancer organoids or organoids from cystic fibrosis tissues of the same patient.

In another exampleembodiment, said operation to be performed with the cells grown in said discrete volumes of said hydrogel matrix array may be the isolation of the grown cells (designated herein as organoids) in order to use said 3D cellular structures in basic scientific research or to implant said cells into a human, for the purposes of regenerative or personalized medicine.

A significant advantage of a preferred exampleembodiment of the method of the present disclosureinvention is that the use of a naturally-derived matrix such as Matrigel® can be completely avoided. It has been surprisingly found that this long-felt need in the art can be achieved by using specifically pre-selected conditions as described hereinafter. Performing the entire method under fully-

defined extracellular matrix conditions provides more precise results for drug screening, since any varying behaviour of a drug can be clearly attributed to a specific extracellular matrix condition. Also, performing the entire method under fully-defined extracellular matrix conditions meets the regulatory approval requirements for personalized and regenerative medicine, in contrast to the methods performed in the prior art.

In another aspect, ~~the present invention-disclosure~~ is also related to a kit of parts for performing an operation on or with one or more tissue type, comprising:

- a) components for preparing a fully defined hydrogel matrix array, so as to create fully defined three-dimensional extracellular matrix conditions that differ from each other in their biological, biophysical and/or biochemical characteristics, said components comprising
 - one or more different hydrogel precursor molecules,
 - optionally at least one crosslinking agent,
 - optionally one or more biologically active molecules,
 - b) one or more different culture media,
- wherein a specific combination of hydrogel features has been pre-selected for the tissue type to be tested.

According to a preferred ~~exampleembodiment~~ of the present ~~invention-disclosure~~, said kit is for testing the influence of drugs on lung cancer cells, preferably non-small cell lung cancer cells, overexpressing c-Met, the hydrogel matrix is preselected as being a non self-degradable PEG hydrogel, wherein the crosslinking agent and said optional bioactive agent do not comprise any RGD motif, and said culture medium preferably comprises FBS (serum) or a Wnt agonist such as R-spondin.

According to another preferred ~~example~~~~embodiment~~ of the present ~~disclosure~~~~invention~~, said kit is for testing the influence of drugs on pancreatic ductal adenocarcinoma (PDAC) cells, the hydrogel matrix is preselected as being a non self-degradable PEG hydrogel having a stiffness in the range of 50 to 3000 Pa, preferably 50 to 2000 Pa and most preferably 50 to 1000 Pa, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif, and said culture medium preferably comprises Wnt agonists such R-spondin and Wnt 3a.

According to another preferred ~~example~~~~embodiment~~ of the present ~~disclosure~~~~invention~~, said kit is for testing the influence of drugs on colorectal cancer (CRC) cells, and the hydrogel matrix is preselected as being PEG hydrogel having at least an initial stiffness in the range of 50 to 2000 Pa, and optionally furthermore comprising one or more biologically active molecules comprising laminin, preferably laminin-111 or laminin-511, and especially preferable natural mouse laminin-111 or recombinant human laminin-511, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif, and wherein said culture medium preferably comprises Wnt agonists such as R-spondin and Wnt 3a.

According to another preferred ~~example~~~~embodiment~~ of the present ~~disclosure~~~~invention~~, said kit is for testing the influence of drugs on breast cancer cells, and the hydrogel matrix is preselected as being preferably an enzymatic-degradable PEG hydrogel, wherein at least one of the crosslinking agent preferably comprises an enzymatically degradable motif, preferably a MMP-sensitive motif, and said hydrogel optionally furthermore comprises one or more biologically active molecules comprising laminin, preferably laminin-111, and especially preferable natural mouse laminin-

111, and wherein said culture medium preferably comprises FBS (serum) or Wnt agonist such as R-spondin.

According to another preferred ~~example~~~~embodiment~~ of the present
5 ~~disclosure~~~~invention~~, said kit is for growing and testing the influence of drugs on cancer cells that grow ex vivo more slowly than their healthy/normal counterparts (e.g. epithelial and/or stromal cells), preferably prostate cancer cells. The hydrogel matrix is preselected as being a PEG hydrogel, preferably having
10 a stiffness in the range of 50 to 2000 Pa, wherein said cross-linking agent and said optional bioactive agent do not comprise any RGD motif.

The kits according to the present ~~disclosure~~~~invention~~ are designated for cells coming from a specific tissue type and can be
15 readily used for performing operations on or with said tissue type, such as testing the influence of drugs on said tissue type, or the isolation of the grown cells in order to use said grown cells in basic scientific research, personalized medicine
20 or to implant said cells into a human, for the purposes of regenerative medicine, or for drug development/discovery or the creation of a cell repository/biobank. The kits according to the present ~~disclosure~~~~invention~~ are correspondingly indicated, e.g.
by instructions provided with said kit, for the said specific
25 tissue type with which it is to be used.