

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 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 disclosure provides an improvement with respect to personalized medicine.

Thus, the first aspect of the present disclosure is related to a drug screening method to be performed with one tissue type, optionally in combination with other cells including 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, 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 (i) a specific combination of hydrogel features has been pre-selected for the said one tissue type to be tested, (ii) freshly isolated or frozen cells from a biopsy or tissue resection of a human or from patient-derived xenograft (PDX) tissue are used, and (iii) in
5 step c) one or more drugs are added to said discrete volumes of said hydrogel matrix.

According to the present disclosure, in step b) of the above method the cells are grown and expanded until a sufficient amount of cells is
10 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 performed manually, wherein increasing the cell number after each passage is important. However, it is also possible
15 to perform step b) automatically and/or in a miniaturized manner.

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

20 In one example, 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 volumes of said hydrogel matrix array. According to said example, the method is a drug screening
25 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 can be used in the field of personalized medicine.

30 If according to a preferred example of the present disclosure said tissue type is derived from a specific patient, e.g. freshly isolated or frozen cells from a biopsy or 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.

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 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,

b) said components comprising

- one or more different hydrogel precursor molecules,
- optionally at least one crosslinking agent,
- optionally one or more biologically active molecules,

c) 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 example of the present 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.

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

5 According to another preferred example of the present disclosure, 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 a stiffness in the range of 50 to 2000 Pa, wherein
10 said crosslinking agent and said optional bioactive agent do not comprise any RGD motif.

The kits according to the present disclosure are designated for cells coming from a specific tissue type and can be readily used for performing operations
15 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 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
20 according to the present disclosure are correspondingly indicated, e.g. by instructions provided with said kit, for the said specific tissue type with which it is to be used. In another aspect, the present disclosure provides a method to be performed with one tissue type, optionally, in combination with other cells including stromal cells or immune cells, comprising the steps of:
25 (a) providing a fully defined hydrogel matrix array with discrete volumes by crosslinking, onto a substrate or into discrete volumes of a substrate, 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
30 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) repeating step b) and performing
35 an operation with the cells grown in said discrete volumes of said hydrogel matrix array to create a cell repository/biobank; and wherein a specific combination of hydrogel features has been pre-selected for the said one tissue type to be tested.

Claims

1. A drug screening method to be performed with one tissue type, optionally, in combination with other cells including 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, 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; andwherein: (i) a specific combination of hydrogel features has been pre-selected for the said one tissue type to be tested, (ii) freshly isolated or frozen cells from a biopsy or tissue resection of a human or from patient-derived xenograft (PDX) tissue are used, and (iii) in step c) one or more drugs are added to said discrete volumes of said hydrogel matrix.
2. The method according to claim 1, wherein the preselection of at least one of said hydrogel precursor molecules, or of said hydrogel features, and said culture media is made on the basis of selecting suitable extracellular matrix conditions from a method using random extracellular matrix conditions.

3. The method according to claim 1 or 2, wherein the tissue type is selected from the group consisting of cancer cells and normal/healthy cells.
- 5 4. The method according to any of the preceding claims, wherein the tissue type is lung cancer cells, overexpressing c-Met, and the hydrogel matrix is preselected as being a non self-degradable polyethylene glycol (PEG) hydrogel, wherein the
10 crosslinking agent and said optional bioactive agent do not comprise any Arginine-Glycine-Aspartic Acid (RGD) motif.
5. The method according to claim 4, wherein said culture medium comprises FBS (Fetal Bovine Serum) or Wnt agonist including
15 R-spondin.
6. The method according to any of claims 1 to 3, wherein the tissue type is pancreatic ductal adenocarcinoma (PDAC) cells, and the hydrogel matrix is preselected as being a non self-degradable PEG hydrogel having a stiffness in the range of 50
20 to 3000 Pa, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif.
7. The method according to claim 6, wherein said culture medium
25 comprises Wnt agonists including R-spondin and Wnt 3a.
8. The method according to any of claims 1 to 3, wherein the tissue type is colorectal cancer (CRC) cells, and the hydrogel matrix is preselected as being a PEG hydrogel having at least
30 an initial stiffness in the range of 50 to 2000 Pa, and optionally furthermore comprising one or more biologically active molecules comprising laminin, wherein at least one of

the crosslinking agent and/or said optional bioactive agent comprise a RGD motif.

- 5 9. The method according to claim 8, wherein said culture medium comprises Wnt agonists including R-spondin and Wnt 3a.
- 10 10. The method according to any of claims 1 to 3, wherein the tissue type is breast cancer cells, and the hydrogel matrix is preselected as being an enzymatic-degradable PEG hydrogel, wherein at least one of the crosslinking agent comprises an enzymatically degradable motif, and said hydrogel optionally furthermore comprises one or more biologically active molecules comprising laminin.
- 15 11. The method according to claim 10, wherein said culture medium comprises FBS (Fetal Bovine Serum) or Wnt agonist including R-spondin.
- 20 12. The method according to any of claims 1 to 3, wherein the tissue type is cancer cells that grow ex vivo more slowly than their normal counterparts, and the hydrogel matrix is preselected as being a PEG hydrogel, 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.
- 25 13. Kit of parts for performing an operation on or with one or more tissue type, comprising:
- 30 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,

b) said components comprising

- one or more different hydrogel precursor molecules,
- optionally at least one crosslinking agent,
- optionally one or more biologically active molecules,

c) one or more different culture media,

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

10 14. Kit according to claim 13, for testing the influence of drugs on 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 comprises FBS (serum) or Wnt agonist including R-spondin.

15 15. Kit according to claim 13, 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, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif, and said culture medium comprises Wnt agonists including R-spondin and Wnt 3a.

20 16. Kit according to claim 13, for testing the influence of drugs on colorectal cancer (CRC) cells, and the hydrogel matrix is preselected as being a 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, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD

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motif, and wherein said culture medium comprises Wnt agonists including R-spondin and Wnt 3a.

5 17. Kit according to claim 13, for testing the influence of drugs on breast cancer cells, and the hydrogel matrix is preselected as being an enzymatic-degradable PEG hydrogel, wherein at least one of the crosslinking agent comprises an enzymatically degradable motif, and said hydrogel optionally further-
10 more comprises one or more biologically active molecules comprising laminin, and wherein said culture medium comprises FBS (serum) or Wnt agonist including R-spondin.

15 18. Kit according to claim 13, for testing the influence of drugs on cancer cells that grow ex vivo more slowly than their normal counterparts, and the hydrogel matrix is preselected as being a PEG hydrogel, 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.

20 19. A method to be performed with one tissue type, optionally, in combination with other cells including stromal cells or immune cells, comprising the steps of:

25 a) providing a fully defined hydrogel matrix array with discrete volumes by crosslinking, onto a substrate or into discrete volumes of a substrate, 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 cross-
30 linking 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) repeating step b) and performing an operation with the cells grown in said discrete volumes of said hydrogel matrix array to create a cell repository/biobank; and

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

20. The method according to claim 19, wherein the preselection of at least one of said hydrogel precursor molecules, or of said hydrogel features, and said culture media is made on the basis of selecting suitable extracellular matrix conditions from a method using random extracellular matrix conditions.

21. The method according to claim 19, wherein the tissue type is a normal/healthy cell.

22. The method according to claim 19, wherein freshly isolated or frozen cells from a biopsy or tissue resection of a human or from patient-derived xenograft (PDX) tissue are used.

23. The method according to claim 19, wherein the tissue type is pancreatic ductal adenocarcinoma (PDAC) cells, and the hydrogel matrix is preselected as being a non self-degradable PEG hydrogel having a stiffness in the range of 50 to 3000 Pa, wherein at least one of the crosslinking agent and/or said optional bioactive agent comprise a RGD motif.