An Insert for Conducting 3D Cell Culture And A Method of Fabricating the Same

FIELD OF INVENTION

[0001] The present invention relates broadly, but not exclusively, to an insert for conducting 3D cell culture and a method of fabricating the same.

BACKGROUND

[0002] Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the physiology and biochemistry of cells. It is also used in drug screening and development, large scale manufacturing of biological compounds, and the production of tissue-like derivatives for creating artificial organs.

[0003] Many methods and devices for culturing, expanding and differentiating cells in vitro have been developed. A conventional and still often used method is growth and maintenance of cells or cell cultures on a suitable growth surface such as a cell culture dish filled with liquid or gelated culture medium. The culture medium may comprise specific constituents which affect the growth and maintenance of the cells or cell culture in desired ways. The main limitation associated with these two dimensional (2D) cell culture models is that the cells grown as a monolayer provides a stiff platform, offering unnatural growth kinetics and cell attachments. Therefore, natural microenvironments of the cells are not fully represented. In the last decade, significant work by researchers produced improvements in the form of better in vitro cell culture models that resemble in vivo conditions. Three-dimensional (3D) cell cultures are such models and better mimic tissue physiology in multicellular organisms.

[0004] Currently available 3D cell culture techniques can be broadly classified into two categories: scaffold-based techniques and scaffold-free techniques. Scaffold-based 3D culture techniques involve culturing cells using a supporting scaffold to allow growth in all directions. Popular types of scaffold include hydrogels and inert matrices. Scaffold-free 3D culture techniques rely on cells to self-assemble into clusters or spheroids. Popular scaffold-free methods include hanging drop method and culturing using low adhesion plates.

[0005] Hanging drop cultures take advantage of self-aggregation of cells into spheroids when a surface is not available for cell attachment. Hanging drops can be created in specialized

plates with open, bottomless wells that are designed for the formation of a small droplet of media. Hanging drop method requires a transfer of the spheroids formed to a different plate for prolonged culture or experimental procedures. Such transfer is a labor-intensive procedure and often results in the loss of cells.

[0006] Culturing cells using low adhesion plates takes advantage of the lack of cell attachment surfaces to promote aggregation of cells and spheroid formation. Low adhesion plates are often made of polystyrene and treated with hydrophilic or hydrophobic coatings like the non-adherent polymer poly-HEMA or natural polymers such as agarose. The coating reduces the attachment of cells to the point that they preferably aggregate with each other to form spheroids. Culturing cells using low adhesion plates usually provides a larger cell culture volume than hanging drop method, thus eliminating the need for transfer of the spheroids formed. However, the use of multiwall culturing plates requires pipetting in each well, which is labor intensive, time consuming, prone to contamination, and results in a higher chance of loss of cells.

[0007] In view of the above, there is a need for a cell culture apparatus that circumvents the problems associated with the existing cell culturing platforms.

SUMMARY

[0008] According to a first aspect of the present invention, there is provided a insert for conducting 3D cell culture comprising:

a plurality of sample wells for containing one or more cells, wherein each of the plurality of sample wells is defined by a peripheral wall and an aperture at a top portion of the peripheral wall for insertion of the one or more cells into the plurality of sample wells,

wherein each of the plurality of sample wells is configured to include a shape that converges from the peripheral wall to the aperture at the top portion, thereby preventing an aggregation of the cells formed in the 3D cell culture from escaping the plurality of sample wells through the aperture,

wherein the peripheral wall of the plurality of sample wells is at least partially provided with a reflective surface to illuminate the one or more cells contained in the plurality of sample wells by reflecting an incident lightsheet, and

wherein the peripheral wall comprises one or more side walls that taper at an angle of 45° from a horizontal plane.

[0009] According to a second aspect of the present invention, there is provided a method of fabricating a insert for conducting 3D cell culture, the method comprising the steps of:

dropping a liquid polymer onto a mould to fill in cavities defined by the mould;

curing the liquid polymer to form a plurality of sample wells, wherein each of the plurality of sample wells is defined by a peripheral wall and an aperture at a top portion of the peripheral wall for insertion of one or more cells into the plurality of sample wells, and

providing a reflective surface to at least a part of the peripheral wall of the plurality of sample wells, wherein the reflective surface is configured to illuminate the one or more cells contained in the plurality of sample well by reflecting an incident lightsheet,

wherein the cavities mould each of the plurality of sample wells into a shape that converges from the peripheral wall to the aperture at the top portion, thereby preventing an aggregation of the cells formed in the 3D cell culture from escaping the plurality of sample wells through the aperture, and

wherein the peripheral wall comprises one or more side walls that taper at an angle of 45° from a horizontal plane.

[0010] According to a third aspect of the present invention, there is provided a microscope assembly comprising a insert as defined in the first aspect.

[0011] According to a fourth aspect of the present invention, there is provided a method for imaging cell cultures, the method comprising the steps of:

seeding a sample comprising one or more cells into the insert as defined in the first aspect;

adding a culture medium to the insert containing the one or more cells; and

capturing an image of the one or more cells contained within the insert using a microscopy technique.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Embodiments of the invention are provided by way of example only, and will be better understood and readily apparent to one of ordinary skill in the art from the following written description and the drawings, in which:

[0013] Figure 1A shows a diagram illustrating a perspective view of an insert for conducting 3D cell culture in accordance with an example embodiment.

[0014] Figure 1B shows an enlarged side view of the insert of Figure 1A.

[0015] Figure 1C shows an enlarged top view of the insert of Figure 1A.

[0016] Figure 2 shows three images of cells on day 0, day 2 and day 15 from the seeding of the cells in the insert of Figures 1A-1C.

[0017] Figure 3A shows the perspective view of the insert of Figures 1A-1C when the cells are released from the insert.

[0018] Figure 3B shows the top view of the insert of Figures 1A-1C when the cells are released from the insert.

[0019] Figure 3C shows an image of the cells upon being released from the insert of Figures 1A-1C.

[0020] Figure 4A shows in-situ confocal images of FoxA2 (endoderm marker, red), actin (Phalloidin, gold) and DNA (DAPI, blue) contained in the insert 102 of Figures 1A-1C.

[0021] Figure 4B shows corresponding images of four spheroids stained with PhalloidinAlexa488 (actin) captured using two different imaging techniques.

[0022] Figure 5 shows two images of inserts with different passivation levels in accordance with an example embodiment.

DETAILED DESCRIPTION

[0023] Figure 1A shows a diagram illustrating a perspective view 100 of an insert 102 for conducting 3D cell culture in accordance with an example embodiment. Figures 1B and 1C show an enlarged side view 104 and an enlarged top view 106 of the insert 102 respectively.

[0024] The insert 102 includes a plurality of sample wells 108 for containing one or more cells 110. Each of the sample wells 108 is defined by a peripheral wall 112 and an aperture 114 at a top portion 116 of the peripheral wall 112 for insertion of the one or more cells 110 into the plurality of sample wells 108. Additionally, each of the plurality of sample wells 108 is configured to include a shape that converges from the peripheral wall 112 to the aperture 114 at the top portion 116, thereby preventing an aggregation of the cells 110 formed in the 3D cell culture from escaping the sample wells 108 through the aperture 114.

[0025] As shown in Figures 1A-1C, the insert 102 is a membrane 118 with through holes formed on an outer surface 120 of the membrane 118. Each sample well

108 is moulded into the shape of a truncated square pyramid, with the aperture 114 formed at the truncated end of the square pyramid for insertion of cells 110 into the sample wells 108. The truncated pyramid has a height *h* equivalent to the thickness of the membrane 118. As such, the peripheral walls 112 of the truncated pyramid include lateral faces 122 of the truncated pyramid defined by the membrane 118.

[0026] As shown in Figure 1B, the lateral faces 122 of the truncated square pyramid converge at an angle from the square base of the pyramid towards the aperture 114. The size of the sample wells 108 can be adapted according to the desired size of the spheroids cultured in the sample wells. For example, an insert 102 including sample wells 108 of the size of 300 µm base (side length), 115 µm height and 70 µm aperture (side length) may be suitable for containing cells 110 to form into spheroids having the size of around 100 µm in diameter.

[0027] The lateral faces 122 of the sample wells 108 tapers at an angle of about 45° from a horizontal plane such as the square base. In an embodiment, the lateral faces 122 is at least partially provided with a reflective surface, e.g. a mirror which can be formed by depositing a (reflective) metal coat such as gold on the lateral faces 122. The reflective surface may advantageously allow selective plane illumination microscopy (SPIM) to be used for imaging the cells 110 by reflecting an incident lightsheet off the reflective surface to illuminate the cells 110 contained in the sample wells 108.

[0028] In use, the insert 102 is placed on a suitable vessel 124 (e.g. a petri dish, a multi well plate or a multi chamber slides) which can contain a culture medium that supplies essential nutrients for the growth of the cells 110. The cells 110 are seeded as single or small cell aggregate and enter the sample wells 108 by the top aperture 114. Over time, the inserted cells 110 aggregate and grow inside the sample wells 108 into spheroids of a size greater than the size of the aperture 114.

[0029] Consequently, the movement of spheroids are confined in the sample wells 108 as the spheroids cannot escape from the top aperture 114. Experimental procedures such as imaging of the cells 110 can be carried out while the spheroids are encaged in the sample wells 108. In addition, the insert 102 can be detached from the vessel 124 to release the cells 110 contained inside the sample wells 108 for analysis of the cells 110 with other procedures such as RNA sequencing, -omic approaches, in vitro experiments, and in vivo transplantation.

[0030] Advantageously, the high density of sample wells 108 of the insert 102 may allow high-content screening (HCS) method to be used for analysing a large number of spheroids at the same time. Thousands of spheroids can be cultured in a single insert 102 for a period of time with nearly no loss of material. As comparison, an area of 4 mm x 2 mm on the insert 102 can contain as many spheroids as a single 384-well plate that occupies the area of 12 cm x 8 cm. Also, when the insert 102 is used, the culture medium in the vessel can be changed with only a single pipetting step. In contrast, when a conventional multi-well plate is used, multiple pipetting steps are required to drop culture medium into each well. The single pipetting step may minimise loss of material due to pipetting and substantially reduces the time required to change the culture medium.

[0031] It should be noted that the samples wells 108 can be moulded into any shape with the peripheral wall 112 of the samples wells 108 converges to the aperture 114 at the top portion 116 of the sample wells 108. For example, the sample wells 108 can be moulded into the shape of a truncated cone or a rectangle or Y shape based pyramids, instead of a truncated pyramid.

[0032] Figure 2 shows three images of cells 110 on day 0, day 2 and day 15 from the seeding of the cells 110 in the insert 102 of Figures 1A-1C. It should be noted that the description below with respect to Figure 2 that explains an example process using the insert 102 is provided by way of example only.

[0033] The first image 202 shows the insert 102 immediately after the seeding of a cell suspension. At the seeding stage, the insert 102 is placed on vessel 124 (e.g. a petri dish, a multi well plate or a multi chamber slides). The cells 110 are detached using a trypsinization process and diluted at a concentration between 100 and 500 cells per µl in the medium to obtain the cell suspension. The cellular suspension is then poured onto the top of the insert 102 at a volume sufficient to cover the entire insert 102. It should be noted that the insert 102 can be used with different types of cells such as human-induced pluripotent stem cells (HiPSC), human embryonic stem cells (hESC), primary cells from rat or cancer cell lines (e.g. HME-1 or HeLa cells).

[0034] Next, the insert 102 including the cell suspension is placed in an incubator (e.g. at 37 °C and 5% CO_2). After a period of time (e.g. 5-15 minutes), cells 110 filled the sample wells 108 of the insert 102, and the insert 102 is rinsed with a warm culture medium to remove the excess of cells 110 on the insert 102. The insert

102 is then refilled with a suitable culture medium and placed back into the incubator.

[0035] Advantageously, the insert 102 may allow single cells or small aggregates of cells to be seeded homogeneously over the insert 102 and to grow into spheroids over a few days or weeks. It is possible that the insert 102 can contain spheroids in a density of about 50 spheroids per mm^2. This density is about 100 times greater than the density of spheroids that can be contained in a conventional 384-well plate.

[0036] The second image 204 and third image 206 shows the cell 110 in two and fifteen days after the cell seeding day respectively. Culture medium, extracellular matrices and differentiation growth factors can be changed or added to the vessel 124 containing the insert 102 in accordance with cell differentiation protocol. On the second day, the cells 110 have grown into a substantial size, at which point the spheroids are encaged in the insert 102. The cells 110 continue to grow into its full development in the insert 102 in fifteen days after the seeding of the cells 110. As shown in the second and third images 204, 206, the cells 110 developed into spheroids inside the sample wells 108 of the insert 102 without any adhesion of the spheroid to the peripheral wall 112 of the insert 102. The sample wells 108 which have the shape of a truncated square pyramid also advantageously prevent the spheroids from escaping the sample wells 108, thus avoiding any loss of the spheroids, e.g. due to pipetting and media exchange. Upon the completion of the experiment in the insert 102, the cells 110 contained inside the sample wells 108 can be released by detaching the insert 102 from the vessel 124. The step of releasing the cells is discussed in further details below in respect of Figures 3A – 3C.

[0037] It should be noted that the insert 102 is compatible with freezing and thawing methods used for cells culture conservation. It is possible to freeze or thaw 3D cell culture directly in the insert 102 as the insert 102 may resist to low temperature without any evident defects. Thus, users can use cryopreservative medium (e.g. CellBanker®2, AMSBIO) with the insert 102 for cell culture conservation.

[0038] For example, after cell seeding on the insert 102, and optionally protocols of differentiation or alginate embedding steps, supernatant medium is aspirated and replaced by cryopreservative medium (e.g. in the volume of about 1 ml). Subsequently, the insert 102 containing the 3D cell culture is placed in a surrounding at a temperature of about -80°C. For thawing, the insert 102 is refilled with adequate warm culture medium

(e.g. in the volume of about 2ml) to rapidly de-freeze and dilute the cryopreservative medium. Once completely thawed, the insert 102 is rinsed a few times with adequate medium and placed in the incubator with appropriate culture medium for further experiments.

[0039] Further, it should be noted that the living cells 110 in the insert 102 can optionally be embedded in alginate. Alginate may provide protection to sensitive cells 110 such as stem cells or differentiated cells during the freezing and thawing processes. Alginate may also avoid any dissociation or unwanted adhesion of cells 110 after being released from the insert 102 and maintain the 3D structure of the cells 110. The preparation of an example of the culture medium containing alginate is discussed in the next paragraph.

[0040] Sodium alginate solution of 300mM sorbitol (Sigma), 2.5% wt/vol sodium alginate (Sigma), and 100mM calcium chloride (Sigma) is prepared and sterilized using filtering (0.45µm, Fisherbrand). The sodium alginate solution is poured in the chip followed by aspiration of the supernatant. Few drops of 100mM $CaCl_2$ are poured directly on the top of the insert 102 to permit the alginate to solidify and immediately followed by dilution (no more than a few second with the 100mM $CaCl^2$ to avoid osmotic choc). Finally, supernatant is aspirated and replaced by culture medium to remove any excess of $CaCl^2$.

[0041] After the process of 3D cell culture, the insert 102 can be cleaned with rapid incubation of bleach (e.g. in less than 5 minutes), followed by several water flushes and rinses of 70% ethanol. After cleaning, the insert 102 can be reused.

[0042] Figures 3A and 3B show the perspective view 302 and top view 304 of the insert 102 of Figures 1A-1C respectively when the cells 110 are released from the insert 102. After the culturing of the cells 110, the cells 110 that have formed into spheroids can be released by simply detaching the insert 102 from the vessel 124. The structure of the insert 102 may advantageously allow for the step of releasing the living spheroids from the sample well 108 to be carried out easily. For example, the insert 102 can be cut with a sterilized scalpel and subsequently peeled with a sterilized tweezer apart from the vessel 124. The insert 102 is then rinsed with culture medium and the spheroids floating in the culture medium can be transferred to another vessel.

[0043] Figure 3C shows an image of the cells 110 upon being released from the insert 102 of Figures 1A-1C. After the peeling of the insert 102, spheroids released from sample wells 108 are transferred to another vessel. The spheroids which

are still alive after the release can be used for other procedures such as RNA sequencing, -omic approaches, in vitro experiments, and in vivo transplantation.

[0044] Figure 4A shows in-situ confocal images of FoxA2 (endoderm marker, red), actin (Phalloidin, gold) and DNA (DAPI, blue) contained in the insert 102 of Figures 1A-1C. As described above in respect of Figures 1A-1C and Figure 2, the insert 102 contains the cells 110 for a period of time. A microscope assembly can be used in conjunction with the insert 102 for analysis of the cells 110 by imaging the cells 110.

[0045] Several high-resolution imaging methods can be used during the morphogenesis of the living 3D spheroids in the insert 102. For example, fluorescence microscopy such as single-objective selective plane illumination microscopy (soSPIM) can be used in the imaging of the spheroids. The reflective surface in the sample wells 108 is slanted at an angle of 45° from a horizontal plane to reflect the incident lightsheet from soSPIM to illuminate spheroid contained in the sample wells 108 of the insert 102. soSPIM can be used for imaging live samples with fixation and immunostaining standards protocols, live samples expressing fluorescents proteins (i.e. GFP) of interest or with medium containing fluorescents dyes (i.e calcein or alexa dyes). Light sheet microscopy such as soSPIM is suitable for live imaging as it has a low photobleaching and phototoxicity effect.

[0046] Other imaging methods can also be applied for the imaging of cells 110 such as brightfield, widefield and confocal microscopies using a standard inverted microscope (Nikon Eclipse Ti-E) that may be equipped with the following:

- air objective (4X, 10X, 20X)
- water-immersion objective (40X, 60X) (Nikon)
- oil-immersion objective (63X, 100X) (Nikon)
- a brightfield lamp
- a fluorescent widefield illumination arm (Nikon)
- a confocal scanner unit (CSU W1, Yogogawa)
- a motorized scanning stage (Physik Instrumente)
- a digital camera (ORCAFlash4.0, Hamamatsu)
- an incubator chamber (Okolab) for live imaging
- Metamorph software (Molecular Devices) to control the x-y-z stage position and camera settings
- imageJ or Imaris software for visualizing the 3D Z-stacks of the spheroids

[0047] Figure 4B shows corresponding images of four spheroids stained with PhalloidinAlexa488 (actin) captured using two different imaging techniques. The four spheroids are contained in the insert 102 and the images of the spheroids are captured using single-objective selective-plane illumination microscopy (soSPIM) and confocal spinning disk techniques, shown as the top row images and the bottom row images in Figure 4B respectively. As shown in the images, soSPIM technique generate better quality images of the spheroids as no optical signals is detected by the confocal spinning disk technique for spheroids at the size of 50µm.

[0048] Figure 5 shows two images 502, 504 of inserts with different passivation levels in accordance with an example embodiment. The passivation of the inserts are achieved by coating lipidure solution in different concentrations and can prevent adhesion of cells to the wall of inserts. The first image 502 shows a first insert coated with about 4-7 µm of lipidure coat 506 formed using a solution including 1% lipidure that is dissolved in 2% w/v in pure ethanol. The second image 504 shows a second insert coated with about 8-13 µm of lipidure coat 508 formed using a solution including 2% lipidure that is dissolved in 2% w/v in pure ethanol. Fabrication of inserts including the passivation stage are discussed in further details below.

[0049] FABRICATION PROCEDURES

[0050] The description below with respect to Steps 1 to 4 explains an example process of fabricating a insert 102 in accordance with the example embodiment and is provided by way of example only.

Materials: Optical mask(s), (100) SEMI II standard single side polished 4" silicon wafers, with thermally grown silicon oxide (low stress, 300 nm thick, double side), 25% water solution of TMAH, Water soluble surfactant (Triton X), Solvents (Acetone and IPA, AZ300T), AZ5214E positive tone photoresist, HMDS, AZ400K developer, Dow Corning 184 PDMS, 1*H*,1*H*,2*H*,2*H*-Perfluorooctyl-trichlorosilane, NOA73 UV-curable optical glue, Au, Water based Au etchant, Buffered Oxide Etching (BOE).

Equipment: UV lithography tools (e.g. MJB4 mask aligner), UV flood-exposure lamps (e.g. Kloe' KUV), Reactive Ion Etching reactor (e.g. Samco 10NR RIE), Hot plates, Glassware, Fume hood and laminar flow hood, Vacuum jar(s), Spin-coater, Optical microscope, Stylus profiler.

Step 1: Silicon Primary Mould Fabrication: Etching Along (110) Directions

The fabrication of silicon primary mould involves multiple lithographic procedures and is carried out in a clean environment. The processes involved in silicon primary mould fabrication include the following:

1. **Silicon oxide wafers (300 nm thick) may be cleaned prior to photoresist coating:** O_2 plasma is preferred to solvent cleaning, such as Acetone/IPA or industry standard RCA-cleaning

2. **Spin-coating:** AZ5214E or equivalent thin positive photoresist is used. If adhesion promoter is required (e.g. HMDS), prime the wafer accordingly. Spin-coating parameters are dependent on the equipment used in the process. The final thickness for the resist should be compatible with the selectivity of the oxide etching process.

3. **Alignment marks:** This step is required only if a high quality alignment to crystal plans in the wafer is achievable. An optical mask with alignment marks is used to expose the photo-resist; align the mask and wafer in the Mask Aligner exposure system using the primary flat as a reference and expose to UV (energy dose/exposure dose may vary depending on mask aligner used and baking conditions).

4. **Oxide etching:** Use plasma etching to open the oxide layer using the photoresist as etching mask. Short O_2 plasma may be applied to remove hexamethyldisilazane (HMDS) and any residual photoresist before the oxide etching.

5. **Resist stripping:** After oxide etching, remove the photoresist and clean the residual organic contaminations.

6. **Markers wet etching:** Use tetramethylammonium hydroxide (TMAH) or other basic solution to etch the silicon along crystal directions. Only features aligned with (111) directions are left, which will then be used as alignment markers.

7. **Mirrors layer lithography:** Spin-coat the oxide wafer with AZ5214E. Use the optical mask with the mirrors lay-out and align precisely the pattern with the markers etched in the previous step. Expose to UV light in the Mask Aligner and develop the photo-resist.

8. **Silicon oxide etching:** Reactive Ion Etching of the oxide using the photo-resist as a mask (same as step 4 above).

9. **Wafer cleaning in preparation of wet etching:** Resist stripping and O_2 plasma cleaning for organic residual removal.

10. **Mirrors wet etching:** Use TMAH solution and surfactant (e.g. 75-120 ppm Triton X) for anisotropic etching. After the required depth is reached, the wafer is removed from etching bath and placed in deionized water for rinsing and dried with N_2 blow.

11. **Oxide removal:** Remove oxide mask, e.g. with BOE selective wet etching.

12. **Silicon Mould anti-sticking coating**: Anti-sticking coating can be applied to the silicon mould by e.g. vapour phase deposition. Activate the silicon surface with O_2 plasma then place the activate mould in a vacuum jar, add a small quantity of e.g. 1*H*,1*H*,2*H*,2*H*-Perfluorooctyl-trichlorosilane and evacuate the jar to a few mbar. Hold for at least 1-3 hr before ventilating and retrieve the silanized mould.

Step 2: Polydimethylsiloxane (PDMS) Working Mould Fabrication

1. **PDMS preparation**: Mix PDMS base resin and curing agent (e.g. Dow Corning 184) in e.g. 10:1 ratio and de-gas until there are no more air bubbles.

2. **PDMS pouring:** Place the silanized silicon mould in a suitable container, e.g. a petri dish or a moulding container, and pour the PDMS until the whole surface is covered. De-gas for a second time to make sure the cavities in the mould are filled with silicone rubber.

3. **Curing:** Thermally cure the PDMS, e.g. 1 hr at 80 °C. After curing, let the PDMS cool down to room temperature and peel off from the Si mould.

4. **PDMS mould silanization:** The PDMS mould may be silanized for replica-moulding or, if required, by the device fabrication protocol. Activate the mould surface with O_2 plasma then place the activate mould in a vacuum jar, add a small quantity of e.g. 1*H*,1*H*,2*H*,2*H*-Perfluorooctyl-trichlorosilane and evacuate the jar to a few mbar. Hold for at least 1-3 hr before ventilating and retrieve the silanized mould.

Step 3: Fabrication of Inserts

1. **Mould Preparation:** Cut a piece of the mould to a suitable size.

2. **Mould-substrate assembly:** Place the PDMS mould face-down on a flat cut of freshly cured PDMS slab. Make sure that only the protruding features in the mould touch the flat cut.

3. **Polymer casting:** At one edge of the mould, drop a small quantity of liquid polymer (e.g. NOA73) and let the liquid fill the cavities by Spontaneous Capillarity Filling (SCF). The liquid polymer comprises at least one UV curable polymer selected from a group consisting of: acrylate-based polymer, polycarbonate-based polymer, polystyrene polymer and elastomeric polymer.

4. **UV exposure:** Use UV exposure to cure the liquid polymer. Light intensity and wavelength depend on the materials used. After curing, peel-off the PDMS mould. At this stage, the insert including a plurality of sample wells is formed. The sample wells include a shape that converges from the peripheral wall to the aperture at the top portion (such as truncated pyramid or cone), thereby preventing an aggregation of the cells formed in the 3D cell culture from escaping the sample wells.

5. **Coating metal layer (Optional for light sheet microscopy):** Coat the sample with a metal layer to provide a reflective surface to at least a part of the peripheral wall of the plurality of sample wells. The reflective surface is configured to illuminate the one or more cells contained in the plurality of sample well by reflecting an incident lightsheet.

6. **Glass coverslip preparation:** Coat a glass coverslip with a thin layer of e.g NOA73 and cure it partially.

7. **Membrane transfer:** Gently press the coated coverslip to the membrane so that the pre-cured NOA makes contact with the upper frame of the membrane. Terminate the curing of the NOA73 on the glass by UV exposure and peel-off the flat PDMS carrier substrate.

Step 4: Passivation of inserts

Passivation of the insert can avoid any adhesion of cells to the chips during the culturing of the cells and can allow 3D culture of the cells. Lipidure® (CM5206, NOF America) at 2% (w/v) in dissolved pure ethanol and stored in 4°C.

1. Place the coverslip into a bigger container to avoid menisque effect. (e.g. Petri of 60mm for coverslip of 25mm). Optionally, plasma cleaning of the coverslip can be carried out.

2. Pour the lipidure solution to fully cover coverslip and degas it for 5-15 minutes.

3. Take out the coverslip from the lipidure mixture and leave it to dry at room temperature under a cell culture hood (>1 hour).

4. Just before cell seeding, replace the PBS with culture medium and sterilize the insert with UV light for 30 minutes under the cell culture hood.

[0051] Organoids, or 3D organotypic cultures, or spheroids, or tumoroids (3D culture of cancer cells) are three-dimensional tissue cultures that consists of multiple cell types derived from one or a few cells that are used as starting material. Examples of such cells are, but not limited to, embryonic stem cells, pluripotent stem cells, induced pluripotent stem cells (iPSCs), multipotent cell, totipotent stem cells, primary cells, adult stem cells, cord blood stem cells, cancer cells. The multiple cell types arise from the self-renewal and differentiating capability of the cells that are used as starting material, wherein the multiple cell types can self-organize in three-dimensional and incorporate some of the key features of an organ to form an organoid. Uses of the insert for organoid growth and analysis are exemplified in the following proof of concept experiments.

[0052] The insert of the example embodiments includes arrays of samples wells to grow and image thousands of spheroids or organoids. The shape of the sample wells converges towards the top portion of the wells and includes apertures large enough to allow seeding of the initial small cell aggregates but small enough to block the larger cell aggregates that grew inside the wells to escape from the wells. The apertures are also large enough to allow the diffusion of culture medium inside the sample wells, thus eliminating the step of pipetting each well to change the medium as required for conventional well plates. As a result, it minimizes the loss of material due to pipetting. The inserts of the example embodiment are also compatible with different techniques of imaging. Hence the same insert can be used from the cell seeding to 3D imaging of the

mature spheroids or organoids. The mature spheroids or organoids can also be released from the inserts at the end of the experiment for other procedures.

[0053] It will be appreciated by a person skilled in the art that numerous variations and/or modifications may be made to the present invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

CLAIMS

1. An insert for conducting 3D cell culture comprising:

a plurality of sample wells for containing one or more cells, wherein each of the plurality of sample wells is defined by a peripheral wall and an aperture at a top portion of the peripheral wall for insertion of the one or more cells into the plurality of sample wells,

wherein each of the plurality of sample wells is configured to include a shape that converges from the peripheral wall to the aperture at the top portion, thereby preventing an aggregation of the cells formed in the 3D cell culture from escaping the plurality of sample wells through the aperture,

wherein the peripheral wall of the plurality of sample wells is at least partially provided with a reflective surface to illuminate the one or more cells contained in the plurality of sample wells by reflecting an incident lightsheet, and

wherein the peripheral wall comprises one or more side walls that taper at an angle of 45° from a horizontal plane.

2. The insert as claimed in claim 1, wherein the one or more cells form into a spheroid during the 3D cell culture.

3. The insert as claimed in claim 1 or 2, wherein the shape of the plurality of sample wells comprises a shape of a truncated pyramid.

4. The insert as claimed in claim 1 or 2, wherein the shape of the plurality of sample wells comprises a shape of a truncated cone.

5. The insert as claimed in any one of the preceding claims, wherein each of the plurality of sample wells comprises a through hole formed within the insert.

6. The insert as claimed in claim 5, wherein the insert is configured to be detachably adhered to a vessel, such that the insert and vessel are detachable from each other to release the one or more cells contained inside the plurality of sample wells.

7. The insert as claimed in claim 1, wherein the reflective surface comprises a metal coat deposited on the peripheral wall.

8. The insert as claimed in any one of the preceding claims, wherein the peripheral wall of the plurality of sample wells is treated with a long term passivation technique to avoid an adhesion of the one or more cells to the peripheral wall.

9. The insert as claimed in any one of the preceding claims, wherein the insert is fabricated using at least one UV curable polymer selected from a group consisting of: acrylate-based polymer, polycarbonate-based polymer, polystyrene polymer, glass, glass-like or glass particles filled resins and elastomeric polymer.

10. A method of fabricating a insert for conducting 3D cell culture, the method comprising the steps of:

dropping a liquid polymer onto a mould to fill in cavities defined by the mould;

curing the liquid polymer to form a plurality of sample wells, wherein each of the plurality of sample wells is defined by a peripheral wall and an aperture at a top portion of the peripheral wall for insertion of one or more cells into the plurality of sample wells; and

providing a reflective surface to at least a part of the peripheral wall of the plurality of sample wells, wherein the reflective surface is configured to illuminate the one or more cells contained in the plurality of sample well by reflecting an incident lightsheet,

wherein the cavities mould each of the plurality of sample wells into a shape that converges from the peripheral wall to the aperture at the top portion, thereby preventing an aggregation of the cells formed in the 3D cell culture from escaping the plurality of sample wells through the aperture, and

wherein the peripheral wall comprises one or more side walls that taper at an angle of 45° from a horizontal plane.

11. The method as claimed in claim 10, wherein the cavities provide each of the plurality of sample wells with a shape of a truncated pyramid.

12. The method as claimed in claim 10, wherein the cavities provide each of the plurality of sample wells with a shape of a truncated cone.

13. The method as claimed in any one of claims 10 to 12, wherein each of the plurality of sample wells comprises a through hole formed within the insert.

14. The method as claimed in claim 13, wherein the insert is configured to be detachably adhered to a vessel, such that the insert and vessel are detachable from each other to release the one or more cells contained inside the plurality of sample wells.

15. The method as claimed in claim 10, wherein the step of providing a reflective surface comprises depositing a metal coat onto the peripheral wall.

16. The method as claimed in any one of claims 10 to 15, further comprises the step of:

treating the peripheral wall of the plurality of sample wells with a long term passivation technique to avoid an adhesion of the one or more cells to the peripheral wall.

17. The method as claimed in any one of claims 10 to 16, wherein the liquid polymer comprises at least one UV curable polymer selected from a group consisting of: acrylate-based polymer, polycarbonate-based polymer, polystyrene polymer and elastomeric polymer.

18. A microscope assembly comprising the insert according to any one of claims 1 to 9.

19. The microscope assembly according to claim 18, wherein the microscope assembly comprises a fluorescence microscopy.

20. The microscope assembly according to claim 19, wherein the fluorescence microscopy is a selective plane illumination microscopy (SPIM).

21. A method for imaging cell cultures, the method comprising the steps of:

seeding a sample comprising one or more cells into the insert as claimed in any one of claims 1 to 9;

adding a culture medium to the insert containing the one or more cells; and

capturing an image of the one or more cells contained within the insert using a microscopy technique.

22. The method for imaging cell cultures as claimed in claim 21, wherein the microscopy technique is fluorescence microscopy.

23. The method for imaging cell cultures as claimed in claim 22, wherein the fluorescence microscopy is selective plane illumination microscopy (SPIM).