

in this disclosure) are structured and arranged to provide an environment that is conducive to the formation of spheroids in culture. That is, in embodiments, the microwells have spheroid-inducing geometry. In addition, the wells are structured and arranged to provide for the movement of liquid into and out of the wells without trapping air between the substrate and liquid or liquid droplets that are introduced into the wells. That is, in embodiments, the microwells have capillary structures. For example, the wells in which cells are grown can be non-adherent to cells to cause the cells in the wells to associate with each other and form spheres. The spheroids expand to size limits imposed by the geometry of the wells. In some embodiments, the wells are coated with an ultra-low binding material to make the wells non-adherent to cells.

[0010] In some embodiments, the cell culture devices have frames comprising the footprint of the device, the substrate of which is configured such that cells cultured in the devices form spheroids. For example, the cell culture substrate in the devices is non-adherent to cells to cause the cells to associate with each other instead of the substrate. The cell culture substrate is further comprised of a plurality of microwells (or wells), the geometry of which enable cells grown in the wells to form similar-sized cell aggregates or spheroids. The spheroids expand to size limits imposed by the geometry of the microwells. In some embodiments, the wells are have a low-binding treatment or are coated with an ultra-low binding material to make the wells non-adherent to cells.

[0010a] In an aspect, there is provided a cell culture substrate comprising an array of microwells, each microwell comprising an opening into the microwell defined by a rounded top well edge, at least one microwell side-wall, and a rounded well-bottom, wherein at least the well-bottom comprises gas permeable material.

[0011] Examples of non-adherent material include perfluorinated polymers, olefins, or like polymers or mixtures thereof. Other examples include agarose, non-ionic hydrogels such as polyacrylamides, polyethers such as polyethylene oxide and polyols such as polyvinyl alcohol, or like materials or mixtures thereof. The combination of, for example, non-adherent wells, well geometry (e.g., size and shape), and/or gravity induce cells cultured in the wells to self-assemble into spheroids. Some spheroids maintain differentiated cell function indicative of a more *in vivo*-like, response relative to cells grown in a monolayer. Other cells types, such as mesenchymal stromal cells, when cultured as spheroids retain their pluripotency,

CLAIMS

What is claimed is:

1. A cell culture substrate comprising an array of microwells, each microwell comprising an opening into the microwell defined by a rounded top well edge, at least one microwell side-wall, and a rounded well-bottom, wherein at least the well-bottom comprises gas permeable material.
2. The cell culture substrate of claim 1 wherein each microwell, in cross section, has a sinusoidal well shape.
3. The cell culture substrate of claim 1, further comprising one or more ridges, wherein the ridge is round, angular, needle-shaped or hexagonal.
4. The cell culture substrate of claim 1, further comprising one or more fissures, wherein the fissure is round, angular, needle-shaped or hexagonal.
5. The cell culture substrate of any one of claims 1-4, wherein the rounded well bottom has a thickness of between 10 and 100 μm .
6. The cell culture substrate of any one of claims 1-4, wherein walls of the microwells are relatively thicker proximate to the opening into the microwell and relatively thinner at the bottom of the microwell.
7. The cell culture substrate of any one of claims 1-6 wherein the substrate comprises 2-10000 of said microwells per square centimeter of surface of said cell culture substrate.

8. The cell culture substrate of any one of claims 1-7 wherein at least the rounded well-bottom comprises a non-adherent surface.
9. The cell culture substrate of any one of claims 1-8, wherein the microwell sidewalls are discontinuous.
10. The cell culture substrate of claim 9 wherein the microwell sidewalls are corrugated.

11. The cell culture substrate of any one of claims 1-10 wherein the cell culture substrate comprises at least a portion of a cell culture container.
12. The cell culture substrate of claim 11 wherein the cell culture container is selected from the group consisting of a multiwell plate, a dish, a flask, a tube, a multi-layer flask, a soft-sided flask and a bag.
13. The cell culture substrate of any one of claims 1-12, wherein microwells are configured to allow fluid communication between at least one of said microwells and a single liquid reservoir.
14. The cell culture substrate of claim 11 or 12, wherein the cell culture container further comprises a mesh placed on top of the array of microwells.
15. The cell culture substrate of any one of claims 1-14 wherein the opening into the microwell
has an effective diameter D_{top} , the rounded well-bottom has a nadir, and each microwell has one or more sidewalls, the one or more sidewalls have a height H extending from the nadir of the rounded well-bottom to the opening into the microwell, wherein each microwell has an effective diameter halfway between the opening into the microwell and the nadir of the rounded well-bottom of $D_{half-way}$, and wherein the ratio of $D_{top} : D_{half-way}$ is 1.5 : 2.5.
16. The cell culture substrate of claim 15, wherein $H = 0.7$ to $1.3 D_{half-way}$.
17. The cell culture substrate of claim 15, wherein $D_{half-way}$ is 200 to 1000 μm .
18. The cell culture substrate of any preceding claim, wherein the gas permeable material is selected from polystyrene, polycarbonate, ethylene vinyl acetate, polysulfone, polymethyl pentene (PMP), polytetrafluoroethylene (PTFE) or compatible fluoropolymer, a silicone rubber or copolymer, poly(styrene-butadiene-styrene), or polyolefin, such as polyethylene or polypropylene, or combinations thereof.

19. The cell culture substrate of claim 18, wherein the gas permeable material is selected from the following:

PE or PS and has a thicknesses of up to 0.2 mm; PDMS and has a thickness of up to 40 μm ; or PMP having a thickness in a range of about 0.02 to 1 mm.

20. The cell culture substrate of any preceding claim, where the wells have an oxygen transmission rate through the gas permeable material of 2000 $\text{cc}/\text{m}^2/\text{day}$ or greater.

21. The cell culture substrate of any preceding claim, wherein at least the bottom of well has a thickness of between 10 and 100 μm .

22. The cell culture substrate of any preceding claim, wherein the at least one side-wall of each microwell is relatively thicker proximate to the opening into the well and relatively thinner at the bottom of the well.