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 treatement 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.

[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,

[**0012**] In some embodiments, the systems, devices, and methods herein comprise one or more cells. In some embodiments, the cells are cryopreserved. In some embodiments, the cells are in three dimensional culture. In some such embodiments, the systems, devices, and methods

comprise one or more spheroids. In some embodiments, one or more of the cells are actively dividing. In some embodiments, the systems, devices, and methods comprise culture media (e.g., comprising nutrients (e.g., proteins, peptides, amino acids), energy (e.g., carbohydrates), essential metals and minerals (e.g., calcium, magnesium, iron, phosphates, sulphates), buffering agents (e.g., phosphates, acetates), indicators for pH change (e.g., phenol red, bromo-cresol purple), selective agents (e.g., chemicals, antimicrobial agents), etc.). In some embodiments, one or more test compounds (e.g., drug) are included in the systems, devices, and methods.

[0013] A wide variety of cell types may be cultured. In some embodiments, a spheroid contains a single cell type. In some embodiments, a spheroid contains more than one cell type. In some embodiments, where more than one spheroid is grown, each spheroid is of the same type, while in other embodiments, two or more different types of spheroids are grown. Cells grown in spheroids may be natural cells or altered cells (e.g., cell comprising one or more non-natural genetic alterations). In some embodiments, the cell is a somatic cell. In some embodiments, the cell is a stem cell or progenitor cell (e.g., embryonic stem cell, induced pluripotent stem cell) in any desired state of differentiation (e.g., pluripotent, multi-potent, fate determined, immortalized, etc.). In some embodiments, the cell is a disease cell or disease model cell. For example, in some embodiments, the spheroid comprises one or more types of cancer cells or cells that can be induced into a hyper-proliferative state (e.g., transformed cells). Cells may be from or derived from any desired tissue or organ type, including but not limited to, adrenal, bladder, blood vessel, bone, bone marrow, brain, cartilage, cervical, corneal, endometrial, esophageal, gastrointestinal, immune system (e.g., T lymphocytes, B lymphocytes, leukocytes, macrophages, and dendritic cells),liver, lung, lymphatic, muscle (e.g., cardiac muscle), neural, ovarian, pancreatic (e.g., islet cells), pituitary, prostate, renal, salivary, skin, tendon, testicular, and thyroid. In some embodiments, the cells are mammalian cells (e.g., human, mice, rat, rabbit, dog, cat, cow, pig, chicken, goat, horse, etc.).

[0014] The cultured cells find use in a wide variety of research, diagnostic, drug screening and testing, therapeutic, and industrial applications.

[0015] In some embodiments, the cells are used for production of proteins or viruses. Systems, devices, and methods that culture large numbers of spheroids in parallel are

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.

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 at least the rounded well-bottom comprises gas permeable material.

10. The cell culture substrate of any one of claims 1-9, wherein the microwell sidewalls are discontinuous.

11. The cell culture substrate of claim 10 wherein the microwell sidewalls are corrugated.

12. The cell culture substrate of any one of claims 1-11 wherein the cell culture substrate comprises at least a portion of a cell culture container.

13. The cell culture substrate of claim 12 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.

14. The cell culture substrate of any one of claims 1-13, wherein microwells are configured to allow fluid communication between at least one of said microwells and a single liquid reservoir.

15. The cell culture substrate of claim 12 or 13, wherein the cell culture container further comprises a mesh placed on top of the array of microwells.

16. The cell culture substrate of any one of claims 1-15 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 $D_{half\text{-}way}$, and wherein the ratio of D_{top} : $D_{half\text{-}way}$ is 1.5 : 2.5.

17. The cell culture substrate of claim 16, wherein H = 0.7 to 1.3 $D_{half\text{-}way}$.

18. The cell culture substrate of claim 16, wherein $D_{half\text{-}way}$ is 200 to 1000 μm.