

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 extending from the opening of the well to the well-bottom, wherein the ridge is round, angular, needle-shaped or hexagonal.
4. The cell culture substrate of claim 1, further comprising one or more fissures extending from the opening of the well to the well-bottom, 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.

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 $D_{\text{half-way}}$, and wherein the ratio of $D_{\text{top}} : D_{\text{half-way}}$ is 1.5 - 2.5:1.
16. The cell culture substrate of claim 15, wherein $H = 0.7$ to $1.3 D_{\text{half-way}}$.
17. The cell culture substrate of claim 15, wherein $D_{\text{half-way}}$ 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), polydimethylsiloxane (PDMS), 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:

polyethylene (PE) or polystyrene (PS) and has a thicknesses of up to 0.2 mm; polydimethylsiloxane (PDMS) and has a thickness of up to 40 mm; or polymethylpentene (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 cc/m²/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.