

BRIEF SUMMARY

[0005] In accordance with various embodiments of the present disclosure, cell culture apparatuses having one or more plates, which can be stacked, are described. The plates have a major surface defining a structured surface. The structured surface defines a plurality of wells in which cells can be cultured. The wells can have dimensions on the micrometer scale and can be configured to promote formation of spheroid cell clusters. The apparatuses described herein also have a plurality of spacers to maintain the space between stacked plates. In embodiments, such spacers include posts or spaced apart rails that extend from the major surface along the length of the plate. A plurality of flow channels are formed between adjacent plates and/or rails for perfusion of cell culture media. The structured surfaces can support very high cell density cell growth, which can be enhanced by spheroid formation. Spheroid formation should also enhance cell functional stability, as spheroids can maintain differentiated cell function indicative of a more in vivo like response relative to cells grown in a monolayer. In some embodiments, the wells in which cells are cultured are non-adherent to the cells, which can facilitate harvesting of the cells. In various embodiments described herein, the apparatuses are designed to allow for passive diffusion of metabolic gases, which can allow for lower media perfusion rates and thus subject the cultured cells to less shear.

[0005a] In an aspect, there is provided a cell culture apparatus comprising:

a plurality of plates having a first major surface and an opposing second major surface, wherein the first major surface comprises a plurality of microwells, wherein the plurality of plates are stacked on one another; and a plurality of spacers extending from the first major surface, wherein a plurality of cell culture media flow channels are defined between adjacent plates, wherein the plates are gas permeable via the microwells.

[0006] In various embodiments, this disclosure describes a cell culture apparatus. The cell culture apparatus includes one or more plates having a first major surface and an opposing second major surface. The first major surface comprises a structured surface defining a plurality of wells. Each well has an interior surface defining an upper aperture and a nadir, wherein the upper aperture of each well has a diametric dimension

in a range from 200 micrometers to 500 micrometers, or a range from 100 micrometers to 2000 micrometers. The apparatus also includes a plurality of spaced apart rails (spacers) extending from the first major surface along a length of the first major surface. A plurality of flow channels are defined between adjacent rails.

[0006a] In another aspect, there is provided a cell culture system comprising:

a cell culture apparatus as defined above; and

an oxygenator coupled to the inlet.

[0007] In some embodiments, provided herein are methods of producing protein (e.g., a therapeutic protein, such as a therapeutic antibody or antibody fragment), comprising: a) culturing cells expressing a protein in the wells of a cell culture apparatus as described herein; and b) isolating the protein from the cells.

[0007a] In another aspect, there is provided a method comprising:

introducing cells into the wells of a cell culture apparatus as defined above ; and perfusing a cell culture medium through the media flow channels of the cell culture apparatus at a first rate to culture the cells in the wells.

[0007b] A method of producing protein, comprising:

a) culturing cells expressing a protein in the microwells of a cell culture apparatus as defined above; and

b) isolating said protein from said cells.

What is claimed is:

1. A cell culture apparatus comprising:
a plurality of plates having a first major surface and an opposing second major surface, wherein the first major surface comprises a plurality of microwells, wherein the plurality of plates are stacked on one another; and
a plurality of spacers extending from the first major surface, wherein a plurality of cell culture media flow channels are defined between adjacent plates,
wherein the plates are gas permeable via the microwells.
2. The cell culture apparatus of claim 1, wherein the spacers extend from the first major surface along a length of the plate, wherein a plurality of cell culture media flow channels are defined between adjacent spacers.
3. The cell culture apparatus of claim 1 or 2, wherein each microwell has an interior surface defining an upper aperture and a nadir, wherein the upper aperture of each microwell has a diametric dimension in a range from 100 micrometers to 2000 micrometer.
4. The cell culture apparatus of claim 1, wherein at least one pair of adjacent stacked plates defines a cell culture chamber having a bottom surface defined by the first major surface of one of the stacked plates and a top surface defined by the second major surface of the adjacent stacked plate.
5. The cell culture apparatus according to any one of claims 1 or 4, further comprising a top plate disposed over a top-most cell culture plate of the plurality of stacked plates.

6. The cell culture apparatus according to any one of claims 1-5, further comprising an inlet and an outlet, wherein each of the plurality of media flow channels is in fluid communication with the inlet and the outlet.
7. The cell culture apparatus according to claim 6, further comprising an inlet manifold that defines the inlet and defines a plurality of inlet flow channel apertures, wherein each of the plurality of inlet flow channel apertures is in fluid communication with a respective media flow channel.
8. The cell culture apparatus according to claim 6, further comprising an outlet manifold that defines the outlet and defines a plurality of outlet flow channel apertures, wherein each outlet flow channel apertures is in fluid communication with a respective media flow channel.
9. The cell culture apparatus according to any one of claims 1-8, wherein at least one spacer comprises an air channel extending the length of the spacer, wherein the air channel is in gaseous communication with an exterior of the cell culture apparatus and is in gaseous communication with at least one of the plurality of media flow channels defined by the spacer.
10. The cell culture apparatus according to any one of claims 1-9, wherein the plates are stacked to align spacers of adjacent plates, the aligned spacers defining walls extending from a bottom-most plate to a top-most plate.
11. The cell culture apparatus according to claim 10, wherein at least one wall defines an air channel extending from the bottom-most plate to the top-most plate, wherein the air channel is in gaseous communication with an exterior of the cell culture apparatus and is in gaseous communication with respective media flow channels defined by the spacers of the wall.

12. The cell culture apparatus according to any one of claims 1-11, wherein the said plurality of microwells comprises an array of hexagonal close-packed microwells.
13. The cell culture apparatus according to claim 3, wherein the microwells have depth defined from the upper aperture to the nadir, wherein the depth is in a range from 200 micrometers to 2000 micrometers.
14. The cell culture apparatus of any one of claims 1-13, wherein each of the wells are non-adherent to microcells.
15. A cell culture system comprising:
a cell culture apparatus according to any one of claims 6-8; and
an oxygenator coupled to the inlet.
16. The cell culture system of Claim 15, further comprising a pump coupled to the inlet.
17. A method comprising:
introducing cells into the microwells of a cell culture apparatus according to claim 1; and
perfusing a cell culture medium through the media flow channels of the cell culture apparatus at a first rate to culture the cells in the microwells.
18. The method according to claim 17, further comprising perfusing the cell culture medium through the media flow channels at a second rate higher than the first rate to remove the cells from the microwells.

19. A method of producing protein, comprising:
- a) culturing cells expressing a protein in the microwells of a cell culture apparatus according to any one of claims 1-14; and
 - b) isolating said protein from said cells.