SUMMARY

[0005] In accordance with 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 examples, such spacers include spaced apart rails that extend from the major surface along the length of the plate. A plurality of flow channels are formed between adjacent 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 examples, the wells in which cells are cultured are non-adherent to the cells, which can facilitate harvesting of the cells. In various examples 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.

[0006] In various examples, 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. 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 one aspect, the present disclosure provides 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, and 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.

[0006b] In another aspect, the present disclosure provides a cell culture system comprising:

a cell culture apparatus as described herein; and

an oxygenator coupled to the inlet.

[0006c] In another aspect, the present disclosure provides a method comprising:

introducing cells into the microwells of a cell culture apparatus as described herein; 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.

[0007] Provided herein are methods of producing proteins (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, the present disclosure provides a method of producing protein, comprising:

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

b) isolating said protein from said cells.

[0008] In some examples, wells (e.g., microwells) have a cross-sectional shape approximating a sine wave. In such examples, the bottom of the well is rounded (e.g., hemispherically round), the side walls increase in diameter from the bottom of the well to the top and the boundary between wells is rounded. As such the top of the wells does not terminate at a right angle. In some examples, a well has a diameter D at the half-way point (also termed $D_{half\text{-}way}$) between the bottom and the top, a diameter D_{top} at the top of the well and a height H from the bottom to top of the well. In these examples, D_{top} is greater than D. Additional examples are shown in Fig. 15, where the width of the well is greater than the width of the barrier between contiguous wells. Such an embodiment permits a greater number of wells within a given area of a culture surface.

[0009] Additional features and advantages of the subject matter of the present disclosure will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the subject matter of the present disclosure as described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0010] It is to be understood that both the foregoing general description and the following detailed description present examples of the subject matter of the present disclosure, and are intended to provide an overview or framework for understanding the nature and character of the subject matter of the present disclosure as it is claimed. The accompanying drawings are included to provide a further understanding of the subject matter of the present disclosure, and are incorporated into and constitute a part of this specification. The drawings illustrate various examples of the subject matter of the present disclosure and together with the description serve to explain the principles and operations of the subject matter of the present disclosure. Additionally, the drawings and descriptions are meant to be merely illustrative, and are not intended to limit the scope of the claims in any manner.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The following detailed description of specific embodiments of the present disclosure can be best understood when read in conjunction with the following drawings, where like structure is indicated with like reference numerals and in which: