

PERFUSION BIOREACTOR PLATFORM

PRIORITY CLAIM

The present disclosure claims priority to U.S. Provisional Application Serial number 62/072039, filed October 29, 2014, which is herein incorporated by reference in its entirety.

FIELD

[0001] The present disclosure relates to apparatuses, systems and methods for culturing cells.

TECHNICAL BACKGROUND

[0002] The viable cell density (VCD) that can be maintained is a primary variable driving productivity and cost in bioreactor vessels. In bioreactors one limitation to achieving the highest possible VCD is the shear force that cells experience as bags are rocked faster or impeller velocities increased to accommodate elevated nutrient and gas exchange requirements due to the high density of cells in culture. Additionally, for applications where attachment dependent cells are the product, such as for production of cells for screening, cellular therapy, or regenerative medicine applications, it can be difficult to harvest the cells from the reactor.

[0003] In recent years there have been efforts to run bioreactors in a steady state, continuously perfused mode in an effort to greatly reduce production costs while maintaining consistent quality in an automated process. Although this method has been shown to be a powerful method to maintain high cell densities, there are difficulties regarding its suitability for commercial production of therapeutic proteins. One risk is unstable expression levels after a prolonged period in culture, with cells exhibiting fastest growth potential rather than those expressing the protein of interest being selected over time.

[0004] Accordingly, new cell culture apparatuses that provide for one or more of increased stability of cells of interest, ease of harvest of attachment dependent cells, or increased viable cell density would be desirable.

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.
- [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.
- [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.

[0008] In some embodiments, wells (e.g., microwells) have a cross-sectional shape approximating a sine wave. In such embodiments, 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 embodiments, a well has a diameter D at the half-way point (also termed $D_{\text{half-way}}$) between the bottom and top, a diameter D_{top} at the top of the well and a height H from bottom to top of the well. In these embodiments, D_{top} is greater than D . Additional embodiments 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 embodiments 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 embodiments 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:

- [0012] **FIG. 1** is a schematic side view of an embodiment of a substrate having a structured surface having wells for culturing cells.
- [0013] **FIG. 2** is a schematic cross-sectional view of an embodiment of a well of a structured surface.
- [0014] **FIG. 3** is a schematic cross-sectional view of an embodiment of a well of a structured surface.
- [0015] **FIG. 4** is an image of an embodiment of a substrate having a structured surface.
- [0016] **FIG. 5** is a schematic top view of cells grown in wells of an embodiment of a structured surface.
- [0017] **FIGS. 6A-C** are schematic perspective views of an embodiments of plates and rails.
- [0018] **FIG. 7** is a schematic cross-sectional view of an embodiment of a cell culture apparatus.
- [0019] **FIG. 8** is a schematic side view of an embodiment of a cell culture apparatus.
- [0020] **FIGS. 9A-B** are schematic views of a face of an embodiment of a manifold (**9A**) and an expander (**9B**).
- [0021] **FIGS. 10A-B** is a schematic diagram of an embodiment of a system operating in batch mode (**10A**) and continuous mode (**10B**).
- [0022] **FIGS. 11A-B** are schematic sectional views of an embodiments of wells.
- [0023] **FIG. 12** is a schematic sectional view of embodiments of stacked units of plates and rails.
- [0024] **FIG. 13** is a schematic cut-away perspective view of embodiments of stacked units of plates and rails.
- [0025] **FIG. 14** is a schematic side view of an embodiment of a cell culture apparatus.
- [0026] **FIG. 15A** shows a schematic cut-away perspective view of a cell culture apparatus **600**, where the wells are formed from gas permeable material. In this exemplary embodiment, media enters one end of the apparatus through inlet port **625**, and

circulates through the whole apparatus before exiting (through outlet port **635**), reversing flow through each chamber. **FIG. 15B** shows a system for controlling flow through the cell culture apparatus illustrated in **FIG. 15A**.

[0027] **FIG. 16A** shows a schematic cut-away perspective view of a cell culture apparatus **600**, where the wells are formed from gas permeable material. In this exemplary embodiment, media enters through the inlet port **625** and distributes (via inlet manifold **620**) to all compartments before exiting the opposite end through outlet manifold **630** attached to the outlet port **635**. **FIG. 16B** shows a system for controlling flow through the cell culture apparatus illustrated in **FIG. 16A**.

[0028] **FIG. 17A and B** are schematic drawings of an exemplary embodiment of an array of wells **100**. **FIG. 17A** is an illustration in cross-section. **FIG. 17B** is a top-down drawing of the exemplary embodiment of an array of wells, taken at line B-B of **FIG. 17A**.

[0029] **FIG. 18A and B** are schematic drawings of another exemplary embodiment of an array of wells **100**. **FIG. 18A** is an illustration in cross-section. **FIG. 18B** is a top-down drawing of the exemplary embodiment of an array of wells, taken at line B-B of **FIG. 18A**.

[0030] **FIG. 19A – C** are schematic drawings of another exemplary embodiment of an array of wells **100**. **FIG. 19A** is an illustration in cross-section. **FIG. 19B** is a top-down drawing of the exemplary embodiment of an array of wells, taken at line B-B of **FIG. 19A**. **FIG. 19C** is a drawing of an array of wells having a sinusoidal or parabolic shape.

DETAILED DESCRIPTION

[0031] Reference will now be made in greater detail to various embodiments of the subject matter of the present disclosure, some embodiments of which are illustrated in the accompanying drawings. Like numbers used in the figures refer to like components, steps and the like. However, it will be understood that the use of a number to refer to a component in a given figure is not intended to limit the component in another figure labeled with the same number. In addition, the use of different numbers to refer to components is not intended to indicate that the different numbered components cannot be the same or similar to other numbered components.

- [0032]** The present disclosure describes, among other things, cell culture apparatuses having a plurality of stacked plates, each having a first major surface and an opposing second major surface. The first major surface includes a structured surface defining a plurality of or an array of microwells. In embodiments, a structured surface is a substrate having an array of microwells. 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 in a range from 100 micrometers to 2000 micrometers. The apparatus also includes a plurality of spacers to maintain a gap between stacked plates. The spacers can include plural spaced apart rails extending from the first major surface along a length of the first major surface. A plurality of flow channels are defined between adjacent rails.
- [0033]** In some embodiments, the wells can be configured such that cells cultured in the wells form spheroids. For example, interior surfaces of the well can be non-adherent to cells to cause the cells in the wells to associate with each other and form spheres. The spheroids can expand to size limits imposed by the geometry of the wells. In some embodiments, the wells can be coated with an ultra-low binding material to make the wells non-adherent to cells.
- [0034]** The formation of three-dimensional (3D) cell agglomerates such as spheroids, as opposed to two-dimensional cell culture in which the cells form a monolayer on a surface, can increase the density of cells grown in a cell culture apparatus. Increased cell density can lead to an increased metabolic burden. In various embodiments described herein diffusion of metabolic gasses occurs through the plate material, rather than relying on flow of cell culture medium, are described. Such apparatuses can be better suited to meet the metabolic demands of cells cultured in high density.
- [0035]** Cells cultured in three dimensions, such as spheroids, can exhibit more in vivo like functionality than their counterparts cultured in two dimensions as monolayers. In two dimensional cell culture systems, cells can attach to a substrate on which they are cultured. However, when cells are grown in three dimensions, such as spheroids, the cells interact with each other rather than attaching to the substrate. Cells cultured in three dimensions more closely resemble in vivo tissue in terms of cellular communication and the development of extracellular matrices. Spheroids thus provide a superior model for cell migration, differentiation, survival, and growth and

therefore provide better systems for research, diagnostics, and drug efficacy, pharmacology, and toxicity testing.

- [0036] In some embodiments, the devices are configured such that cells cultured in the devices form spheroids. 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 a non-adherent material. In embodiments, the non-adherent material is an ultra-low binding material to make the wells non-adherent to cells.
- [0037] 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. In embodiments, these may be ultra low binding materials. 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,
- [0038] 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.

- [0039]** 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.).
- [0040]** The cultured cells find use in a wide variety of research, diagnostic, drug screening and testing, therapeutic, and industrial applications.
- [0041]** 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 particularly effective for protein production. Three-dimensional culture allows for increased cell density, and higher protein yield per square centimeter of cell growth surface area. Any desired protein or viruses for vaccine production may be grown in the cells and isolated or purified for use as desired. In some embodiments, the protein is a native protein to the cells. In some embodiments, the protein is non-native. In some embodiments, the protein is expressed recombinantly. Preferably, the protein is overexpressed using a non-native promoter. The protein may be expressed as a fusion protein. In some embodiments, a purification or detection tag is expressed as a fusion partner to a protein of interest to facilitate its purification and/or detection. In some

embodiments, fusions are expressed with a cleavable linker to allow separation of the fusion partners after purification.

- [0042]** In some embodiments, the protein is a therapeutic protein. Such proteins include, but are not limited to, proteins and peptides that replace a protein that is deficient or abnormal (e.g., insulin), augment an existing pathway (e.g., inhibitors or agonists), provide a novel function or activity, interfere with a molecule or organism, or deliver other compounds or proteins (e.g., radionuclides, cytotoxic drugs, effector proteins, etc.). In some embodiments, the protein is an immunoglobulin such as an antibody (e.g., monoclonal antibody) of any type (e.g., humanized, bi-specific, multi-specific, etc.). Therapeutic protein categories include, but are not limited to, antibody-based drugs, Fc fusion proteins, anticoagulants, antigens, blood factor, bone morphogenetic proteins, engineered protein scaffolds, enzymes, growth factors, hormones, interferons, interleukins, and thrombolytics. Therapeutic proteins may be used to prevent or treat cancers, immune disorders, metabolic disorders, inherited genetic disorders, infections, and other diseases and conditions.
- [0043]** In some embodiments, the protein is a diagnostic protein. Diagnostic proteins include, but are not limited to, antibodies, affinity binding partners (e.g., receptor-binding ligands), inhibitors, antagonists, and the like. In some embodiments, the diagnostic protein is expressed with or is a detectable moiety (e.g., fluorescent moiety, luminescent moiety (e.g., luciferase), colorimetric moiety, etc.).
- [0044]** In some embodiments, the protein is an industrial protein. Industrial proteins include, but are not limited to, food components, industrial enzymes, agricultural proteins, analytical enzymes, etc.
- [0045]** In some embodiments, the cells are used for drug discovery, characterization, efficacy testing, and toxicity testing. Such testing includes, but is not limited to, pharmacological effect assessment, carcinogenicity assessment, medical imaging agent characteristic assessment, half-life assessment, radiation safety assessment, genotoxicity testing, immunotoxicity testing, reproductive and developmental testing, drug interaction assessment, dose assessment, adsorption assessment, disposition assessment, metabolism assessment, elimination studies, etc. Specific cells types may be employed for specific tests (e.g., hepatocytes for liver toxicity, renal proximal tubule epithelial cells for nephrotoxicity, vascular endothelial cells for vascular

toxicity, neuronal and glial cells for neurotoxicity, cardiomyocytes for cardiotoxicity, skeletal myocytes for rhabdomyolysis, etc.). Treated cells may be assessed for any number of desired parameters including, but not limited to, membrane integrity, cellular metabolite content, mitochondrial functions, lysosomal functions, apoptosis, genetic alterations, gene expression differences, and the like.

[0046] In some embodiments, the cell culture devices are a component of a larger system. In some embodiments, the system comprises a plurality (e.g., 2, 3, 4, 5, ..., 10, ..., 20, ..., 50, ..., 100, ..., 1000, etc.) of such cell culture devices. In some embodiments, the system comprises an incubator for maintaining the culture devices at optimal culture conditions (e.g., temperature, atmosphere, humidity, etc.). In some embodiments, the system comprises detectors for imaging or otherwise analyzing cells. Such detectors include, but are not limited to, fluorimeters, luminometers, cameras, microscopes, plate readers (e.g., PERKIN ELMER ENVISION plate reader; PERKIN ELMER VIEWLUX plate reader), cell analyzers (e.g., GE IN Cell Analyzer 2000 and 2200; THERMO/CELLOMICS CELLNSIGHT High Content Screening Platform), and confocal imaging systems (e.g., PERKIN ELMER OPERAPHENIX high throughput content screening system; GE INCELL 6000 Cell Imaging System). In some embodiments, the system comprises perfusion systems or other components for supplying, re-supplying, and circulating culture media or other components to cultured cells. In some embodiments, the system comprises robotic components (e.g., pipettes, arms, plate movers, etc.) for automating the handling, use, and/or analysis of culture devices.

[0047] Referring now to **FIG. 1** a schematic side view of an embodiment of a plate **110**, or portion thereof, having a first major surface **112** and an opposing second major surface **114** is shown. The first major surface **112** defines a structured surface for culturing cells. The structured surface of the first major surface **112** is a substrate having a plurality of wells **115**. Spheroids **200** are shown residing inside wells **115**.

[0048] A structured surface of a plate as described herein may define any suitable number of wells that may have any suitable size or shape. The wells define a volume based on their size and shape. In many embodiments, one or more or all of the wells are symmetric around a longitudinal axis. In some embodiments, the longitudinal axes of one or more or all of the wells are parallel with one another. The wells may be uniformly or non-uniformly spaced. In embodiments, the wells are uniformly spaced.

One or more or all the wells can have the same size and shape or can have different sizes and shapes.

[0049] Referring now to **FIG. 2 and FIG. 3**, schematic cross-sectional views of some embodiments of wells **115** are depicted. As described above with regard to **FIG. 1**, the wells **115** are defined by the structured surface of plate **110**. The second major surface **114** or non-culture surface of plate **110** can have any suitable shape. As depicted in **FIG. 2**, the plate **110** forming the well **115** has a thickness along the well that is substantially constant and is relatively thin. In contrast, the thickness of the plate **110** defining the well **115** in **FIG. 3** varies along the well, such that proximate to upper aperture **118** of the well, the thickness is relatively large, and at nadir **116** the thickness is relatively thin.

[0050] In some embodiments, the wells **115** are gas permeable through the plate **110**, i.e., through the well wall. The gas permeability of the wells **115** through the plate **110** to second major surface **114** will depend in part on the material of the plate and the thickness of the plate along the well. All else being equal, the well in **FIG. 2** would be more gas permeable than the well in **FIG. 3**. However, depending on the material used and the thickness employed, plate **110** shaped in either **FIG. 2** or **FIG. 3** may be sufficiently gas permeable for purposes of the present disclosure.

[0051] In embodiments, the wells **115** have an oxygen transmission rate through the plate **110** of 2000 cc/m²/day or greater. In embodiments, the wells have a gas permeability through the plate of 3000 cc/m²/day or greater. In embodiments, the wells have a gas permeability through the plate of 5000 cc/m²/day or greater.

[0052] Plate **110** may be formed of any material having a suitable gas permeability over at least a portion of the well. Examples of suitable materials include polydimethylsiloxane (PDMS), (poly)4-methylpentene (PMP), polyethylene (PE), and polystyrene (PS). PDMS can have a high degree of gas permeability and can be achieve sufficient gas permeabilities at thicknesses up to three to four centimeters. PMP can achieve sufficient gas permeabilities at thicknesses up to about 0.03 inches. In some embodiments, PMP having a thickness in a range of about 0.001 inch to about 0.025 inches is used to form wells. PE or PS can achieve sufficient gas permeabilities at thicknesses up to 0.005 inches, such as 0.003 inches or less. Some thinner plates may not have sufficient structural integrity. To compensate for poor

structural integrity, an open frame, standoffs, or the like can be used to support the plate, or a structured surface portion thereof, from the bottom.

- [0053] In embodiments, where the wells **115** are not gas permeable through the plate **110**, thicker or non-gas permeable materials can be used. Examples of suitable materials for forming plates or other cell culture components described herein include polystyrene, polymethylmethacrylate, polyvinyl chloride, polycarbonate, polysulfone, polystyrene copolymers, fluoropolymers, polyesters, polyamides, polystyrene butadiene copolymers, fully hydrogenated styrenic polymers, polycarbonate PDMS copolymers, and polyolefins such as polyethylene, polypropylene, polymethyl pentene, polypropylene copolymers and cyclic olefin copolymers, and the like.
- [0054] Still with reference to **FIGS. 2** and **3**, the wells **115** have a depth **d** defined by the height from nadir **116** to upper aperture **118**. The wells **115** also have a diametric dimension **w**, such as a diameter, width, etc., across the well defined by the upper aperture **118**. The wells may have any suitable depth **d** and diametric dimension **w**. In some embodiments, the depth **d**, diametric dimension **w** and shape of the well, along with the material forming the well, serve to define a volume in which cells can grow.
- [0055] In some embodiments, the inner surface of the wells **115** is non-adherent to cells. The wells may be formed from non-adherent material or may be coated with non-adherent material to form a non-adherent well. 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, and gravity can induce cells cultured in the wells to self-assemble into spheroids. Some spheroids can maintain differentiated cell function indicative of a more *in vivo* like response relative to cells grown in a monolayer.
- [0056] The combination of, for example, non-adherent wells, well geometry, and gravity can define a confinement volume in which growth of cells cultured in the wells is limited.
- [0057] In some embodiments, one or more wells have a concave surface, such as a hemispherical surface, a conical surface having a rounded bottom, and the like surface geometries or a combination thereof. The well and well bottom can ultimately

terminate, end, or bottom-out in a spheroid conducive rounded or curved surface, such as a dimple, a pit, and like concave frusto-conical relief surfaces, or combinations thereof. Other shapes and construction of gas-permeable spheroid-conductive wells are described in commonly-assigned U.S. Patent Application No. 14/087,906, which application is hereby incorporated herein by reference in its entirety to the extent that it does not conflict with the present disclosure.

- [0058] In some embodiments, well bottoms are flat or come to a point. Well bottoms may have any other suitable shape or dimension.
- [0059] In some embodiments, the wells **115** described herein have a diametric dimension **w** in a range from about 200 micrometers to about 500 micrometers, e.g., 200, 250, 300, 350, 400, 450 or 500 micrometers, including ranges between any of the foregoing. Such diametric dimensions can control the size of a spheroid grown therein such that cells at the interior of the spheroid are maintained in a healthy state. In some embodiments, the wells **115** have a depth **d** in a range from about 200 micrometers to about 500 micrometers, e.g., 200, 250, 300, 350, 400, 450 or 500 micrometers, including ranges between any of the foregoing. Of course, other suitable dimensions may also be employed.
- [0060] In some embodiments, the structured surface defining the wells includes an array of hexagonal close-packed well structures. An image of an embodiment of a first major surface **112** having such a structured surface is shown in **FIG. 4**, showing the surface **112** forming wells **115**. **FIG. 5** is a schematic drawing showing cells **200** grown in wells **115** of an embodiment of a plate having a first major surface **112** including a structured surface defining a hexagonal close-packed well structure. In some embodiments, the cells **200** within each well **115** form a single spheroid.
- [0061] A structured surface as described herein can be formed in any suitable matter. For example, a plate or film can be molded or embossed to form the structured surface. In a further example, a heated reform tool may be applied to a molded plate. Or, a structured surface may be formed by injection molding.
- [0062] A plate having a structured surface as described above may be incorporated into a cell culture apparatus. The plates may be stacked to form a cell culture apparatus. In embodiments, the stacked plates are separated by rails, with flow channels formed above a structured surface between adjacent rails.

- [0063] Referring now to **FIGS. 6A-C**, schematic perspective views of embodiments of a single stackable unit **500** having rails **300A**, **300B**, **300C**, **300D** and plates **110** are shown. The rails **300A**, **300B**, **300C**, **300D** extend from the plate **110** along the length of the plate. Flow channels **400A**, **400B**, **400C** are formed between adjacent rails. In the embodiment depicted in **FIG. 6A**, rails **300A**, **300B**, **300C**, **300D** are disposed on the first major surface of the plate **110**. In the embodiment depicted in **FIG. 6B**, the rails **300A**, **300B**, **300C**, **300D** extend below the first major surface of the plate. In the embodiment depicted in **FIG. 6C**, the plate **110** and rails **300A**, **300B**, **300C**, **300D** comprise a unitary part. The rails and plates can be assembled in any suitable manner. For example, the plate and rails can be molded as a single part. In embodiments, the plate or a portion thereof is over-molded to form the rails. In embodiments, the plate and rails are welded (e.g., thermal, laser, long IR or ultrasonic welding, or the like), adhered, solvent-bonded or the like. The plates and rails can form a unit **500**, which can be stacked during assembly of a cell culture apparatus as described herein.
- [0064] Referring now to **FIG. 7**, a schematic cross-section of an embodiment of a cell culture apparatus **600** having a plurality of stacked units **500A-L** is shown. Each unit includes a plate **110** and a plurality of rails **300**. Flow channels **400** are formed between adjacent rails **300** above a first major surface **112** of the plate **110** and below an opposing second major surface **114** of an adjacent plate **110** of the above-stacked unit. A top plate **610** can be disposed above the top-most unit **500A**. Each unit as a plurality of wells **115**.
- [0065] Referring now to **FIG. 8**, a schematic side view of an embodiment of cell culture apparatus **600** having an inlet manifold **620** defining an inlet port **625** and an outlet manifold **630** defining an outlet port **635** is shown. The apparatus **600** includes a plurality of stacked units **500** (e.g., units having a plate and rails as depicted in **FIGS. 6A-C** and **FIG. 7**). Each flow channel of each unit is in fluid communication with inlet port **625** via manifold **620** and is in fluid communication with outlet port **635** via manifold **630**. In some embodiments, an expander having inlet port **625** can be used rather than a manifold. In some embodiments, a reducer having outlet port **635** can be used rather than a manifold. The top face of the apparatus is also shown **610**.
- [0066] For example and with reference to **FIGS. 9A-B**, schematic views of the inside face of an embodiment of a manifold **620** (**FIG. 9A**) and the top face **622** of an embodiment

of a manifold **620** (**FIG. 9B**) are shown. The manifold **620** in **FIG. 9A** defines a plurality of apertures **621**, each of which can be aligned with, and welded to a respective flow channel to create a liquid tight seal. **Fig. 9B** shows the top face of the manifold with the port **625**. Alternatively, because a face of the assembled units can essentially form a manifold with access to each flow channel a simplified expander **620** can be used to deliver cell culture fluid to the flow channels via the inlet port.

- [0067] The inlet port and the outlet port can be coupled to tubing, which can be coupled to a pump for delivery of cell culture media to the flow channels of the cell culture apparatus.
- [0068] Final assembly of a cell culture apparatus described herein can be performed in any suitable manner. For example, units can be welded, bonded, adhered or otherwise joined together. Manifolds, reducers or expanders can be welded, bonded, adhered or otherwise joined to assembled units. By way of example, the units can be inserted into a sleeve onto which inlet and outlet manifolds are joined; stacked and manifolded units could be assembled and overmolded; bags can be heat-shrunk to form a tight fit around the stacked units; units are stacked and packed into a formed chamber or formed bag; and the like.
- [0069] Referring now to **FIGS. 10A-B**, schematic diagrams of embodiments of systems employing embodiments of cell culture apparatuses **600** as described herein are shown. The diagram depicted in **FIG. 10A** illustrates the system in batch mode. Flow of media enters the culture apparatus **600** through input **625**. The media is oxygenated by an optional oxygenator **660**. The force of the flow is generated and controlled by recirculation pump **650**, operably coupled to source of cell culture media. Media flows out of the culture apparatus **600** through output **635**. The flow (e.g., flow rate) is managed by a controller **640** (e.g., via control of valves). The controller may also control gas flow to the oxygenator and control flow of supplemental nutrients or pH modifiers as desired or required. Cultured cells are harvested by directing media from the output **635** to a cell collection cartridge **670**. Each of the components is connected by tubing, channels (e.g., microchannels) or any other desired fluid connectivity. The diagram in **FIG. 10B** illustrates the system in continuous mode. The system further includes an input flow channel **680** through which fluid flows into the cell culture apparatus **600** and an output flow channel **690** through which fluid can flow out of the cell culture apparatus **600**. In some

embodiments described below in which the cell culture apparatus is configured to allow diffusion of metabolic gases, an oxygenator can be omitted.

- [0070] In embodiments where the inner surfaces of the wells of the structured surface of the plates are non-adherent to cells, the flow rate of cell culture media through the flow channels of the apparatus **600** can be increased to dislodge the cultured cells, which can be spheroids, from the wells to be carried with the flow of cell culture medium to cell collection cartridge. Lower flow rates can be used to grow and maintain cells within the wells. It will be understood that the shape and dimensions of the wells can be modified to alter flow rates for maintaining the cells in the wells or to dislodge the cells from the wells.
- [0071] By way of example and with reference to **FIGS. 11A-11B**, schematic sectional views of alternative well geometries are shown. The wells **115** in **FIG. 11B** are deeper than the wells **115** in **FIG. 11A** and thus the spheroids **200** are deeper in the wells **115** in **FIG. 11B** than the wells **115** in **FIG. 11A**. Accordingly, the wells **115** in **FIG. 11B** allow for higher flow rates through the cell culture apparatus without dislodging the spheroids **200**. Similarly, the wells **115** in **FIG. 11A** allow for dislodgment of the spheroids **200** at lower flow rates to facilitate harvesting. As discussed above, wells of structured surfaces of plates described herein can have any suitable geometry. The shape and size and characteristics of the well can be tuned for purposes of tuning cell properties (e.g., spheroid formation), for purposes of facilitating cell dislodgement, for purposes of ensuring sufficient nutrient and gas distribution to cultured cells, and the like, depending on the needs of the user.
- [0072] In some embodiments, gas flow channels can be formed in the rails that coextend with the plates. In such embodiments the rails may be formed from gas permeable materials having sufficient thicknesses to allow metabolic gas exchange from a flow channel adjacent to the rail. Examples of suitable gas permeable materials and thicknesses are described above. In some embodiments, the rails are formed from PDMS, which is relatively gas permeable. By way of example, the rails can be formed from extruded PDMS that has air channel running therethrough. PDMS can be either thermoset or thermoplastic PDMS, such as Geniomer 145 from Wacker which can have higher gas exchange rates than thermoset PDMS. PDMS, however, is well known to bind small molecules, which can be undesirable for cell culture. This could be avoided with multilayer extrusion, overmolding, coatings or other treatments

to create barriers to absorption which can also act as a non-cell adhesive layer. The air channels within the rails can run the length or height of the rails. Multiple air channels can be formed in the rails

- [0073] For example and with reference to **FIG. 12**, a schematic sectional view of stacked units **500A**, **500B**, **500C**, **500D**, each having a plate **110** and rails **300** with flow channels **400** formed between rails (e.g., as described above) is depicted. Spheroids **200** are shown with wells **115** of the structured surface of plate **110**. In the depicted embodiment, rails **300** define air channels **310** that run the length of the rail. The thickness of the rail **300** between the channel **310** and an adjacent media flow channel **400** is sufficiently small to allow exchange of metabolic gases through the material of the rail.
- [0074] Referring now to **FIG. 13**, a schematic cut-away perspective view of stacked units **500A**, **500B**, **500C**, **500D**, each having a plate **110** and rails (e.g., rails **300A-D**) with flow channels **400** formed between rails (e.g., as described above) is depicted. In this embodiment, each rail (e.g., rails **300A-D**) forms a plurality of air channels **310** that extend the height of the rail. The air channels **310** of respective rails (e.g., rails **300A-D**) are oriented such that when the units **500A**, **500B**, **500C**, **500D** are stacked, the air channels **310** align. When the units **500A**, **500B**, **500C**, **500D** are stacked, the rails form a wall **350** from the bottom-most unit (e.g., unit **500D**) to the top-most unit (e.g., unit **500A**). Columns of aligned air channels **310** formed by the rails (e.g., rails **300A-D**) extend throughout the height of the assembled apparatus.
- [0075] Regardless of the orientation of the air channels (e.g., along the length of the rails as depicted in **FIG. 12** or along the height of the rails or wall as depicted in **FIG. 13**), the air channels can be connected to a manifold, expander or reducer to form single supply and vent port exiting the apparatus. In some embodiments, separate manifolds, expanders or reducers are coupled to both ends of the air channels of the apparatus to allow gas flow to be controlled through the stacked unit.
- [0076] For example and with reference to **FIG. 14**, a schematic side view of an embodiment of a cell culture apparatus **600** including first **370** and second **380** vent manifolds is depicted. Many of the components depicted in the apparatus of **FIG. 14** are depicted in, and described above with regard to the apparatus of **FIG. 8**. The apparatus **600** depicted in **FIG. 14** can be envisioned with an assembly of units as depicted in **FIG.**

13 where the rails form walls that form air channel columns spanning the height of the apparatus. Vent manifolds 370 and 380 respectively form ports 375 and 385. Ports can be vented to atmosphere or can be coupled to tubing to provide a controlled gaseous environment to the apparatus.

[0077] Referring now to **FIG. 15A**, which shows a schematic cut-away perspective view of a cell culture apparatus 600, where the wells are formed from gas permeable material. In this exemplary embodiment, media enters one end of the apparatus through inlet port 625, and circulates through the whole apparatus, feeding spheroids 200 residing in microwells 115, before exiting (through outlet port 635), reversing flow through each chamber. The chambers/vessels may also have support bosses or posts (not shown) to maintain the appropriate structure. Air channels 310 run along the bottom of the gas permeable wells, allowing air exchange to feed the spheroids in the wells. Rails 300 allow formation of various air channels 310. **FIG. 15B** shows the cell culture apparatus of **FIG. 15A**, and specifically shows how air may infiltrate into the device via air channels 310 (white lines on front of apparatus). In this regard, as shown in **FIG 15B**, no oxygenator is required since the air channels 310 (also called tracheal spaces) provide gas exchange through gas permeable well material. **FIG. 15B** further shows system component to manage media. A controller pump 1600 pumps fresh media from a fresh medium source 1610 to the cell culture apparatus 600. A waste containing 1620 collects used media. A valve 1630 may also be used to collect media and harvest compounds that might exist in the media, as the media is exiting the apparatus.

[0078] Referring now to **FIG. 16A**, which shows a schematic cut-away perspective view of a cell culture apparatus 600, where the wells are formed from gas permeable material. In this exemplary embodiment, media enters through the inlet port 625 and distributes (via inlet manifold 620) to all compartments before exiting the opposite end through outlet manifold 630 attached to the outlet port 635. The chambers/vessels may also have support bosses or posts (not shown) to maintain the appropriate structure. Air channels 310 run along the bottom of the gas permeable wells, allowing air exchange to feed the spheroids in the wells. Rails 300 allow formation of various air channels 310. **FIG 16B** shows the cell culture apparatus of **FIG 16A**, and specifically shows how air may infiltrate into the device via air channels 310 (white lines on front of apparatus). In this regard, as shown in **FIG 16B**, no oxygenator is required since the

air channels **310** (also called tracheal spaces) provide gas exchange through gas permeable well material. **FIG. 16B** further shows system component to manage media. A controller pump **1600** pumps fresh media from a fresh medium source **1610** to the cell culture apparatus **600**. A waste containing **1620** collects used media.

[0079] Additional embodiments and geometries are shown in Figs. 18, 19, and 20. **FIG. 17** is a schematic drawing of an exemplary embodiment of an array of wells **100**, showing individual wells **115**. In the embodiment illustrated in **FIG. 17**, well **115** has a mouth **101**. Mouth **101** is a region at the top part of the well, adjacent the top opening **111** of the well **115**, which provides a more open area, before the well constricts to form a well-bottom where cells settle to form spheroids. In embodiments, mouth **101** can be conical (wider at the top of the mouth than at the bottom of the mouth) and annular in shape (as shown in **FIG. 17A** and **FIG. 18A**, where the well is round). In additional embodiments, as shown in, for example, **FIG. 19A**, where the well has a round opening, but is parabolic in shape, mouth **101** may be parabolic.

[0080] In some embodiments, well geometries comprise capillary structures (including, for example, a mouth, ridge fissure, rounded or parabolic top opening, etc.) in the well walls to facilitate the escape of air upon introduction of liquid into the well. **FIG. 17B** is a top-down view, taken at line B-B of **FIG. 17A**, illustrating a ridge **170**. As shown in **FIG. 17B**, the ridge is a bump or a protuberance from the mouth **101** or the sidewall **113** of the well. In embodiments, the ridge extends the length of the microwell from the top opening **111** to the well bottom **116**. In additional embodiments, the ridge extends from the top of the mouth **111** to the bottom of the mouth **112**. The sharp angles formed on either side of the ridge **170** create a capillary force on the aqueous fluid to provide for fluid entry to the microwell without air entrapment.

[0081] **FIG. 18B** is a top-down view of an array of wells **100** shown in cross section in **FIG. 18A**. **FIG. 18B** illustrates a fissure **270**. As shown in **FIG. 18B**, the fissure is an indentation in the sidewall **113** of the well **115**. The sharp angles formed on either side of the fissure **270** create a capillary force to allow aqueous fluid flow into the microwell.

- [0082] **FIG. 20A and B** are schematic drawings of another exemplary embodiment of an array of wells **100**. **FIG. 19A** is an illustration in cross-section. **FIG. 19B** is a top-down drawing of the exemplary embodiment of an array of wells, taken at line B-B of **FIG. 19A**. **FIG. 19A and B** illustrate that each well **115** may have more than one ridge **170** or fissure **270**, and that ridges **170** or fissures **270** may be arranged in an array within the well **115**. As shown in **FIG. 19A and B**, in embodiments, a radial distribution of ridges and/or fissures is envisioned. The number of capillary structures is not limited to one per microwell. In some embodiments, greater numbers of capillaries increase the rate of fluid entry into the microwell.
- [0083] **FIG. 19A-C** demonstrates inclusion of multiple (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20, 24, 28, 32, or any ranges therein) vertically-oriented capillary structures within a single well. Features may be regularly-spaced (as depicted in **FIG. 19**), irregularly spaced, grouped/bunched, etc. In some embodiments, capillary structures extend from the top opening of the well to the well bottom. When multiple capillary structures are present in a single well, the multiple features may be of different types (e.g., ridgelines and/or fissures) and may comprise different shapes (e.g., square, rounded, etc.)
- [0084] **Fig. 19C** illustrates that the array of wells **100** may have a sinusoidal or parabolic shape. This shape creates a rounded top edge or well edge which, in embodiments, reduces the entrapment of air at a sharp corner or 90 degree angle at the top of a well. This sinusoidal or parabolic well shape, or rounded top well edge, is also a capillary structure. As shown in **FIG. 19C**, the well **115** has a top opening having a top diameter D_{top} , a height from the bottom of the well **116** to the top of the well H , and a diameter of the well at a height half-way between the top of the well and the bottom **116** of the well $D_{half-way}$.
- [0085] Both the relative and absolute dimensions of the wells may be selected for the desired culturing conditions. For spheroid growth, the diameter D is preferably one to three times the desired diameter of the 3D cellular aggregate to be cultured in the well. The height H is preferably 0.7 to 1.3 times D . The diameter D_{top} is preferably 1.5 to 2.5 times D . D is preferably 100 micrometers (μm) to about 2000 micrometers (e.g., 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, or 2000 micrometers, including ranges between any two of the foregoing values (e.g., 200-1000 μm , 200-750 μm , 300-750 μm , 400-600 μm , etc.)). However,

alternative relative or absolute dimensions may be employed. For example, D may be from 1 to 10 times (e.g., 2, 3, 4, 5, 6, 7, 8, 9) the desired diameter of the cellular aggregate or any value or range therein between (e.g., 1, 1 to 1.5, 1 to 2, 2, 1 to 2.5, 1 to 3, 2 to 3, 1 to 5, 3 to 5, 2 to 7, etc.). D may be from 100 μm to 10,000 μm or any value (e.g., 100, 200, 500, 1000, 2000, 5000) or range therein between (e.g., 100-2000, 200-1000, 300-700, 400-600, 500, etc.). H may be from 0.5 to 10 times D (e.g., 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 or any values or ranges therein between). D_{top} may be from 1.1 to 5 times D (e.g., 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5 or any values or ranges therein between).

[0086] In certain embodiments, the gas permeable cell culture apparatus (e.g., spheroid perfusion bioreactor) of this disclosure permits continuous media replenishment and gas exchange for all of the spheroids on each surface in each culture compartment. In general, media replenishment and gas exchange will also be equivalent, or approximately equivalent, for all the spheroids in each culture compartment. Employing spheroid forming geometry in the wells/compartments, allows the cells added to these bioreactors to form spheroids that are all approximately the same size. Due to the gas permeability of the culture compartments an oxygenator that is typically required for bioreactors will not be required for this system to function optimally.

[0087] In certain embodiments, the gas permeable cell culture apparatus allows growing large quantities of cells in a small footprint. For example, a 400 micrometer diameter spheroid may contain an average of 30,000 cells in 3D. In a 2D surface of 400 micrometers in diameter, ~305 cells can attach. So roughly 100 times more cells can be grown in the same unit area in 3D as can be grown in 2D. Growing this many cells in a compact footprint generally requires more nutrients and makes more waste products that need to be removed. The perfusion systems disclosed herein provide such a solution without requiring constant intervention. Moreover, the quality of the cells growing in 3D is generally higher since they function more similarly to cells in vivo.

[0088] In certain embodiments, bioreactor chambers/wells can be molded, or thermoformed from gas permeable materials, or constructed from a combination of gas permeable and non-gas permeable materials. Structural support can come in many forms, including, for example, bosses and columns. The chambers/wells may receive fresh

medium in parallel through a manifold that distributes equally to all chambers, or the chambers may be fed in series, with the flow reversing from one chamber to the next.

- [0089] In general, the gas exchange requirements for the cells in the vessel dictate the perfusion rate and by allowing for respiration requirements to be met via gas permeable materials of construction it allows the perfusion rate to be significantly reduced. This allows for reduced shear flow over the spheroids, which makes it easier to retain spheroids within spheroid formation/retention features in the substrates.
- [0090] In embodiments where the wells are non-adherent to cells, cells cultured in the apparatuses described herein may be harvested by inverting the apparatus to allow gravity to displace the cells from the wells. Alternatively, flow rates of fluid through media flow channels can be increased to dislodge cells from wells as described above.
- [0091] All scientific and technical terms used herein have meanings commonly used in the art unless otherwise specified. The definitions provided herein are to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.
- [0092] As used herein, singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a “structured surface” includes examples having two or more such “structured surfaces” unless the context clearly indicates otherwise.
- [0093] As used in this specification and the appended claims, the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. The term “and/or” means one or all of the listed elements or a combination of any two or more of the listed elements.
- [0094] As used herein, “have”, “has”, “having”, “include”, “includes”, “including”, “comprise”, “comprises”, “comprising” or the like are used in their open ended inclusive sense, and generally mean “include, but not limited to”, “includes, but not limited to”, or “including, but not limited to”.
- [0095] “Optional” or “optionally” means that the subsequently described event, circumstance, or component, can or cannot occur, and that the description includes instances where the event, circumstance, or component, occurs and instances where it does not.

- [0096] The words “preferred” and “preferably” refer to embodiments of the disclosure that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the inventive technology.
- [0097] Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, examples include from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.
- [0098] Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.). Where a range of values is “greater than”, “less than”, etc. a particular value, that value is included within the range.
- [0099] Any direction referred to herein, such as “top,” “bottom,” “left,” “right,” “upper,” “lower,” “above,” “below,” and other directions and orientations are described herein for clarity in reference to the figures and are not to be limiting of an actual device or system or use of the device or system. Many of the devices, articles or systems described herein may be used in a number of directions and orientations. Directional descriptors used herein with regard to cell culture apparatuses often refer to directions when the apparatus is oriented for purposes of culturing cells in the apparatus.
- [00100] Unless otherwise expressly stated, it is in no way intended that any method set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not actually recite an order to be followed by its steps or it is not otherwise specifically stated in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that any particular order be inferred. Any recited single or multiple feature or aspect in any one claim can be combined or permuted with any other recited feature or aspect in any other claim or claims.

[00101] It is also noted that recitations herein refer to a component being “configured” or “adapted to” function in a particular way. In this respect, such a component is “configured” or “adapted to” embody a particular property, or function in a particular manner, where such recitations are structural recitations as opposed to recitations of intended use. More specifically, the references herein to the manner in which a component is “configured” or “adapted to” denotes an existing physical condition of the component and, as such, is to be taken as a definite recitation of the structural characteristics of the component.

[00102] While various features, elements or steps of particular embodiments may be disclosed using the transitional phrase “comprising,” it is to be understood that alternative embodiments, including those that may be described using the transitional phrases “consisting” or “consisting essentially of,” are implied. Thus, for example, implied alternative embodiments to a cell culture apparatus comprising a plate defining a structured surface, one or more rails, a top and one or more manifolds include embodiments where a cell culture apparatus consists of a plate defining a structured surface, one or more rails, a top and one or more manifolds and embodiments where a cell culture apparatus consists essentially of a plate defining a structured surface, one or more rails, a top and one or more manifolds.

[00103] It will be apparent to those skilled in the art that various modifications and variations can be made to the present inventive technology without departing from the spirit and scope of the disclosure. Since modifications, combinations, sub-combinations and variations of the disclosed embodiments incorporating the spirit and substance of the inventive technology may occur to persons skilled in the art, the inventive technology should be construed to include everything within the scope of the appended claims and their equivalents.

What is claimed is:

1. A cell culture apparatus comprising:
one or more plates having a first major surface and an opposing second major surface,
wherein the first major surface comprises a plurality of microwells; 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.
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 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 100 micrometers to 2000 micrometer.
4. The cell culture apparatus according to any one of claims 1-3, wherein the cell culture
apparatus comprises a plurality of said plates stacked on one another.
5. The cell culture apparatus according to any one of claims 1-4, 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.
6. The cell culture apparatus according to claim 5, 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.
7. The cell culture apparatus according to claim 5, 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.

8. The cell culture apparatus of any one of claims 1-7, 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.
9. The cell culture apparatus according to any one of claims 1-8, further comprising a top plate disposed over a top-most cell culture plate of the plurality of stacked plates.
10. The cell culture apparatus according to any one of claims 1-9, 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.
11. The cell culture apparatus according to any one of claims 1-10, 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.

12. The cell culture apparatus according to claim 11, 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.
13. The cell culture apparatus according to any one of claims 1-12, wherein the said plurality of wells comprises an array of hexagonal close-packed wells.
14. The cell culture apparatus according to any one of claims 1-13, wherein the wells have depth defined from the upper aperture to the nadir, wherein the depth is in a range from 200 micrometers to 2000 micrometers.
15. The cell culture apparatus of any one of claims 1-14, wherein each of the wells are non-adherent to cells.
16. The cell culture apparatus of any one of claims 1-15, wherein the plates are gas permeable via the wells.
17. A cell culture system comprising:
a cell culture apparatus according to any one of claims 1-16; and
an oxygenator coupled to the inlet.
18. The cell culture system of Claim 17, further comprising a pump coupled to the inlet.

19. A method comprising:
introducing cells into the wells 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 wells.
20. The method according to claim 19, 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 wells.
21. A method of producing protein, comprising:
 - a) culturing cells expressing a protein in the wells of a cell culture apparatus according to any one of claims 1-17; and
 - b) isolating said protein from said cells.