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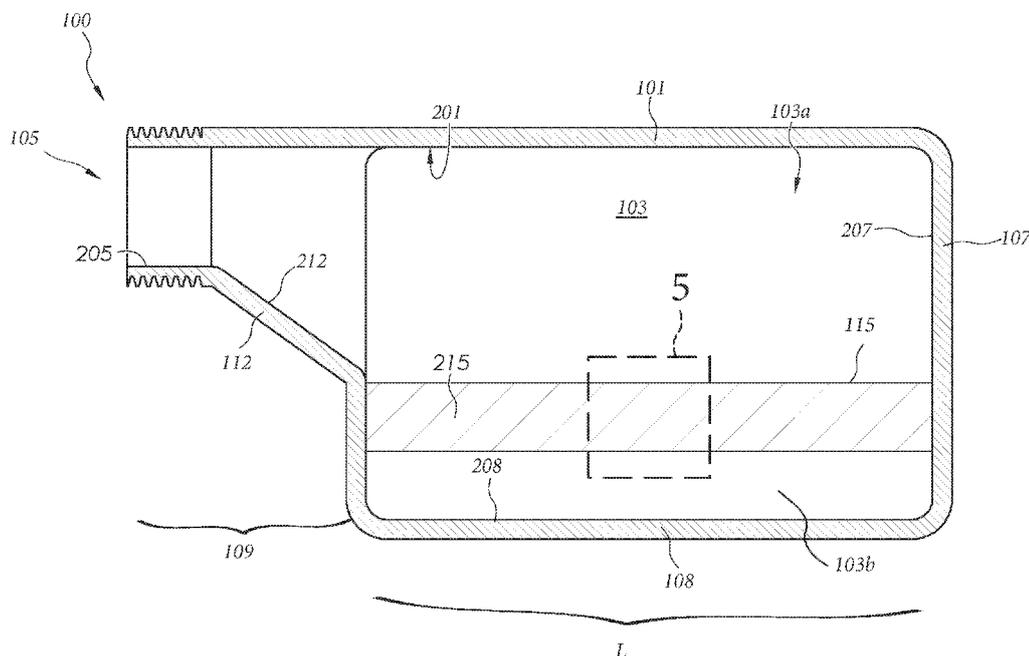


FIG. 3

(57) Abstract: A cell culture vessel (100) includes a wall including an inner surface defining a cell culture chamber of the vessel. A substrate (215) of non-porous material is positioned in the cell culture chamber between a first region of the cell culture chamber and a second region of the cell culture chamber. The substrate includes a plurality of microcavities (220), each microcavity of the plurality of microcavities includes a concave surface defining a well and an opening, the concave surface of each microcavity includes at least one aperture including a dimension less than or equal to about 15 microns defining a path from the well to the second region. A cell culture vessel including a substrate including a plurality of microcavities and a layer of porous material is also provided. Methods of culturing cells in the cell culture vessel are also provided.



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CELL CULTURE VESSEL

CROSS REFERENCE TO RELATED APPLICATION

[0001] The application claims the benefit of priority of U.S. Provisional Application Serial No. 62/532,671 filed on 07/14/2017, entitled “Cell Culture Containers and Methods of Culturing Cells”, the content of which is relied upon and incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure relates generally to a cell culture vessel and methods of culturing cells, and more particularly, to a cell culture vessel having a porous support for culturing three-dimensional cells, and methods of culturing three-dimensional cells in the cell culture vessel.

BACKGROUND

[0003] It is known to contain three-dimensional cells in a cell culture vessel. It is also known to culture three-dimensional cells in a cell culture vessel.

SUMMARY

[0004] The following presents a simplified summary of the disclosure in order to provide a basic understanding of some exemplary embodiments described in the detailed description.

[0005] In embodiments, the disclosure provides a cell culture vessel having a necked opening, a cell culture chamber, a top, a bottom, sidewalls an endwall opposite the necked opening and a surface for culturing cells that has a microcavity array. In embodiments, the surface for culturing cells having a microcavity array is formed, partially or entirely from porous material. In embodiments, the substrate for culturing cells has an array of microcavities on one surface and a porous material on a second surface, and a portion of the walls of the microcavities are provided by porous material.

In embodiments, the microcavities have openings to allow gas to pass through the wall of the microcavities.

[0006] In additional embodiments, the cell culture vessel has structures for introducing and removing media below the porous material or through the porous material without disturbing the media residing above cells resting in the microcavities of the microcavity array. In additional embodiments, the disclosure provides methods for culturing cells in the cell culture vessel, and methods of introducing and removing cells and media from the vessel.

[0007] The above embodiments are exemplary and can be provided alone or in any combination with any one or more embodiments provided herein without departing from the scope of the disclosure. Moreover, it is to be understood that both the foregoing general description and the following detailed description present embodiments of the present disclosure, and are intended to provide an overview or framework for understanding the nature and character of the embodiments as they are described and claimed. The accompanying drawings are included to provide a further understanding of the embodiments, and are incorporated into and constitute a part of this specification. The drawings illustrate various embodiments of the disclosure, and together with the description, serve to explain the principles and operations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] These and other features, embodiments, and advantages of the present disclosure can be further understood when read with reference to the accompanying drawings in which:

[0009] **FIG. 1** schematically illustrates a side view of a first exemplary cell culture vessel in accordance with embodiments of the disclosure;

[0010] **FIG. 2** shows a plan view of the first exemplary cell culture vessel along line **2-2** of **FIG. 1** in accordance with embodiments of the disclosure;

[0011] **FIG. 3** shows an exemplary cross-sectional view of a cell culture vessel including a cell culture chamber having a first region and a second region in accordance with embodiments of the disclosure;

[0012] **FIG. 4** illustrates an enlarged schematic representation of an exemplary embodiment of the microcavity array of cell culture vessel of **FIG. 3** including a substrate including a plurality of microcavities including at least one aperture in accordance with embodiments of the disclosure;

[0013] **FIG. 5** illustrates an enlarged schematic representation of an exemplary embodiment of a portion of cell culture vessel taken at view **5** of **FIG. 3** including a substrate including a plurality of microcavities including at least one aperture in accordance with embodiments of the disclosure;

[0014] **FIG. 6** shows an alternative embodiment of the portion of the cell culture vessel portion of cell culture vessel taken at view **5** of **FIG. 3** including a substrate including a plurality of microcavities and a layer of porous material in accordance with embodiments of the disclosure

[0015] **FIG. 7** shows a method of culturing cells in at least one microcavity of the plurality of microcavities including at least one aperture of the substrate of the portion of the cell culture vessel in accordance with embodiments of the disclosure;

[0016] **FIG. 8** shows an alternative embodiment of the portion of the cell culture vessel portion of cell culture vessel taken at view **5** of **FIG. 3** including a substrate having a plurality of microcavities and a layer of porous material in accordance with embodiments of the disclosure;

[0017] **FIG. 9** shows an alternative embodiment of the portion of the cell culture vessel including a substrate having a plurality of microcavities and a layer of porous material in accordance with embodiments of the disclosure;

[0018] FIG. 10 shows an alternative embodiment of the portion of the cell culture vessel including a substrate having a plurality of microcavities and a layer of porous material in accordance with embodiments of the disclosure

[0019] FIG. 11 shows a method of culturing cells in at least one microcavity of the plurality of microcavities of the portion of the substrate including a plurality of microcavities and a layer of porous material in accordance with embodiments of the disclosure;

[0020] FIG. 12 shows an alternative embodiment of the cross-sectional view of the cell culture vessel including a port and a gas-permeable filter in accordance with embodiments of the disclosure;

[0021] FIG. 13 shows an alternative embodiment of the cross-sectional view of the cell culture vessel including a conduit connecting a first aperture to the second region in accordance with embodiments of the disclosure;

[0022] FIG. 14 shows an alternative embodiment of the cross-sectional view of the cell culture vessel including a second aperture and a conduit connecting the second aperture to the second region in accordance with embodiments of the disclosure;

[0023] FIG. 15 shows an alternative embodiment of the cross-sectional view of the cell culture vessel including a conduit connecting the first region to the second region in accordance with embodiments of the disclosure.

DETAILED DESCRIPTION

[0024] Features will now be described more fully hereinafter with reference to the accompanying drawings in which exemplary embodiments of the disclosure are shown. Whenever possible, the same reference numerals are used throughout the drawings to refer to the same or like parts. However, this disclosure can be embodied in many different forms and should not be construed as limited to the embodiments set forth herein.

[0025] A cell culture vessel (e.g., flask) can provide a sterile chamber for culturing cells. In some embodiments, culturing cells can provide information related to

the study of diseases and toxicology, the efficacy of medications and treatments, characteristics of tumors, organisms, genetics, and other scientific, biological, and chemical principles of and relating to cells. As compared to two-dimensional cell cultures, in some embodiments, three-dimensional cell cultures can produce multicellular structures that are more physiologically accurate and that more realistically represent an environment in which cells can exist and grow in real life applications as compared to two-dimensional cell culture. For example, three-dimensional cell cultures have been found to more closely provide a realistic environment simulating “in vivo” (i.e. within the living, in a real-life setting) cell growth; whereas two-dimensional cell-cultures have been found to provide an environment simulating “in vitro” (i.e., within the glass, in a laboratory setting) cell growth that is less representative of a real-life environment occurring outside of a laboratory. By interacting with and observing the properties and behavior of three-dimensional cell cultures, advancements in the understanding of cells relating to, for example, the study of diseases and toxicology, the efficacy of medications and treatments, characteristics of tumors, organisms, genetics, and other scientific, biological, and chemical principles of and relating to cells can be achieved.

[0026] In embodiments, the cell culture vessel **100** can include a bottom **108**, a top **101**, and endwall **107** and sidewalls **106**, each having internal surfaces that contact liquid media and cells in culture. These internal surfaces define the cell culture chamber **103**. At least one of these surfaces can be more particularly adapted for cell growth. For example, a cell culture surface may be treated with a coating to encourage or discourage cells to stick to a surface. Or, to support the culture of spheroid cells, the cell growth surface can include a plurality of microcavities or compartments (e.g., micron-sized wells, submillimeter-sized wells) arranged, for example, in an array. The cell growth surface can be integral to the flask or can be a separate surface having a microcavity array placed or affixed in the cell growth chamber. The top surface, the bottom surface, one or more side surfaces or a combination of these can include microcavities in an array.

[0027] For example, in some embodiments, a single spheroid can form in each microcavity of the plurality of microcavities. Cells introduced into the vessel in liquid media will settle into a microcavity by gravity. One or more cells suspended in liquid media will fall through the liquid and settle within each microcavity. The shape of the microcavity (e.g., a concave surface defining a well), and a surface coating of the microcavity that prevents the cells from attaching to the surface can also facilitate growth of cells into three-dimensional form, forming a spheroid in each microcavity.

[0028] Microcavities can be, for example, formed in an undulating or sinusoidal shape forming microcavities or microwells having rounded tops and rounded bottoms. These rounded edges may prevent the formation of bubbles when liquid media fills the vessel. In some embodiments, the flask can be filled with a material (e.g., media, solid, liquid, gas) that facilitates growth of three-dimensional cell cultures (e.g., cell aggregates, organoids or spheroids). For example, a media including cells suspended in a liquid can be added to the cell culture chamber or vessel. The suspended cells can collect in the plurality of microcavities by gravity and can form (e.g., grow) into grouping or cluster of cells. The grouped or clustered cells grow in three dimensions to form cells in 3D, otherwise known as a spheroid or an organoid. A single cluster of cells or spheroid forms in a single microcavity. Thus, a vessel, or a cell culture chamber, having a cell culture surface having an array of microcavities, can be used to culture an array of spheroids, each residing in its own microcavity.

[0029] During culturing, the spheroids can consume media (e.g., food, nutrients) and produce metabolites (e.g., waste) as a byproduct. Thus, in some embodiments food in the form of media can be added to the cell culture chamber during culturing and waste media can be removed from the cell culture chamber during culturing. This ability to change the media to feed cells and remove waste products, is important for the long-term culture of cells. However, adding and removing media may create turbulence which may disrupt or displace spheroids resting in microcavities. This is especially true when the microcavities are coated with a low binding coating to prevent the cells from sticking to

the microcavity surface. The spheroids are loose (not attached to the surface) and may be dislodged and float free of their microcavity resting place. It is not preferable to dislodge spheroids growing in culture for many reasons. The spheroids may be removed from the culture with the removal of spent media. Dislodged spheroids may settle into occupied microcavities, and may merge with other spheroids to form non-uniform 3D cellular structures. That is, after a media change, some spheroids may be bigger than others in the culture. This reduces the uniformity of the cell culture and may affect results of assays or other tests carried out on 3D cells. In this disclosure, structures are disclosed which reduce turbulence, reducing the risk of displacing spheroids from the microcavities, thus promoting the long-term culture of spheroids.

[0030] Embodiments of a cell culture vessel **100** and **200** and methods of culturing cells in the cell culture vessel **100** and **200** are described with reference to **FIGS. 1-15**.

[0031] **FIG. 1** schematically illustrates a side view of the exemplary cell culture vessel **100**. As shown in **FIG. 1**, an embodiment of a cell culture vessel **100** is shown. The cell culture vessel **100** has a port or aperture **105** (shown in **FIG. 1** covered by a cap **104**) and a neck **112** connecting the port or aperture **105** to the cell culture chamber **103**. In embodiments the aperture can be releasably sealed. For example, in embodiments, the aperture **105** section of the neck **112** can have threads (either interior or exterior) that allow a cap **104** to be releasably sealed **105** by a cap **104** having complimentary threaded structure. Or, the necked opening **105** can be releasably sealed by any other mechanism known in the art to close a vessel. The aperture **105** combined with the neck **112** is the necked opening **109** (See **FIG. 3**). The necked opening **109** extends through a wall of the cell culture chamber **103** and is in fluid communication with the cell culture chamber **103**. The necked opening **113** allows liquid to be introduced and removed from the cell culture chamber (the interior) of the vessel.

[0032] The cell culture surface **200** of the vessel **100** is, in embodiments, the interior surface **208** bottom **108** of the vessel **100** when the vessel **100** is oriented for cell

growth. In embodiments, the vessel **100** is oriented for cell growth when the vessel **100** is placed with the bottom **108** of the vessel **100** flat on a surface. The vessel **100** may also have sidewalls **106** and an endwall **107** opposite the necked opening **109**, a top **101** and bottom **108**. In embodiments the top **101** is opposite the cell culture surface **200** of the vessel **100**. In embodiments, the necked opening **109** is opposite the endwall **107** of the vessel **100**. In embodiments, the cell culture surface **200** has a microcavity array **115**. Each of these structures (the necked opening **109**, the top **101**, the bottom **108**, the sidewalls **106** and the endwall **107**) of the vessel **100** have internal surfaces facing inside the vessel **100**. That is, the top **101** has an interior surface **201**. The end wall **107** has an interior surface **207**. The sidewalls **106** have interior surfaces **206**. The neck **112** has an interior surface **212**. The inside of the vessel is the cell culture chamber **103**, the space inside the vessel **100**, defined by the top **101**, the bottom **108**, the sidewalls **106** and the endwall **107** where cells reside inside the vessel **100**. For example, in some embodiments, the cell culture chamber **103** can include an internal spatial volume of the vessel **103**.

[0033] **FIG. 2** shows a plan view of the vessel **100** along line **2-2** of **FIG. 1**. In some embodiments, the cell culture vessel **100** can be manufactured from a material including, but not limited to, polymer, polycarbonate, glass, and plastic. In an embodiment, the vessel **100** is illustrated as being manufactured from a clear (e.g., transparent) material; although, in some embodiments, the vessel **100** may, alternatively, be manufactured from a semi-transparent, semi-opaque, or opaque material without departing from the scope of the disclosure.

[0034] Turning back to **FIG. 1** and **FIG. 2**, in some embodiments, the vessel **100** can include a cap **104** oriented to cover the aperture **105** to at least one of seal and block the aperture **105**, thereby obstructing a path into the cell culture chamber **103** from outside the vessel **100** through the aperture **105**. For clarity purposes, the cap **104** is removed and, therefore, not shown in other drawing figures, although it is to be understood that the cap **104** can be provided and selectively added to or removed from the aperture **105** of the vessel **100**, in some embodiments, without departing from the

scope of the disclosure. In some embodiments, the cap **104** can include a filter that permits the transfer of gas in to and/or out of the cell culture chamber **103** of the vessel **100**. For example, in some embodiments, the cap **104** can include a gas-permeable filter oriented to regulate a pressure of gas within the cell culture chamber **103**, thereby preventing pressurization (e.g., over-pressurization) of the cell culture chamber **103** relative to a pressure of the environment (e.g., atmosphere) outside the vessel **100**.

[0035] As shown in **FIG. 3**, which shows a cross-sectional view along line **3-3** of **FIG. 2**, in some embodiments, a substrate **215** spans a length “**L**” of the cell culture chamber **103**. Thus, referring to **FIG. 3**, in some embodiments, the cell culture vessel **100** can include a top **101**, a sidewall (not shown in this view), and endwall **107**, a bottom **108**, a port or aperture **105**, a neck **112** and a necked opening **109**. Each of these has an internal surface. That is, the top **101** has an internal surface **201**, the sidewall (not shown) has an internal surface (also not shown), the endwall **107** has an internal surface **207**, the bottom **108** has an internal surface **207**, the port or aperture **105** has an internal surface **205**, and the neck **112** has an internal surface **212**. Also shown is the cell culture chamber **103**. Additionally, in embodiments, the vessel **100** can include a substrate **215** of porous material positioned in the cell culture chamber **103** between a first region **103a** of the cell culture chamber **103** and a second region **103b** of the cell culture chamber **103**. That is, the substrate **215** separates the cell culture chamber **103** into an area above the porous material **103a**, and an area below the porous material **103b**. The vessel **100** can include a first aperture **105** in fluid communication with the first region **103a**. In some embodiments, the substrate **215** can include a plurality of microcavities **220** (See **FIG. 4-9**). In some embodiments, the substrate **215** can be positioned entirely within the cell culture chamber **103** of the vessel **100** and can, therefore, be entirely isolated within a sterile environment of the cell culture chamber **103** with access into and out of the cell culture chamber **103** limited, for example, by the first aperture **105**.

[0036] **FIG. 4** shows an enlarged schematic representation of a portion of the substrate **215** shown in **FIG. 3**, having a microcavity array **115**. Additionally, **FIG. 5**

shows a cross-sectional view of the portion of the surface shown as **5** in **FIG. 3** having a microcavity array **115**. As shown in **FIGS. 4** and **5**, in some embodiments, each microcavity **120** (shown as **120a**, **120b**, **120c**) in the array of microcavities **115** in the top surface **120** of the substrate **216** has an opening **123a**, **123b**, **123c** at the top of each microcavity **120**. And, each microcavity **120** in the array of microcavities **115** can include a concave surface **121a**, **121b**, **121c** defining a well **122a**, **122b**, **122c**. Further, each microcavity **120a**, **120b**, **120c** can include a well **122a**, **122b**, **122c**. These structures are present whether the microcavity array is integral to the bottom **108** of the vessel **100** or whether the microcavity array is provided by an insert **215** having a microcavity array **315**.

[0037] In some embodiments, the substrate **215** can be a polymeric material including, but not limited to, 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. Additionally, in some embodiments, at least a portion of the well **122a**, **122b**, **122c** defined by the concave surface **121a**, **121b**, **121c** can be coated with an ultra-low binding material, thereby making the at least a portion of the well **122a**, **122b**, **122c** non-adherent to cells. For example, in some embodiments, one or more of perfluorinated polymers, olefins, agarose, non-ionic hydrogels such as polyacrylamides, polyethers such as polyethyleneoxide, polyols such as polyvinylalcohol or mixtures thereof can be applied to at least a portion of the well **122a**, **122b**, **122c** defined by the concave surface **121a**, **121b**, **121c**.

[0038] Moreover, in some embodiments, each microcavity **120a**, **120b**, **120c** of the plurality of microcavities **120** can include a variety of features and variations of those features without departing from the scope of the disclosure. For example, in some embodiments the plurality of microcavities **120** can be arranged in an array including a

linear array (shown), a diagonal array, a rectangular array, a circular array, a radial array, a hexagonal close-packed arrangement, etc. Additionally, in some embodiments, the opening **123a**, **123b**, **123c** can include a variety of shapes. In some embodiments, the opening **123a**, **123b**, **123c** can include one or more of a circle, an oval, a rectangle, a quadrilateral, a hexagon, and other polygonal shapes. Additionally, in some embodiments, the opening **123a**, **123b**, **123c** can include a dimension (e.g., diameter, width, diagonal of a square or rectangle, etc.) from about 100 microns (μm) to about 5000 μm . For example, in some embodiments, the opening **123a**, **123b**, **123c** can include a dimension of 100 μm , 150 μm , 200 μm , 250 μm , 300 μm , 350 μm , 400 μm , 450 μm , 500 μm , 550 μm , 600 μm , 650 μm , 700 μm , 750 μm , 800 μm , 850 μm , 900 μm , 950 μm , 1000 μm , 1500 μm , 2000 μm , 2500 μm , 3000 μm , 3500 μm , 4000 μm , 4500 μm , 5000 μm , and any dimension or ranges of dimensions encompassed within the range of from about 100 μm to about 5000 μm .

[0039] In some embodiments, the well **122a**, **122b**, **122c** defined by the concave surface **121a**, **121b**, **121c** can be any shape. In some embodiments, the well **122a**, **122b**, **122c** defined by the concave surface **121a**, **121b**, **121c** can include one or more of a circular, elliptical, parabolic, hyperbolic, chevron, sloped, or other cross-sectional profile shape. Additionally, in some embodiments, a depth of the well **122a**, **122b**, **122c** (e.g., depth from a plane defined by the opening **123a**, **123b**, **123c** to the concave surface **121a**, **121b**, **121c**) can include a dimension from about 100 microns (μm) to about 5000 μm . For example, in some embodiments, the depth of the well **122a**, **122b**, **122c** can include a dimension of 100 μm , 150 μm , 200 μm , 250 μm , 300 μm , 350 μm , 400 μm , 450 μm , 500 μm , 550 μm , 600 μm , 650 μm , 700 μm , 750 μm , 800 μm , 850 μm , 900 μm , 950 μm , 1000 μm , 1500 μm , 2000 μm , 2500 μm , 3000 μm , 3500 μm , 4000 μm , 4500 μm , 5000 μm , any dimension or ranges of dimensions encompassed within the range of from about 100 μm to about 5000 μm .

[0040] FIG. 5 illustrates that in some embodiments, three-dimensional cells **150** (e.g., spheroids, organoids **150a**, **150b**, **150c**) that can be cultured in at least one

microcavity **120a**, **120b**, **120c** of the plurality of microcavities **120** can include a dimension (e.g., diameter) of from about 50 μm to about 5000 μm , and any dimension or ranges of dimensions encompassed within the range of from about 50 μm to about 5000 μm . In some embodiments, dimensions greater than or less than the explicit dimensions disclosed can be provided and, therefore, unless otherwise noted, dimensions greater than or less than the explicit dimensions disclosed are considered to be within the scope of the disclosure. For example, in some embodiments, one or more dimensions of the opening **123a**, **123b**, **123c**, the depth of the well **122a**, **122b**, **122c**, and the dimension of the three-dimensional cells **150** (e.g., spheroids **150a**, **150b**, **150c**) can be greater than or less than the explicit dimensions disclosed without departing from the scope of the disclosure.

[0041] As shown in **FIG. 6**, which illustrates an enlarged schematic representation of another exemplary embodiment of a microcavity array **220** in a vessel **200**, in some embodiments, each microcavity **220a**, **220b**, **220c** of the plurality of microcavities **220** can include a concave surface **221a**, **221b**, **221c** defining a well **222a**, **222b**, **222c** and an opening **223a**, **223b**, **223c** defining a path from the first region **103a** (as shown in **FIG. 3**) into the well **222a**, **222b**, **222c**. That is, liquid media and cells can enter the microcavities through the openings **223a**, **223b**, **223c** in the top of each microcavity **220a**, **220b**, **220c**. In some embodiments, the concave surface **221a**, **221b**, **221c** of each microcavity **220a**, **220b**, **220c** can include at least one aperture **229a**, **229b**, **229c** including a dimension **d2a**, **d2b**, **d2c** less than or equal to about 15 microns. The at least one aperture **229a**, **229b**, **229c** can extend from a top side **225** of the substrate **215** through the substrate **215** to a bottom side **226** of the substrate **215** and can define a path from the well **222a**, **222b**, **222c** to the second region **103b**. For example, in some embodiments, a dimension **d2a**, **d2b**, **d2c** less than or equal to about 15 microns (e.g., 5-15 microns, 5-10 microns, 10-15 microns, 5 microns, and all ranges and subranges therebetween) can provide one or more apertures **229a**, **229b**, **229c** through which gas can pass and through which liquid and cells cannot pass. In some embodiments, each microcavity **220a**, **220b**, **220c** can include a plurality of apertures (not shown).

Additionally, the apertures **229a**, **229b**, **229c** can include a profile including one or more of a circle, an oval, a rectangle, or other quadrilaterals. Likewise, in some embodiments, the apertures **229a**, **229b**, **229c** can include a dimension **d2a**, **d2b**, **d2c** (e.g., diameter, width, diagonal of a square or rectangle) less than or equal to about 15 microns.

[0042] In some embodiments, the apertures **229a**, **229b**, **229c** can be formed by operation of, for example, laser drilling or laser ablation, machining, manufacturing, forming, or other technique to provide the apertures **229a**, **229b**, **229c** in the microcavities **220a**, **220b**, **220c** of the substrate **215**. Thus, in some embodiments, providing the microcavity **220a**, **220b**, **220c** with at least one aperture **229a**, **229b**, **229c** can prevent gas from becoming entrapped in the microcavities **220a**, **220b**, **220c** when a solution media (e.g., liquid containing cells) is deposited in the microcavities **220a**, **220b**, **220c**. Additionally, because the dimension **d2a**, **d2b**, **d2c** is less than or equal to about 15 microns, while gas can pass through the apertures **229a**, **229b**, **229c**, the liquid and cells suspended in the liquid would not pass through the apertures **229a**, **229b**, **229c** without applied force as it is considered that the liquid and the cells (taking into account surface tension of the liquid, in some embodiments) include dimensions greater than or equal to about 15 microns. Further, as compared to a drop-style or hanging cell culture, where cells fall through a hole formed in the bottom of a well and are suspended by surface tension below the profile of the well and are prone to falling away from the well in their suspended droplet of liquid, it is to be understood that features of the disclosure permit containment and culturing of the cells within the well **222a**, **222b**, **222c**, while permitting the passing of gas through the apertures **229a**, **229b**, **229c**, and do not permit cells or spheroids to pass through the apertures **229a**, **229b**, **229c**.

[0043] For example, as shown in **FIG. 7**, in some embodiments, a method of culturing cells in the cell culture vessel **200** can include introducing liquid, containing cells into the microcavities **220a**, **220b**, **220c**. As the microcavities **220a**, **220b**, **220c** fill with liquid, gas passes down through the at least one aperture **229a**, **229b**, **229c** in the concave surface **221a**, **221ba**, **221c** of at least one microcavity **220a**, **220b**, **220c** of the

plurality of microcavities **220**. Because of the surface tension of media, which contains proteins and other ingredients which lend media a relatively high surface tension, and because of the small size of the microcavities **220a**, **220b**, **220c**, bubble formation is a concern. Bubbles interfere with cell culture. In embodiments, by allowing gas to leave the microcavities as liquid enters the microcavities, bubbles do not form in the microcavities. The method shown in FIG. 7 also includes culturing cells or spheroids **250** (e.g., three-dimensional cell **250a**, three-dimensional cell **250b**, three-dimensional cell **250c**) in the at least one microcavity **220a**, **220b**, **220c**. For example, as represented by arrows **251a**, **251b**, **251c**, gas can pass from the at least one microcavity **220a**, **220b**, **220c** through the at least one aperture **229a**, **229b**, **229c** in the concave surface **221a**, **221b**, **221c** of the at least one microcavity **220a**, **220b**, **220c** into the second region **103b** of the cell culture chamber **103**. Alternatively, as represented by arrows **252a**, **252b**, **252c** gas can pass from the second region **103b** of the cell culture chamber **103** through the at least one aperture **229a**, **229b**, **229c** in the concave surface **221a**, **221b**, **221c** of the at least one microcavity **220a**, **220b**, **220c** into the at least one microcavity **220a**, **220b**, **220c**.

[0044] In some embodiments, liquid **270** including cells **250a**, **250b**, **250c** can be contained and cultured in the microcavities **220a**, **220b**, **220c** while gas can pass through the apertures **229a**, **229b**, **229c**. Allowing gas to pass through the apertures **229a**, **229b**, **229c** while containing liquid **270** and culturing cells **250a**, **250b**, **250c** in the at least one microcavity **220a**, **220b**, **220c**, not only prevents gas entrapment (e.g., during initial filling of the at least one microcavity **220a**, **220b**, **220c** with liquid **270**) but also permits the passage of gas into and out of the at least one microcavity **220a**, **220b**, **220c** during culturing. For example, in some embodiments, gas produced by the cells **250a**, **250b**, **250c** as a byproduct of cell culturing can be removed from the microcavities **220a**, **220b**, **220c** by passing through the apertures **229a**, **229b**, **229c** while culturing the cells **250a**, **250b**, **250c**. Similarly, in some embodiments, gas to aid in cell culturing of the cells

250a, 250b, 250c can be added to the microcavities **220a, 220b, 220c** by passing through the apertures **229a, 229b, 229c** while culturing the cells **250a, 250b, 250c**.

[0045] Alternatively, as shown in **FIGS. 8-11**, in some embodiments, the substrate **215** can include a layer of porous material **216**. In some embodiments, each microcavity **220a, 220b, 220c** of the plurality of microcavities **220** can include a microcavity sidewall surface **224a, 224b, 224c** extending from the opening **223a, 223b, 223c** to the concave surface **221a, 221b, 221c**. As shown in **FIG. 8**, in some embodiments, a first side **216a** of the layer of porous material **216** can define at least a portion of the concave surface **221a, 221b, 221c** of each microcavity **220a, 220b, 220c** of the plurality of microcavities **220**, and a second side **216b** of the layer of porous material **216** can face the second region **103b**. In some embodiments, the first side **216a** of the layer of porous material **216** can define the entirety of the concave surface **221a, 221b, 221c** of each microcavity **220a, 220b, 220c** of the plurality of microcavities **220**.

[0046] As shown in **FIG. 9**, in some embodiments, the first side **216a** of the layer of porous material **216** can define at least a portion of the sidewall surface **224a, 224b, 224c** of each microcavity **220a, 220b, 220c** of the plurality of microcavities **220**. In some embodiments, the first side **216a** of the layer of porous material **216** can define the entirety of the sidewall surface **224a, 224b, 224c** of each microcavity **220a, 220b, 220c** of the plurality of microcavities **220**. As shown in **FIG. 10**, the layer of porous material **216** can define the entirety of the substrate **215**, where the first side **216a** of the layer of porous material defines the first side **225** of the substrate **215** and the second side **216b** of the layer of porous material defines the second side **226** of the substrate **215**.

[0047] As shown in **FIG. 11**, in some embodiments, a method of culturing cells **250** in the cell culture vessel **100** can include passing gas into the at least a portion of the concave surface **221a, 221b, 221c** defined by the first side **216a** of the layer of porous material **216** of at least one microcavity **220a, 220b, 220c** of the plurality of microcavities **220** and culturing cells in the at least one microcavity **220a, 220b, 220c**. For example, as represented by arrows **253a, 253b, 253c**, gas can pass from the at least

one microcavity **220a**, **220b**, **220c** into the layer of porous material **216**, and then, the gas can pass from the layer of porous material **216** into the second region **103b** of the cell culture chamber **103**. Alternatively, gas can pass from the second region **103b** of the cell culture chamber **103** into the layer of porous material **216**, and then, as represented by arrow **254a**, **254b**, **254c** gas can pass from the layer of porous material **216** into the at least one microcavity **220a**, **220b**, **220c**.

[0048] In some embodiments, liquid **270** including cells **250a**, **250b**, **250c** can be contained and cultured in the microcavities **220a**, **220b**, **220c** while gas can pass through the layer of porous material **216**. Allowing gas to pass through the layer of porous material **216** while containing liquid **270** and culturing cells **250a**, **250b**, **250c** in the at least one microcavity **220a**, **220b**, **220c**, not only prevents gas entrapment (e.g., during initial filling of the at least one microcavity **220a**, **220b**, **220c** with liquid **270**) but also permits the passage of gas into and out of the layer of porous material **216** during culturing. For example, in some embodiments, gas produced by the cells **250a**, **250b**, **250c** as a byproduct of cell culturing can be removed from the microcavities **220a**, **220b**, **220c** by passing through the layer of porous material **216** while culturing the cells **250a**, **250b**, **250c**. Similarly, in some embodiments, gas to aid in cell culturing of the cells **250a**, **250b**, **250c** can be added to the microcavities **220a**, **220b**, **220c** by passing through the layer of porous material **216** while culturing the cells **250a**, **250b**, **250c**.

[0049] Additionally, as shown in **FIGS. 12-15**, which show additional exemplary embodiments of the cross-sectional view of a vessel **200**, a method of culturing cells **250** in the cell culture vessel **200** can include passing liquid **270** through the first aperture **205**, and culturing cells **250a**, **250b**, **250c** in at least one microcavity **220a**, **220b**, **220c** of the plurality of microcavities **220** (See **FIG. 5** and **FIG. 7**). Unless otherwise noted, the substrate **215** described with respect to features of **FIGS. 12-15** can include one or more features of the substrate **215** shown in **FIG. 6** and **FIG. 7** including the apertures **229a**, **229b**, **229c** as well as one or more features of the substrate **215** shown in **FIGS. 8-11**. In

some embodiments, as represented by arrow **260**, the method can include passing liquid **270** through the first aperture **205** from outside the vessel **200** into the first region **103a**.

[0050] As shown in **FIG. 10**, in some embodiments, the vessel **200** can include a port **230** extending through the wall **101** in fluid communication with the second region **103b**. In embodiments, the port **231** may include gas-permeable material **231**. In some embodiments, the method can include passing gas **271** through the gas-permeable material **231** of the port **230**, and culturing cells in the plurality of microcavities **220**. In embodiments, the gas permeable material **231** may be a gas permeable filter. For example, as represented by arrow **262a**, gas **271** can pass from the second region **103b** into the gas-permeable material **231** of the port **230**, and then, for example, gas can pass from the gas-permeable material **231** to outside the vessel **200**. Likewise, as represented by arrow **262b**, gas can pass from outside the vessel **200** through the gas-permeable material **231** of the port **230**, and then, for example, gas can pass from the gas-permeable filter **231** in to the second region **103b**. Providing the second region **103b** with a port **230** including a gas-permeable filter **231** can allow gas exchange between, for example, the second region **103b** and the environment outside the vessel **200**, thereby preventing pressurization (e.g., over-pressurization) of gas **271** in the second region **103b** of the cell culture chamber **103** of the vessel **200**. Additionally, in some embodiments, gas produced by the cells as a byproduct of cell culturing can be removed from the cell culture chamber **103** (e.g., second region **103b**) by passing through the port **230** while culturing the cells. Similarly, in some embodiments, gas to aid in cell culturing of the cells can be added to the cell culture chamber **103** (e.g., second region **103b**) by passing through the port **230** while culturing the cells **250a**, **250b**, **250c**.

[0051] As shown in **FIG. 13**, in some embodiments, the vessel **200** can include a conduit **232** connecting the first aperture **205** to the second region **103b**. For example, in some embodiments, the conduit **232** can include tubing positioned in the cell culture chamber **103** defining a path **273** that is in fluid communication with the first aperture **205** and the second region **103b**. In some embodiments, the conduit **232** can provide

fluid communication along the path **273** between the outside of the vessel **200** (e.g., via the first aperture **205**) and the second region **103b**. In some embodiments, the method can include passing liquid **272** through the conduit **232** connecting the first aperture **205** to the second region **103b**, and culturing cells in the plurality of microcavities **220**. For example, as represented by arrow **263a**, liquid **272** can pass from outside the vessel **200** into the conduit **232**, along the path **273**, and then, as represented by arrow **263b**, the liquid **272** can pass from the conduit **232** into the second region **103b**. In addition or alternatively, as represented by arrow **264a**, liquid **272** can pass from the second region **103b** into the conduit **232**, along the path **273**, and then, as represented by arrow **264b**, the liquid **272** can pass from the conduit **232** to outside the vessel **200**. In addition, liquid can be introduced into the first region **103a** as shown by arrow **260** through the first aperture **205** and removed from the first region **103a** as shown by arrow **261**.

[0052] As shown in **FIG. 14**, in some embodiments, the vessel **200** can include a second aperture **233** extending through the wall **101** in fluid communication with the second region **103b**. Additionally, in some embodiments, the vessel **200** can include a conduit **234** connecting the second aperture **233** to the second region **103b**. For example, at least one of the second aperture **233** and the conduit **234** can define a path **274** that is in fluid communication with the second aperture **233** and the second region **103b**. In some embodiments, the conduit **234** can provide fluid communication along the path **274** between the outside of the vessel **200** (e.g., via the second aperture **233**) and the second region **103b**. Additionally, in some embodiments, the second aperture **233** can include a cap (not shown, See **FIG. 1** and **FIG. 2**) oriented to cover the second aperture **233** to seal or block the second aperture **233**, thereby obstructing a path into the cell culture chamber **103** from outside the vessel **200** through the second aperture **233**. In some embodiments, the cap (not shown) of the second aperture **233** can be similar to or the same as the cap **104** of the aperture **105** discussed above with respect to the cell culture vessel **200**. In some embodiments, the method can include passing liquid **272** through the second aperture **233** and through the conduit **234**, and culturing cells in at least one microcavity

220a, 220b, 220c of the plurality of microcavities **220**. For example, as represented by arrow **265a**, liquid **272** can pass from outside the vessel **200** into the second aperture **233** and the conduit **234**, along the path **274**, and then, as represented by arrow **265b**, the liquid **272** can pass from the conduit **234** into the second region **103b**. In addition or alternatively, as represented by arrow **266a**, liquid **272** can pass from the second region **103b** into the conduit **234**, along the path **274**, and then, as represented by arrow **266b**, the liquid **272** can pass from the second aperture **233** to outside the vessel **200**. In addition, liquid can be introduced into the first region **103a** as shown by arrow **260** through the first aperture **205** and removed from the first region **103a** as shown by arrow **261**.

[0053] Turning back to **FIG. 13**, providing the conduit **232** can allow liquid **272** to be added to or removed from the second region **103b** without disturbing the first region **103a** of the cell culture chamber **103**, and therefore, without disturbing (e.g., dislodging, dislocating, damaging) one or more cells **250a, 250b, 250c** of the plurality of cells **250** being cultured in the microcavities **220a, 220b, 220c** of the plurality of microcavities **220** of the substrate **215**. Likewise, referring to **FIG. 14**, providing the second aperture **233** and the conduit **234** can allow liquid **272** to be added to or removed from the second region **103b** without disturbing the first region **103a** of the cell culture chamber **103**, and therefore, without disturbing (e.g., dislodging, dislocating, damaging) one or more cells **250a, 250b, 250c** of the plurality of cells **250** being cultured in the microcavities **220a, 220b, 220c** of the plurality of microcavities **220** of the substrate **215**. Additionally, providing liquid **272** in the second region **103b** can permit diffusion of nutrients and waste to and from the cells **250a, 250b, 250c** through the substrate **215**, further reducing or eliminating the possibility of disturbing one or more cells **250a, 250b, 250c** being cultured in the microcavities **220a, 220b, 220c**. Moreover, providing liquid **272** in the second region **103b**, in some embodiments, a rate of gas exchange to and from the cells **250a, 250b, 250c** can be selected and controlled based at least on, for example, a height (e.g., head height) of the liquid **270** in the first region **103a**. For example, in some

embodiments, the resistance of the liquid **270** to gas diffusion through the liquid **270** can be based on a height of the liquid **270** relative to one or more features of the substrate **215**. Thus, by providing liquid **272** in the second region **103b** and selecting a particular height of liquid **270** in the first region **103a**, in some embodiments, the rate of gas diffusion through the liquid **270** can be controlled and improved control over the cell culturing process can be achieved.

[0054] Further, when the microcavity array **220** has the features illustrated in **FIGs. 6** and **7** (wells with openings) and **FIGs. 8-11** (porous material) gas and liquid can pass from the second region **103b** to the microcavities **220** through either the well openings or the porous material **216**.

[0055] As shown in **FIG. 15** in some embodiments, the vessel **200** can include a conduit **235** connecting the first region **103a** to the second region **103b**. For example, the conduit **235** can define a path **275** that is in fluid communication with the first region **103a** and the second region **103b**. In some embodiments, the conduit **235** can provide fluid communication along the path **275** between the first region **103a** and the second region **103b**. Additionally, in some embodiments, an end **236** of the conduit **237** leading from the first region **103a** to the second region **103b** can be positioned in the first region **103a** spaced (e.g., distance “**d3**”) from the opening **223a**, **223b**, **223c** of each microcavity **220a**, **220b**, **220c** of the plurality of microcavities **220**. In some embodiments, the method can include passing gas **271** through the conduit **235** connecting the first region **103a** to the second region **103b**, and culturing cells in at least one microcavity **220a**, **220b**, **220c** of the plurality of microcavities **220**. For example, as represented by arrow **267a**, gas **271** can pass from the first region **103a** into the conduit **235**, along the path **275**, and then, as represented by arrow **267b**, the gas **271** can pass from the conduit **235** into the second region **103b**. In addition or alternatively, as represented by arrow **268a**, gas **271** can pass from the second region **103b** into the conduit **235**, along the path **275**, and then, as represented by arrow **268b**, the gas **271** can pass from the conduit **235** to the first region **103a**. In addition, liquid can be introduced into the first region **103a** as shown by

arrow **260** through the first aperture **205** and removed from the first region **103a** as shown by arrow **261**.

[0056] Providing the vessel **200** with a conduit **235** can allow gas exchange between, for example, the first region **103a** and the second region **103b**, thereby preventing pressurization (e.g., over-pressurization) of gas **271** in the second region **103b** of the cell culture chamber **103** of the vessel **100**. Additionally, in some embodiments, gas produced by the cells **250a**, **250b**, **250c** as a byproduct of cell culturing can be removed from the second region **103b** by passing through the conduit **235** into the first region **103a** while culturing the cells **250a**, **250b**, **250c**. Similarly, in some embodiments, gas to aid in cell culturing of the cells **250a**, **250b**, **250c** can be added to the second region **103b** by passing from the first region **103a** through the conduit **235** and into the second region **103b** while culturing the cells **250a**, **250b**, **250c**. Additionally, by spacing the end **236** of the conduit **235** leading from the first region **103a** to the second region **103b** a distance “**d3**” from the opening **223a**, **223b**, **223c** of each microcavity **220a**, **220b**, **220c** of the plurality of microcavities **220**, liquid **270** can be contained within the first region **103a** without flowing, splashing, or entering the end **236** of the conduit **235** and flowing into the second region **103b** that contains gas **271**.

[0057] Throughout the disclosure, the terms “material”, “liquid”, and “gas” can be used to describe properties of a material employed when, for example, culturing cells in the cell culture vessel. Unless otherwise noted, for purposes of the disclosure, “material” can include fluid material (e.g., liquid or gas). Additionally, material can include a culture solution or media including a liquid including solid particles (e.g., cells) suspended in the liquid. Unless otherwise noted, for purposes of the disclosure, “liquid” can include cleaning or rinsing solutions, aqueous solutions, or other liquid that can be added to or removed from the vessel to, for example, clean the cell culture chamber, sterilize one or more features of the substrate and the vessel, prepare the substrate for cellular growth and other uses of liquid. Additionally, liquid can include a culture solution or media including a liquid including solid particles (e.g., cells) suspended in the

liquid. Unless otherwise noted, for purposes of the disclosure, “gas” can include air, filtered or treated air, or other gases.

[0058] Throughout the disclosure, the terms “non-permeable”, “gas-permeable”, and “porous” can be used to describe properties (e.g., material properties, characteristics, parameters) of one or more features of a substrate.

[0059] Unless otherwise noted, for purposes of the disclosure, a “non-permeable” substrate (e.g., material of a non-permeable substrate) is considered to be impermeable to solid, liquid, and gas under normal conditions (e.g., no external influence including but not limited to pressure and force) and, therefore, does not permit the transfer of solid, liquid, or gas in to, through, or out of, the non-permeable substrate under normal conditions. In some embodiments, a non-permeable substrate can form a portion of the wall of the vessel. Additionally, the cell culture chamber of the vessel is considered to be sterile when a non-permeable substrate forms a portion of the wall of the vessel because bacteria, for example, cannot pass through the non-permeable substrate. However, when filling the plurality of microcavities of the substrate with material, gas can become trapped within the microcavity of a non-permeable substrate based on surface tension of the liquid, thereby, in some embodiments, preventing material from filling the microcavities and preventing growth of a spheroid.

[0060] Unless otherwise noted, for purposes of the disclosure, a “gas-permeable” substrate (e.g., material of a gas-permeable substrate) is considered to be impermeable to solid and liquid, and permeable to gas under normal conditions. Therefore, a gas-permeable substrate does not permit the transfer of solid and liquid in to, through, or out of, the gas-permeable substrate and does permit the transfer of gas in to, through, or out of, the gas-permeable substrate. In some embodiments, a gas-permeable substrate can form a portion of the wall of the vessel. Additionally, the cell culture chamber of the vessel is considered to be sterile when a gas-permeable substrate forms a portion of the wall of the vessel because bacteria, for example, cannot reasonably pass through the gas-permeable substrate. However, although the substrate is gas-permeable, gas can still

become trapped in the microcavity during filling with material because gas-permeation rates through the gas-permeable substrate can be slower than the rate required to displace gas from the cavity under ordinary operating conditions and can therefore take an unacceptably long amount of time to permeate through the substrate. Thus, in some embodiments, slowly filling the microcavities allows the liquid front to enter each microcavity at an angle, thereby displacing gas as the liquid fills the microcavity. In some embodiments, after filling the cavity with liquid, gas can permeate (slowly) through the gas-permeable substrate.

[0061] Unless otherwise noted, for purposes of the disclosure, a “porous” substrate (e.g., material of a porous substrate) is considered to be impermeable to solid and permeable to liquid and gas under normal conditions. Therefore, a porous substrate does not permit the transfer of solid in to, through, or out of, the porous substrate and does permit the transfer of liquid and gas in to, through, or out of, the porous substrate. A porous substrate cannot form a portion of the vessel because bacteria can pass through a porous substrate, thus causing sterility issues in the cell culture chamber. Thus, when using a porous substrate, the substrate must be enclosed (entirely enclosed) in the sterile cell culture chamber of the vessel. During filling of the microcavities with material, however, gas can escape (e.g., pass) through the porous substrate. Thus, filling of the microcavities can be performed rapidly without concern for entrapping gas in the microcavities. In some embodiments, liquid can only pass through the porous substrate with added pressure or physical contact and disturbance of the substrate. Thus, in some embodiments, material including liquid can be contained in the microcavities of the substrate so long as the substrate is not exposed to added pressure or physical contact and disturbance. For example, in some embodiments, the porous substrate can be supported in the cell culture chamber to allow gas to pass through the substrate during filling as well as during culturing and to isolated the substrate from added pressure or physical contact and disturbance from external forces (e.g., outside the cell culture chamber).

[0062] A number of aspects of cell culture vessels and methods of culturing cells have been disclosed herein. A summary of some selected aspects is presented below.

[0063] In a first aspect, a cell culture vessel is provided, wherein the cell culture vessel has a wall comprising inner surfaces defining a cell culture chamber of the vessel; a substrate of non-porous material positioned in the cell culture chamber between a first region of the cell culture chamber and a second region of the cell culture chamber, the substrate comprising a plurality of microcavities, each microcavity of the plurality of microcavities comprises a concave surface defining a well and an opening defining a path from the first region into the well, the concave surface of each microcavity comprises at least one aperture comprising a dimension less than or equal to about 15 microns defining a path from the well to the second region; and a first aperture extending through the wall in fluid communication with the first region.

[0064] In a second aspect, the disclosure provides the features of aspect one, and further provides a port comprising a gas-permeable filter extending through the wall in fluid communication with the second region.

[0065] In a third aspect, the disclosure provides the features of aspect one, and further provides a conduit connecting the first aperture to the second region.

[0066] In a fourth aspect, the disclosure provides the features of aspect one and further provides a second aperture extending through the wall in fluid communication with the second region.

[0067] In a fifth aspect, the disclosure provides the features of aspect four and further provides a conduit connecting the second aperture to the second region.

[0068] In a sixth aspect, the disclosure provides the features of aspect one or two and further provides a conduit connecting the first region to the second region.

[0069] In a seventh aspect, the disclosure provides the features of aspect six and further provides an end of the conduit leading from the first region to the second region is positioned in the first region spaced from the opening of each microcavity of the plurality of microcavities.

[0070] In an eighth aspect, the disclosure provides a method of culturing cells in the cell culture vessel of any one of aspect one through seven, and further provides passing gas through the at least one aperture in the concave surface of at least one microcavity of the plurality of microcavities; and culturing cells in the at least one microcavity.

[0071] In a ninth aspect, the disclosure provides a method of culturing cells in any one of aspects one through seven comprising passing liquid through the first aperture; and culturing cells in at least one microcavity of the plurality of microcavities.

[0072] In a tenth aspect, the disclosure provides a method of culturing cells in any one of aspects one through seven comprising passing liquid through the first aperture from outside the vessel into the first region; depositing at least a portion of the liquid in at least one microcavity of the plurality of microcavities; and culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the liquid in the at least one microcavity.

[0073] In an eleventh aspect, the disclosure provides a method of culturing cells in a cell culture vessel according to aspect two, comprising passing gas into the gas-permeable filter of the port, and culturing cells in at least one microcavity of the plurality of microcavities.

[0074] In a twelfth aspect, the disclosure provides a method of culturing cells in a cell culture vessel according to aspect two, comprising passing liquid through the conduit connecting the first aperture to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

[0075] In a thirteenth aspect, the disclosure provides a method of culturing cells in a cell culture vessel according to aspect four comprising passing liquid through the second aperture; and culturing cells in at least one microcavity of the plurality of microcavities.

[0076] In a fourteenth aspect, the disclosure provides a method of culturing cells in a cell culture vessel according to aspect five comprising passing liquid through the

conduit connecting the second aperture to the second region; and culturing cells in at least one microcavity of the plurality of microcavities.

[0077] In a fifteenth aspect, the disclosure provides a method of culturing cells in a cell culture vessel according to aspect six comprising passing gas through the conduit connecting the first region to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

[0078] In a sixteenth aspect, the disclosure provides cell culture vessel having a wall comprising an inner surface defining a cell culture chamber of the vessel; a substrate positioned in the cell culture chamber between a first region of the cell culture chamber and a second region of the cell culture chamber, the substrate comprising a plurality of microcavities and a layer of porous material, each microcavity of the plurality of microcavities comprises a concave surface defining a well and an opening defining a path from the first region into the well, a first side of the layer of porous material defines at least a portion of the concave surface of each microcavity of the plurality of microcavities, and a second side of the layer of porous material faces the second region; and a first aperture extending through the wall in fluid communication with the first region.

[0079] In a seventeenth aspect, the disclosure provides the cell culture vessel of aspect sixteen the first side of the layer of porous material defines the entirety of the concave surface of each microcavity of the plurality of microcavities.

[0080] In an eighteenth aspect, the disclosure provides the cell culture vessel of aspect sixteen or seventeen each microcavity of the plurality of microcavities comprises a sidewall surface extending from the opening to the concave surface, and the first side of the layer of porous material defines at least a portion of the sidewall surface of each microcavity of the plurality of microcavities.

[0081] In a nineteenth aspect, the disclosure provides the cell culture vessel of aspect eighteen the first side of the layer of porous material defines the entirety of the sidewall surface of each microcavity of the plurality of microcavities.

[0082] In a twentieth aspect, the disclosure provides the cell culture vessel of any one of aspects sixteen through nineteen the layer of porous material defines the entirety of the substrate.

[0083] In a twenty-first aspect the disclosure provides the cell culture vessel of any one of aspect sixteen through twenty comprising a port comprising a gas-permeable filter extending through the wall in fluid communication with the second region.

[0084] In a twenty-second aspect, the disclosure provides the cell culture vessel of any one of aspect sixteen through twenty including a conduit connecting the first aperture to the second region.

[0085] In a twenty-third aspect, the disclosure provides the cell culture vessel of any one of aspect sixteen through twenty including a second aperture extending through the wall in fluid communication with the second region.

[0086] In a twenty-fourth aspect, the disclosure provides the cell culture vessel of twenty-three including a conduit connecting the second aperture to the second region.

[0087] In a twenty-fifth aspect, the disclosure provides the cell culture vessel according to any one of aspects sixteen to twenty one including comprising a conduit connecting the first region to the second region.

[0088] In a twenty-sixth aspect, the disclosure provides the cell culture vessel according to aspect twenty-five including an end of the conduit leading from the first region to the second region is positioned in the first region spaced from the opening of each microcavity of the plurality of microcavities.

[0089] In a twenty-seventh aspect, the disclosure provides the cell culture vessel of any one of aspects sixteen to twenty-six including passing gas into the at least a portion of the concave surface defined by the first side of the layer of porous material of at least one microcavity of the plurality of microcavities; and culturing cells in the at least one microcavity.

[0090] In a twenty-eighth aspect, the disclosure provides a method of culturing cells in the cell culture vessel of any one of aspects sixteen to twenty-six including

passing liquid through the first aperture; and culturing cells in at least one microcavity of the plurality of microcavities.

[0091] In a twenty-ninth aspect, the disclosure provides a method of culturing cells in the cell culture vessel of any one of aspects sixteen to twenty-six including passing liquid through the first aperture from outside the vessel into the first region; depositing at least a portion of the liquid in at least one microcavity of the plurality of microcavities; and culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the liquid in the at least one microcavity.

[0092] In a thirtieth aspect, the disclosure provides a method of of culturing cells in the cell culture vessel of aspect twenty-one passing gas into the gas-permeable filter of the port, and culturing cells in at least one microcavity of the plurality of microcavities.

[0093] In a thirty-first aspect, the disclosure provides a method of culturing cells in the cell culture vessel of any aspect twenty-two including passing liquid through the conduit connecting the first aperture to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

[0094] In a thirty-second aspect, the disclosure provides a method of culturing cells in the cell culture vessel of aspects twenty-three including passing liquid through the second aperture; and culturing cells in at least one microcavity of the plurality of microcavities.

[0095] In a thirty-third aspect, the disclosure provides a method of culturing cells in the cell culture vessel of aspect twenty-four including passing liquid through the conduit connecting the second aperture to the second region; and culturing cells in at least one microcavity of the plurality of microcavities.

[0096] In a thirty-fourth aspect, the disclosure provides a method of culturing cells in the cell culture vessel of any one of aspects twenty-five or twenty-six including passing gas through the conduit connecting the first region to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

[0097] In an embodiment, the disclosure provides, a cell culture vessel includes a substrate including a plurality of microcavities; a wall, the substrate and an inner surface of the wall define a cell culture chamber of the vessel; an aperture extending through the wall in fluid communication with the cell culture chamber; a first portion of the inner surface positioned opposite the aperture along an axis of the vessel, the substrate spans a length of the cell culture chamber that extends along the axis of the vessel; a second portion of the inner surface extending from the aperture to the substrate; and a third portion of the inner surface extending from the first portion to the substrate.

[0098] It will be appreciated that the various disclosed embodiments can involve particular features, elements or steps that are described in connection with that particular embodiment. It will also be appreciated that a particular feature, element or step, although described in relation to one particular embodiment, can be interchanged or combined with alternate embodiments in various non-illustrated combinations or permutations.

[0099] It is to be understood that, as used herein the terms "the," "a," or "an," mean "at least one," and should not be limited to "only one" unless explicitly indicated to the contrary. Thus, for example, reference to "a component" includes embodiments having two or more such components unless the context clearly indicates otherwise.

[00100] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, embodiments 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.

[00101] 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.

[00102] While various features, elements or steps of particular embodiments can be disclosed using the transitional phrase "comprising," it is to be understood that alternative embodiments, including those that can be described using the transitional phrases "consisting" or "consisting essentially of," are implied. Thus, for example, implied alternative embodiments to an apparatus that comprises A+B+C include embodiments where an apparatus consists of A+B+C and embodiments where an apparatus consists essentially of A+B+C.

[00103] It will be apparent to those skilled in the art that various modifications and variations can be made to the present disclosure without departing from the spirit and scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

CLAIMS

What is claimed is:

1. A cell culture vessel comprising:
 - a wall comprising inner surfaces defining a cell culture chamber of the vessel;
 - a substrate of non-porous material positioned in the cell culture chamber between a first region of the cell culture chamber and a second region of the cell culture chamber, the substrate comprising a plurality of microcavities, each microcavity of the plurality of microcavities comprises a concave surface defining a well and an opening defining a path from the first region into the well, the concave surface of each microcavity comprises at least one aperture comprising a dimension less than or equal to about 15 microns defining a path from the well to the second region; and
 - a first aperture extending through the wall in fluid communication with the first region.
2. The cell culture vessel of claim 1, wherein the wall comprises a port comprising gas-permeable material in fluid communication with the second region.
3. The cell culture vessel of claim 1, comprising a conduit connecting the first aperture to the second region.
4. The cell culture vessel of claim 1, comprising a second aperture extending through the wall in fluid communication with the second region.
5. The cell culture vessel of claim 4, comprising a conduit connecting the second aperture to the second region.

6. The cell culture vessel of claim 1 or claim 2, comprising a conduit connecting the first region to the second region.

7. The cell culture vessel of claim 6, an end of the conduit leading from the first region to the second region is positioned in the first region spaced from the opening of each microcavity of the plurality of microcavities.

8. A method of culturing cells in the cell culture vessel of any one of claims 1-7, comprising:

passing gas through the at least one aperture in the concave surface of at least one microcavity of the plurality of microcavities; and
culturing cells in the at least one microcavity.

9. A method of culturing cells in the cell culture vessel of any one of claims 1-7, comprising:

passing liquid through the first aperture; and
culturing cells in at least one microcavity of the plurality of microcavities.

10. A method of culturing cells in the cell culture vessel of any one of claims 1-7, comprising:

passing liquid through the first aperture from outside the vessel into the first region;
depositing at least a portion of the liquid in at least one microcavity of the plurality of microcavities; and
culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the liquid in the at least one microcavity.

11. A method of culturing cells in the cell culture vessel of claim 2, comprising passing gas into the gas-permeable filter of the port, and culturing cells in at least one microcavity of the plurality of microcavities.
12. A method of culturing cells in the cell culture vessel of claim 3, comprising passing liquid through the conduit connecting the first aperture to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.
13. A method of culturing cells in the cell culture vessel of claim 4, comprising:
passing liquid through the second aperture; and
culturing cells in at least one microcavity of the plurality of microcavities.
14. A method of culturing cells in the cell culture vessel of claim 5, comprising:
passing liquid through the conduit connecting the second aperture to the second region; and
culturing cells in at least one microcavity of the plurality of microcavities.
15. A method of culturing cells in the cell culture vessel of claim 6 or claim 7, comprising passing gas through the conduit connecting the first region to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.
16. A cell culture vessel comprising:
a wall comprising an inner surface defining a cell culture chamber of the vessel;
a substrate positioned in the cell culture chamber between a first region of the cell culture chamber and a second region of the cell culture chamber, the substrate comprising a plurality of microcavities and a layer of porous material, each microcavity of the plurality of microcavities comprises a concave surface defining a well and an opening defining a path from the first region into the well, a first side of the layer of porous

material defines at least a portion of the concave surface of each microcavity of the plurality of microcavities, and a second side of the layer of porous material faces the second region; and

a first aperture extending through the wall in fluid communication with the first region.

17. The cell culture vessel of claim 16, the first side of the layer of porous material defines the entirety of the concave surface of each microcavity of the plurality of microcavities.

18. The cell culture vessel of claim 16 or claim 17, each microcavity of the plurality of microcavities comprises a sidewall surface extending from the opening to the concave surface, and the first side of the layer of porous material defines at least a portion of the sidewall surface of each microcavity of the plurality of microcavities.

19. The cell culture vessel of claim 18, the first side of the layer of porous material defines the entirety of the sidewall surface of each microcavity of the plurality of microcavities.

20. The cell culture vessel of any one of claims 16-19, the layer of porous material defines the entirety of the substrate.

21. The cell culture vessel of any one of claims 16-20, comprising a port comprising a gas-permeable filter extending through the wall in fluid communication with the second region.

22. The cell culture vessel of any one of claims 16-20, comprising a conduit connecting the first aperture to the second region.

23. The cell culture vessel of any one of claims 16-20, comprising a second aperture extending through the wall in fluid communication with the second region.

24. The cell culture vessel of claim 23, comprising a conduit connecting the second aperture to the second region.

25. The cell culture vessel of any one of claims 16-21, comprising a conduit connecting the first region to the second region.

26. The cell culture vessel of claim 25, an end of the conduit leading from the first region to the second region is positioned in the first region spaced from the opening of each microcavity of the plurality of microcavities.

27. A method of culturing cells in the cell culture vessel of any one of claims 16-26, comprising:

passing gas into the at least a portion of the concave surface defined by the first side of the layer of porous material of at least one microcavity of the plurality of microcavities; and

culturing cells in the at least one microcavity.

28. A method of culturing cells in the cell culture vessel of any one of claims 16-26, comprising:

passing liquid through the first aperture; and

culturing cells in at least one microcavity of the plurality of microcavities.

29. A method of culturing cells in the cell culture vessel of any one of claims 16-26, comprising:

passing liquid through the first aperture from outside the vessel into the first region;

depositing at least a portion of the liquid in at least one microcavity of the plurality of microcavities; and

culturing cells in the at least one microcavity of the plurality of microcavities after depositing the at least a portion of the liquid in the at least one microcavity.

30. A method of culturing cells in the cell culture vessel of claim 21, comprising passing gas into the gas-permeable filter of the port, and culturing cells in at least one microcavity of the plurality of microcavities.

31. A method of culturing cells in the cell culture vessel of claim 22, comprising passing liquid through the conduit connecting the first aperture to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

32. A method of culturing cells in the cell culture vessel of claim 23, comprising:
passing liquid through the second aperture; and
culturing cells in at least one microcavity of the plurality of microcavities.

33. A method of culturing cells in the cell culture vessel of claim 24, comprising:
passing liquid through the conduit connecting the second aperture to the second region; and
culturing cells in at least one microcavity of the plurality of microcavities.

34. A method of culturing cells in the cell culture vessel of claim 25 or claim 26, comprising passing gas through the conduit connecting the first region to the second region, and culturing cells in at least one microcavity of the plurality of microcavities.

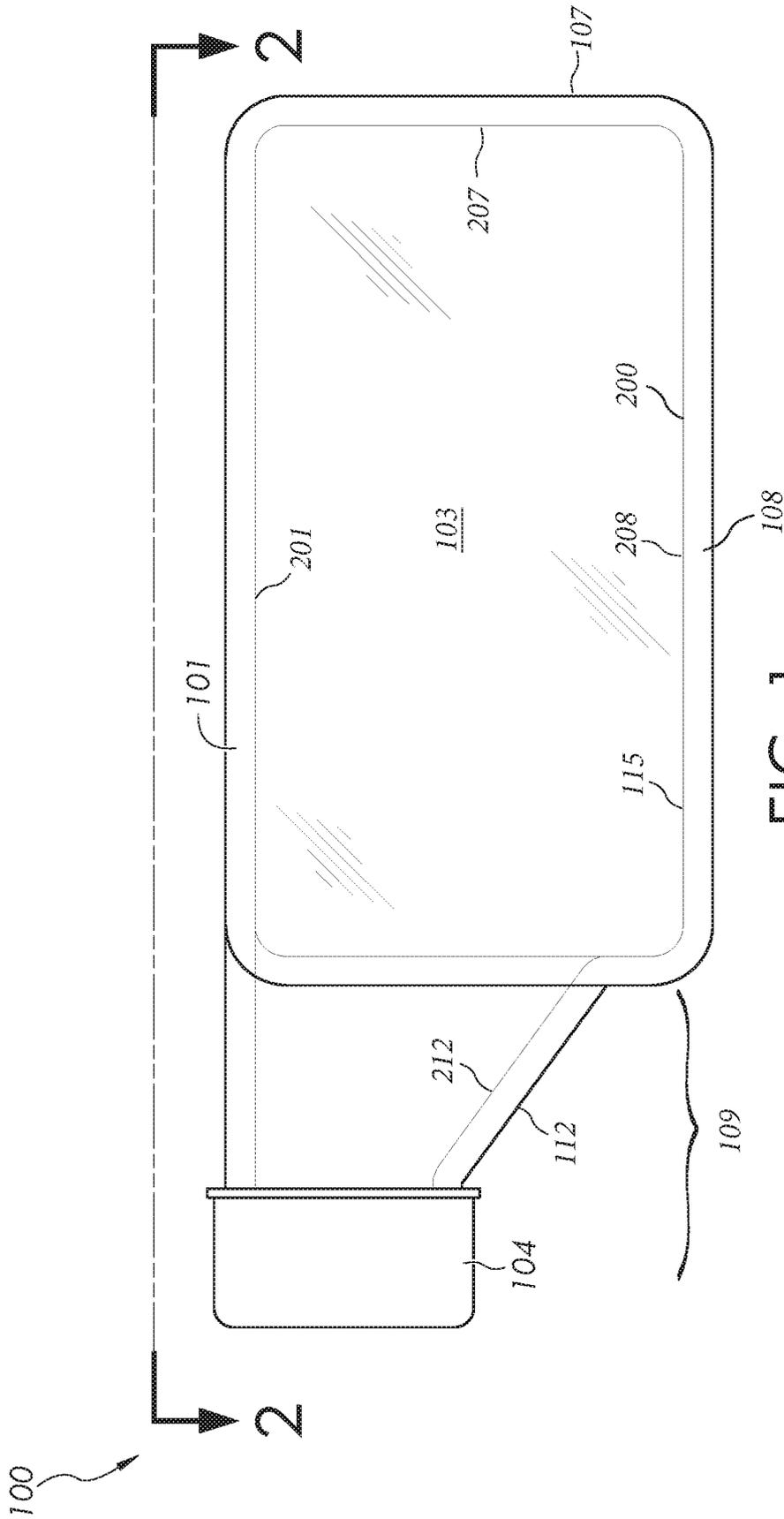


FIG. 1

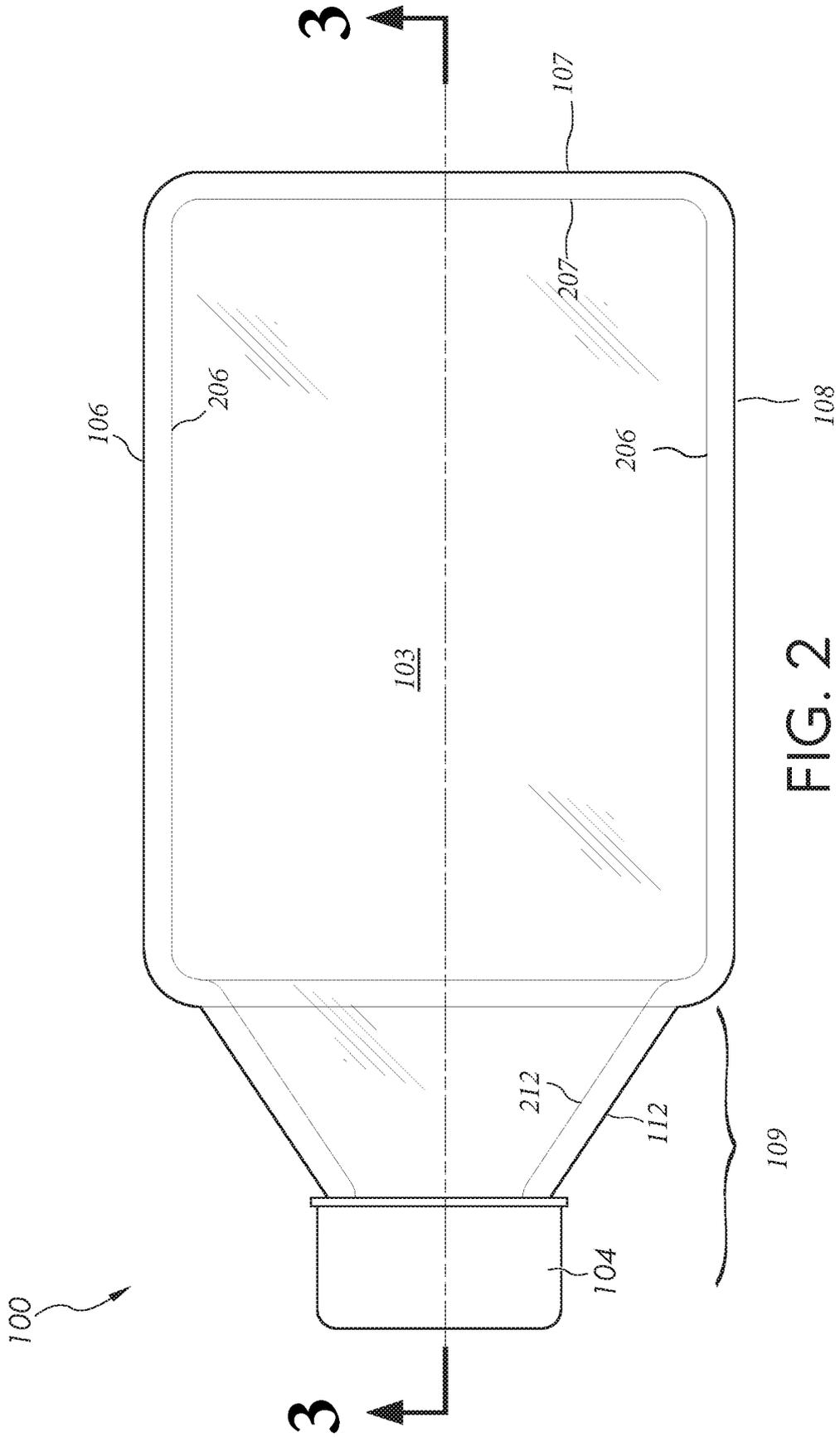


FIG. 2

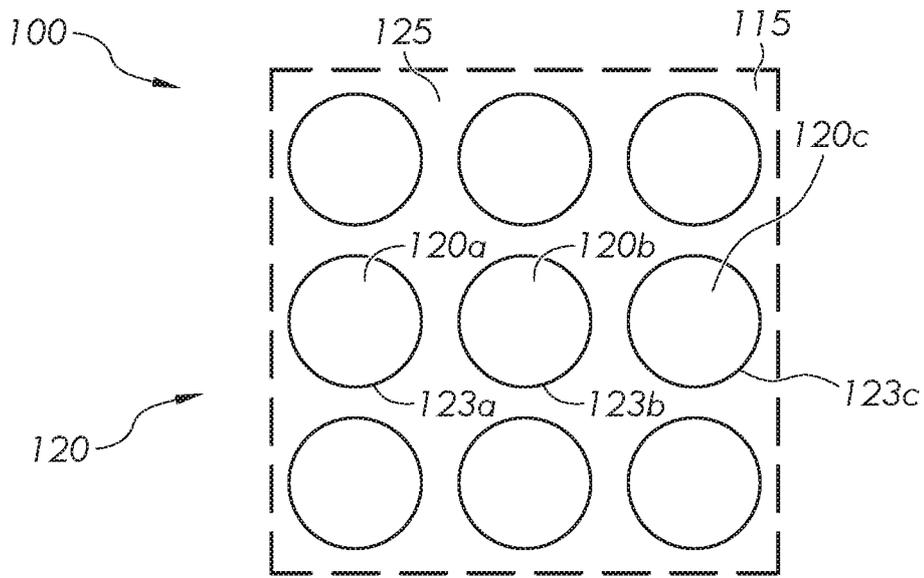


FIG. 4

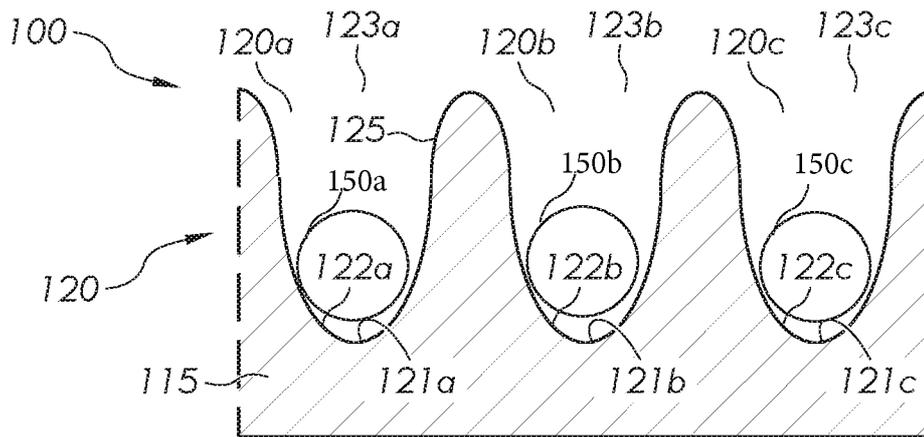


FIG. 5

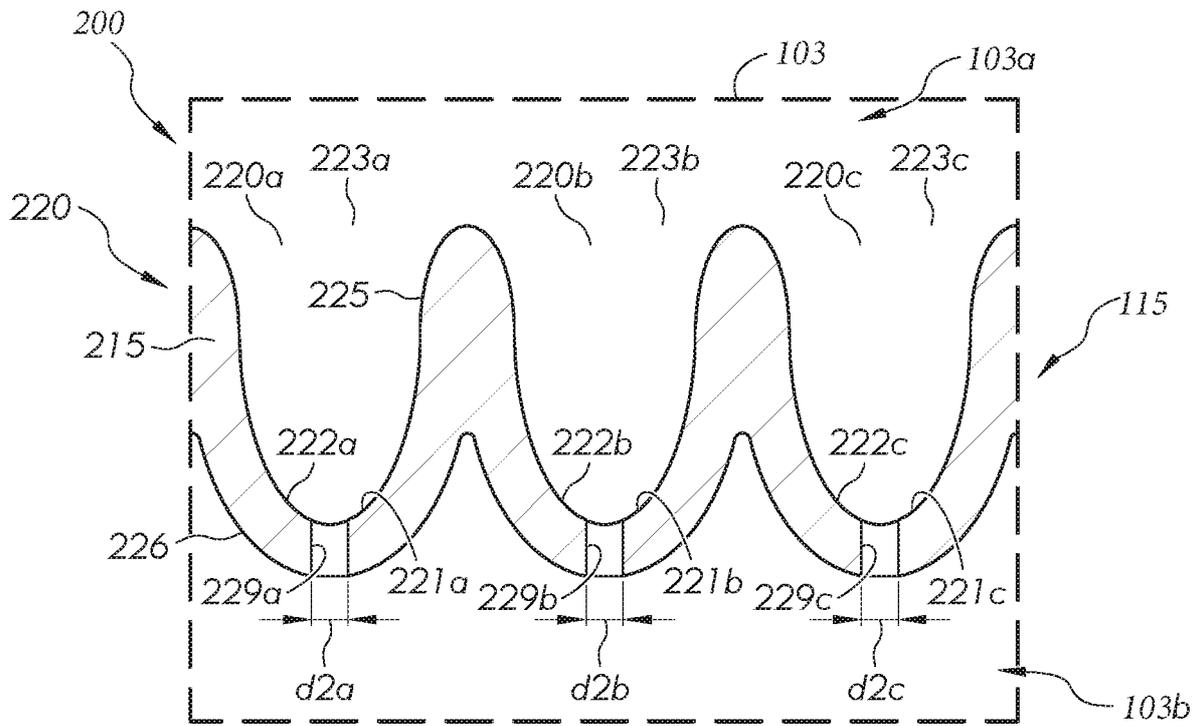


FIG. 6

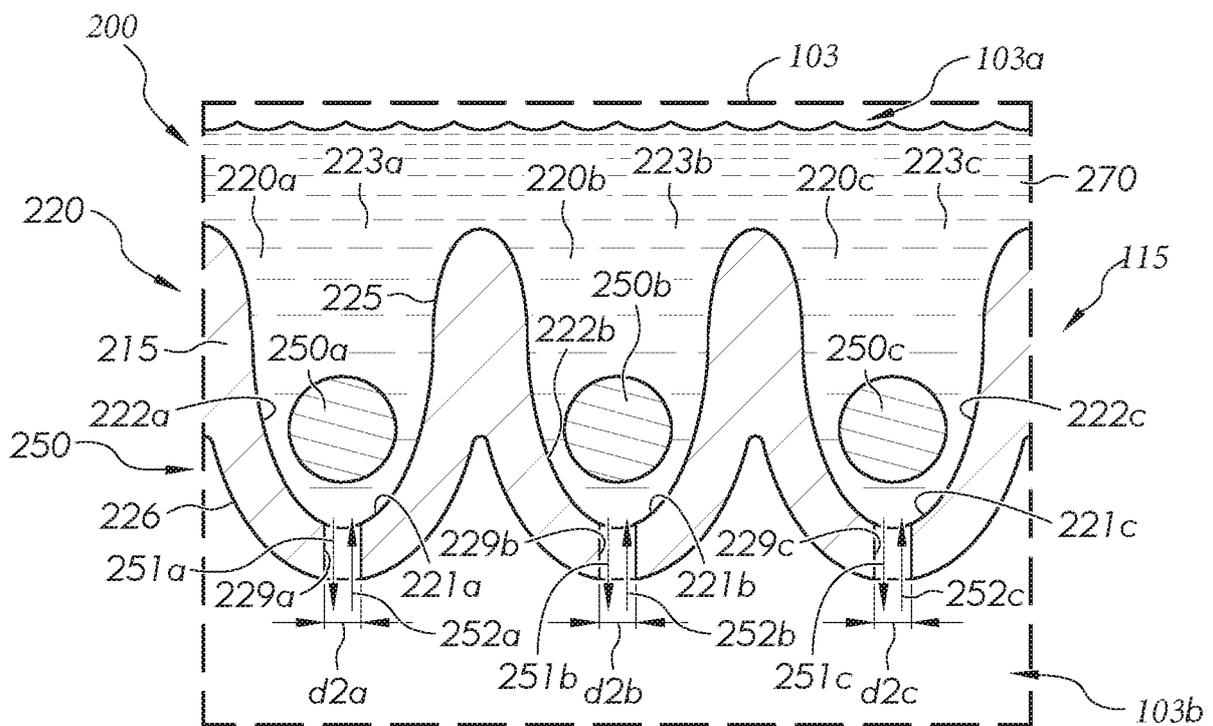


FIG. 7

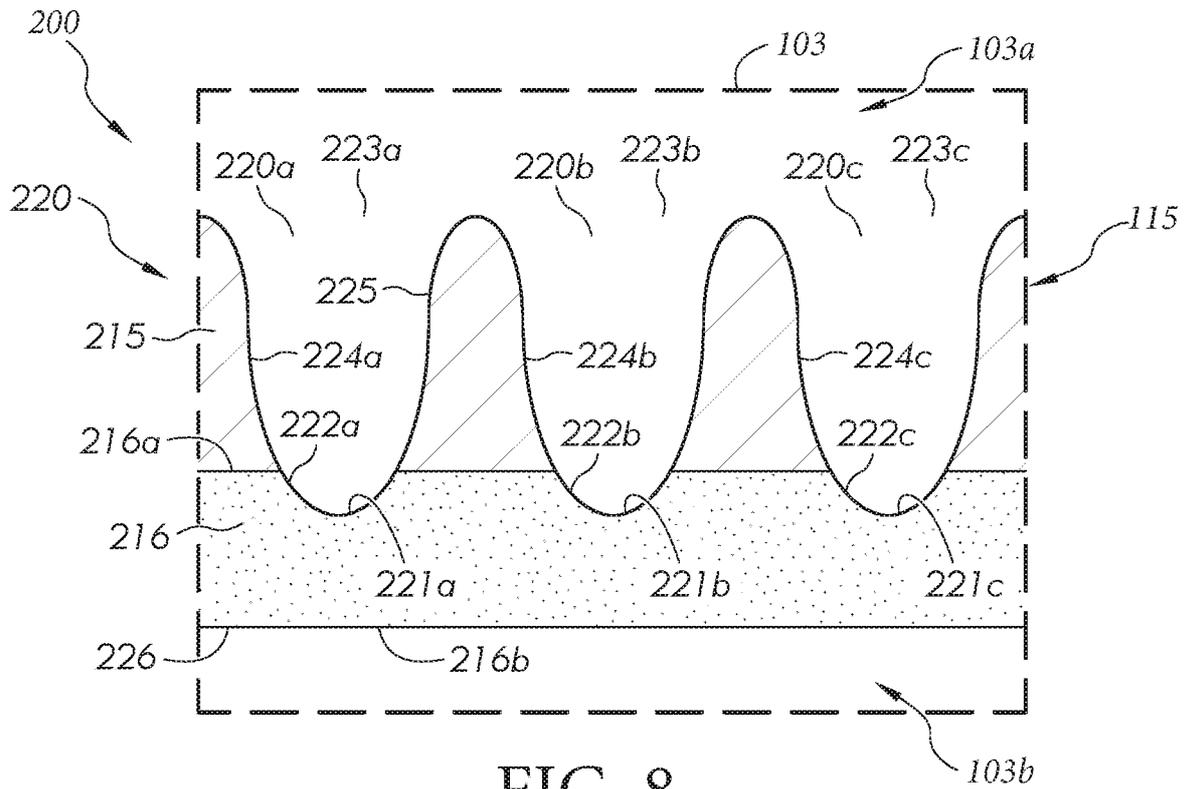


FIG. 8

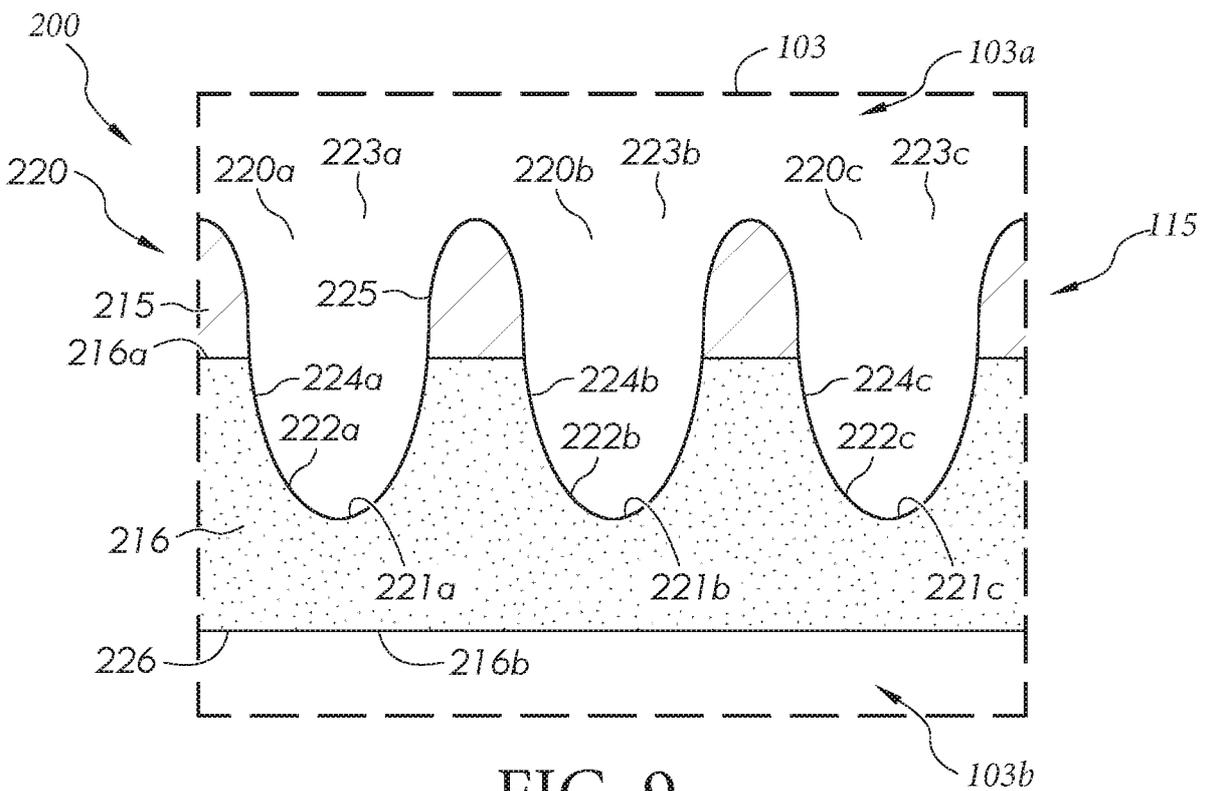


FIG. 9

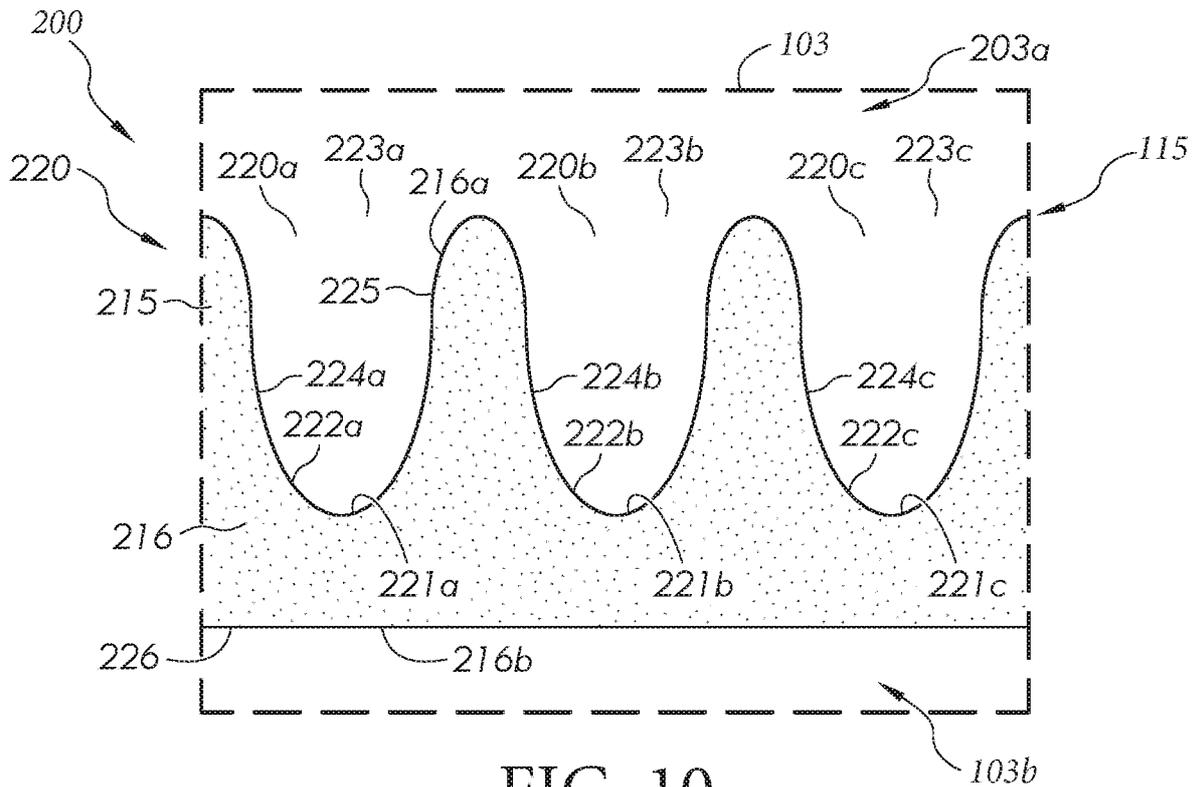


FIG. 10

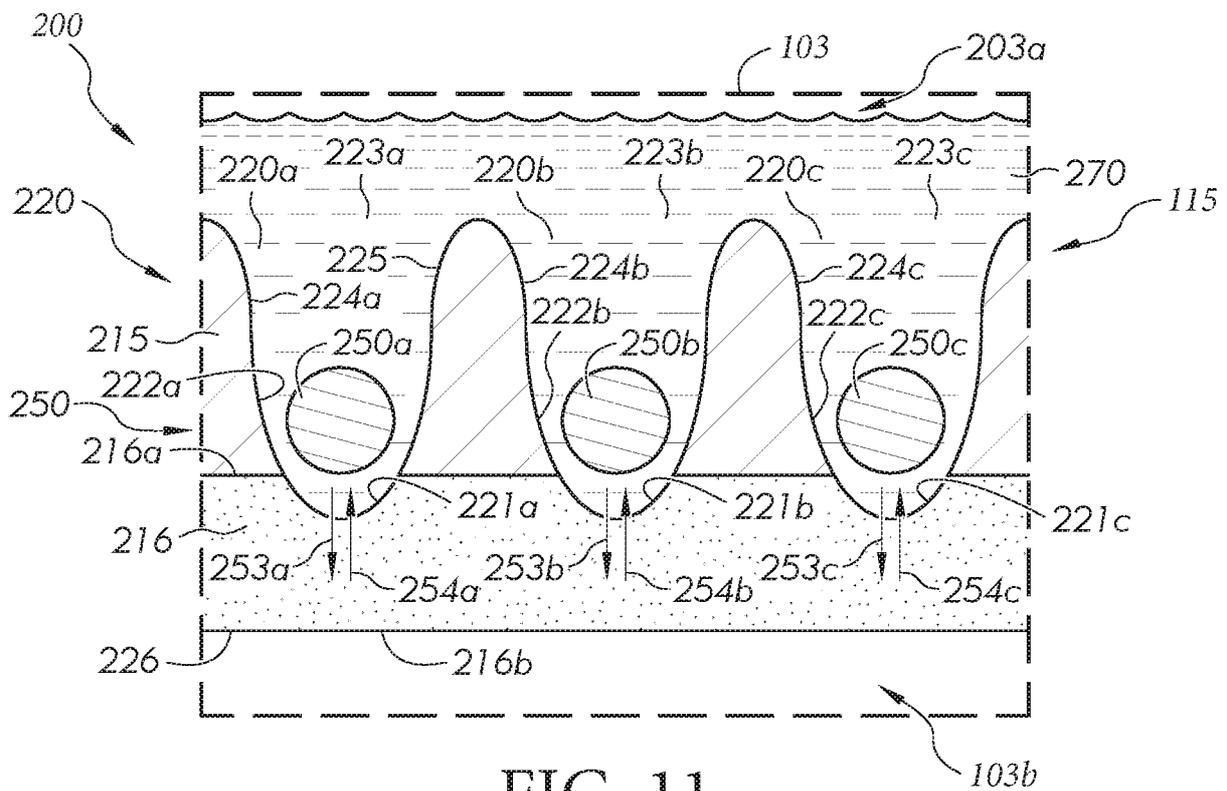


FIG. 11

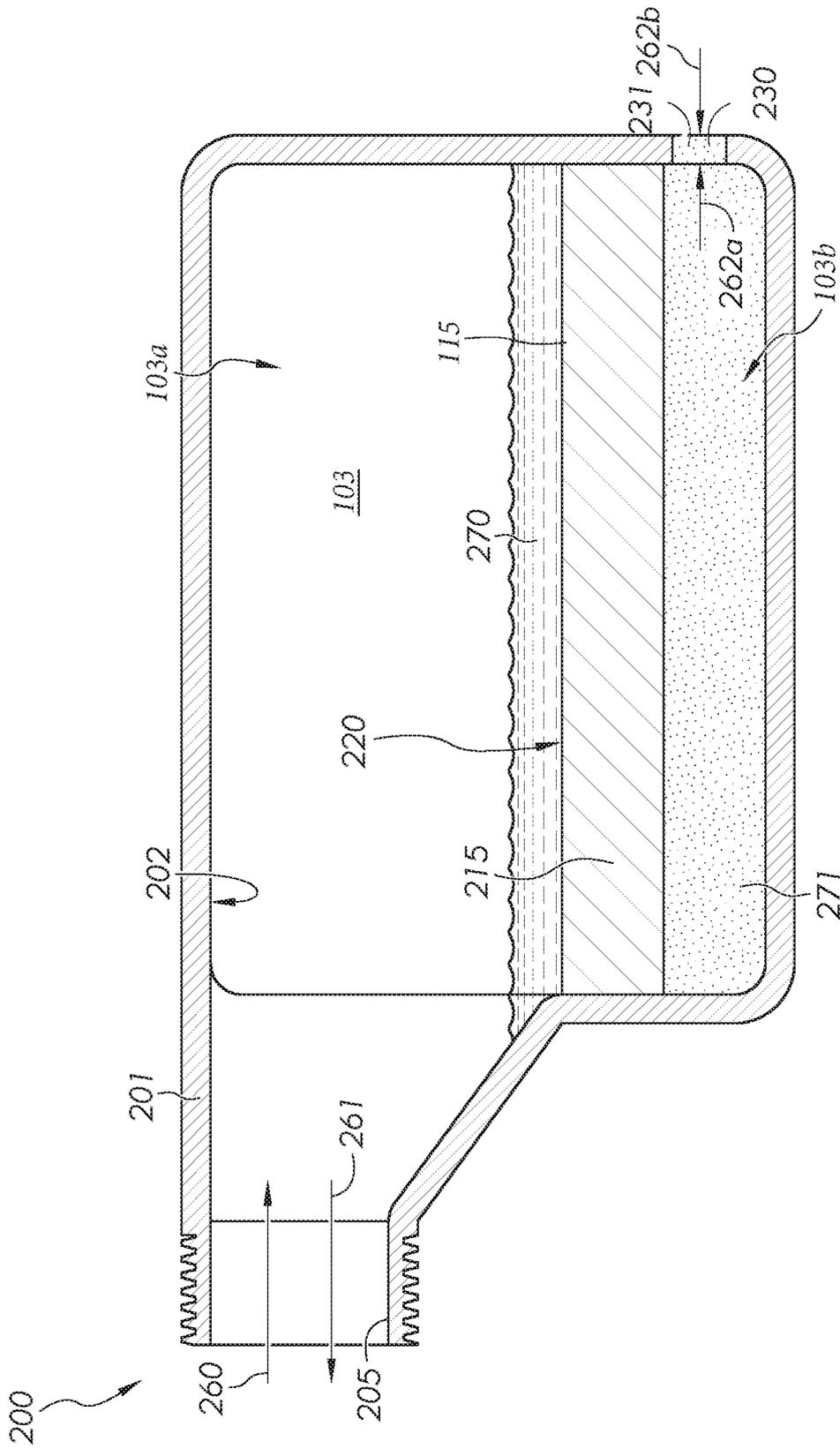


FIG. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/042161

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12M1/24 C12M1/32 C12M1/04 C12M1/00
 ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 081 627 A1 (YAMAHA MOTOR CO LTD [JP]) 19 October 2016 (2016-10-19)	1,8-10
Y	paragraph [0014]; figure 2 paragraph [0018]	2-7, 11-15
X	----- WO 2016/069895 A1 (CORNING INC [US]) 6 May 2016 (2016-05-06)	16-20, 27-29
Y	paragraph [0030]	21-26, 30-34
Y	----- US 5 047 347 A (CLINE MARTIN J [US]) 10 September 1991 (1991-09-10) the whole document	2,11,21, 30
Y	----- DE 10 2014 214077 A1 (HAMILTON BONADUZ AG [CH]) 21 January 2016 (2016-01-21) the whole document	3,12,22, 31
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Further documents are listed in the continuation of Box C.

See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search 30 November 2018	Date of mailing of the international search report 12/12/2018
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Busuiocescu, Bogdan
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/042161

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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