(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
04 April 2024 (04.04.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/072655 A1

(51) International Patent Classification:
C12M 1/24 (2006.01) *C12N 5/00* (2006.01)
C12M 1/00 (2006.01)

(21) International Application Number:
PCT/US2023/033095

(22) International Filing Date:
19 September 2023 (19.09.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/411,210 29 September 2022 (29.09.2022) US

(71) Applicant: **CORNING INCORPORATED** [US/US]; 1 Riverfront Plaza, Corning, New York 14831 (US).

(72) Inventors: **CLOUTIER, Thomas Albert**; 30 Equestrian Way, Arundel, Maine 04046 (US). **LACEY, William Joseph**; 16 Ogunquit Road, North Andover, Massachusetts 01845 (US). **PARDO, Ana Maria del Pilar**; 55 Meadow Rd., Portsmouth, New Hampshire 03801 (US). **SEYMOUR, James Mark**; 89 Maine Ave., Portland, Maine 04103 (US). **TANNER, Allison Jean**; 380 Greenleaf Avenue, Portsmouth, New Hampshire 03801 (US).

(74) Agent: **PANIAN, Michael G.**; Corning Incorporated, Intellectual Property Department, SP-TI-03-1, Corning, New York 14831 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG,

(54) **Title:** MICROCAVITY CELL CULTURE VESSELS INCLUDING CANTED SURFACE WITH DIVERTER

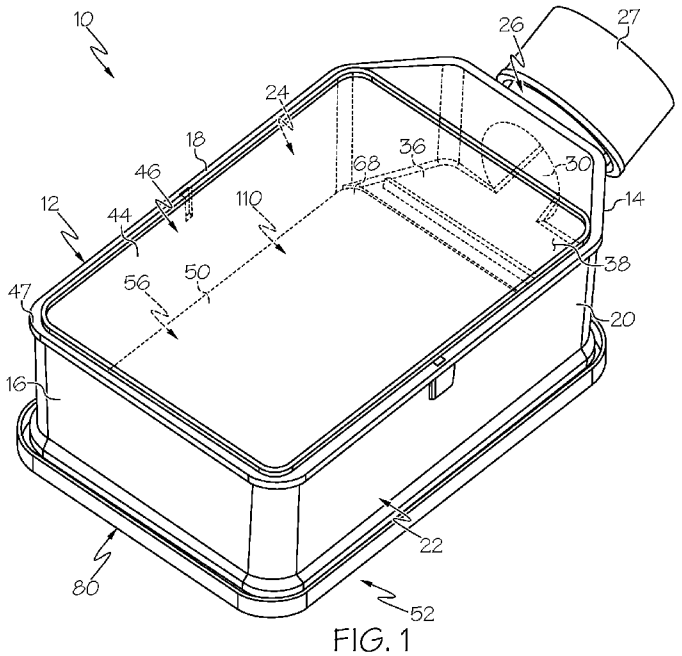

FIG. 1

(57) **Abstract:** A cell culture vessel includes a vessel body including a first end wall, a second end wall opposite the first end wall and a pair of side walls that extend between the first end wall and the second end wall. The first end wall, second end wall and pair of side walls define a perimeter of a cell culture chamber. An inner fill wall extends inward from the first end wall and the pair of sidewalls into the cell culture chamber. The inner fill wall has a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface. A diverter extends along the canted inner surface at least partially transverse to a fill direction between the pair of sidewalls. The diverter provides passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation.

WO 2024/072655 A1

[Continued on next page]

KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

MICROCAVITY CELL CULTURE VESSELS INCLUDING CANTED SURFACE WITH DIVERTER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119 of U.S. Provisional Application Serial No. 63/411,210 filed on September 29, 2022, the content of which is relied upon and incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present specification generally relates to cell culture vessels used for growing cells, more specifically, to microcavity cell culture vessels with diverters to restrain and control liquid flow down a canted surface of the cell culture vessels.

BACKGROUND

[0003] Generally, three-dimensional (3D) cell cultures can be better suited for simulating an environment of natural tissues and organs than two-dimensional (2D) cell cultures grown in monolayers. Cells grown in 3D cell cultures are able to form spheroids or cell aggregates by attaching to other deposited cells within the three-dimensional environment, thereby creating a more natural interaction between the cells than 2D cells grown in a monolayer. This arrangement of cells provides a flexible configuration, similar to that of natural tissues. Providing an accurate exemplification of a tissue microenvironment is desirable In order to increase accuracy when conducting experimental research for developing therapies against diseases, it is desirable to develop the therapies in a 3D culture instead of 2D culture, as 3D culture more closely resembles the environment in which the developed drug will ultimately be applied in.

[0004] However, the formed spheroids or cell aggregates are susceptible to damage, such as when transporting the cell culture vessel or during media exchange. Transporting a 3D cell culture vessel generally causes the liquid contained therein to move unintentionally, resulting

in turbulence within the vessel. Turbulence may also occur when liquid media is added or removed from the vessel during the 3D cell culture process. The turbulence may cause the spheroids or cell aggregates to slosh around or be displaced from the respective microcavities in which they are formed within the 3D culture vessel, since the cells in a 3D cell culture are not attached to any surface of the vessel. If spheroids are displaced from their respective microcavities, the formed spheroids may attach to other formed spheroids, resulting in loss of uniformity of the spheroids within the vessel and the size thereof. Accordingly, a need exists for stabilizing liquid motion within 3D spheroid culture vessels.

SUMMARY

[0005] According to an embodiment, a cell culture vessel includes a vessel body including a first end wall, a second end wall opposite the first end wall and a pair of side walls that extend between the first end wall and the second end wall. The first end wall, second end wall and pair of side walls define a perimeter of a cell culture chamber. An inner fill wall extends inward from the first end wall and the pair of sidewalls into the cell culture chamber. The inner fill wall has a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface. A diverter extends along the canted inner surface at least partially transverse to a fill direction between the pair of sidewalls. The diverter provides passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation.

[0006] According to another embodiment, a method of forming a cell culture vessel is provided. The method includes forming a vessel body as a single, monolithic part such that the vessel body includes a first end wall, a second end wall opposite the first end wall and a pair of side walls that extend between the first end wall and the second end wall. The first end wall, second end wall and pair of side walls define a perimeter of a cell culture chamber. An inner fill wall extends inward from the first end wall and the pair of sidewalls into the cell culture chamber. The inner fill wall has a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface. A diverter extends along the canted inner surface at least partially transverse to the fill direction

between the pair of sidewalls. The diverter provides passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation. A protective carrier is formed separately from the vessel body. The protective carrier includes a plate that is sized and configured to cover a cell culture face of the vessel body.

[0007] Additional features and advantages of the cell culture vessels described herein 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 embodiments described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0008] It is to be understood that both the foregoing general description and the following detailed description describe various embodiments and are intended to provide an overview or framework for understanding the nature and character of the claimed subject matter. The accompanying drawings are included to provide a further understanding of the various embodiments and are incorporated into and constitute a part of this specification. The drawings illustrate the various embodiments described herein, and together with the description serve to explain the principles and operations of the claimed subject matter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 shows a perspective view of a cell culture vessel including vessel body with a diverter and a protective carrier, according to one or more embodiments shown and described herein;

[0010] FIG. 2 shows a side section view of the vessel body of FIG. 1 in isolation, according to one or more embodiments shown and described herein;

[0011] FIG. 3 shows an end section view of the vessel body along lines 3-3 of FIG. 2, according to one or more embodiments shown and described herein;

[0012] FIG. 4 shows a top perspective view of the protective carrier of FIG. 1 in isolationr, according to one or more embodiments shown and described herein;

[0013] FIG. 5 shows a bottom perspective view of the protective carrier of FIG. 4, according to one or more embodiments shown and described herein;

[0014] FIG. 6 shows a side perspective view of a portion of a cell culture substrate for use in the cell culture vessel of FIG. 1, according to one or more embodiments shown and described herein; and

[0015] FIG. 7 shows the cell culture vessel of FIG. 1 in use, according to one or more embodiments shown and described herein.

DETAILED DESCRIPTION

[0016] Reference will now be made in detail to various embodiments of cell culture vessels with diverters located therein, examples of which are illustrated in the accompanying drawings. Whenever possible, the same reference numerals will be used throughout the drawings to refer to the same or like parts. Directional terms as used herein - for example up, down, right, left, front, back, top, bottom, distal, and proximal - are made only with reference to the figures as drawn and are not intended to imply absolute orientation.

[0017] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes 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 embodiment. 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.

[0018] 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, nor that with any apparatus specific orientations be required. Accordingly, where a method claim does not

actually recite an order to be followed by its steps, or that any apparatus claim does not actually recite an order or orientation to individual components, or it is not otherwise specifically stated in the claims or description that the steps are to be limited to a specific order, or that a specific order or orientation to components of an apparatus is not recited, it is in no way intended that an order or orientation be inferred, in any respect. This holds for any possible non-express basis for interpretation, including: matters of logic with respect to arrangement of steps, operational flow, order of components, or orientation of components; plain meaning derived from grammatical organization or punctuation, and; the number or type of embodiments described in the specification.

[0019] As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a" component includes aspects having two or more such components, unless the context clearly indicates otherwise.

[0020] Liquid in cell culture vessels with an open expanse of volume has freedom to move somewhat uncontrolled throughout the open area. If the cell culture vessel is moved or transported, the movement can cause turbulence in the liquid, leading to "sloshing" and potentially spilling of the liquid. In contrast to cells cultured in two dimensional (2D) culture that attach to the culture surface of the vessel, cells, such as spheroids in 3D culture, may not attach to the culture surface and thus may be susceptible to sloshing and may dislocate from the microcavities in which they are being cultured. Such turbulence and dislocation of spheroids from microcavities may cause loss of spheroids. Because dislocated spheroids may end up settling in microcavities with other spheroids, the size of the spheroids can become heterogeneous, resulting in the undesirable loss of uniformity of spheroids.

[0021] Further, there is a degree of skill that is needed to fill the microcavities with medium without trapping air, and to change media after spheroids are formed. Trapped air can cause the cells to not settle in the microcavities. Also, since the cells in the cell culture vessels are growing in 3D, many more cells can be contained in the vessel than are normally contained within a 2D cell growth surface area. This increased number of cells can require either more frequent media changes or to hold a larger fluid volume to meet the metabolic needs of the cells.

[0022] Embodiments described herein are directed to cell culture vessels that include fill structures that can be used to control filling of the cell culture vessels with liquid media. The fill structures can also control movement of the media after the cell culture vessels are filled. The cell culture vessels include a vessel body that includes a first end wall, a second end wall opposite the first end wall and a pair of side walls that extend between the first end wall and the second end wall. The first end wall, second end wall and pair of side walls form a perimeter of a cell culture chamber when a floor and a lid are connected to the vessel body. The vessel body includes a neck that extends outward from the first end wall in a fill direction to an opening. The neck includes a ramped filling surface having an outer edge at the opening that is angularly offset from an inner edge of the ramped filling surface at the first end wall. An inner fill wall extends inward from the first end wall and the pair of sidewalls into the cell culture chamber. The inner fill wall has a canted inner surface that has an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface. A diverter extends along the canted inner surface at least partially transverse to the fill direction between the pair of side walls. The diverter has opposite ends that terminate inside the pair of sidewalls to form passageways through which liquid flows by the diverter during a filling operation.

[0023] Referring to FIGS. 1 and 2, an embodiment of a cell culture vessel 10 (FIG. 1) is a microcavity flask that includes a vessel body 12 (FIG. 2). The vessel body 12 includes a first end wall 14 and a second end wall 16 that is opposite the first end wall 14. Side walls 18 and 20 extend between the first end wall 14 and the second end wall 16. In some embodiments, the first and second end walls 14 and 16 or portions thereof are substantially parallel and the pair of side walls 18 and 20 or portions thereof are substantially parallel forming a somewhat rectangular-shaped perimeter 22 of a cell culture chamber 24. While a rectangular perimeter 22 is illustrated, any suitable perimeter shape may be used.

[0024] The vessel body 12 includes a neck 26 that extends outward from the first end wall 14 in a fill direction to an opening 28. The opening 28 provides a point of ingress for the liquid medium to enter the cell culture vessel 10 and flow into the cell culture chamber 24 during a filling operation. The opening 28 also provides a point of egress for liquid medium to exit the cell culture chamber 24 during an emptying operation. The neck 26 may optionally configured

to mate with a cap 27, for example, using a threaded, snap fit, or any suitable releasable connection.

[0025] The neck 26 includes a ramped filling surface 30 that forms part of the neck 26 having an outer edge 32 at the opening 28 that is angularly offset from an inner edge 34 of the ramped filling surface 30 at the first end wall 14. An inner fill wall 36 extends inward from the first end wall 14 and the pair of side walls 18 and 20 into the cell culture chamber 24. The inner fill wall 36 has a canted inner surface 38 that has an outer edge 40 at the first end wall 14 that is angularly offset from an inner terminal edge 42 of the canted inner surface 38. In some embodiments, the canted inner surface 38 is an extension of the ramped filling surface 30 of the neck 26 wherein both the canted inner surface 38 and the ramped filling surface 30 intersect and extend at a same angle θ to horizontal (e.g., 10 degrees or more, 15 degrees or more, 20 degrees or more, between 10 and 20 degrees, between 15 and 20 degrees) with the cell culture vessel 10 in a horizontal incubation position, as illustrated in FIGS. 1 and 2.

[0026] The cell culture vessel 10 further includes a lid 44 that covers a viewing face 46 of the vessel body 12. The lid 44 includes a lip 47 that engages an edge 48 of the first and second end walls 14, 16 and side walls 18, 20 to provide an interlocked connection. The lid 44 may be formed of a clear material, such as a clear or translucent plastic such that the contents within the cell culture chamber 24 can be visually inspected.

[0027] The cell culture vessel 10 may include a cell culture substrate 50 positioned along an opposite cell culture face 52 vessel body 12. Cell culture substrate 50 may comprise a surface 54 that includes a plurality of microcavities 56 sized and shaped to receive at least one cell or spheroid therein. Accordingly, cell culture surface 54 is a cell culturing area that is configured to facilitate growth and development of the cells within cell culture chamber 24.

[0028] Referring to FIG. 2, the vessel body 12 forms a frame 58 at the cell culture face 52 of the vessel body 12 that is sized and configured to receive the cell culture substrate 50 (FIG. 1). In particular, the frame 58 may be formed at one edge 61 thereof between a ledge 62 extending outwardly from the inner fill wall 36 away from the cell culture chamber 24 and the inner terminal edge 42 of the inner fill wall 36 offset underneath the canted inner surface 38. An opposite edge 63 of the frame 58 may be formed between another ledge 64 extending

outwardly from the second end wall 16 away from the cell culture chamber 24 and an edge 66 of the second end wall 16. Sides of the frame 58 may also be formed along the side walls 18 and 20 including ledges 67 and 69 in a similar fashion (see FIG. 3). The cell culture substrate 50 can be held within the frame 58 during an incubation process. As can be seen, an edge 68 of the cell culture substrate 50 may be offset underneath the inner terminal edge 42 of the inner fill wall 36.

[0029] Referring particularly to FIG. 3, a diverter 70 extends along the canted inner surface 38. The diverter 70 extends at least partially transverse to the fill direction between the pair of side walls 18 and 20. The diverter 70 is formed as a partial height wall in that a height H_1 of the diverter is less than (e.g., 50 percent or less, 60 percent or less, 70 percent or less, 80 percent or less, 90 percent or less) a height H_2 of the cell culture chamber 24. The diverter 70 has opposite ends 72 and 74 that terminate inside the pair of side walls 18 and 20 to form passageways 76 and 78 through which liquid medium flows by the diverter 70 during filling and emptying operations.

[0030] The diverter 70 extends widthwise along the canted inner surface 38. In some embodiments, the diverter 70 is formed integrally with the inner fill wall 36 as a single, monolithic piece of the inner fill wall 36. In other embodiments, the diverter 70 may be formed separately and connected to the canted inner surface 38. The diverter 70 may be in contact and contiguous with the canted inner surface 38 along its entire length in order to direct the liquid medium through the passageways 76 and 78. While the diverter 70 is illustrated as being rectangular in cross sectional shape, it may be formed having other shapes, such as curved, triangular, etc. Also, the diverter 70 may extend in other directions across the width of the canted inner surface 38. For example, the diverter 70 may extend at an oblique angle to the fill direction.

[0031] The diverter 70 may extend only partially along a width W of the canted inner surface 38 between ends 72 and 74 to provide the passageways 76 and 78. For example, the diverter 70 may extend no more than 99 percent, such as no more than 95 percent, such as no more than 90 percent, such as no more than 85 percent, such as no more than 80 percent, such as no more than 75 percent, such as no more than 70 percent, such as no more than 65 percent, such as no more than 60 percent, such as no more than 55 percent, such as no more than 50 percent, such

as no more than 45 percent, such as no more than 40 percent, such as no more than 35 percent, such as no more than 30 percent of the width W. in some embodiments, the diverter 70 extends at least about 50 percent and no more than about 90 percent of the width W, such as about 80 percent of the width W.

[0032] Referring to FIG. 4, a protective carrier 80 is a rigid plate that may protect the gas permeable microcavities of the cell culture substrate 50 during shipping and that may also act as a protective carrier for the cell culture vessel 10 during use for cell culture. The protective carrier 80 may be comprised of a rigid plate 82 that is slightly larger than a footprint of the cell culture vessel 10. An outer lip 84 may extend around a perimeter of the plate 82 and be sized and configured to extend about the ledges 62, 64, 67, 69 of the inner fill wall 36 and second end wall 16 and the side walls 18 and 20. At corners of the protective carrier 80 are standoff ribs 86. The standoff ribs 86 extend along both the plate 82 and the outer lip 84 to provide clearance and air gaps between the protective carrier 80 and the cell culture substrate 50. Referring to FIG. 5, a stacking groove or rib 88 may be provided along an underside of the plate 82. The stacking groove or rib 88 is provided to mate with a corresponding stacking rib or groove 90 (FIG. 1) provided on the lid 44.

[0033] Referring now to FIG. 6, gas permeability is a property that contributes to the 3D cell culture environment. By allowing for gas permeability within microcavities 100 of the cell culture vessel, cell culture growth media may need to be changed out less frequently and cell growth may be encouraged. Microcavity vessels are unique in their geometry and formation in that they are formed from the gas permeable substrate 50 with micron-scale wells 100, also referred to as a microcavities. Such microcavity vessels enjoy gas permeability due to the thickness of the microcavity substrate 50, wherein gas permeability occurs because the microcavity substrate may be formed from a very thin polystyrene material of manufacture, which has a thickness of about 28 micrometers to about 72 micrometers. Though gas permeability may be an asset for culturing cell aggregates, the thinness of the microcavity substrate material makes microcavity cell culture vessels 10 susceptible to damage during shipping and use.

[0034] Each microcavity 100 may include an inner cavity with a rounded bottom 102 that is non-adherent to cells. Thus, microcavity vessels as described herein are cell culture devices

facilitate 3D cell culture by allowing cells seeded into the microcavities to self-assemble or attach to one another to form a spheroid in each microcavity. Microcavities may be shallow and permit cell culture medium to cover the spheroids, organoids, or 3D cell aggregates in all cavities at once to make manual handling easy.

[0035] In an embodiment, a top plane 104 of the microcavities 100 may be recessed to a location close to a bottom of the side walls 18 and 20 (FIG. 3). Individual microcavities may hold a small volume of medium. The individual microcavities may have any suitable dimensions. For example, the diameter or width of individual microcavities may be in a range of about 500 microns to about 5 millimeters. The depth of individual microcavities may be in a range of about 500 microns to about 6 millimeters. In some embodiments, a depth of the individual microcavities may be about 500 microns to about 650 microns. In some embodiments, a depth of the individual microcavities may be about 1.6 millimeters. An excess of culture medium may be added to the microcavity vessel so that the spheroids, organoids, or 3D cell aggregates do not need to rely only on the small amount of medium in the individual microcavities.

[0036] In some embodiments, the microcavity substrate 50 may have a cross-sectional shape that is undulating or is a shape approximating a sine wave. In such embodiments, the bottom of the microcavity well 100 is rounded (e.g., hemispherically round), the side walls increase in diameter from the bottom of the well to the top and the boundary or barrier between wells is rounded. As such, the top of the microcavity wells does not terminate at a right angle. In some embodiments, the width of the well is greater than the width of the barrier between adjacent wells. Such an embodiment permits a greater number of wells within a given area of culture surface.

[0037] In some embodiments, the plurality of microcavities are arranged in a hexagonal close-pack pattern. In some embodiments, each microcavity comprises a rounded bottom. In some embodiments, each microcavity is configured such that cells cultured in the microcavity vessel form three-dimensional (3D) cell aggregates. In some embodiments, an interior surface of the microcavity substrate is non-adherent to cells. In some embodiments, the interior surface of the microcavity substrate comprises a cell non-adherent surface coating comprising perfluorinated polymers, olefins, lipids, agarose, non-ionic hydrogels, polyethers, polyols,

polymers that inhibit cell attachment, or a combination thereof. In some embodiments, the cell non-adherent surface coating comprises an ultra-low attachment (ULA) surface coating.

[0038] The microcavity substrate, microcavity vessel, and diverter may be formed from the same material or a similar material. In some embodiments, the microcavity substrate may be molded or formed separately from the rest of the microcavity vessel and bonded subsequently through thermal-bonding, ultrasonic welding, or any other method of plastic joining. The material of construction for the microcavity vessel, microcavity substrate, and/or baffle may comprise a "plastic" polymer, co-polymer, or polymer blend. Nonlimiting examples include silicone rubber, polystyrene, polypropylene, polyethylene, polyethylene terephthalate, polymethylpentene, polycarbonate, polymethyl methacrylate, styrene-ethylene-butadiene-styrene, other such polymers, or a combination thereof. In some embodiments, the microcavity substrate is formed from polydimethylsiloxane (PDMS), polymethylpentene, (poly)4-methylpentene (PMP), polyethylene (PE), polystyrene (PS), polypropylene, polyethylene terephthalate, polycarbonate, polymethyl methacrylate, styrene-ethylene-butadiene-styrene, a silicone rubber or copolymer, ethylene vinyl acetate, polysulfone, polytetrafluoroethylene, poly(styrene-butadiene-styrene), or a combination thereof. Any suitable construction method may be used to form the microcavity substrate, microcavity vessel, and diverter, such as nonlimiting examples including injection molding, thermoforming, 3D printing, or any other method suitable for forming a plastic part.

[0039] In some embodiments, the protective carrier 80 is formed from a polymer, metal, or glass. In some embodiments, the polymer comprises polystyrene, polypropylene, polyethylene, polyethylene terephthalate, polymethylpentene, polycarbonate, polymethyl methacrylate, styrene-ethylene-butadiene-styrene, other such polymers, or a combination thereof. In some embodiments, the metal comprises aluminum, stainless steel, zinc, or a combination thereof. In some embodiments, the glass comprises a borosilicate glass. In some embodiments, the protective carrier is formed from a recyclable material. In some embodiments, the protective carrier is formed from a biodegradable material. In some embodiments, the protective carrier is opaque. In some embodiments, the protective carrier is translucent.

[0040] In use, referring to FIGS. 1-3, a plurality of cells may be deposited within the cell culture vessel 10 such that cell culture chamber 24 is operable to house the cells within the

plurality of microcavities 100 of a cell culture surface 110 of the cell culture substrate 50. First, it may be desirable to pre-wet the microcavity surfaces. A liquid medium can be provided through the neck 26, along the ramped filling surface 30 and then over the canted inner surface 38 of the inner fill wall 36. The liquid medium then can impinge upon the diverter 70, which diverts the liquid medium along its length toward the passageways 76 and 78 such that the flow is slowed as it approaches the cell culture surface 110. The liquid medium can be allowed to enter the microcavities 100 without assistance. Should liquid not enter the microcavities, trapped air may appear as opaque areas on the cell culture surface 110. If there are microcavities 100 with trapped air, cells in suspension after the filling process may not settle into those microcavities 100. With the plurality of cells received along the cell culture substrate 50 of the cell culture surface 110, the development of the cells is facilitated by exposing the cell culture surface 110 to various nutrients and growth fluids during a liquid culture medium filling operation.

[0041] The microcavities may be deep enough to permit medium exchange with gentle handling, but not too deep so as to be difficult to recover the spheroids when desired. During medium exchanges, the cell culture vessel 10 may remain in the incubation position to inhibit the loss of spheroids. The canted inner surface 38 and the diverter 70 can facilitate gentle flow of medium out of the cell culture vessel 10.

[0042] During medium exchange step, lifting the second end wall 16 three to four degrees can push the liquid medium toward the diverter 70 and canted inner surface 38 to enable full liquid removal, and can slow the flow of new liquid addition into the cell culture vessel 10 to reduce sloshing. It may be desirable for the angle of lift of the second end wall 16 to not exceed 10 degrees when aspirating and dispensing liquids to inhibit liquid from sloshing and wetting out the cap 27. Referring to FIG. 7, the protective carrier 80 or item of similar height (e.g., between 6 and 7 mm) may be used to elevate the second end wall 16 during medium exchange steps. In particular, the ledges 67 and 69 may include stacking indents 114 that can serve as a stop feature that catches the outer lip 84 of the protective carrier 80 and places the cell culture vessel at the proper angle. To remove the spent medium, a pipette tip may be placed up against the diverter 70 to aspirate out the medium. To replace medium, the pipette tip can be placed against the diverter 70 to slowly add fresh medium into the cell culture vessel 10. Once the

medium exchange is complete, the protective carrier 80 can be removed and the cell culture vessel 10 can be placed in the flat incubation position.

[0043] The above-described cell culture vessels including diverters located within the respective cell culture chambers of microcavity cell culture vessels, and systems for microcavity cell culture including diverts located within the respective cell culture chambers of microcavity cell culture vessels reduce the amount of movement of liquid culture medium within a cell culture chamber. The diverters can also improve filling by controlling liquid flow and reducing trapped air within the microcavities. The diverters can be formed (e.g., molded) along with the vessel body such that the diverters and vessel bodies are formed as a single, monolithic piece of material. The diverters can also provide a location against which a tip of a pipette can be placed and used to remove and dispense liquid medium from and into the cell culture chamber which can improve fill effectiveness and repeatability between fill and empty operations.

[0044] Embodiments can be described with reference to the following numbered clauses, with preferred features laid out in the dependent clauses:

[0045] Clause 1: A cell culture vessel comprising: a vessel body comprising: a first end wall; a second end wall opposite the first end wall; a pair of side walls that extend between the first end wall and the second end wall, the first end wall, second end wall and pair of side walls defining a perimeter of a cell culture chamber; an inner fill wall that extends inward from the first end wall and the pair of sidewalls into the cell culture chamber, the inner fill wall having a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface; and a diverter that extends along the canted inner surface at least partially transverse to a fill direction between the pair of sidewalls, the diverter providing passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation.

[0046] Clause 2: The cell culture vessel of clause 1, wherein an entire length of the diverter is contiguous with the canted inner surface of the inner fill wall.

[0047] Clause 3: The cell culture vessel of clause 2, wherein the diverter is formed as an integral, monolithic part of the inner fill wall.

[0048] Clause 4: The cell culture vessel of any of clauses 1-3, wherein the diverter has opposite ends that terminate inside the pair of side walls to provide the passageways.

[0049] Clause 5: The cell culture vessel of any of clauses 1-4 further comprising a lid sized and configured to cover a viewing face of the vessel body.

[0050] Clause 6: The cell culture vessel of any of clauses 1-5 further comprising a protective carrier comprising a plate that is sized and configured to cover a cell culture face of the vessel body.

[0051] Clause 7: The cell culture vessel of clause 6, wherein the protective carrier comprises a lip extending about a perimeter of the plate that is sized and configured to extend along the first end wall, the end side wall and the pair of side walls.

[0052] Clause 8: The cell culture vessel of clause 6 or 7, wherein the protective carrier comprises a standoff rib that is arranged and configured to be positioned between the plate and the vessel body to provide space therebetween.

[0053] Clause 9: The cell culture vessel of any of clauses 1-8, wherein the diverter extends at least about 50 percent and no more than about 90 percent of a width of the canted inner surface between ends of the diverter.

[0054] Clause 10: The cell culture vessel of any of clauses 1-9, wherein the vessel body further comprises a neck that extends outward from the first end wall in the fill direction to an opening, the neck including a ramped filling surface having an outer edge at the opening that is angularly offset from an inner edge of the ramped filling surface at the first end wall.

[0055] Clause 11: The cell culture vessel of clause 10, wherein the ramped filling surface and the canted inner surface intersect.

[0056] Clause 12: A method of forming a cell culture vessel, the method comprising: forming a vessel body as a single, monolithic part such that the vessel body comprises: a first end wall; a second end wall opposite the first end wall; a pair of side walls that extend between the first end wall and the second end wall, the first end wall, second end wall and pair of side walls defining a perimeter of a cell culture chamber; an inner fill wall that extends inward from

the first end wall and the pair of sidewalls into the cell culture chamber, the inner fill wall having a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface; and a diverter that extends along the canted inner surface at least partially transverse to the fill direction between the pair of sidewalls, the diverter providing passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation; and forming a protective carrier separately from the vessel body, the protective carrier comprising a plate that is sized and configured to cover a cell culture face of the vessel body.

[0057] Clause 13: The method of clause 12, wherein an entire length of the diverter is contiguous with the canted inner surface of the inner fill wall.

[0058] Clause 14: The method of clause 12 or 13, wherein the diverter has opposite ends that terminate inside the pair of side walls to provide the passageways.

[0059] Clause 15: The method of any of clauses 12-14 further comprising forming a lid that is configured to cover a viewing face of the vessel body.

[0060] Clause 16: The method of any of clauses 12-15, wherein the protective carrier comprises a lip extending about a perimeter of the plate that is sized and configured to extend along the first end wall, the end side wall and the pair of side walls.

[0061] Clause 17: The method of any of clauses 12-16, wherein the protective carrier comprises a standoff rib that is arranged and configured to be positioned between the plate and the vessel body to provide space therebetween.

[0062] Clause 18: The method of any of clauses 12-17, wherein the diverter extends at least about 50 percent and no more than about 90 percent of a width of the canted inner surface between ends of the diverter.

[0063] Clause 19: The method of any of clauses 12-18, wherein the vessel body further comprises a neck that extends outward from the first end wall in the fill direction to an opening, the neck including a ramped filling surface having an outer edge at the opening that is angularly offset from an inner edge of the ramped filling surface at the first end wall.

[0064] Clause 20: The method of clause 19, wherein the ramped filling surface and the canted inner surface intersect.

[0065] It will be apparent to those skilled in the art that various modifications and variations can be made to the embodiments described herein without departing from the spirit and scope of the claimed subject matter. Thus, it is intended that the specification cover the modifications and variations of the various embodiments described herein provided such modification and variations come within the scope of the appended claims and their equivalents.

[0066] What is claimed is:

CLAIMS

1. A cell culture vessel comprising:

a vessel body comprising:

a first end wall;

a second end wall opposite the first end wall;

a pair of side walls that extend between the first end wall and the second end wall, the first end wall, second end wall and pair of side walls defining a perimeter of a cell culture chamber;

an inner fill wall that extends inward from the first end wall and the pair of sidewalls into the cell culture chamber, the inner fill wall having a canted inner surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface; and

a diverter that extends along the canted inner surface at least partially transverse to a fill direction between the pair of sidewalls, the diverter providing passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation.

2. The cell culture vessel of claim 1, wherein an entire length of the diverter is contiguous with the canted inner surface of the inner fill wall.

3. The cell culture vessel of claim 2, wherein the diverter is formed as an integral, monolithic part of the inner fill wall.

4. The cell culture vessel of claim 1, wherein the diverter has opposite ends that terminate inside the pair of side walls to provide the passageways.

5. The cell culture vessel of claim 1 further comprising a lid sized and configured to cover a viewing face of the vessel body.

6. The cell culture vessel of claim 1 further comprising a protective carrier comprising a plate that is sized and configured to cover a cell culture face of the vessel body.

7. The cell culture vessel of claim 6, wherein the protective carrier comprises a lip extending about a perimeter of the plate that is sized and configured to extend along the first end wall, the end side wall and the pair of side walls.

8. The cell culture vessel of claim 6, wherein the protective carrier comprises a standoff rib that is arranged and configured to be positioned between the plate and the vessel body to provide space therebetween.

9. The cell culture vessel of claim 1, wherein the diverter extends at least about 50 percent and no more than about 90 percent of a width of the canted inner surface between ends of the diverter.

10. The cell culture vessel of claim 1, wherein the vessel body further comprises a neck that extends outward from the first end wall in the fill direction to an opening, the neck including a ramped filling surface having an outer edge at the opening that is angularly offset from an inner edge of the ramped filling surface at the first end wall.

11. The cell culture vessel of claim 10, wherein the ramped filling surface and the canted inner surface intersect.

12. A method of forming a cell culture vessel, the method comprising:

forming a vessel body as a single, monolithic part such that the vessel body comprises:

a first end wall;

a second end wall opposite the first end wall;

a pair of side walls that extend between the first end wall and the second end wall, the first end wall, second end wall and pair of side walls defining a perimeter of a cell culture chamber;

an inner fill wall that extends inward from the first end wall and the pair of sidewalls into the cell culture chamber, the inner fill wall having a canted inner

surface having an outer edge at the first end wall that is angularly offset from an inner terminal edge of the canted inner surface; and

a diverter that extends along the canted inner surface at least partially transverse to the fill direction between the pair of sidewalls, the diverter providing passageways between the diverter and the pair of side walls through which liquid flows by the diverter during a filling operation; and

forming a protective carrier separately from the vessel body, the protective carrier comprising a plate that is sized and configured to cover a cell culture face of the vessel body.

13. The method of claim 12, wherein an entire length of the diverter is contiguous with the canted inner surface of the inner fill wall.

14. The method of claim 12, wherein the diverter has opposite ends that terminate inside the pair of side walls to provide the passageways.

15. The method of claim 12 further comprising forming a lid that is configured to cover a viewing face of the vessel body.

16. The method of claim 12, wherein the protective carrier comprises a lip extending about a perimeter of the plate that is sized and configured to extend along the first end wall, the end side wall and the pair of side walls.

17. The method of claim 12, wherein the protective carrier comprises a standoff rib that is arranged and configured to be positioned between the plate and the vessel body to provide space therebetween.

18. The method of claim 12, wherein the diverter extends at least about 50 percent and no more than about 90 percent of a width of the canted inner surface between ends of the diverter.

19. The method of claim 12, wherein the vessel body further comprises a neck that

extends outward from the first end wall in the fill direction to an opening, the neck including a ramped filling surface having an outer edge at the opening that is angularly offset from an inner edge of the ramped filling surface at the first end wall.

20. The method of claim 19, wherein the ramped filling surface and the canted inner surface intersect.

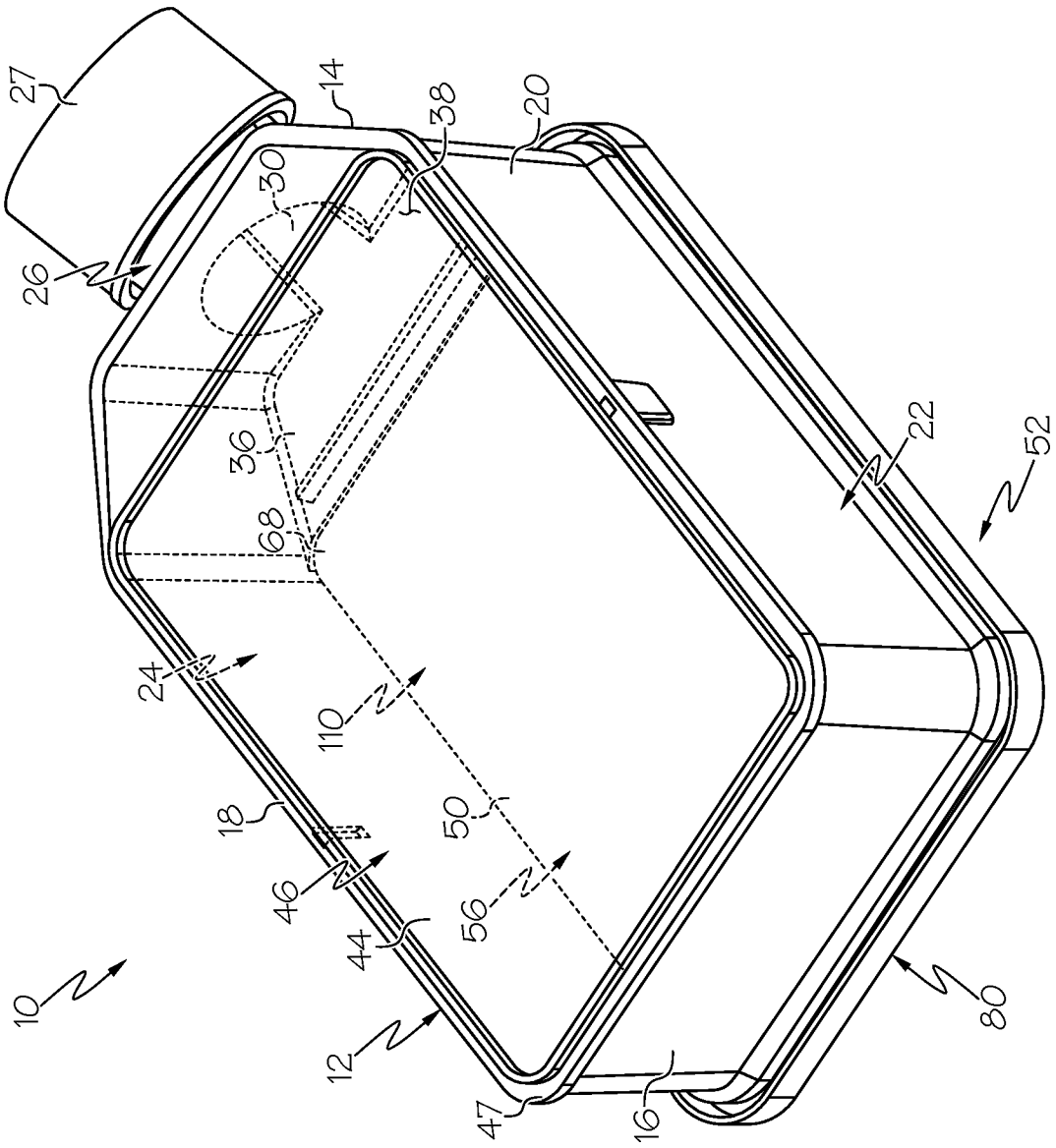

FIG. 1

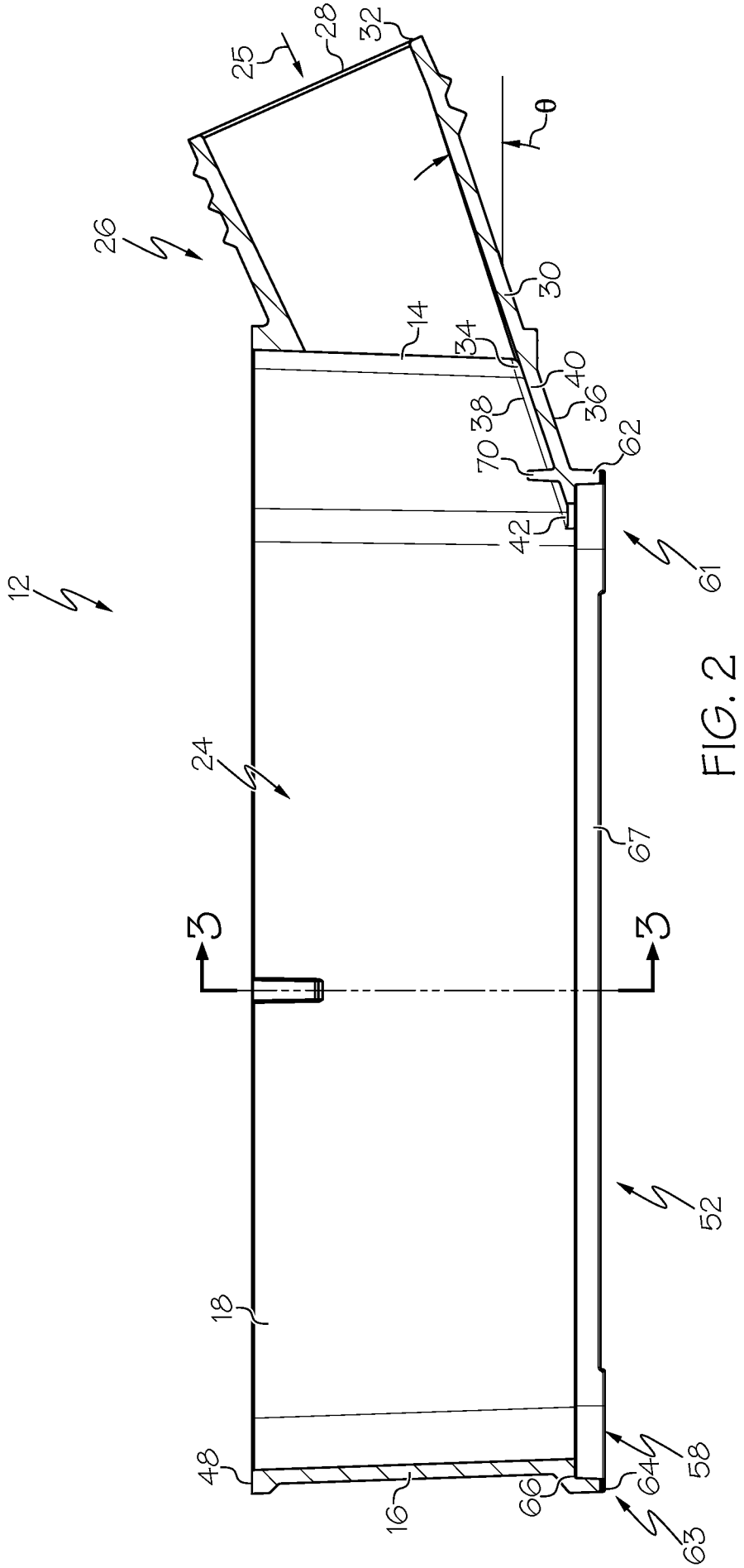
25
28
32
θ
26
30
14
34
40
36
38
70
62
42
61
12
24
67
3
3
52
18
48
16
66
64
58
63

FIG. 2

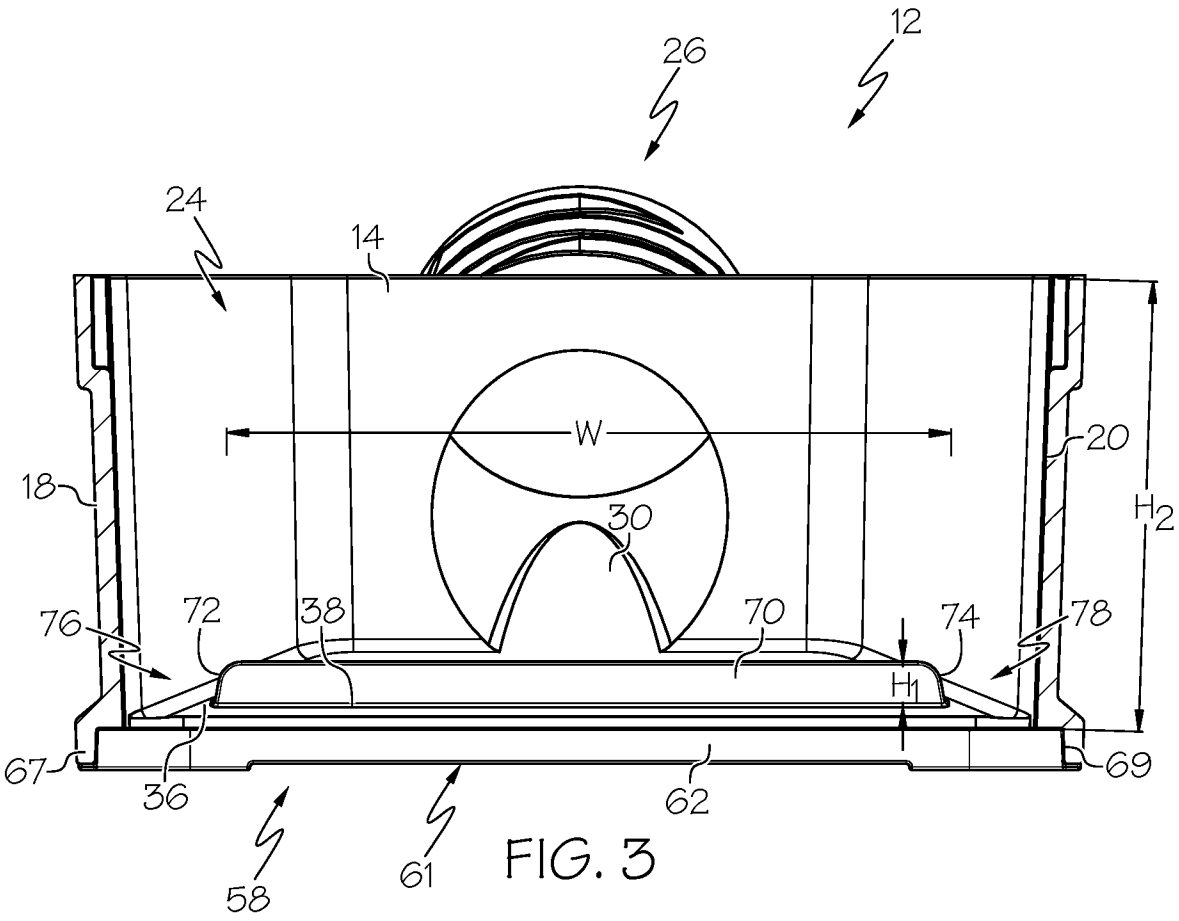

FIG. 3

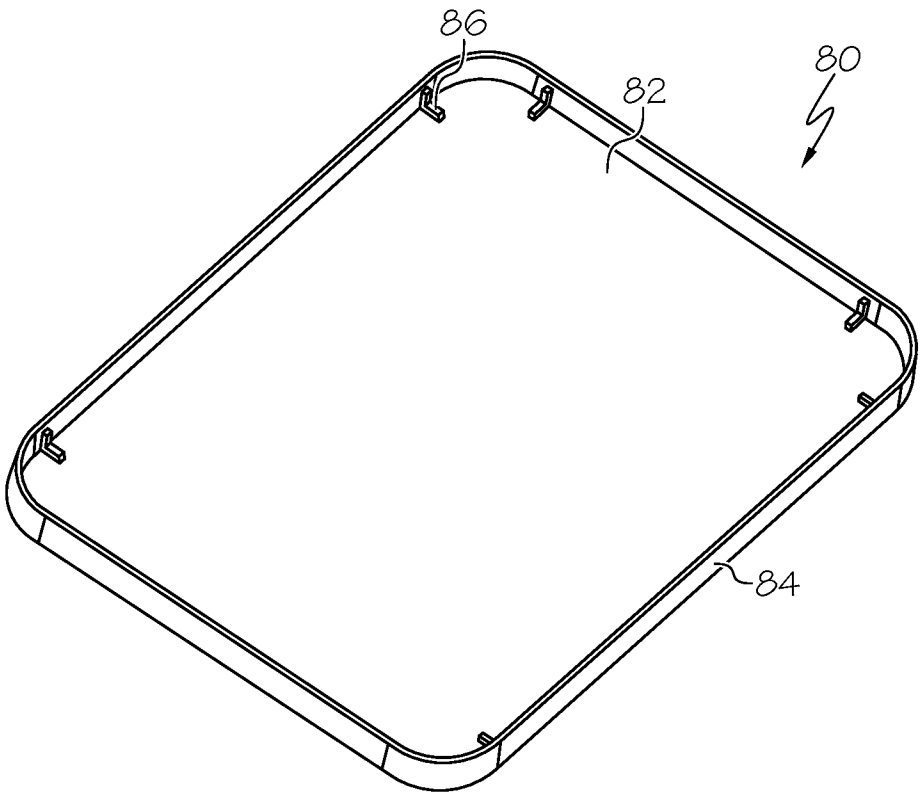

FIG. 4

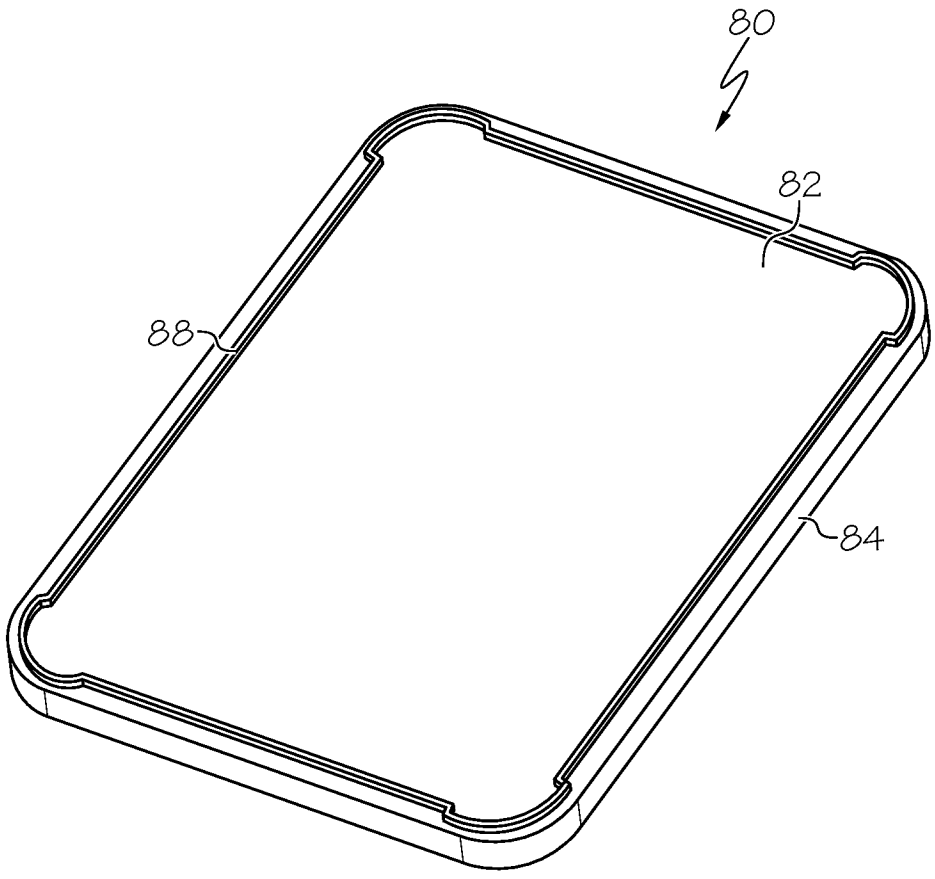

FIG. 5

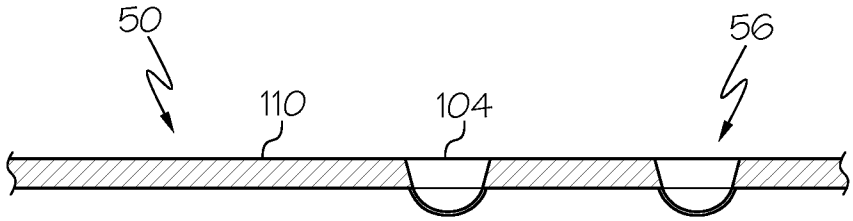

FIG. 6

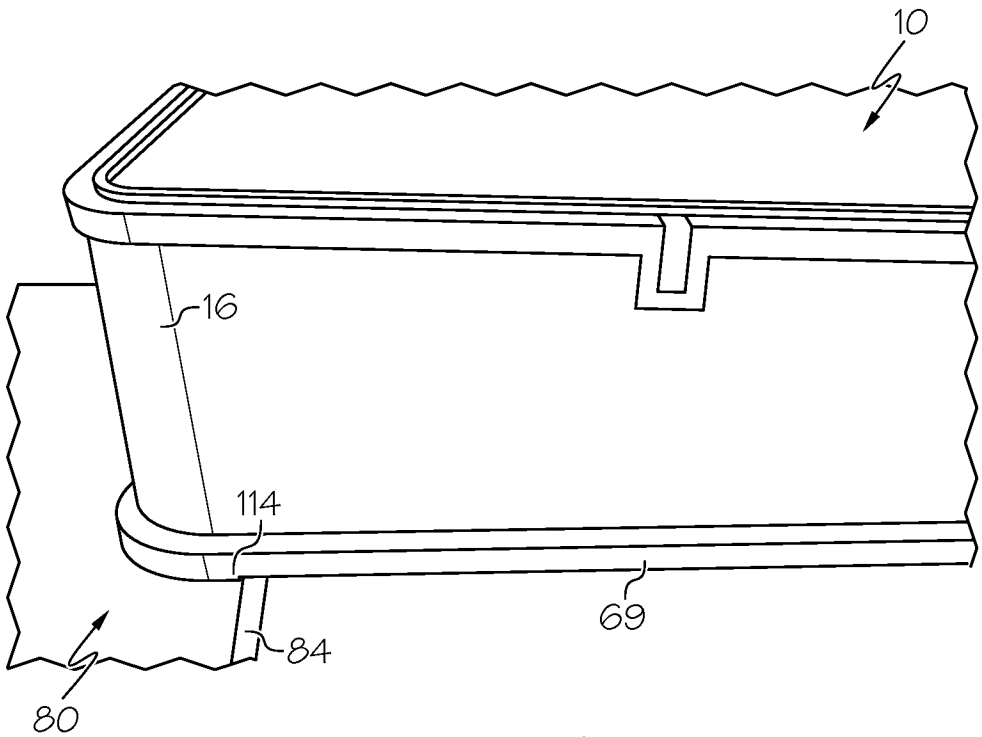

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/033095

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/24 C12M1/00 C12N5/00
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 C12N

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	US 2008/003671 A1 (MARTIN GREGORY ROGER [US]) 3 January 2008 (2008-01-03) paragraphs [0015], [0016]; figures 1,2	1-20
X	WO 2019/014627 A1 (CORNING INC [US]) 17 January 2019 (2019-01-17) paragraphs [0005], [0033] - [0038], [0040], [0041], [0045], [0047], [0048]; figures 1-7	1-20
A	EP 1 405 904 A1 (BECTON DICKINSON CO [US]) 7 April 2004 (2004-04-07) paragraphs [0013] - [0019]; figures 1-6	1-20

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2023	08/01/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pantelidis, D

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/033095

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2023/009772 A1 (CORNING INC [US]) 2 February 2023 (2023-02-02) paragraphs [0051] - [0054], [0059], [0060], [0062], [0063], [0067], [0075] - [0081]; figures 1-6,11	1,2,4-6, 9-15, 18-20

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/033095

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008003671 A1	03-01-2008	NONE	
WO 2019014627 A1	17-01-2019	CN 111094534 A	01-05-2020
		EP 3652289 A1	20-05-2020
		JP 2020526217 A	31-08-2020
		US 2020181552 A1	11-06-2020
		WO 2019014627 A1	17-01-2019
EP 1405904 A1	07-04-2004	AT E384116 T1	15-02-2008
		DE 60224673 T2	15-01-2009
		EP 1405904 A1	07-04-2004
		ES 2295275 T3	16-04-2008
		JP 2004141072 A	20-05-2004
		US 2004106191 A1	03-06-2004
WO 2023009772 A1	02-02-2023	NONE	