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(54) **Title:** REINFORCED GAS-PERMEABLE MEMBRANE AND REINFORCED GAS-PERMEABLE WELL UTILIZING SAME

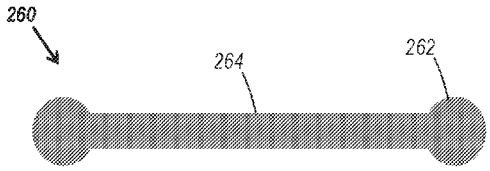


FIG. 2A

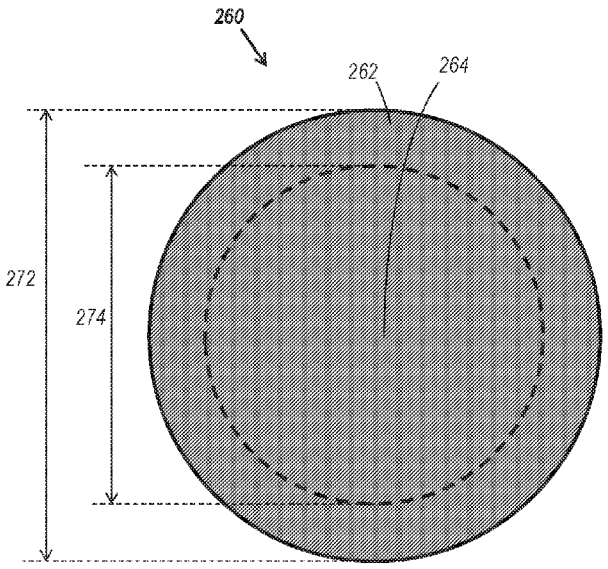


FIG. 2B

(57) **Abstract:** A gas-permeable well for use with a bioreactor may include a reinforced gas-permeable membrane. The gas-permeable membrane may include an integrated reinforcing structure such as a stainless steel mesh or perforated screen, and may include an integrated gasket or O-ring, or other regions of increased thickness. A sensor brought into close proximity to the reinforced gas-permeable membrane may take an indirect measurement of a condition of process medium on the other side of the gas-permeable membrane, such as a measurement of dissolved oxygen or carbon dioxide.

[Continued on next page]

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REINFORCED GAS-PERMEABLE MEMBRANE AND REINFORCED GAS-PERMEABLE WELL UTILIZING SAME

BACKGROUND

Technical Field

[0001] Embodiments described herein relate to sensor systems and associated components, and in particular are directed to gas-permeable wells into which sensors can be inserted, and gas-permeable reinforced membranes for use with the gas-permeable wells.

Description of the Related Art

[0002] In biopharmaceutical manufacturing processes, gas-permeable membranes can be used to allow indirect measurements to be taken of process media such as cell culture media within bioreactors, allowing measurements of the media using sensors such as dissolved oxygen (DO) sensors or carbon dioxide (CO_2) sensors without breaching the sterility of the bioreactor. However, when integrated into a bioreactor, these gas-permeable membranes represent a possible point of failure, and limit the pressure differential or other conditions to which the bioreactor can be exposed.

SUMMARY

[0003] In one aspect, a reinforced gas-permeable well for use with a bioreactor is provided, the reinforced gas-permeable well including a reinforced gas-permeable membrane, the reinforced gas-permeable membrane including a gas-permeable membrane, and a gas-impermeable reinforcing structure at least partially embedded within the gas-permeable membrane, the gas-impermeable reinforcing structure including at least one aperture extending therethrough, and a retaining structure configured to retain the gas-permeable membrane.

[0004] The gas-impermeable reinforcing structure can include an interwoven mesh. The gas-impermeable reinforcing structure can include a perforated screen. The gas-impermeable reinforcing structure can include a plurality of reinforcing elements extending across a central portion of the reinforced gas-permeable membrane.

[0005] The gas-impermeable reinforcing structure can further include a ring disposed at or near the periphery of the gas-impermeable reinforcing structure. The ring

can be fully embedded within the gas-permeable membrane. The ring can be partially embedded within the gas-permeable membrane.

[0006] The gas-impermeable reinforcing structure can include stainless steel. The gas-permeable membrane can include silicone. The reinforced gas-permeable well can additionally include a sealing structure configured to allow the reinforced gas-permeable well to be installed in a wall of a bioreactor.

[0007] The reinforced gas-permeable well can additionally include a channel configured to receive a sensor therein, to allow the sensor to be positioned adjacent the reinforced gas-permeable membrane. The retaining structure can include at least one flange extending inwardly from the interior wall of the channel and configured to inhibit translation of the gas-permeable membrane in at least one direction along the channel. The reinforced gas permeable membrane can be oriented at an angle to an axis of the channel.

[0008] The reinforced gas-permeable well can be configured to be installed such that the reinforced gas-permeable membrane is positioned generally coplanar with a wall of the bioreactor. The reinforced gas-permeable membrane can be oriented at an angle to the sealing structure.

[0009] In one aspect, a bioreactor can include a reinforced gas-permeable well as described herein. The bioreactor can be a single-use bioreactor having flexible walls. The bioreactor can include an internal volume of greater than 2 liters or greater than 30 liters. The bioreactor can include a reusable bioreactor having rigid walls.

[0010] In another aspect, a reinforced gas-permeable well for use with a bioreactor is provided, the reinforced gas-permeable well including a reinforced gas-permeable membrane, the reinforced gas-permeable membrane including a reinforcing structure which decreases the permeability of the gas-permeable membrane in an area adjacent the reinforcing structure, and a retaining structure configured to retain the reinforced gas-permeable membrane.

[0011] The reinforcing structure can include at least one region of increased thickness. The at least one region of increased thickness can extend around the periphery of the reinforced gas-permeable membrane. The at least one region of increased thickness can include an integrated O-ring or gasket.

[0012] The reinforcing structure can include a gas-impermeable reinforcing structure at least partially embedded within the gas-permeable membrane and including at

least one aperture extending therethrough. The gas-impermeable reinforcing structure can include an interwoven mesh. The gas-impermeable reinforcing structure can include a perforated screen. The gas-impermeable reinforcing structure can include a plurality of reinforcing elements extending across a central portion of the reinforced gas-permeable membrane.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1A** is a side cross-sectional view schematically illustrating a bioreactor having an integrated gas-permeable well for sensor access.

[0014] **FIG. 1B** is a side cross-sectional view schematically illustrating the bioreactor of **FIG. 1A**, with a dissolved oxygen sensor inserted into the gas-permeable well.

[0015] **FIGS. 2A** and **2B** are cross-sectional and top plan views, respectively, of a gas-permeable membrane design with a cross-sectional shape that provides additional reinforcement.

[0016] **FIG. 3** is a cross-sectional view of another embodiment of a gas-permeable membrane design with an alternate cross-sectional shape that provides additional reinforcement.

[0017] **FIGS. 4A** and **4B** are cross-sectional and top plan views, respectively, of a gas-permeable membrane design with a plurality of integrated reinforcing members.

[0018] **FIG. 5A** is a cross-sectional view of a reinforced membrane including the peripheral reinforcing structure of **FIG. 2A** and the reinforcing mesh of **FIG. 4**.

[0019] **FIG. 5B** is a cross-sectional view of a reinforced membrane including the peripheral reinforcing structure of **FIG. 3** and the reinforcing mesh of **FIG. 4**.

[0020] **FIG. 6A** is a cross-sectional view of a reinforced membrane including a discrete peripheral reinforcing structure internal to the reinforced membrane. **FIG. 6B** is a top plan view of the reinforced membrane of **FIG. 6A**.

[0021] **FIG. 7** is a cross-sectional view of a reinforced membrane including a discrete peripheral reinforcing structure at least partially external to the reinforced membrane.

[0022] **FIG. 8** is a side cross-section of a reinforced gas-permeable well including a reinforced gas-permeable membrane.

[0023] **FIG. 9** is a cross-sectional view schematically illustrating an embodiment of a bioreactor having a low-profile reinforced gas-permeable well installed therein, where the reinforced gas-permeable membrane substantially flush with the wall of the bioreactor.

DETAILED DESCRIPTION

[0024] By using a gas-permeable membrane, indirect measurements of a process medium within a bioreactor or other container can be made. A dissolved oxygen (DO) sensor, CO_2 sensor, or similar sensor can be configured to provide a signal indicative of the parameter of the medium to be measured. For example, a DO sensor can generate a signal, depending on its sensing mechanism, where the signal indicative of the amount of oxygen in a process medium to which the sensor is exposed. However, indirect measurement of the medium is also possible, without direct contact between the sensor and the medium to be measured.

[0025] Due to diffusion of gas through a gas-permeable membrane, an accurate measurement of the oxygen content or other parameter of the medium can be obtained even when a sensor is not brought into direct contact with the medium. A sensing surface of a sensor such as a DO sensor can be brought adjacent or into contact with a gas-permeable membrane when a measurement is to be taken. The measurement taken will be indicative of conditions within the process medium despite the lack of actual contact between the process medium and the sensing surface of the sensor.

[0026] In some embodiments, the walls of a bioreactor may be rigid, and the bioreactor may be reusable, with sterilization occurring between uses of the bioreactor. In such embodiments, a sensor may be directly installed into or through a wall of a rigid bioreactor. The rigidity of the bioreactor wall can provide mechanical support for the installed sensor, which can remain sealed in place during sterilization of the bioreactor and subsequent use of the bioreactor.

[0027] In other embodiments, however, the bioreactor can include a bag or other flexible structure, which is filled with and retains the process medium. Flexible bioreactor bags may in some embodiments be single-use bioreactors, which can be disposed of rather than being re-sterilized and re-used. Such a flexible bioreactor may itself be seated within a rigid retaining vessel, but as the walls of the actual containment vessel retaining the process medium are flexible, sensors and other components can be

configured to be insertable through ports in the bioreactor wall or may be installed within the bioreactor prior to sterilization and/or filling of the bioreactor with sterile components or media.

[0028] In other embodiments, certain sensors and other components may interact with a bioreactor which has already been sterilized or filled with process media. For example, sensors may be configured to be insertable into gas-permeable wells in the flexible bioreactor wall, such that the sterility and integrity of the flexible bioreactor are not compromised by the insertion of the sensor. In some embodiments, a gas-permeable well may be built into the flexible bioreactor bag or otherwise installed prior to the assembled bioreactor being sterilized or filled with a process medium. This allows a sensor to be freely inserted into the well and removed during the bioreactor process as needed, and does not require that the sensor be coupled to a single bioreactor for the duration of a process in that bioreactor. The freedom to insert and remove the sensor as desired also allows validation of the measurements, and reuse of a single sensor with multiple single-use bioreactors.

[0029] For indirect measurements, such as the measurement of dissolved oxygen through a gas-permeable membrane, which itself is a sterile barrier, the sensor itself need not be installed in the bioreactor during sterilization, shipment, and a setup process at the end-user's site. Thus, the sensor need not be installed during sterilization or prior to the deployment of the bioreactor bag. However, to maintain the sterility of the bioreactor bag, the gas-permeable well including a gas-permeable membrane must be installed in the bioreactor bag prior to the sterilization process and remain in place thereafter. Similarly, the gas-permeable membrane must remain in place after the bioreactor is deployed, and through the conclusion of the process, to maintain the integrity of the bioreactor.

[0030] **FIG. 1A** is a side cross-sectional view schematically illustrating a bioreactor having an integrated gas-permeable well for sensor access. The bioreactor **100** includes a wall **110** and a process medium **120** retained therein. A gas-permeable well **150** including a channel **180** dimensioned to accommodate a sensor (not shown) that extends inwardly from the wall **110** of the bioreactor **100** and is canted downward, terminating in a gas-permeable membrane **160**.

[0031] The gas-permeable membrane **160** may include a thin layer of silicone, although other gas-permeable materials may also be used. The gas-permeable membrane

160 is sealed to the channel **180** of the well **150** to provide a fluid-tight seal, allowing certain gases to pass through the gas-permeable membrane while preventing leakage of the process medium **120** through or between the components of the well **150**. In particular, gas-permeable membrane **160** can allow gases such as oxygen to diffuse through the membrane **160**. Thus, the concentration of such gases in the area of well **150** immediately adjacent the gas-permeable membrane **160** will be representative of the concentration of those gases in the process medium **150**.

[0032] Any suitable sealing method can be used to seal the gas-permeable membrane **160** to the channel **180**, including compression fits, adhesives, O-rings or other gaskets, or any other sealing method or structure, as well as any combination of such methods and structures. In addition to the seal formed between the gas-permeable membrane **160** and the channel **180**, a sealing structure **190** surrounds the open proximal end of the channel **180**, and can be adhered or otherwise secured to the wall **110** of the bioreactor **100** to install the gas-permeable well **150** through a hole or port in the wall **110** of the bioreactor **100**.

[0033] **FIG. 1B** is a side cross-sectional view schematically illustrating the bioreactor of **FIG. 1A**, with a dissolved oxygen sensor inserted into the gas-permeable well. By bringing the sensor **130** into close proximity with the gas-permeable membrane **160**, an indirect measurement of the amount of dissolved oxygen in the process medium **120** can be obtained, without the need to bring the sensor **130** into actual contact with the process medium **120**.

[0034] In the embodiment of **FIGS. 1A** and **1B**, the gas-permeable well **150** including the gas-permeable membrane **160** are integral parts of the bioreactor **100** as used. Even if the gas-permeable well **150** does not form a part of the bioreactor **100** as originally manufactured, and is installed at a later point in time, prior to use, the gas-permeable well **150** and the gas-permeable membrane **160** will remain installed and in place from a point in time prior to the sterilization process to a point in time after the process within the bioreactor **100** is complete. The gas-permeable well **150** and gas-permeable membrane **160** represent a potential point of mechanical failure, both because the gas-permeable membrane**160** itself could be ruptured or damaged, and because a seal between gas-permeable membrane **160** and another portion of the gas-permeable well **150** could fail, such as through dislodgement of the gas-permeable membrane **160**. The potential for such modes of mechanical failure may place constraints on the conditions to

which a bioreactor **100** including a gas-permeable well such as gas-permeable well **150** can be exposed.

[0035] In some embodiments, exposure of the gas-permeable membrane **160** to pressure differentials may rupture or dislodge the gas-permeable membrane **160**. While a thicker membrane may be less likely to rupture, increasing the overall thickness of the gas-permeable membrane **160** as a whole will reduce the rate at which gas diffuses through the gas-permeable membrane **160**, which may increase the time required to obtain an accurate measurement of the process medium. If control of the process within the bioreactor **100** is based in part on the measurement of the process medium, an increase in the time required to obtain an accurate measurement may render the process difficult or impossible to control via feedback from the delayed measurements.

[0036] **FIGS. 2A** and **2B** are cross-sectional and top plan views, respectively, of a gas-permeable membrane design with a cross-sectional shape that provides additional reinforcement. **FIG. 2A** shows a membrane **260** that includes a thicker perimeter region **262** surrounding a thinner central region **264**. In the embodiment of **FIGS. 2A** and **2B**, the perimeter region **262** takes the form of a toroidal shape, and may be integrally molded with the thinner, planar, central region **264**. In some embodiments, the membrane **260** may be considered to include an integrated O-ring. In some embodiments, the integrated O-ring or similar toroidal structure defining the thicker perimeter region **262** may be formed from the same material as the central region **264**, while in other embodiments, the O-ring may be formed from a material that may less permeable to gas and may be more resilient than the material of the integrated O-ring.

[0037] By integrating an O-ring or similar feature within the membrane **260**, the integrity of the seal between the membrane **260** and a surrounding retaining structure, such as a channel of a gas-permeable well, can be increased. Because the gasket-type structure is integrated into the membrane itself, the number of joints between elements of the well can be reduced in comparison to a well design which utilizes one or more additional gaskets or O-rings adjacent a gas-permeable membrane. This can increase, for example, the reliability of a compression seal between the membrane **260** and an adjacent retaining structure configured to retain the membrane **260** in place.

[0038] In addition, the use of a thicker perimeter region **262** can increase the resistance of the membrane **260** to rupture while under load. By increasing the thickness of the perimeter region **262**, without necessarily increasing the thickness of the central

region **264**, the gas-permeable membrane **260** may be reinforced without significantly slowing diffusion of gas through the gas-permeable membrane **260**. Because the diameter **274** of the central region **264** is smaller than the overall diameter **270** of the gas-permeable membrane **260**, the thinner central region **264** can be resistant to pressure differential than a similarly dimensioned membrane of constant thickness. The thicker perimeter region **262** may also facilitate more secure retention of the membrane within a surrounding retention structure.

[0039] In other embodiments, other shapes may be used for the thicker perimeter shape, as well. **FIG. 3** is a cross-sectional view of another embodiment of a gas-permeable membrane design with an alternate cross-sectional shape that provides additional reinforcement. Like the membrane **260** of **FIG. 2A**, the membrane **360** includes a thicker perimeter region **362** surrounding a thinner central region **364**. The thicker perimeter region **364** of the membrane **360** is in the form of a flat ring, with substantially rectangular cross-sectional shapes in profile. The generally flat surfaces of the perimeter region **362** provide a larger surface area in contact with adjacent surfaces, which may be helpful when sealing mechanisms such as compression seals and adhesives are used.

[0040] In other embodiments, any suitable profile or cross-sectional shape may be used for a thicker perimeter section of a gas-permeable membrane. For example, the profile may be chosen to be complementary with a retaining structure to enable a more secure fit or fluid seal. As another example, although depicted as vertically symmetric in cross-section in the embodiments shown in the figures, the thinner central portion may be located closer to either the inner side or the outer side of the thicker perimeter region.

[0041] The use of a reinforced gas-permeable membrane can expand the range of conditions to which a bioreactor containing a gas-permeable membrane can be exposed. As the volume of process media within a bioreactor increases, the hydrostatic pressure on a gas-permeable membrane will increase as well, increasing the likelihood of rupture or dislodgement of the gas-permeable membrane. The use of reinforced gas-permeable membranes such as the reinforced gas-permeable membranes described herein can allow the use of, or increase the reliability of, gas-permeable wells in larger bioreactors. For example, in addition to inclusion of gas-permeable wells in smaller bioreactors, such as 1-liter and 2-liter bioreactors, gas-permeable wells with reinforced

membranes can be used in much larger bioreactors, such as 30-liter bioreactors and bioreactors as large as 2,000 liters or larger. In addition, this can enable placement of the gas-permeable wells at lower heights on the wall of a given bioreactor, where hydrostatic pressures may be greater due to the increased volume of process medium above the gas-permeable well.

[0042] In addition, the reinforced membrane may provide additional protection against other modes of mechanical failure of the membrane. As the use of a dedicated gas-permeable well allows the insertion and removal of a sensor into the gas-permeable well during the bioreactor process, reinforcement of the membrane protects against accidental puncturing or other rupture of the membrane, which could breach the sterility of the bioreactor and result in a loss of significant time and money.

[0043] A wide variety of other structures, designs, and configurations can be used to provide additional reinforcement to a gas-permeable membrane. In some embodiments, integrated reinforcing members may be included within the gas-permeable membrane. **FIGS. 4A** and **4B** are cross-sectional and top plan views, respectively, of a gas-permeable membrane design with a plurality of integrated reinforcing members. **FIG. 4A** shows a membrane **460** that includes a first plurality of reinforcing members **472** extending generally parallel to the plane of the figure and a second plurality of reinforcing members **474** extending generally perpendicular to the plane of the figure. The first plurality of reinforcing members **472** and the second plurality of reinforcing members **474** are interwoven with each other to form a mesh **470**, which can best be seen in **FIG. 4B**.

[0044] The mesh **470** provides a reinforcing structure which can be integrated within the gas-permeable membrane material to provide a reinforced gas-permeable membrane. Because the mesh **470** is non-contiguous, and includes a plurality of spaces **476** or apertures between the interwoven first and second pluralities **472** and **474** of reinforcing members forming the mesh **470**, the mesh **470** can be formed from a non-permeable material while allowing the reinforced gas-permeable membrane **460** as a whole to be permeable to oxygen and other gases. In some embodiments, the mesh **470** may be formed from a material which is partially or completely gas-impermeable, stainless steel or a similar material, and the mesh **470** may be at least partially encased in a gas-permeable material such as silicone.

[0045] Like the reinforcing structures of membranes **230** and **360**, the mesh **470** will also reduce the likelihood of mechanical failure of the membrane. In particular, because the mesh **470** extends across at least a central region of the membrane **460**, it can provide significant protection against failure of the integrity of the membrane itself, such as rupture due to a pressure differential across the membrane. In contrast to the membranes **230** and **360**, which include peripheral reinforcing structures and reduce the size of the thinner central sections, the reinforcing structure of the mesh **470** extends across the entire area of the membrane **460**, and increases the overall stiffness of the membrane. By increasing the stiffness of the membrane **460**, the deformation of the membrane **460** under load can be reduced, making it less likely that the membrane **460** will undergo rupture or other mechanical failure.

[0046] In some embodiments, the internal reinforcing structures can include a structure other than a plurality of discrete reinforcing elements extending across the membrane. For example, a perforated screen or similar structure could be formed by etching apertures into a solid layer of non-permeable material. In some embodiments, this etching could be accomplished via laser etching or another suitable etching process.

[0047] A perforated sheet, layer, or screen could provide a reinforcement structure having a lower profile in cross-section than an interwoven mesh, which could decrease the overall thickness of the membrane, and could maintain a smoother outer profile of the membrane when deformed under load. Because discrete reinforcing structures can be embedded within the gas-permeable membrane, or otherwise designed to provide a largely planar profile in contact with the process media, buildup that may interfere with the rapid measurement of conditions within the bioreactor can be minimized or prevented.

[0048] In some embodiments, the peripheral reinforcing structures of membranes **260** and **360** may be used in conjunction with the mesh **470** of membrane **460** or a similar structure. **FIG. 5A** is a cross-sectional view of a reinforced membrane including the peripheral reinforcing structure of **FIG. 2A** and the reinforcing mesh of **FIG. 4**. The reinforced membrane **560a** includes a thicker perimeter region **562a** in the form of an integrated O-ring surrounding a thinner central region **564a**. The reinforced membrane also includes a reinforcing mesh **570a** extending across the thinner central region **564a** and into the thicker perimeter region **562a**. The thicker perimeter region

562a may increase the integrity of the seal, while the reinforcing mesh **570a** may increase the stiffness of the membrane **560a** and reduce the likelihood of rupture.

[0049] Similarly, **FIG. 5B** is a cross-sectional view of a reinforced membrane including the peripheral reinforcing structure of **FIG. 3** and the reinforcing mesh of **FIG. 4**. The reinforced membrane **560b** includes a thicker perimeter region **562b** in the form of an integrated flat ring that is thicker than and surrounding a thinner central region **564b**. The reinforced membrane also includes a reinforcing mesh **570b** extending across the thinner central region **564b** and into the thicker perimeter region **562b**.

[0050] In other embodiments, a peripheral reinforcing structure can include a discrete reinforcing element, rather than being formed only from of a thicker section of gas-permeable material. **FIG. 6A** is a cross-sectional view of a reinforced membrane including a discrete peripheral reinforcing structure internal to the reinforced membrane. **FIG. 6B** is a top plan view of the reinforced membrane of **FIG. 6A**. The reinforced membrane **660** includes an internal reinforcing structure in the form of a mesh **670** that extends across a thinner central portion **664** and into a thicker peripheral structure **662**. In addition, the reinforced membrane **660** includes a discrete peripheral reinforcing structure **678** that in the illustrated embodiment is a ring extending around the edge of the reinforced membrane **660**. The peripheral reinforcing structure **678** may in some embodiments be securely connected to the mesh **670** by welding, wrapping of the mesh **670** or the individual elements of mesh **670** around the peripheral reinforcing structure **678**, or any other suitable means of secure attachment, such that the mesh **670** and peripheral reinforcing structure **678** form a single generally rigid structure.

[0051] Like the mesh **670**, the peripheral reinforcing structure **678** can be made from stainless steel or another suitable material. The mesh **670** may be connected to and extend across the interior of the peripheral reinforcing structure **678**. In the illustrated embodiment, the gas-permeable material completely envelops the mesh **670** and the peripheral reinforcing structure **678.** The use of a rigid peripheral reinforcing structure **678** can further increase the stiffness of the reinforced membrane **660**, and can support the integrated O-ring or similar gasket feature of the reinforced membrane **660** to increase the integrity of the seal between the reinforced membrane **660** and one or more other components of a reinforced gas-permeable well including the reinforced membrane **660**.

[0052] **FIG. 7** is a cross-sectional view of a reinforced membrane including a discrete peripheral reinforcing structure at least partially external to the reinforced membrane. Like the membrane **660**, the membrane **760** includes an internal reinforcing structure in the form of a mesh **770** extending across a thinner central portion **764** and into a thicker peripheral structure **762**, as well as a discrete peripheral reinforcing structure **778** in the form of a ring extending around the edge of the reinforced membrane **760**. An outer portion of the peripheral reinforcing structure **778** is exposed, extending outside of the gas-permeable material. The partially exposed peripheral reinforcing structure **778** can facilitate certain securement arrangements of the membrane within a retaining structure such as the retaining structures discussed in greater detail below.

[0053] Membranes such as the reinforced membranes of **FIGS. 2A – 7** can be formed by any suitable manufacturing process. In some embodiments, the gas-permeable material can be injection molded, with or without discrete internal reinforcing components. In other embodiments, other suitable manufacturing processes can be used.

[0054] A reinforced membrane such as the reinforced membranes of **FIGS. 2A – 7** can be used in conjunction with a retaining structure to provide a reinforced gas-permeable well which is more resilient than a gas-permeable well including an unreinforced membrane. **FIG. 8** is a side cross-section of a reinforced gas-permeable well including a reinforced gas-permeable membrane. In the illustrated embodiment, reinforced gas-permeable well **850** includes a channel **880** having a reinforced membrane **860** at the distal end, and an open proximal end. The membrane **860** forms a fluid-tight seal with the surrounding components of the reinforced gas-permeable well **850**. A generally planar sealing structure **890** surrounds the open proximal end of the channel **880**.

[0055] In the illustrated embodiment, the channel **880** extends along an axis that is oriented at an angle to the normal of the plane defined by the sealing structure **890**. This allows the channel **880** to be canted downward when installed, or in any other desired direction, relative to a sidewall of a bioreactor in which the reinforced gas-permeable well **850** is installed. In other embodiments, the channel **880** may be aligned with the normal, or oriented at a larger or smaller angle to the normal.

[0056] The reinforced membrane **860** may be retained at the distal end of the channel **880** with a fluid-tight seal formed between the reinforced membrane **860** and the channel **880**. The reinforced membrane **860** may be retained there, with a fluid-tight seal

formed there, by any suitable retention mechanism or retention structure. In some embodiments, the reinforced membrane may be compression-sealed, press-fit, adhered, welded or otherwise mechanically secured to the channel **880** or an intermediate structure that is itself secured to the channel **880**. In some embodiments, flanges or other mechanical stops **882**, which may extend inwardly from the walls of channel **880**, may be used to secure the reinforced membrane **860** in place, with a seal formed between the reinforced membrane **860** and contacting surfaces of the stops **882 and**/or channel **880**. Any other suitable retention mechanism, or combination of retention mechanisms, may be used to prevent dislodgement of the reinforced membrane **860** and maintain the integrity of the seal formed by or between the reinforced membrane **860** and one or more adjacent components of the reinforced gas-permeable well **850**.

[0057] The use of a reinforced membrane such as membrane **660** or **760**, having an exposed section of a peripheral ring or other peripheral reinforcing member, may allow for a more robust mechanical connection between the retaining member and the seal. The channel **880** may in some embodiments be integrally formed with the channel **880**, such that no discrete retention mechanism is used.

[0058] The sealing structure **890** may be used to seal the reinforced gas-permeable well **850** to a portion of a bioreactor wall surrounding a port or other aperture in the bioreactor wall, after insertion of the distal end of the channel **880** through the port or other aperture in the bioreactor wall. The sealing structure **890** of the reinforced gas-permeable well **850** may be brought against and sealed to the wall of the bioreactor to form a fluid-tight and gas-tight seal. By using modular reinforced gas-permeable wells such as reinforced gas-permeable well **850**, a single-use bioreactor having a desired configuration can be assembled and sterilized, and provided to an end user.

[0059] The channel **880** need not terminate at the sealing structure **890**, but may in some embodiments extend through the sealing structure **890** and beyond, to provide additional stability to a DO sensor or other sensor seated therein. In other embodiments, the channel **880** may be substantially smaller or non-existent.

[0060] **FIG. 9** is a cross-sectional view schematically illustrating an embodiment of a bioreactor having a low-profile reinforced gas-permeable well installed therein, where the reinforced membrane of the gas-permeable well is substantially flush with the wall of the bioreactor. The bioreactor **900** has a low-profile reinforced gas-permeable well **950** installed in one of the walls **910** of the bioreactor **900**. The low-

profile reinforced gas-permeable well **950** includes a reinforced gas-permeable membrane **960** with a fluid-tight seal formed between the reinforced gas-permeable membrane **960** and at least one other component of the low-profile reinforced gas-permeable well **950.** The reinforced gas-permeable well **950** is secured to the wall **910** of the bioreactor **900** by a sealing structure **990** that is generally coplanar with the membrane **960**. A retention structure **982**, which may extend to one or both sides of the membrane **960**, secures the membrane **960** in place and cooperates with the membrane **960** to form a fluid-tight seal.

[0061] In such an embodiment, additional support (not shown), which may be external to the bioreactor **900**, may be used to support a DO sensor or other sensor brought into contact with or adjacent the membrane **960**. Because the reinforced membrane **960** is substantially flush with the bioreactor wall **910** in the embodiment of **FIG. 9**, the low profile low-profile reinforced gas-permeable well **950** does not reduce the usable interior volume of the bioreactor **900**, and does not interfere with other components which may extend into or be inserted into the interior of the bioreactor **900**, such as agitators or other sensors.

[0062] In some embodiments, the reinforced membranes described above may be used in conjunction with reusable bioreactors, including bioreactors with rigid walls. In such an embodiment, a reinforced gas-permeable well including a reinforced gas-permeable membrane may be built into the rigid bioreactor during the initial construction of the bioreactor. In other embodiments, however, an existing bioreactor can be retrofitted or otherwise modified to include a reinforced gas-permeable well including a gas-permeable membrane.

[0063] In some embodiments, the reinforced gas-permeable membrane may be provided as part of a module that can be inserted into or otherwise secured and sealed to an existing access port, tube, well, or other luminal structure within or extending into a reusable bioreactor. Because the gas-permeable membrane is reinforced, the reinforced membrane may remain in place and maintain the integrity of the seal during sterilization proceses between bioprocesses or other potentially damaging conditions. In some embodiments, these sterilization processes can involve exposure of the bioreactor to high temperatures and pressures.

[0064] In the foregoing description, specific details are given to provide a thorough understanding of the examples. However, it will be understood by one of ordinary skill in the art that the examples may be practiced without these specific details.

Certain embodiments that are described separately herein can be combined in a single embodiment, and the features described with reference to a given embodiment also can be implemented in multiple embodiments separately or in any suitable subcombination. In some examples, certain structures and techniques may be shown in greater detail than other structures or techniques to further explain the examples.

[0065] The previous description of the disclosed embodiments is provided to enable any person skilled in the art to make or use the present invention. Various modifications to these embodiments will be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other embodiments without departing from the spirit or scope of the invention. Thus, the present invention is not intended to be limited to the embodiments shown herein but is to be accorded the widest scope consistent with the principles and novel features disclosed herein.

WHAT IS CLAIMED IS:

1. A reinforced gas-permeable well for use with a bioreactor, the reinforced gas-permeable well comprising:

a reinforced gas-permeable membrane, the reinforced gas-permeable membrane comprising:

a gas-permeable membrane; and

a gas-impermeable reinforcing structure at least partially embedded within the gas-permeable membrane, the gas-impermeable reinforcing structure including at least one aperture extending therethrough; and

a retaining structure configured to retain the gas-permeable membrane.

2. The reinforced gas-permeable well of claim 1, wherein the gas-impermeable reinforcing structure includes an interwoven mesh.

3. The reinforced gas-permeable well of claim 1, wherein the gas-impermeable reinforcing structure includes a perforated screen.

4. The reinforced gas-permeable well of claim 1, wherein the gas-impermeable reinforcing structure includes a plurality of reinforcing elements extending across a central portion of the reinforced gas-permeable membrane.

5. The reinforced gas-permeable well of any of claims 2 to 4, wherein the gas-impermeable reinforcing structure further includes a ring disposed at or near the periphery of the gas-impermeable reinforcing structure.

6. The reinforced gas-permeable well of claim 5, wherein the ring is fully embedded within the gas-permeable membrane.

7. The reinforced gas-permeable well of claim 5, wherein the ring is partially embedded within the gas-permeable membrane.

8. The reinforced gas-permeable well of any of claims 1 to 7, wherein the gas-impermeable reinforcing structure comprises stainless steel.

9. The reinforced gas-permeable well of any of claims 1 to 8, wherein the gas-permeable membrane includes silicone.

10. The reinforced gas-permeable well of any of claims 1 to 9, additionally comprising a channel configured to receive a sensor therein, to allow the sensor to be positioned adjacent the reinforced gas-permeable membrane.

11. The reinforced gas-permeable well of any of claims 1 to 9, additionally comprising a sealing structure configured to allow the reinforced gas-permeable well to be installed in a wall of a bioreactor.

12. The reinforced gas-permeable well of claim 11, additionally comprising a channel extending between the sealing structure and the retaining structure, the channel configured to allow a sensor to be positioned adjacent the reinforced gas-permeable membrane.

13. The reinforced gas-permeable well of claim 12, wherein the retaining structure comprises at least one flange extending inwardly from the interior wall of the channel and configured to inhibit translation of the of the gas-permeable membrane in at least one direction along the channel.

14. The reinforced gas-permeable well of claim 11, wherein the reinforced gas-permeable well is configured to be installed such that the reinforced gas-permeable membrane is positioned generally coplanar with a wall of the bioreactor.

15. The reinforced gas-permeable well of claim 11, wherein the reinforced gas-permeable membrane is oriented at an angle to the sealing structure.

16. The reinforced gas-permeable well of claim 11, additionally comprising a channel extending between the sealing structure and the retaining structure, the channel configured to allow a sensor to be positioned adjacent the reinforced gas-permeable membrane, wherein the reinforced gas-permeable membrane is oriented at an angle to the axis of the channel.

17. A bioreactor comprising the reinforced gas-permeable well of any of claims 1 to 16.

18. The bioreactor of claim 17, wherein the bioreactor comprises a single-use bioreactor having flexible walls.

19. The bioreactor of claim 17, wherein the bioreactor comprises an internal volume of greater than 2 liters or greater than 30 liters.

20. The bioreactor of claim 17, wherein the bioreactor comprises a reusable bioreactor having rigid walls.

21. A reinforced gas-permeable well for use with a bioreactor, the reinforced gas-permeable well comprising:

a reinforced gas-permeable membrane, the reinforced gas-permeable membrane including a reinforcing structure which decreases the permeability of the gas-permeable membrane in an area adjacent the reinforcing structure; and

a retaining structure configured to retain the reinforced gas-permeable membrane.

22. The reinforced gas-permeable well of claim 21, wherein the reinforcing structure includes at least one region of increased thickness.

23. The reinforced gas-permeable well of claim 22, wherein the at least one region of increased thickness extends around the periphery of the reinforced gas-permeable membrane.

24. The reinforced gas-permeable well of claim 23, wherein the at least one region of increased thickness comprises an integrated O-ring or gasket.

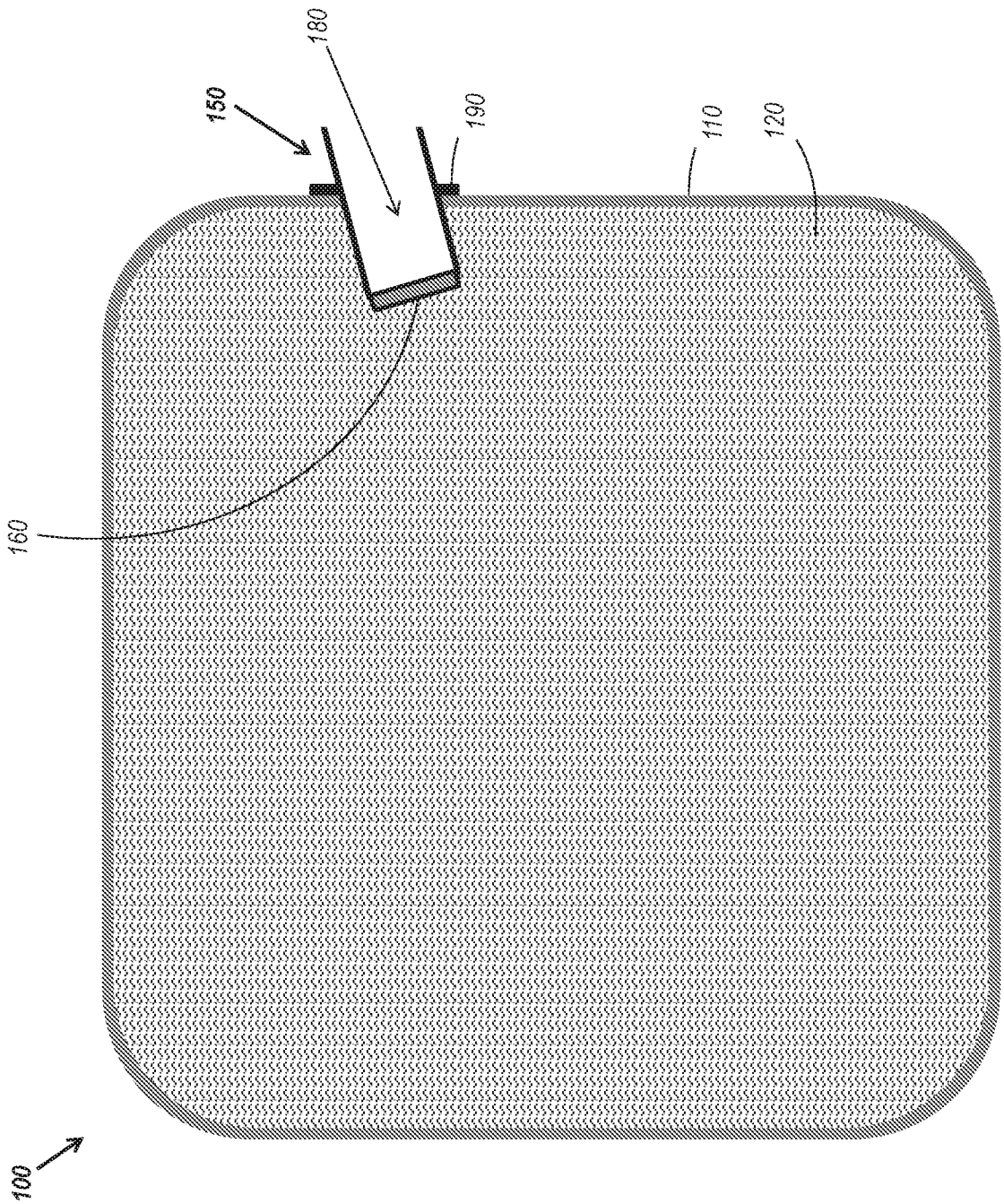
25. The reinforced gas-permeable well of any of claims 21 to 24, wherein the reinforcing structure comprises a gas-impermeable reinforcing structure at least partially embedded within the gas-permeable membrane and comprising at least one aperture extending therethrough

26. The reinforced gas-permeable well of claim 25, wherein the gas-impermeable reinforcing structure includes an interwoven mesh.

27. The reinforced gas-permeable well of claim 25, wherein the gas-impermeable reinforcing structure includes a perforated screen.

28. The reinforced gas-permeable well of claim 25, wherein the gas-impermeable reinforcing structure includes a plurality of reinforcing elements extending across a central portion of the reinforced gas-permeable membrane.

FIG. 1A



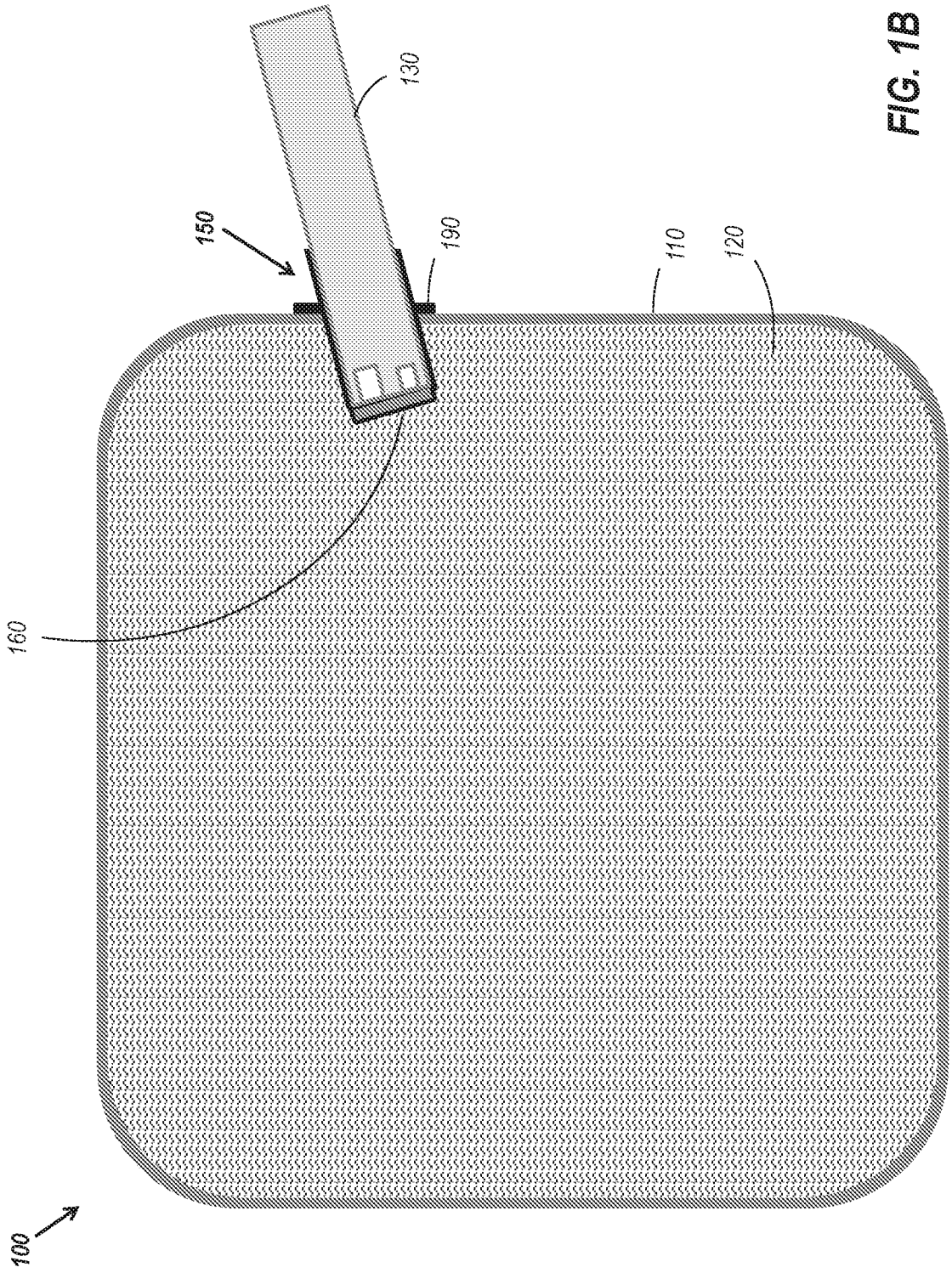


FIG. 1B

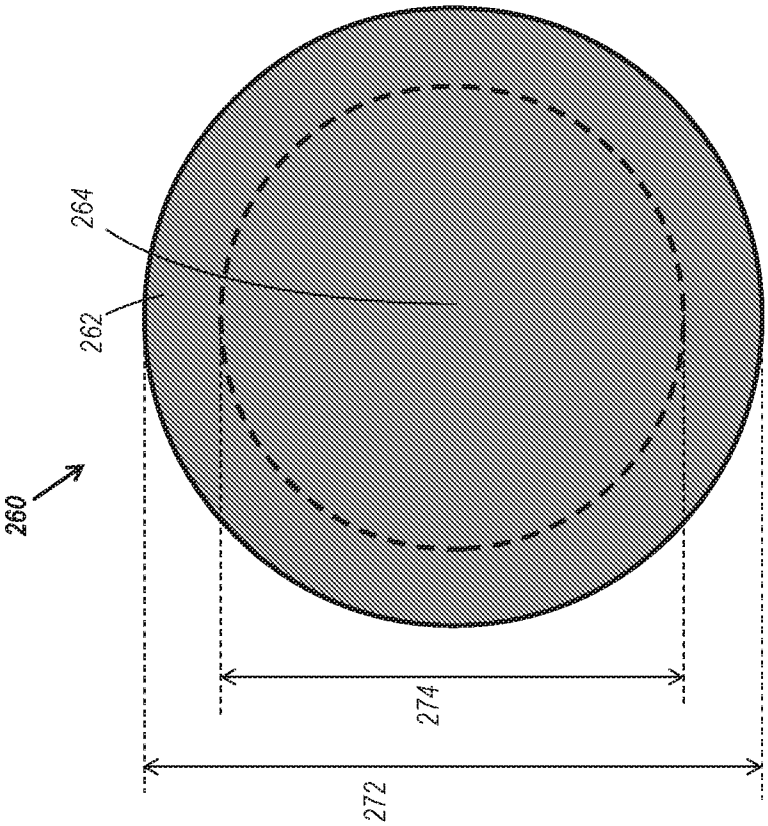


FIG. 2B

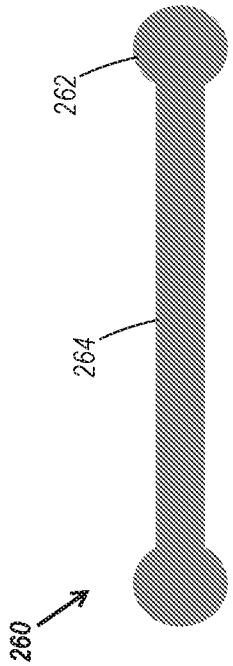


FIG. 2A

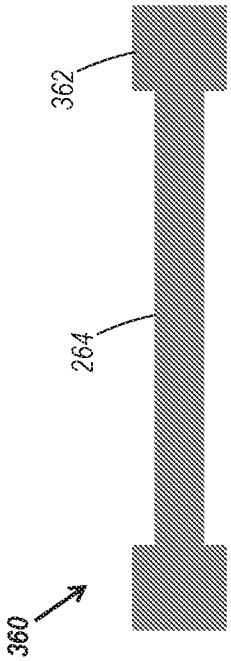


FIG. 3

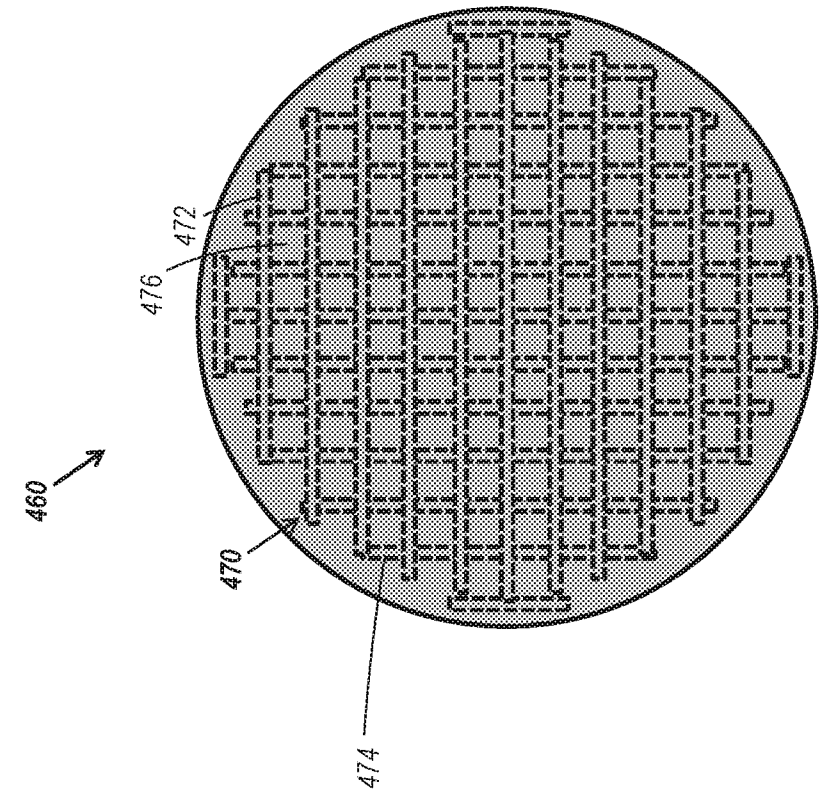


FIG. 4B

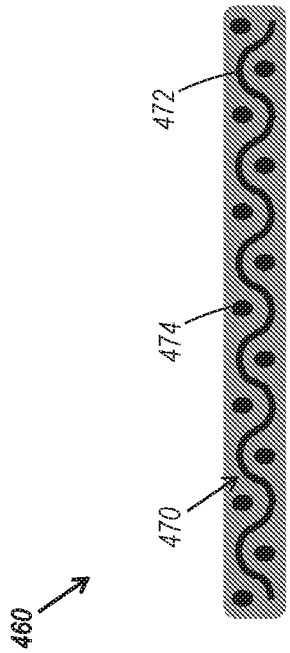


FIG. 4A

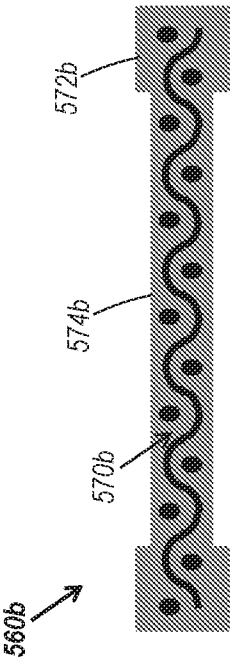


FIG. 5B

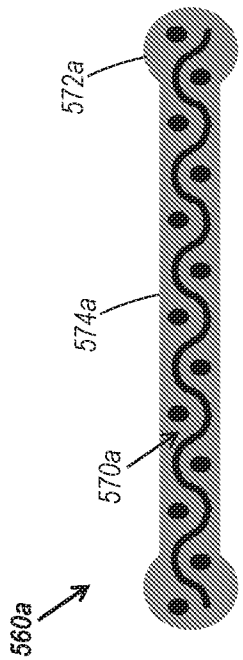


FIG. 5A

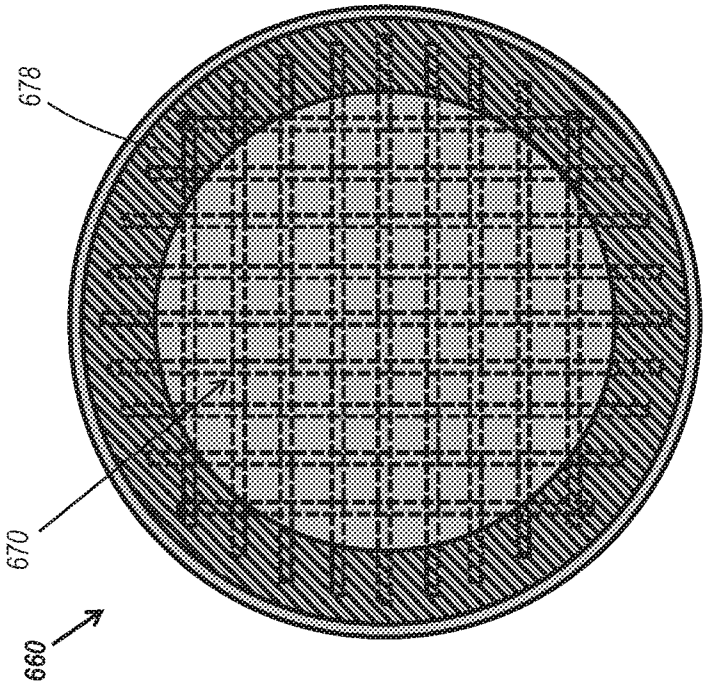


FIG. 6B

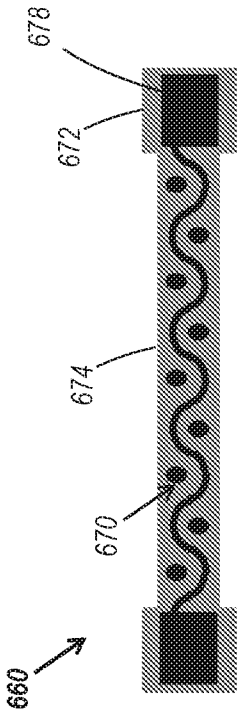


FIG. 6A

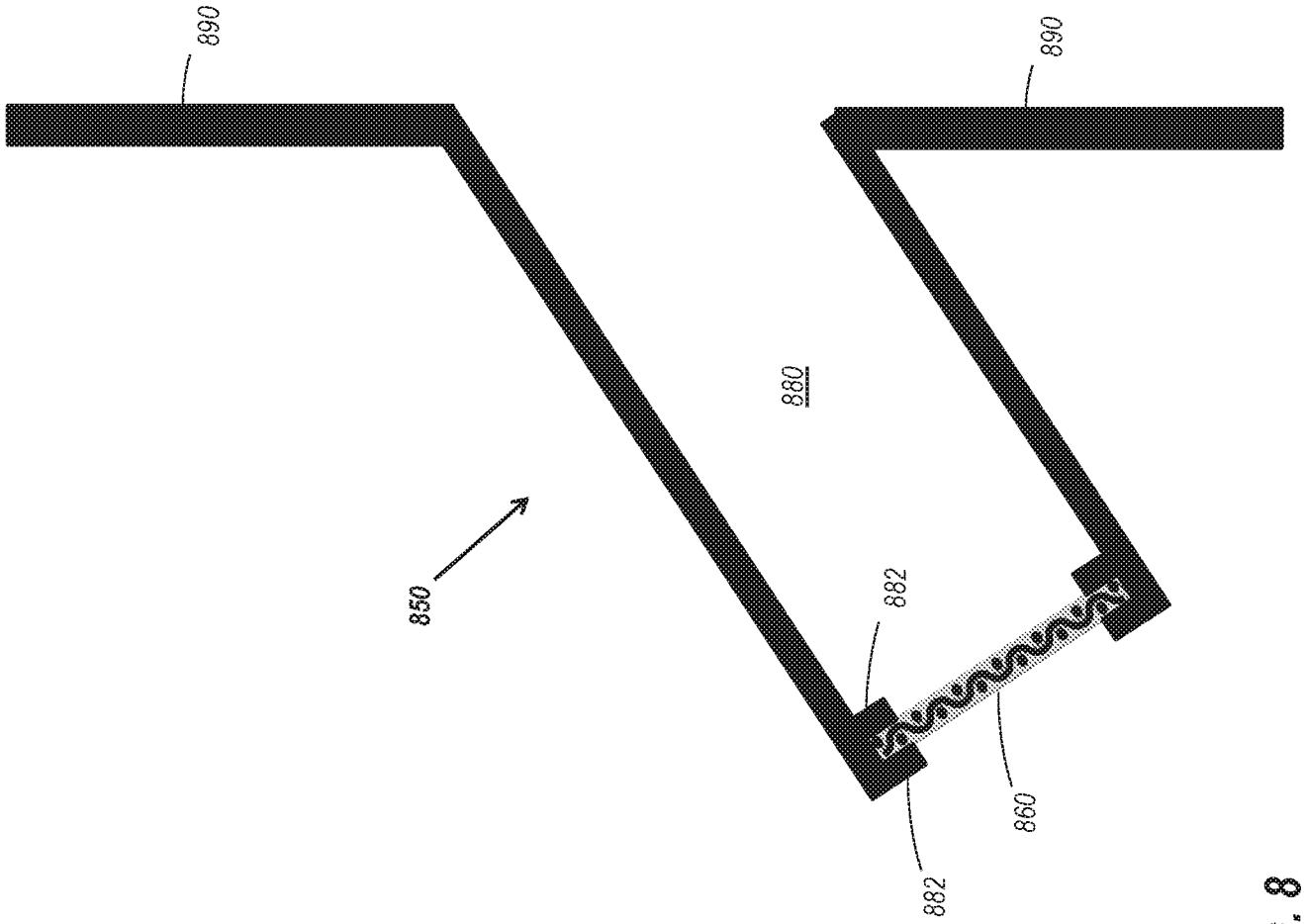


FIG. 8

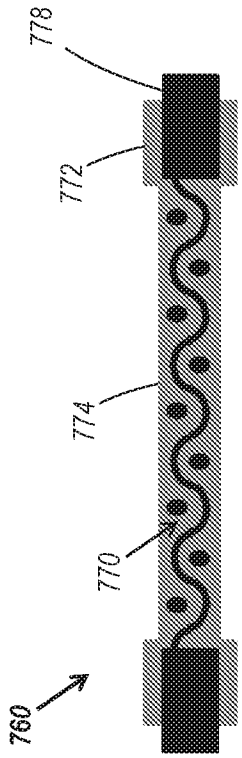


FIG. 7

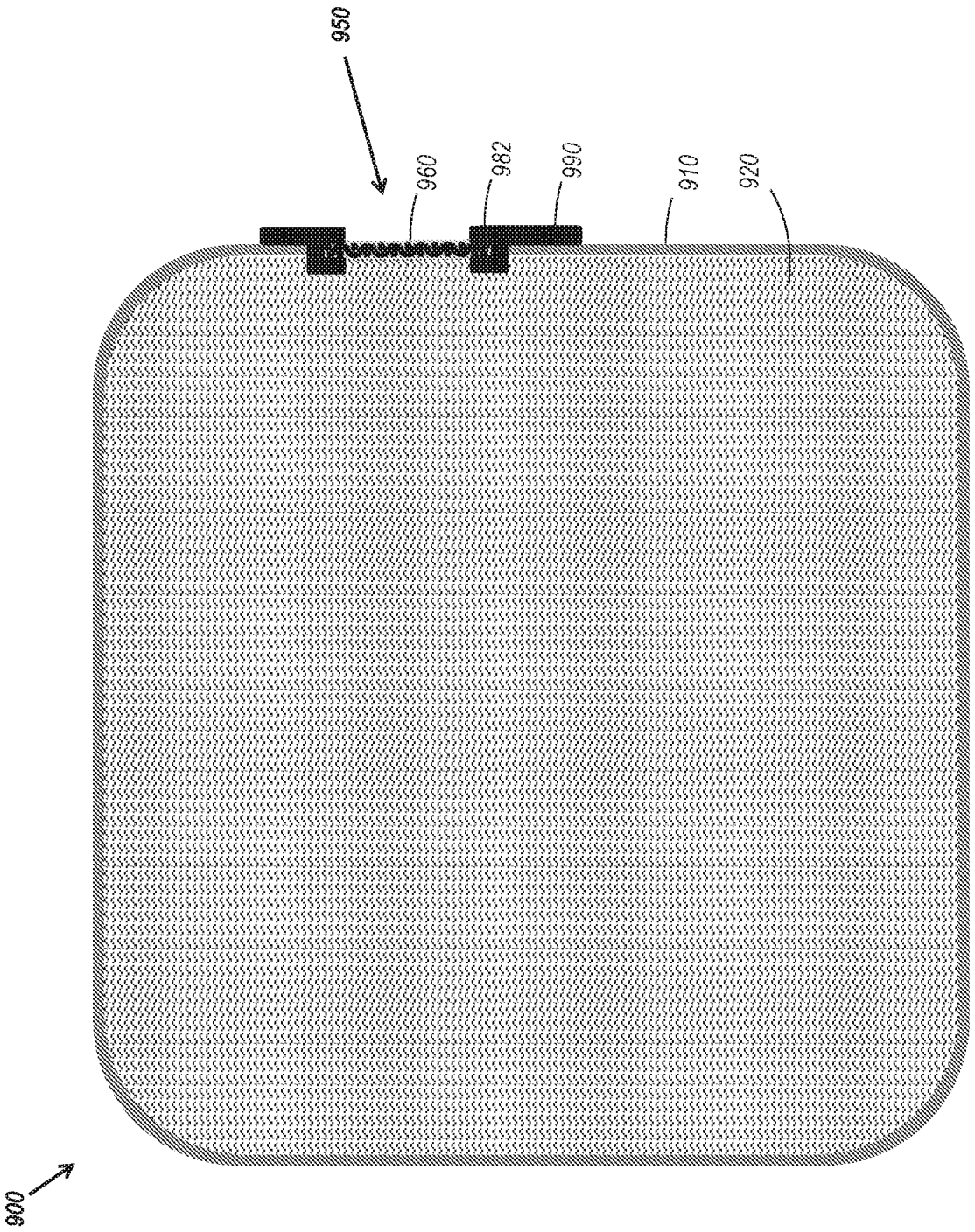


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/022101

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01D65/00 B01L3/00 B01D69/10 B29C70/04 C12M1/34
G01N31/22 A61M39/18 B01D69/02 C12M1/00 G01N27/28
G01D11/24

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)
B01D C12M B29C G01N B01L A61M G01D

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 2012/097557 A1 (BAUMFALK REINHARD [DE] ET AL) 26 April 2012 (2012-04-26) figures 1-6 paragraph [0001] - paragraph [0041] claims 1,11,12,16	1-28
X Y	US 2012/171760 A1 (RAO GOVIND [US] ET AL) 5 July 2012 (2012-07-05) paragraphs [0047] - [0057], [0063] claims 15,16,29	1-28 1-28
Y	US 2018/055985 A1 (STOECKER MARTIN [DE]) 1 March 2018 (2018-03-01) paragraphs [0008] - [0009], [0030] - [0035]	1-28

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

[X] See patent family annex.

* Special categories of cited documents :

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2019	24/06/2019

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	Williams, Jennifer

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/022101

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 2012/023888 A1 (ADIXEN SCANDINAVIA AB [SE]; ENQUIST FREDRIK [SE]; HEBO PETER [SE]) 23 February 2012 (2012-02-23) figures 1,2 page 0047	13

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US2019/022101

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