BIOREACTOR WITH LID FOR EASY ACCESS TO INCUBATION CAVITY

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(57) Abstract: There is provided a bioreactor which is provided with a lid (13) that facilitates access to the incubation cavity. Specifically the end wall of the incubation cavity is constituted by the lid (13) so that removal of the cap renders the incubation cavity fully accessible.

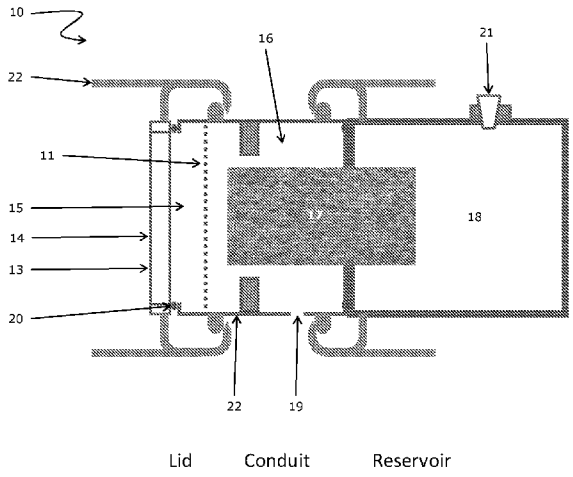

Figure 1

WO 2012/079577 A1

BIOREACTOR WITH LID FOR EASY ACCESS TO INCUBATION CAVITY

Field of the invention

The present invention relates to a bioreactor which is provided with a lid that facilitates easy and rapid access to the incubation cavity. Specifically the end wall of the incubation cavity is constituted by the lid so that removal of this lid renders the incubation cavity fully accessible.

Background of the invention

During "classical" cell culture in an essentially flat culture vessel, primary cells in general and biopsies in particular tend to de-differentiate. Visibly, biopsies exhibit the 'melting ice-cream effect' as cells migrate from a block of tissue out onto the flat supporting surface of the culture vessel. Gene expression is altered in these "migrating" cells, which begin to behave biochemically as isolated cells rather than as cellular components of a differentiated tissue. De-differentiated cells express different biochemical pathways than those normally expressed by corresponding cells in an intact organism. In addition, immortal cells normally have lost some or many of their specialised functions compared to the corresponding mortal cell in the intact organism

In contrast with "classical" cell culture conditions, "microgravity" conditions preserve the differentiation state of many types of cells in culture. Microgravity bioreactors maintain microgravity conditions by continuous rotation of a typically cylindrical or tubular incubation cavity or compartment. This rotation continuously helps to prevent cells from adhering to the walls of the incubation cavity, suspending the cells in a fluid environment using a minimum shear force. This induces them to interact and to aggregate into colonies. These colonies have been given a variety of names including spheroids, cell conglomerates, cell aggregates and ProtoTissue™ (all of which are considered equivalent herein). For microgravity culturing, cells are often initially sown out onto small (ca. 100 µm diameter) beads (this accelerates the formation of microtissue structures) but is not essential and there are several other alternatives published in the literature for example using scaffolds [Lee KW, Wang S, Dadsetan M, Yaszemski MJ, Lu L. Enhanced cell ingrowth and proliferation through three dimensional nano composite

scaffolds with controlled pore structures. Biomacromolecules. 11:682-9, 2010] or cross-linked hydrogels [Villanueva I, Klement BJ, Von Deutsch D, Bryant SJ. Cross-linking density alters early metabolic activities in chondrocytes encapsulated in poly(ethylene glycol) hydrogels and cultured in the rotating wall vessel. Biotechnol Bioeng. 102:1242-50, 2009]. As Spheriods are formed by cell growth around these beads, the beads usually become completely covered with cells. Spheriods formed in this manner become highly differentiated so as to resemble adult tissue [Navran S. The application of low shear modelled microgravity to 3-D cell biology and tissue engineering. Biotechnol Ann Rev. 14: 275-296, 2008] [Freed LE, Vunjak-Novakovic G and Langer R. Cultivation of Cell-Polymer Cartilage Implants in Bioreactors. J Cell Biochem. 51: 257-64, 1993] [Brown LA, Arterburn LM, Miller AP, Cowger NL, Hartley SM, Andrews A, Silber PM, Li AP. Maintenance of Liver Functions in Rat Hepatocytes Cultured as Spheroids in a Rotating Wall Vessel. In Vitro Cell Dev Biol Anim.; 39: 13-20, 2003].

Microgravity bioreactors have been used in a variety of contexts. Early studies showed that microgravity bioreactor systems helped cells form three dimensional structures by reducing shear stress on the cells [Reduced shear stress: a major component in the ability of mammalian tissues to form three-dimensional assemblies in simulated microgravity. Goodwin TJ, Prewett TL, Wolf DA, Spaulding GF. J Cell Biochem. 1993 Mar;51(3):301-11].

Now a significant body of literature demonstrates increased differentiation of cells grown in a microgravity bioreactor system. For reviews see: [[Navran S. The application of low shear modelled microgravity to 3-D cell biology and tissue engineering. Biotechnol Ann Rev. 14: 275-296, 2008] and [Growing tissues in microgravity. Unsworth BR, Lelkes PI. Nat Med. 1998 Aug;4(8):901-7.] For example, microgravity culturing induces neural precursor cells to form cellular clusters or "neurospheres". These neurospheres are characterized by a crude, but organized, architecture, having a surface layer of immature proliferating cells (nestin- and proliferating cell nuclear antigen-positive) that enclose strata of more differentiated cells (beta-tubulin III- and glial fibrillary acidic protein-positive). These "neurospheres" show promise for development of neurotransplantable tissue. See e.g. [Neural precursor cells form rudimentary tissue-like structures in a rotating-wall vessel bioreactor. Low HP, Savarese TM, Schwartz WJ.*In vitro* Cell Dev Biol Anim. 2001 Mar;37(3):141-7.] and see [Rapid differentiation of NT2 cells in Sertoli-NT2 cell tissue constructs grown in the rotating wall bioreactor. Saporta S, Willing AE,

Shamekh R, Bickford P, Paredes D, Cameron DF. Brain Res Bull. 2004 Dec 150;64(4):347-56.].

Or for another example, microgravity culturing of a multipotential human retinal cell line induced expression of a nearly *in vivo* phenotype, which could not be achieved when the cells were grown under other conditions [Generation of 3D retina-like structures from a human retinal cell line in a NASA bioreactor. Dutt K, Harris-Hooker S, Ellerson D, Layne D, Kumar R, Hunt R. Cell Transplant. 2003;12(7):717-31.] Improved differentiation has also been demonstrated in other tissues [Freed LE, Vunjak-Novakovic G and Langer R. Cultivation of Cell-Polymer Cartilage Implants in Bioreactors. J Cell Biochem. 51: 257-64, 1993] [Brown LA, Arterburn LM, Miller AP, Cowger NL, Hartley SM, Andrews A, Silber PM, Li AP. Maintenance of Liver Functions in Rat Hepatocytes Cultured as Spheroids in a Rotating Wall Vessel. In Vitro Cell Dev Biol Anim.; 39: 13-20, 2003].

Some technical problems with microgravity bioreactors have been reported. For example, when temporomandibular joint (TMJ) disc tissues were engineered using either flat culture or a microgravity bioreactor, there were no significant differences in total matrix content and compressive stiffness, notwithstanding marked differences in gross appearance, histological structure, and distribution of collagen types I and II (with the bioreactor discs having more collagen type II). The authors concluded that improvements of the microgravity bioreactor culture system were needed [Detamore MS, Athanasiou KA. Use of a rotating bioreactor toward tissue engineering the temporomandibular joint disc. Tissue Eng. 2005 Jul-Aug;11(7-8):1188-97]. The DNA repair system also seems to be detrimentally influenced [Kumari R, Singh KP, Dumond JW Jr. Simulated microgravity decreases DNA repair capacity and induces DNA damage in human lymphocytes. J. Cell Biochem.107:723-31, 2009]. Although well known and widely used, currently available microgravity bioreactors have significant limitations:

Another significant limitation of microgravity bioreactors of the prior art is moisture loss, which affects cell growth. Dehydration (even only by 5-10%) during incubation can result in changes in pH and other concentration-dependent parameters, such as concentrations of salts, nutrient substances, and the like. Many cell types are highly sensitive to their environment. For such cells, even a small change in such environmental conditions can influence cell growth and gene expression. This problem is especially pronounced in a small volume bioreactor, where small changes in volume can cause relatively large changes in concentration-dependent parameters. Without some solution to this dehydration problem, a small volume bioreactor would experience rapid

loss of moisture, notwithstanding maintenance of humidified conditions (100% relative humidity) in the incubator where the bioreactor was used. This tendency for rapid dehydration in a small volume bioreactor, that is, this tendency for rapid change in relative volume greatly increases the need for time-consuming manual monitoring and manipulation, for example to replenish or exchange culture medium. This tendency effectively renders long-term maintenance of cultures in a small volume bioreactor impractical or impossible. Accordingly, it would be advantageous to provide a microgravity bioreactor with very high relative water retention in the cell incubation compartment.

Still another limitation of microgravity bioreactors of the prior art is that access ports used for adding or removing cells and growth medium have typically relied on conventional "luer lock" closures. These and similar closures have a finite 'dead' volume and this becomes proportionally larger as the volume of the bioreactor is reduced. This disadvantage can be circumvented by using ports of essentially no dead volume.

Luer lock closures can also lead to presence of air bubbles in the incubation compartment. Bioreactors are preferably kept free of air bubbles in the incubation compartment which otherwise have detrimental effects, breaking up the Spheriods. This air bubble problem is especially pronounced in a small volume bioreactor, where a single bubble can represent a relatively significant volume. Some solutions to the air bubble problem are known in the prior art. For example, WO 95/07344 provides a reservoir chamber for entrapping gas bubbles away from the incubation compartment. However, these solutions would be wholly unsuitable for a small volume bioreactor because of the volumes involved. A better solution is thus to provide a closure mechanism for access ports that excludes any possibility of introducing air bubbles into the incubation compartment.

Conventional "luer lock" and similar closures also increase fluid turbulence because they do not have a smooth inner surface and this can lead to increased shear forces which will have a detrimental effect on the Spheriods. Microgravity bioreactors require continuous rotation of the incubation compartment to maintain microgravity conditions for differentiated or differentiating cells and other tissues. If the incubation compartment inner surface is not suitably adapted, it may give rise to turbulence. Such turbulence may lead to tearing or "shearing" of Spheriods. Accordingly, it is advantageous to provide microgravity bioreactors with an access port closure mechanism that avoids turbulence.

WO 95/07344, US 5153131, US 5437998, US 5665594, US 5989913, and US 6,642,019 each disclose improvements of microgravity bioreactors. US 2005/0084965 discloses use of a conventional, commercially available microgravity bioreactor for incubating hepatocyte spheroids. However none of these patents or published applications addresses the problem of dehydration or discloses a microgravity bioreactor having a small incubation compartment volume or having a zero volume access port closure.

Prior art bioreactors of all volumes suffer yet another major problem, namely the difficulty of accessing the incubation chamber/cavity with instruments having dimensions exceeding e.g. a hypodermic syringe or pincers. Thus it is difficult to remove from the bioreactor individual or small amounts of Spheriods without risk of damage (e.g. by shear forces in using a syringe).

US 5576211 and US 5153131 describe cylindrical bioreactors being rotatable around a central axis, the bioreactors comprising a cell culture chamber and a supply chamber separated by a membrane. US 5153131 does not disclose a removable lid, but instead the flange 22 not only constitute the end of the incubator but also the sidewall. Moreover, this flange is by far easy to remove and upon removal it disintegrates from the membrane. US 5576211 is not a micro-gravity bioreactor (60 ml incubation volume), but only a roller-bottle system, and hence it will be difficult to access the entire culture chamber, when removing the screw-on ring. Moreover, even when the screw-on ring has been removed there is still a silicone membrane to cope with. Hence the bioreactor system of US 5576211 does not provide easy access to the incubation cavity. In figure 9 in US 5576211, there does not appear to be anything to hold the membrane support grid 82 in medium container 55. Likewise there is nothing to hold the dialysis membrane 64 against 82. Therefore, the user would have to empty or drain 55 before one can open into the cell culture chamber (between the dialysis membrane 64 and a gas exchange membrane in 72). Otherwise the dialysis membrane might fall out and spill the media.

Since microgravity is used to induce the cells to exhibit differentiated phenotypes, the cessation of rotation results in the Spheriods settling usually to the bottom of the incubation chamber. This can induce Spheriods to stick together or to the walls of the incubation chamber and start to lose the desirable phenotype. Thus if it is necessary to remove Spheriods from the incubation chamber, it must be possible to open the

incubation chamber, remove one or more pieces of Spheriods and close the incubation chamber quite quickly (e.g. within a few minutes).

Accordingly, it is advantageous to provide an improved microgravity bioreactor that addresses these problems.

Summary of the invention

The present invention relates to a bioreactor (10) which is provided with a lid (13) that facilitates access to the incubation cavity. Specifically the end wall of the incubation cavity is constituted by the lid (13) so that removal of the lid renders the incubation cavity fully accessible.

This and several other objectives are obtained in a first aspect of the invention by providing a bioreactor adapted for rotation, the reactor comprising:

- an incubation cavity (15), the incubation cavity providing, in conjunction with, a semipermeable membrane (11 or 12) in the first end of the wall, and a sealable lid (13) which forms the second end of the wall and an essentially cylindrical wall, and provides a substantially closed confinement, said semipermeable membrane (11) being permeable to molecules up to a predetermined molecular weight or size, allowing a gas exchange in the incubation chamber and retention of cells and cellular aggregates in the incubation cavity,

- a reservoir chamber, said reservoir chamber providing a volume of water (or other dilute aqueous solutions) for the maintenance of very high humidity levels close to the semipermeable membrane 11,
- equilibrium chamber providing a conduit from the semipermeable membrane (11) to the reservoir chamber and a narrow connection to the external air surrounding the bioreactor (19),

wherein said lid (13) is removably attached to the second end of the wall so as to provide access to the entire incubation cavity.

Optionally the lid (13) may have one or more ports at different locations.

In a second aspect, embodiments of the invention provide a bioreactor adapted for rotation, the bioreactor comprising

an incubation cavity, the incubation cavity providing, in conjunction with an essentially cylindrical wall, a semipermeable membrane (11) in the first end of the wall, and a sealable lid (13) in the second end of the wall, a substantially closed confinement, wherein said lid (13) is removably attached to the second end of the wall so as to provide access to the entire incubation cavity,

-a reservoir chamber (18) comprising an aqueous liquid, the chamber comprising a humidifier (17) made of a suitable material to enhance high evaporation and
-equilibrium chamber (16) providing a conduit from the semipermeable membrane (12) to the humidifier (17) and a narrow connection to the external air surrounding the bioreactor (19),

wherein the semipermeable membrane (12) is substantially impermeable to water and substantially permeable to oxygen and carbon dioxide so as to facilitate:

a) aeration of the incubation cavity through the semipermeable membrane (12) and
b) substantial retainment of water in the incubation chamber.

In a preferred embodiment the reservoir has a port by which its contents can be replenished (21). Usually a water or aqueous solution will be used, but this solution may contain additives for special purposes (e.g. antibiotics and antimycotics for protection of the incubation chamber and conduit from infection).

In a preferred embodiment of the present invention the lid (13) is attached to the second end of the wall in a easily detachable manner (22). In this respect the incubation cavity may be lockable by virtue of a reversible snap-locking mechanism. In this way it is possible to open the incubation cavity, withdraw a portion of the Spheriods being cultivated, and close the incubation chamber in less than 10 minutes.

For the embodiments of the first and second aspect of the present invention is preferred that one or more of the following characteristics is/are satisfied:

- the incubation cavity and the walls have a substantially cylindrical shape;

- the bioreactor is adapted for rotation around a horizontal, rotational axis by associated rotation means, said rotational axis being substantially coincident with a central axis through the incubation cavity;

- the lid (13) has at least one sealing port accessible with a tube for introducing or removing biological material to/from the incubation cavity;

- removal of a portion of the Spheriods from the incubation cavity does not adversely affect the growth or physiological function of the Spheriods remaining in the incubation cavity;

- it is possible to cultivate cells in the incubation cavity for about a week, more preferably several weeks or most preferably longer than a month (where the growth medium is changed appropriately);

- the semipermeable membrane is permeable to small molecules, but can prevent bacteria, mycoplasma or other living organisms to get through; and

- the incubation cavity has an internal fluid volume selected from the group consisting of: about 25 µl to about 1000 ml, about 50 µl to about 500 ml, about 100 µl to about 200 ml, and about 200µl to about 100ml.

Brief description of the drawing

Some embodiments of the present invention are illustrated by the accompanying Figures, where

Figure 1 is a schematic cross-sectional drawing of a bioreactor according to the first aspect of the invention.

Figure 2 is an exploded version of figure 1 where the three main components are separated.

Detailed description of the invention

Definitions

As used herein, the following terms have the following meanings:

The terms “semipermeable membrane” refer to a membrane that can be penetrated by some, but not all, chemical or biological substances.

The term "incubation cavity" refers to that portion of a bioreactor in which cell cultures, tissue biopsies, cell clusters, spheroids, tissue-like structures, “Spheriods" or similar samples are grown, differentiated, incubated, or otherwise cultured. The term "incubation cavity" is used interchangeably with "incubation chamber" and "incubation compartment."

The term “substantially impermeable to water” is used to describe characteristics of membranes of the present invention and refers to a membrane that exhibits a high degree of repulsion of water and water-like molecules in gas and/or liquid phase.

The term "almost completely impermeable to water" is used to describe characteristics of membranes of the present invention and refers to a membrane across which the water flow rate at 1 bar is not greater than 0.1 mL/min/cm^2.

The term “substantially permeable to oxygen and carbon dioxide” is used to describe characteristics of membranes of the present invention and refers to a membrane across which air will readily pass.

The term “relative retainment” is used to describe conditions arising from operation of a bioreactor of the invention with an aqueous solution or suspension in the incubation cavity and refers to the relative amount of residual substance initially present. For example, the relative retainment of water in the incubation cavity (with a flexible membrane) may be calculated as the volume of the cavity after operating the bioreactor divided by the volume of the cavity at the beginning of operating the bioreactor.

The term “toxic” has the usual meaning known in the art. A "toxic" substance is a substance that in the amount present in the chemical compositions as defined above can impair the functioning of, or cause structural damage to a cell, tissue or organism.

The term “predetermined toxicity” relates to both toxic and non-toxic substances. As Paracelsus stated in the 16th century, “All things are poison and nothing is without poison, only the dose permits something not to be poisonous”. The toxicity type of a substance may e.g. be determined according to the toxicity typing scheme of the Food and Drug Administration (FDA) of the United States of America. According to this scheme, the predetermined toxicity of a substance may belong to toxicity type A, B, etc. or may be non-toxic.

The term “cell cultures” refers to any kind of cells, tissue biopsies, cell clusters, tissue-like structures, “Spheriods" or similar samples obtained or initially cultured by any method known in the art.

The term “cells” refers to primary, immortal or stem cells from any type of living organism, whether archaea, prokaryote or eukaryote, and also includes viruses or other entities that need living cells to replicate.

The term "microgravity bioreactor" refers to a bioreactor adapted for rotation.

The term "incubating under microgravity conditions" refers to growth of cell cultures in a bioreactor adapted for rotation while rotating said bioreactor about a substantially horizontal central axis at a rate that suspends one or more cell cultures in a liquid culture medium and continuing such rotation for a time period that permits growth of said one or more cell cultures.

The term "means of relative retainment of water" is used to describe features of a bioreactor and refers to any means other than perfusion that is used in combination with a membrane that substantially confines the incubation chamber to achieve relative retainment of water in the incubation chamber or, in the alternative, to any single membrane that substantially confines the incubation chamber across which membrane the water flow rate at 1 bar is not greater than 0.1 mL/min/cm^2.

Preferred embodiments

In preferred embodiments, the semipermeable membranes utilised in the present invention allow passage of molecules up to a certain molecular weight or size. Semipermeable membranes with a well-defined pore size are known to the person skilled in the art and are commercially available. In preferred embodiments of the invention, semipermeable membranes may be permeable to molecules up to a predetermined molecular weight, such as 50 kDa, 100kDa, 150 kDa, 200 kDa or 250 kDa. Alternatively, the permeability of semipermeable membranes may be determined by the pore sizes therein. The pore size of semipermeable membranes may be less than or equal to 0.5 µm, such as less than or equal to 0.3 µm, preferably less than or equal to 0.2 µm, even more preferably less than or equal to 0.1 µm, and most preferably less than or equal to 0.05 µm. A membrane with pore sizes of 0.22 µm is generally considered sufficient to prevent bacteria and mycoplasma from crossing the membrane. A wide variety of membranes can be used. These could be made of materials selected from (but not limited to) the group consisting of polytetrafluroethylene (PTFE), Polyvinylidene fluoride (PVDF), silicon rubber, foam plastics, radiation treated plastic, and similar materials. In one preferred embodiment, a TE 35 filter from Whatman or a Zefluor filter (cat. no. 66142 from Pall Life Sciences can be used.

In preferred embodiments of the invention, the water flow rate at 1 bar accross membranes that are "substantially impermeable to water" and "substantially permeable to oxygen and carbon dioxide" is not greater than 50 ml/min/cm^2, preferably not greater than 40 ml/min/cm^2, more preferably not greater than 30 ml/min/cm^2, even more preferably not greater than 20 ml/min/cm^2, most preferably not greater than 10 ml/min/cm^2. It will be readily understood by those skilled in the art that water permeability can be expressed in other units, which can be converted into ml/min/cm^2.

In preferred embodiments of the invention, the air flow rate at 3 mbar accross membranes that are "substantially impermeable to water" and "substantially permeable to oxygen and carbon dioxide" is at least 5 ml/min/cm^2, preferably at least 10 ml/min/cm^2, more preferably at least 15 ml/min/cm^2, even more preferably at least 20 ml/min/cm^2, most preferably at least 25 ml/min/cm^2. It will be readily understood by those skilled in the art air flow can be expressed in other units, which can be converted into ml/min/cm^2.

Membranes comprised of a wide variety of materials can be used, that are "substantially impermeable to water" and "substantially permeable to oxygen and carbon dioxide," including but not limited to membranes well known in the art comprised of polytetrafluoroethylene (PTFE), Polyvinylidene fluoride (PVDF), silicon rubber, foam plastics, radiation treated plastic or similar materials. One example of a suitable membrane is commercially available from Whatman under the trade mark "TE 35®," a PTFE membrane with polyester support having characteristics (quoted by the manufacturer): pore size 0.2μM, thickness 190μM, water flow rate at 0.9bar of 20 ml/min/cm^2 when measured with ethanol, air flow rate 15 ml/min/cm^2 at 3 mbar and bubble point 1.4 bar. Another example of a suitable membrane is commercially available from Millipore under the trade mark "SureVent®," a PVDF membrane having characteristics (quoted by the manufacturer): pore size 0.22μM, thickness 100-150μM, water breakthrough 45 mbar, air flow rate >1 slpm /cm^2 at 10psi. In some embodiments, the membranes can be Millipore 0.22μm "Durapel" membranes or Whatman TE 35 and TE36 membranes.

In preferred embodiments of the invention, the water flow rate at 1 bar accross membranes that are "almost completely impermeable to water" while "substantially permeable to oxygen and carbon dioxide" is not greater than 0.1 ml/min/cm^2, even more preferably not greater than 0.05 mL/min/cm^2, still more preferably not greater than 0.04 ml/min/cm^2, even more preferably not greater than 0.03 ml/min/cm^2, still more preferably not greater than 0.02 ml/min/cm^2, most preferably not greater than 0.01 ml/min/cm^2. It will be readily understood by those skilled in the art that water permeability can be expressed in other units, which can be converted into ml/min/cm^2.

In preferred embodiments of the invention, the air flow rate at 3 mbar accross membranes that are "almost completely impermeable to water" while "substantially permeable to oxygen and carbon dioxide" is at least 5 ml/min/cm^2, preferably at least 10 ml/min/cm^2, more preferably at least 15 ml/min/cm^2, even more preferably at least 20 ml/min/cm^2, most preferably at least 25 ml/min/cm^2.

Membranes comprised of a wide variety of materials can be used, that are "almost completely impermeable to water" while "substantially permeable to oxygen and carbon dioxide" including but not limited to membranes initially prepared for ultrafiltration purposes that have very low water permeabilities at atmospheric pressures, for example, due to low porosity and high hydrophobicity. Such membranes include ultrafiltration

membranes commercially available from Amicon under the trademark "YM1®" and from Pall Corp. under the trademark "Omega 1K. ®". Other suitable membranes include thermoplastic ultrafiltration membranes prepared by thermally induced phase inversion process of semi-crystalline materials such as poly(ether ether ketone)(PEEK) and poly(phenylene sulfide)(PPS), as described by [Micro- and ultrafiltration film membranes from poly(ether ether ketone)(PEEK). Sonnenschein M, Journal of Applied Polymer Science 1999 74:1146]. Immobilized, stable supported liquid membranes (SLM) can also be used comprising a suitable oligomeric or polymeric liquid membrane material immobilized within a solid, microporous, hydrophobic support, such as the system disclosed in US 5507949.

In a preferred embodiment of the invention, cells that can be applied in the context of the present invention are selected from the group consisting of hepatocytes, adipocytes, kidney cells, muscle cells, or similar cells, liver tissue, fat tissue (brown or white), liver biopsies, kidney biopsies, muscle biopsies, ovarian follicles, islets of Langerhans, and all cancer cells derived therefrom.

In a particularly preferred embodiment of the invention, cells that can be applied in the context of the present invention are hepatocytes, in particular human hepatocytes.

Figure 1 is a schematic cross-sectional drawing of a bioreactor 10 according to the first aspect of the invention. The bioreactor 10 has a high degree of rotational symmetry around a horizontal axis as viewed in Figure 1. The reactor comprises an incubation cavity 15 for incubation of cells, tissues etc. The incubation cavity provides, in conjunction with an essentially cylindrical wall, a semipermeable membrane (11) in the first end of the wall, and a sealable lid (13) in the second end of the wall, a substantially closed confinement (using optionally an O-ring (22). The incubation cavity (15) provides, in conjunction with a semipermeable membrane (12) (also known as a sterile filter) (11), a substantially closed confinement for incubation of cells etc. In order to provide nutrients and/or fresh fluid culture medium, the semipermeable membrane 11 is permeable to molecules up to a predetermined molecular weight, such as 50 kDa, 100kDa, 150 kDa, 200 kDa or 250 kDa. Standard dialysis membranes can fulfil these requirements. Alternatively, the permeability of the semipermeable membrane 11 is determined by the pore sizes therein. The pore size of the semipermeable membrane 11 may be less than

or equal to 0.5 µm, such as less than or equal to 0.3 µm, preferably less than or equal to 0.2 µm, even more preferably less than or equal to 0.1 µm, and most preferably less than or equal to 0.05 µm. Sizes less than or equal to 0.2µm are preferable because of the need to prevent infections (e.g. bacteria, mycoplasma or yeasts) entering through the membrane. In this preferred embodiment the main purpose of the semipermeable membrane is to allow exchange of nutrients and waste products while excluding cells and bacteria from entering (or leaving) the incubation cavity. Thus a wide variety of membranes could be used for 11. These could be made of polytetrafluroethylene (PTFE), Polyvinylidene fluoride (PVDF), silicon rubber, foam plastics, radiation treated plastic or similar materials. Thereby an inflow of nutrients and fluid culture medium into the incubation chamber is provided while at the same time providing retainment of cells and cellular aggregates and their protection from external infection in the incubation cavity 15. The incubation cavity 15 has an internal fluid volume of about 25 µl to about 1,000 ml. Preferably, the fluid volume of the incubation cavity 15 is about 50 µl to about 500 ml, more preferably about 0.1 to about 200 ml. Small sizes significantly reduces the cost of use and the amount of materials (both organic and inorganic) necessary for successful operation. Small size will facilitate close-up monitoring of the cells (e.g. by remote camera or microscope), and automated processing.

The larger sizes will allow for the preparation of larger amounts of spheriods which have uniform characteristics which may be used for regenerative medicine, the preparation of large amounts of metabolites (e.g. from drugs or other compounds) or for the subdivision into small aliquots for further experimentation.

In the front of the bioreactor 10, a transparent section 14 is located so that the cultivation of cells etc. may be monitored and assessed visually, either manually or automatically with e.g. a camera, from outside of the bioreactor 10. The transparent section 14 could be made of glass, plastic or any other suitable materials being both transparent and biologically and chemically inert with respect to the cell cultivation process. Preferred materials would include (but not be limited to) various types of glass, polystyrene, polycarbonate, polypropylene, polyethylene and Polymethyl methacrylate (PMMA). Suitable variants of polymethyl methacrylate (PMMA) are available commercially including products marketed under the trademarks/trade names Perspex®, Plexiglas®, Lucite®, Acrylite®, Rhoplex®, and Oroglas®. Any embodiment of the bioreactor could be made in whole or in part from such transparent materials.

The incubation cavity 15 preferably has a substantially cylindrical shape but other shapes are also possible, e.g. elliptical shapes, spherical shapes etc. Preferably, the bioreactor 10 is adapted for rotation around a horizontal, rotational axis by associated rotation means (not shown) to facilitate growth of the cells in the cavity 15. The rate of rotation is adjusted to maintain the cells or Spheriods in suspension and this rate has to be varied as the size of the Spheriods increases. The person skilled in the art will know how to adjust the rotation speed in order to maintain the cells or Spheriods in suspension.

Figure 2 is an exploded version of figure 1 where the three main components are separated.

Claims

1. A bioreactor adapted for rotation, the reactor comprising

- an incubation cavity having a wall section, and a first and second end, the incubation cavity providing, a semipermeable membrane (11) constituting the first end of the incubation cavity, and a sealable lid (13) consituting the second end of the incubation cavity, thus providing a substantially closed confinement, said semipermeable membrane (11) being permeable to molecules up to a predetermined molecular weight or size, allowing a gas exchange into and out of the incubation chamber and retainment of aqueous media, cells and cellular aggregates in the incubation cavity,

- an equilibrium chamber (16), said equilibrium chamber providing a volume for an exchange of gasses like oxygen and carbon dioxide for one or more cell cultures, tissue biopsies, or cell clusters resident in the incubation cavity,

wherein said lid (13) is removably attached to the wall section so as to provide access to the entire incubation cavity.

2. A bioreactor according to claim 1, wherein the lid (13) is attached to the wall section in a lockable manner.

3. A bioreactor according to claim 1, wherein the incubation cavity and the wall section have a substantially cylindrical shape.

4. A bioreactor according to claim 3, wherein the bioreactor is adapted for rotation around a horizontal, rotational axis by associated rotation means, said rotational axis being substantially coincident with a central axis through the incubation cavity.

5. A bioreactor according to any one of the claims 1-4, wherein the lid (13) has a sealing port accessible with a tube for introducing or removing biological material to/from the incubation cavity.

6. A bioreactor according to claim 1, wherein the semipermeable membrane is permeable to small molecules, but can prevent bacteria, mycoplasma or other living organisms to get through.

7. A bioreactor adapted for rotation, the bioreactor comprising

an incubation cavity, the incubation cavity providing, in conjunction with an essentially cylindrical wall, a semipermeable membrane (11) in the first end of the wall, and a sealable lid (13) in the second end of the wall, a substantially closed confinement, wherein said lid (13) is removably attached to the second end of the wall so as to provide access to the entire incubation cavity,

-a reservoir chamber comprising an aqueous liquid, the chamber comprising a device to enhance evaporation from the reservoir chamber into the equilibrium chamber and
-equilibrium chamber providing a substantially closed conduit from the semipermeable membrane (11) to the evaporation enhancing device and walls of the aqueous reservoir chamber,

wherein the semipermeable membrane (11) is substantially impermeable to water and substantially permeable to oxygen and carbon dioxide so as to facilitate:

a) aeration of the incubation cavity through the semipermeable membrane (11) and the equilibrium chamber, and
b) substantial retainment of water in the incubation chamber.

8. A bioreactor according to claim 7, wherein evaporated water from the reservoir chamber and/or the incubation chamber provides a relative humidity in the equilibrium chamber selected from the group consisting of:
at least 50%, at least 70%, and at least 90%.

9. A bioreactor according to claim 7, wherein the bioreactor, when being operated at 37°C with an aqueous solution or suspension in the incubation cavity and in the reservoir chamber, has a relative retainment of water in the incubation cavity after 3 days selected from the group consisting of:
at least 80%, at least 90%, and at least 95%.

10. A bioreactor according to claim 7, wherein the incubation cavity and the wall has a substantially cylindrical shape.

11. A bioreactor according to claim 10, wherein the bioreactor is adapted for rotation around a horizontal, rotational axis by associated rotation means, said rotational axis being substantially coincident with a central axis through the incubation cavity.

12. A bioreactor according to claim 7, wherein the incubation cavity has an internal fluid volume selected from the group consisting of:
about 25 µl to about 1000 ml, about 50 µl to about 500 ml, and about 100 µl to about 200 ml.

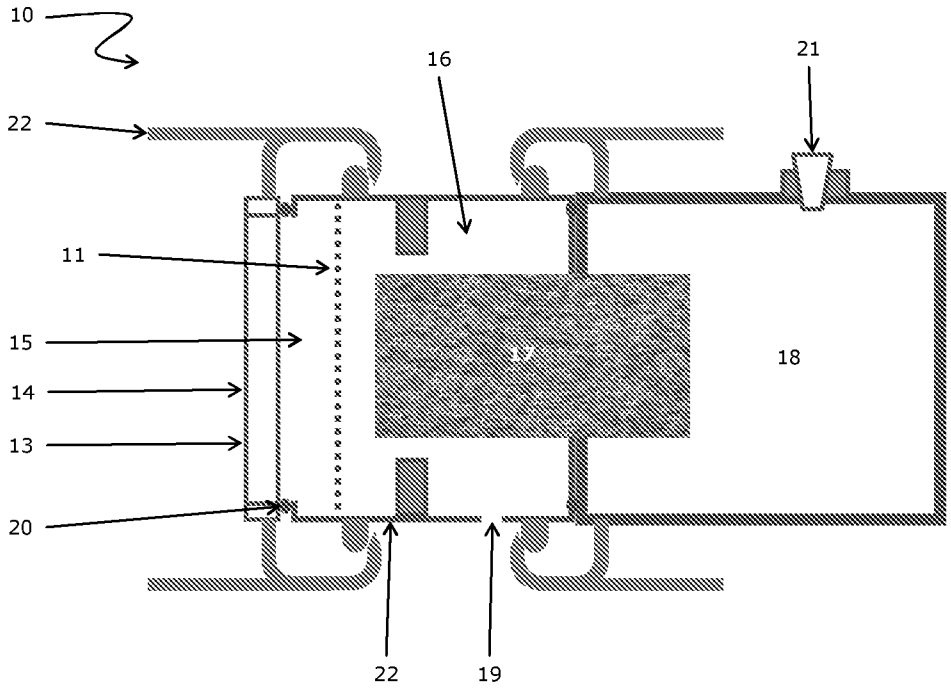

Figure 1

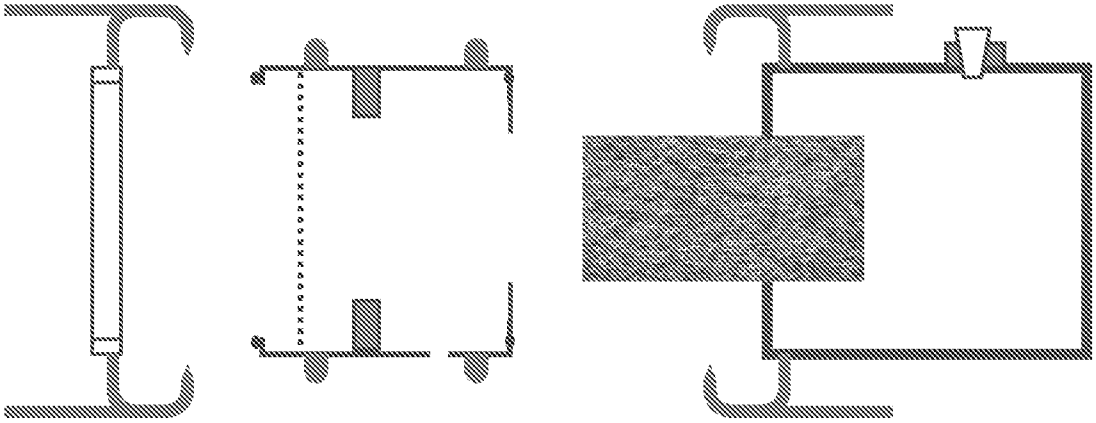

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2011/050466

A. CLASSIFICATION OF SUBJECT MATTER
C12M 3/06 (2006.01), C12M 1/10 (2006.01), C12M 1/12 (2006.01)

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)
IPC: B01D, C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
DK, FI, NO, SE: C12B, C12M

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPODOC, WPI, ENGLISH FULLTEXT PATENT DATABASES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US4142940 A (MODOLELL et. al) 1979.03.06, see whole document.	1-3, 6
X	US4839292 A (CREMONESE) 1989.06.13, see whole document, especially fig. 2-3, and col. 4.	1-2, 6
X	US5576211 A (FALKENBERG et al.) 1996.11.19, see whole document, especially fig. 1 and col. 4.	1-6
X	US5153131 A (WOLF et al.) 1992.10.06, see whole document, especially fig. 5 and col. 9-10.	1-6
X	WO2008073313 A2 (WILSON WOLF MANUFACTURING CORPORATION) 2008.06.19, see whole document, especially p, 11-12 and 24 and fig. 1A-1B.	1-2, 6
X	JP2009273399 A (OMORI TAKASHI) 2009.11.26, see PAJ abstract and figures.	1-6

☒ Further documents are listed in the continuation of Box C. ☒ 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
05/03/2012	07/03/2012
Name and mailing address of the ISA/ Nordic Patent Institute, Helgeshoj Allé 81, 2630 Taastrup, Denmark Facsimile No. +45 43 50 80 08	Authorized officer Isabelle Rivas Telephone No. +45 43 50 81 86

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2011/050466

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2011/050466

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US5935845 A (KOONTZ) 1999.08.10, see whole document, especially col. 7, l. 1-13.	1-6
A	JP2009118820 A (DEUCE:KK) 2009.06.04, see PAJ abstract and figures.	-
A	US2005148068 A1 (LACEY et al.) 2005.07.07, see whole document	-

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/DK2011/050466

Patent document Cited in search report	Publication date	Patent family member(s)	Publication date
US4142940 A 19790306		FR2323759 A1 19770408 FR2323759 B1 19810109 GB1498557 A 19780118 CH603794 A5 19780831 SE7609531 A 19770314 SE7609531L L 19770314 SE421075 B 19811123 SE421075 C 19820304 JP52038082 A 19770324 JP55038112B B 19801002 JP1045899C C 19810528 DE2541000 B1 19761111	
US4839292 A 19890613		CA1306714 C 19920825 EP0307048 A2 19890315 EP0307048 A3 19891018 EP0307048 B1 19931222 DE3886483T T2 19940714 AT98988T T 19940115 US4839292 B1 19940913	
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US5153131 A 19921006		US5846807 A 19981208 US6117674 A 20000912 US5962324 A 19991005 US5851816 A 19981222 US5496722 A 19960305	

Continuation of Box III:
The separate groups of inventions are considered to be:

A: Claims 1-6 relating to a bioreactor adapted for rotation comprising an incubation cavity and an equilibrium chamber; the incubation cavity being defined by a wall section with one end and another end, a semipermeable membrane constituting one end of the wall section and a sealable lid constituting the other end of the wall section wherein the lid is removably attached in a lockable manner to the rest of the wall section; the membrane being permeable to molecules up to a predetermined molecular weight, allowing exchanges of gasses such as oxygen and carbon dioxide into and out of the incubation cavity and retainment of water in the incubation cavity; and the equilibrium chamber providing a volume for gas exchange with the incubation cavity.

B: Claims 7-12 relating to a bioreactor adapted for rotation comprising an incubation cavity, a reservoir chamber and an equilibrium chamber; the incubation cavity being defined by a cylindrical wall with one end and another end, a semipermeable membrane at one end of the cylindrical wall and a sealable lid removably attached to the other end of the cylindrical wall; the membrane being substantially permeable to oxygen and carbon dioxide and substantially impermeable to water; the reservoir chamber comprising aqueous liquid and a device for enhancing evaporation from the reservoir chamber into the equilibrium chamber; and the equilibrium chamber providing a closed conduit from the semipermeable membrane to the evaporation enhancing device and walls of the reservoir chamber.

The common subject matter of inventions A and B is a rotatable bioreactor comprising an incubation cavity and an equilibrium chamber; the incubation cavity being defined by a wall, a semipermeable membrane at one end of the wall section and a sealable lid removably attached to the other end of the wall section; and the membrane allowing exchanges of gasses such as oxygen and carbon dioxide and retainment of water in the incubation cavity. Such a bioreactor is known from US 4142940 A and therefore not patentable.

As there are no common special technical features between inventions A and B the requirement of unity is not fulfilled, according to Rule 13.2 PCT.

Patent Family Annex
Information on patent family members

International application No.
15/12/2010

Patent document Cited in search report	Publication date	Patent family member(s)	Publication date
WO2008073313 A2	20080619	WO2008073314 A3 WO2008073314 A2 WO2008073313 A3 US2008176318 A1 US2008227176 A1 JP2010512149 A JP2010512148 A EP2089507 A2 EP2092052 A2 CN101611133 A CN101611132 A CA2671967 A1 CA2671812 A1 AU2007332870 A1 AU2007332869 A1	20080731 20080619 20080731 20080724 20080918 20100422 20100422 20090819 20090826 20091223 20091223 20080619 20080619 20080619 20080619
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US2005148068 A1	20050707	WO2005066328 A1 JP2007516726 A EP1704222 A1 EP1704222 B1 US7078228 B2	20050721 20070628 20060927 20090715 20060718