

TITLE: "DEVICE FOR CULTURING AND TRANSPORTING CELLS."**FIELD OF THE INVENTION:**

This invention relates to the field of biology and more precisely to the field of the cell cultures. The present invention relates to the disposable device for culturing and transport of cells. The present invention involves a new disposable device, which can be used to support a film, membrane or sponge for the purpose of isolation, expansion, transportation and/or transplantation of cells

BACKGROUND OF THE INVENTION

Cell culture is a process of growing and maintaining cells under laboratory conditions. This process involves isolating the cells from animals or plants, which are then grown and maintained alive in laboratory devices using artificial culture media to support their basic biological functions. Cell cultures are derived from either primary tissue explants or from cell suspensions. Depending on the nature of the isolated cells, the cells could be either of adherent or suspension type. Various methods are known in the art for growing cells in culture, both on large scale and small scales.

A lot of culture devices have been designed or fabricated for adaptability to the challenging needs of maintaining and transport of cell cultures.

The cell culture devices are commercially available in several shapes and designs, the conventional form being culture flasks and dishes. The flasks are provided with caps, while the dishes are available as individual or multi well dishes, which are provided with lids.

Typically available individual culture dishes contain a base for receiving the cultures and a lid to cover it. The primary aim of the culture dish is to support cell growth. In the cell growth application, the cells isolated from the tissues are seeded in the culture device along with the nutrient media to nourish the cells, followed by its incubation in a controlled environment. A transparent material like

polystyrene is used in the manufacture of the cell culture dishes to enable visualization of the cultured cells under the microscope.

Contamination being one of the principal challenges for successful cell and tissue culture techniques; cell culture dishes are required to provide protection against microbial contamination. In case of culture dishes the contamination is minimized by typically using sterilized dishes. The lid serves as a barrier to microbes, and prevents evaporation of the media. Cells are maintained in liquid media that support their viability. Transport of live cells in commercially available dishes is not feasible as the absence of a tight seal in the dishes would result in high risk of contamination, as well as media spillage would result in cell death. Specific cells are cultured in dishes under sterile conditions to a desired state of growth for specialized purposes as in the case of medical, scientific or industrial requirements. However, following growth, these cells have to be removed from their growth environment, cryopreserved and shipped to the required centers in liquid nitrogen. At the centers, the cells have to be revived and re-cultured. Besides, the time and resources required in re-culturing the cells results in significant delays. The hostile transport conditions results in considerable loss in viability of cells as well as high costs in shipment.

Current cell based therapies as in the case of skin, cornea, cartilage etc, require the delivery of cells to the site of transplantation. This is feasible by growing the cells either as monolayers on suitable membranes as described in US patent No 5,693,332) and/or differentiating into multi layers as published in US patent publication 2003/0208266A1) before transplantation, or the cells can be cultured on foams or sponges which can then be transplanted. The important factors involved in membrane based cell delivery system include, cell attachment, cell viability, cell density and control over differentiation. The delicate relationship between the cell layers and the membrane can be disturbed during shipment due to movement shear, tear and/or otherwise mechanical damage to the individual cells and their contact with each other, thus destroying the graft and rendering them useless. Although the cells can be cultured on membranes in culture

dishes, their transport in the same dishes is not feasible because of the design of the dishes.

To overcome the contamination with culture dishes, culture flasks were developed. These flasks typically have a culture chamber, a small tubular opening located at one end of the flask and a corresponding closure. The principal teachings of various designs of culture flasks are described in U.S. Pat. Nos. 4,334,028, 4,851,351 and 5,398,837. As these culture flasks require a relatively large amount of expensive media in order to be filled completely, these flasks are not cost effective. These flasks are also not suitable for culturing cells on films /membranes as they cannot be placed inside the flask to grow cells for transplantation.

Further to these above dishes and flasks, the technology of roller bottles was developed. The roller bottles offer a larger surface area for cell attachment and growth. However, due to the presence of high shear forces associated with these roller bottles, this technology was suitable only for those cells that are capable of remaining adhered to the wall of the roller bottle. Also the maintenance of cell cultures in roller bottles for longer periods poses a challenge due to constant high shear force environment and possible contamination. US. Patent Nos. 3,450,598 involves attaching and growing cells on the interior surface of plastic or glass roller tubes and bottles. The patents directed to the roller bottles include U.S. Pat. Nos. 5,527,705 and 4,962,033. The improved technology of roller bottles with increased surface area for cell attachment is described in U.S. Pat. No 5,010,013 wherein corrugated channels are added to the interior surface area of the roller bottle. However, the roller bottle technology does not provide for sufficient aeration to the cells thus posing a limitation to their viability.

More recently, the use of hollow fibers or capillaries has been disclosed as a support matrix for the propagation of cells. The teachings of this technology is described in U.S. Pat Nos. 3,821,087, 3,883,393, 3,997,396, 4,184,822,

4,200,689, 4,206,015, 4, 220,725, 4,391,912 wherein various apparatus and methods for the in-vitro growth of cells on semi-permeable tubular membranes or capillaries are disclosed. The cells are initially allowed to settle onto the surfaces of the capillary walls in a nutrient medium. The nutrients diffuse from the perfusing medium through the capillary walls and are utilized by the cells. Cell products diffuse from the cells through the capillary walls and into the perfusate, from which cell products may be recovered. However, it is found that the nutrient media flow through the hollow capillaries prevents complete penetration of the capillary bundle by the cells and the resultant undesirable gradient of the medium flow leads to incomplete utilization of the available capillary surface for cell attachments and cells become unevenly distributed along the surface. Further these devices require high media circulation rates to supply adequate oxygen to the cells. Also these devices have an additional disadvantage of being mechanically complex, difficult to assemble and being unduly large.

Considering the disclosures of the prior art, there is a continuing need for an efficient and economical device for culturing, expansion and easier transport of cell cultures.

Accordingly, it would be desirable to provide a method and disposable device suitable for culturing and transporting cells attached to membranes, sponges/foams that are ready to use upon delivery.

The present invention solves the above setbacks and for the first time, provides a specially designed device, which is compact, portable, commercially viable and easy-to-use device with a provision to load membrane's of choice into the device for the purpose of culturing the cells and transporting it to the recipients location for transplantation of cells which aid in regenerating damaged tissues.

OBJECT OF THE INVENTION

It is the object of the present invention to design a device for cell cultures, which is disposable, sterile and safe for culturing and transporting adherent cells.

It is the object of the present invention to design a device in a manner where the chances of the membrane crinkling are avoided.

The rings of the device of this present invention are designed in the manner to maintain the tautness of the membranes during culture.

Since the prior art devices such as dishes and flasks are not suitable for transport under sterile conditions, it is the primary objective of the present invention to design a device, which is convenient to handle and ensures transportation of cells free from microbial contamination.

The device of the present invention aims to employ a low volume of media. Hence it is a cost effective device and poses to be beneficial for transport of the cells without any spillage.

The device of the present invention is designed in such a manner that it accommodates a gasket that prevents leakage during the transportation of the cells thus maintaining the viability of the cells. Thus the device of the present invention serves a dual purpose for culturing of cells and also for transport of cells

The device of the present invention has been fabricated with a safe, non-toxic, biocompatible material made of polyethylene, polystyrene, polyethylene terephthalate, polycarbonate. These medical grade materials are transparent thus allowing visualizing of the cells under microscope.

The device of the present invention has been designed in such a way with minimum space requirements for easy and cheaper transport.

Still further the object of this invention is to present a device suitable and adaptable for transplantation of cells in cell based therapies.

The device of the present invention is a commercially useful means to transport cells that maintain their viability, that it is still useful as a transplant, implant, or graft after transport.

SUMMARY OF THE INVENTION

The present invention provides a disposable device for culturing and/or transporting cells in mono- or multi layers. One illustrative embodiment of the invention, a square shaped culture device is provided.

The device has two components:

- 1) a base plate and
- 2) a top lid.

The base plate is provided with an outer wall extending downwards and a flat top portion carrying a circular housing device, which also extends downwards. The housing device in the base plate, which is contiguous with an extended groove, has a bottom wall, a rim and a sidewall extending downwardly from the flat portion of the base plate. The depth of the housing device is in the same plane as that of the exterior wall of the base plate. The base of each wall in the middle of the base plate is provided with a notch for fixation of the snap.

The desired membrane/sponge is disposed in the interior of the housing device and held in place with a ring. A groove, which is provided for a silicon gasket runs just outside the housing device in the base component.

The ring of the device of the present invention serves multipurpose functions as follows:

The membranes made of biological materials like amniotic membrane or polymers would tend to crinkle leading to uneven distribution of cells on the membrane. The provided ring would ensure that the membranes are held taut during seeding of cells (if culturing is part of the purpose to which the device is meant for.)

Some membranes such as chitosan / PLA have a tendency to float in the dish resulting in the cells coming directly in contact with the lid of the housing device. The provided ring ensures that the membranes are fixed to the bottom of the housing device.

Prior to transplantation, the membranes/sponges are generally washed with buffered solutions to remove traces of transport media. The presence of the ring ensures that the membrane does not crinkle and damage the cells.

It is generally advantageous in clinical setting that these membranes are held taut so as to maintain a smooth surface enabling uniform cell attachment and growth to avoid crinkling of the membranes as such crinkling would result in cells attaching in the crevices leading to non-uniform cell attachment.

The illustrated square lid component is provided with an outer wall, extending downwardly from the top. The flat portion of the lid is provided with a well, which extends downwardly. The circular base of the well is contiguous with a groove. The well and the groove provided in the lid fits exactly into the housing device provided in the base plate component and serves as the lid of the housing device. A snap is provided at the middle of each wall of the lid that fits the notch in the base plate. The inner side of the lid is provided with a protrusion running around the well, which during closure of the device aligns with the circular groove present on the flat base plate.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1: Shows a perspective assembly view of the invention for culturing/transport of cells on membranes.

Fig.2: Top view of the base.

Fig.3: Side view of the base plate.

Fig.4: View of the ring

Fig.5: Side view of the lid.

Fig.6: Lower view of the lid.

Fig.7: Illustrated view of the notch

Fig.8: Completely assembled view of the device.

DETAILED DESCRIPTION

Several terms used in the invention are defined as follows.

Definitions:

The term "device" as used herein describes any container, apparatus, or vessel, which are intended for cell culture growth and transport.

Throughout the specification of the application, various terms are used such as "top", "bottom", "upper", "lower", diagonal and the like. These terms are words of convenience in order to distinguish between different elements. While such terms are provided to explain the cell culture device relative to positions in which the cell culture device may normally be used, such terms are not intended to be limiting as to how the different elements may be utilized.

The term "cells" is used herein, for the purposes of the specification and claims, to mean one or more of live cells, cells comprising cellular aggregates, or an organized structure or network of cells forming a tissue, as apparent to those skilled in the art.

The term "medium or media" is used herein, for the purposes of the specification and claims, to mean a liquid solution which is used to provide sufficient nutrients

(e.g., vitamins, amino acids, essential nutrients, salts, and the like) and properties (e.g., osmolarity, buffering) to maintain living cells (or living cells in a tissue) and support their growth.

The term "membrane" is used herein for the purpose of the specification and the claims, to mean sheet, film, foam, sponge or the like. The membrane used in the invention may either be of Biological Material, Semi-synthetic material or Synthetic material. These membranes can be easily detached or removed from the device.

The term "Biological Material" include but not limited to Human amniotic membrane, acellularised dermis, small intestinal submucosal (SIS) membrane and/or combination thereof. The term "Semi synthetic Material" includes but not limited to Poly Lactic Acid (PLA), Poly Glycolic Acid (PGA), Hyaluronic Acid and/or a combination thereof.

The present disclosure relates to a cell culture device for culturing cells that may be used for transplantation.

The illustrated embodiment of the present invention is not only restricted to a square shape but could also be circular, hexagonal or triangular. The device comprises of moldable parts, produced from a variety of medical grade materials, selected from polyethylene, polystyrene, polyethylene terephthalate, polycarbonate and the like. The grade of the material chosen is crystal clear that enables visualization of the cells cultured on membranes, and allows inspection and photography of the cells throughout the experiments using high-end microscope.

A device of the present invention for culturing and/or transporting cells on membranes or sponges/gels is shown in Fig 1. The device assembly includes the

following components, a base plate component (1), a ring (2), a silicon ring (3) and a lid (4). Membrane with the cells (5) is not a part of the device.

Fig 2: Illustrates the top view of the base plate component of the device comprising a housing device (6). The bleb like region (7), which is seen contiguous with the housing device, is a provision for changing the media easily wherein a pipette would be used to drain or add the media to the housing device, without disturbing the cells. The bleb portion can also be utilized for rinsing off the transport media from the final membrane or sponge before transplantation. The bleb like area(8) ensures that air trapped in the area above the ring, moves into the groove thereby ensuring that no air bubbles are present in the housing portion of the device. The groove (8) running around the housing device is provided for fixing the silicon gasket, to ensure airtight as well as leak proof conditions are maintained during transportation of the cells. The rim (9) skirts the outer wall (10) of the base plate and serves as a support during the opening and closing of the device. The notch (11) seen in the middle of each wall is for fixing of the snaps (15) to the base plate.

The side view of the base plate is illustrated in Fig.3, wherein the outer wall (10) of the base plate along with the rim (9) is indicated. The base (12) of the housing device (6) is in the same plane as the base of the wall (13) of the bottom device but higher than the rim(9). The notch (11) is provided in the middle of each wall of the base device for locking the corresponding snaps (15) provided in the lid.

Fig. 4 shows an illustrated view of the ring. The height of the ring is such that on placing the ring inside the housing device and closing the lid, the base of the well located on the lid sits flush with the ring. The primary aim of the ring is to hold the membrane taut during cell culture and/or transport. The width of the ring is minimum to ensure larger area of cell coverage on the membrane/sponge.

Fig.5 shows the side view of the top lid. The design of the top lid is similar to the base plate component so that the well (14) in the lid sits flush on the ring inside the housing device. In the middle of each wall of the lid is a snap (15). This snap is designed to lock firmly with the notch (11) provided in the base plate.

The angle (16) seen in the illustrated fig.6 of the snap is for its easy locking and unlocking. The height of the snap (15) extends from the top of the lid to the base of the base plate. The number of snaps (15) provided per each device would vary with the geometric design of the plate for e.g. a (15) triangular device would have three snaps.

In one embodiment of the invention, the base and the lid include orientation features that are used to orient the lid on the base to ensure that the lid fits on the base. The well in the lid is contiguous with an extension that would fit in the bleb like area (8) present in the base. During closure, the lid is oriented in such a way that the extended portion on the well in the lid is aligned with the bleb like area (8) in the housing device.

Fig 7 illustrates the inner view of the top lid. The protrusion (17) skirting around the well (14) seen in the lid fits on the silicon ring, which is fixed in the groove (8) of the base plate. The pressure inserted by this on the silicon ring ensures an airtight and leak proof closure of the lid to the base.

A complete assembled view of the device is shown in Fig.8. A frosted area (18) is provided on the outer surface of the lid for the purpose of marking by the user.

The base, lid and the ring can be molded using standard molding techniques (e.g. injection molding) generally known in the art. Sterilization of the device can be done either by standard gamma radiation or by autoclaving. In some case, as in the case of laboratory research, the device can be reused after thorough washing and sterilization.

UTILITY OF THE CELL CULTURE DEVICE

For culturing the cells using the present device, a suspension of the cells of interest is added to the membrane/sponge supported by the ring inside the housing device. Alternatively, a suspension of the cells in the gelling solution can be added to the culturing device. After the setting of the gel in the device, the cells can be cultured in the following manner.

The quantity of the media added to the device is such that it fills the space provided inside the ring. The lid is placed loosely over the base plate and the device is incubated under environmentally controlled conditions. For changing the media, the lid is lifted, the base plate gently angled and media is drained with the tip of the pipette introduced into the bleb like area provided in the housing device. Fresh media can similarly be added to the housing device as mentioned above.

For transporting the cells using the present device, the cultured cells directly in the device or cultured in another device can be shipped after adding appropriate transport media to the cells. The silicon gasket is placed inside the groove and the lid snapped tightly to the base to ensure that air bubbles are not entrapped. The device of the present invention is leak proof and airtight. This device with the cells can be shipped under proper shipping conditions to the recipient.

On receiving the cells, the physician breaks the snaps and drains the transport media (as mentioned above for media change). The membrane/sponge is rinsed with buffered solution, the ring is lifted and the underlying membrane/sponge is transplanted directly on the patient.

The device of the present invention has the following features:

It aims to provide a disposable and ready to use single device for culturing and transport of cells.

It can be sterilized by conventional autoclaving or gamma radiation techniques.

The media required for this device is less than 10 ml, as compared to 25ml required by conventional cell culture flasks or dishes. Hence, the present device is cost effective.

The lid of the present invention prevents the microbial contamination as well as provides aeration for optimum cell growth.

A silicon gasket provided with this device, ensures airtight seal thereby preventing leakage during transportation of the cells thus maintaining the viability of the cells.

For culturing, the cells in the dishes, the dishes without silicon gasket can be incubated in normal carbon-dioxide incubators. This would provide for gas exchange to take place.. Further the composition matter of this device is of transparent material, which enables inspection of the cells under microscope throughout the experiments.

There is no delay in transplantation as this device provides ready to use cells for transplantation after washing of the cells.

The design of the present device is simple and occupies less space thus reducing the freight charges

Having thus described several illustrative embodiments of the invention, various alterations, modifications and improvements will occur to those skilled in the art. Such alterations, modifications and improvements are intended to be within the spirit and scope of the invention.

CLAIMS:

1. A device for culturing and transportation of the cells for therapeutic purpose comprising a

- i) a base with an outer wall, which includes
 - a) a cell-housing device with a wall at the sides and bottom,
 - b) an area for media exchange being contiguous with the housing device,
 - c) a groove for the silicon gasket running external to the housing device, d) atleast one notch in each wall of the base.
- ii) a ring to hold the membrane taut.
- iii) a lid with outer dimensions same as that of the base which is provided with snaps corresponding to the number of notches in the base.

2. The device as per claim 1, is of any shape such as circular, hexagonal, square, triangular or any other shape.

3. The device as per claim 1, comprises of moldable parts produced by injection molding techniques comprising a crystal clear, non toxic, inert and biocompatible material selected from the group of polyethylene, polystyrene, polyethylene terephthalate, polycarbonate, polypropylene and/or combinations thereof.

4. The device as per claim 1, wherein the device is sterilized by gamma radiation and/or by autoclaving .

5. The device as per claim 1, wherein the device is compact, portable, commercially viable and easy-to-use device with reduced installation accessories.

6. The device as per claim 1, wherein the culturing of cells comprises
 - a) Snap of the device is lifted to detach the lid.
 - b) Loading of the cells onto the device supported by the ring of the housing device,
 - c) Culture media is filled sufficient to the space provided inside the ring and
 - d) the lid is placed loosely.
 - e) The device is incubated under controlled conditions for culturing of the cells.
7. The cell culture device as per claim 7, wherein the cells can be loaded as a suspension in the gelling solution to the housing device and allowing the gel to set.
8. The cell culture device as per claim 7, wherein the cells can be loaded as a suspension on a membrane or sponge which is then fixed on to the housing device supported by the ring.
9. The device as per claim 1, wherein the transportation of cells comprises
 - a) Snap of the device is lifted to detach the lid.
 - b) Loading of the cells onto the device supported by the ring of the housing device,
 - c) Transport media is filled sufficient to prevent the formation of air bubbles inside the dish,
 - d) Silicon gasket is placed in the groove so as to ensure tight sealing of the lid.
 - e) the lid is fixed tightly.
 - f) The device is shipped to the recipient.
10. The device as per claim 9 for transport of cells wherein the membrane with the cells is placed in the housing device secured firmly with the ring and

silicon gasket and then lid is snapped tightly to the base; thereby minimizing the cell being damaged during transportation.

11. The device as per claim 1, comprising of the detachable ring ensuring that the membrane is fixed taut.
12. The device as per claim 1, wherein the device has a provision to load membrane's of choice for the purpose of culturing and transporting cells to the recipients location for transplantation of cells which aid in regenerating damaged tissues.
13. The device as per claim 1, wherein the cells are selected from the one or more of live cells, cell comprising cellular aggregates or an organized structure or network of cells forming a tissue.
14. The device as per claim 1, wherein the membrane is selected from the group consisting of sheet, film, foam, sponge or a combination thereof.
15. The device as per claim 14, wherein the membrane is selected from the group consisting of biological, semi-synthetic or synthetic material.
16. The device as per claim 15, wherein the biological membrane is selected from the group consisting of Human amniotic membrane, acellularised dermis, small intestinal submucosal (SIS) membrane and/ or combination thereof.
17. The device as per claim 15, wherein the semisynthetic material is selected from the group consisting of poly lactic acid (PLA), polyglycolic acid (PGA), Hyaluronic acid and/ or a combination thereof.

18. The device as per claim 15, wherein the synthetic material is selected from the group consisting of polyethylene, polypropylene, polybutylene, polystyrene, polycarbonate, polyethylene-terephthalate and/or a combination thereof.
19. The device as per claim 15, wherein the membrane can be transparent, translucent, or opaque, which can be optionally coated.
20. The device as per claim 1, wherein the ring has a thickness of about 1-4mm.
21. The device as per claim 1, wherein the silicon gasket has a thickness of about 1-2mm
22. The device as per claim 1 for culturing, maintaining and transport of cell on membrane of choice for therapeutic purpose substantially as herein described with reference to and as illustrated in figures 1 to 8 of the accompanying drawings.