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(54) **Title**: MICROFLUIDIC PLATFORM FOR DEVELOPING IN-VITRO CO-CULTURES OF MAMMALIAN TISSUES.

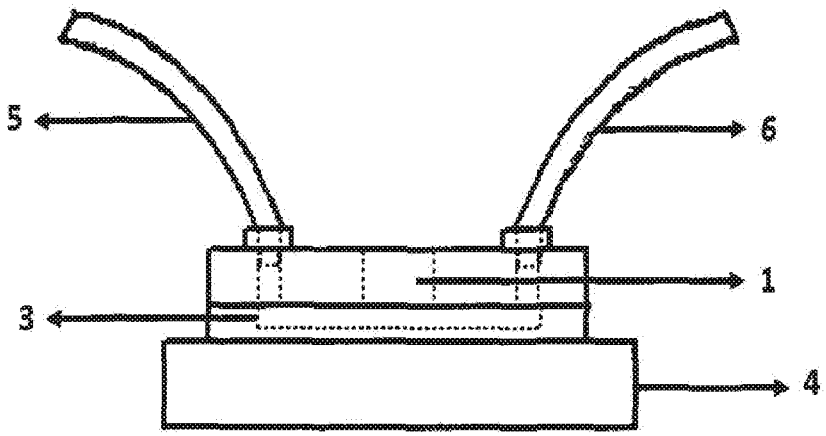

Figure 1

(57) **Abstract**: A microfluidic device for the construction of media and scaffold guided three dimensional tissue or organ like cellular orientation, similar to in vivo conditions, comprising. a. culture chamber layer; b. porous membrane; c. microchannel layer comprising a single channel; and d. optionally baffle or wedge layer.

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MICROFLUIDIC DEVICE FOR THE DEVELOPMENT OF *IN-VITRO* CO-CULTURES OF MAMMALIAN TISSUES

The present invention relates to a microfluidic device for the construction of media and scaffold guided three dimensional, tissue or organ like cellular orientation, similar to in vivo conditions, for preclinical or biomedical applications of pharmaceutical or cosmetic products.

Background of the invention

Testing of pharmaceuticals and biological compounds in humans or in animals is not always possible, at least not in the early stage. Moreover, while in vivo animal studies can provide data more relevant to human responses, animal tests are expensive, labor-intensive, and time consuming. Moreover, growing ethical concerns regarding animal usage has increased the complexity of conducting in vivo evaluations. Accordingly, sometimes decisions need to be made based on in vitro data. However, extrapolating in vitro data (e.g., cell culture data) to the in vivo relevant conditions is often difficult. Several innovations have been carried out to overcome this difficulty.

The process of drug discovery stems from selecting few potential candidates from a library of thousands of active pharmaceutical molecules. These candidates are screened through pre-clinical and clinical trials prior to approval. The pre-clinical trials involved during the process of drug discovery consist of assessment of active pharmaceutical molecules and/or their formulations on suitable animal models (Referred from: Drug Development Process, US-FDA Website). However, in addition to the involvement of stringent regulatory guidelines and ethical considerations, these models suffer from disadvantages related to animal experimentation such as requirement of skilled manpower, time consuming protocols and high cost. Thus, a need has been realized to develop a suitable alternative-to-animal models that may provide safe and effective models for screening pharmaceutical ingredients and/or their formulations or cosmetics and/or excipients (Doke S.K., et. al. 2015).

Moreover, the current invention also possesses the ability to provide a realistic model to test the effects of cosmetic ingredients and/or cosmetic products and/or excipients and actives with applications in healthcare and allied fields and/or their formulations.

Several cell or tissue-based *in-vitro* models have been used or are being proposed as potential alternatives for animal-based testing. Apart from the organotypic approach, these models are mainly focused at reproducing *in-vivo* micro-environments by co-culturing individual cells types of the

desired tissue. For example, several attempts of constructing *in-vitro* models of liver are being tried to test drug efficacy and toxicity, using liver tissue slices, isolated microsomes, perfused liver, immortalized cell lines, primary hepatocytes, stem cells, etc. However, the conventional *in-vitro* models, aimed at building 2D culture of the target tissues, often lead to poor outcomes such as low throughput, less physiological relevance, absence of involvement of cell-cell and cell-extracellular matrix interactions etc. Hence, they do not provide a realistic depiction of the anatomical layout and spatial organization, molecular and cellular events and physiological functions of different tissues and/or organs of mammals including humans and animals. Hence in recent years, there has been an inclination towards reconstructing physiologically relevant 3D co-culture based bioartificial constructs and/or equivalents of mammalian tissues including human tissues. This has facilitated models which are more physiologically mimicking, exhibiting high throughput capability, assisting in generation of metabolic and proliferative gradients during culture and involving interplay of cell-cell and cell-extracellular matrix interactions (Miki, Yasuhiro, et al., 2012; Wu, Min-Hsien, et. al., 2010; 3D Biomatrix Web Page; Hickman, John A., et al., 2014; Astashkina, Anna, et.al., 2012 ; Allen, David D., et al., 2005; Soldatow, Valerie Y., et al. 2013).

Several platforms are available for constructing the 3D co-culture based *in-vitro* models of mammalian including human tissues. These methods include 3D scaffolds, hydrogels, hanging-drop technique, magnetic levitation methods, bioreactor based platforms and microengineering approaches, etc. The microengineering-based methods usually involve use of a microbioreactor or microreactor or microfluidic assemblies to construct 3D environments for the growth and differentiation of cells and/or tissues. In contrast to the other approaches of building 3D co-cultures, microfluidic based models generally provide advantages such as lower consumption of media and buffers, production of considerable cell density, high surface to volume ratio and rate of mass transfer, high gas permeability, laminar flow conditions of cell culture media, provision of perfusion based physiologically relevant systems, allows local micro-environmental control, provides flexibility for fabrication and combination with other approaches, offers real time analysis of cells being cultured, provides precise operational control and adaptability to high throughput and scale up. The potential of microfluidic assemblies for developing *in-vitro* models, thus, lies in their ability to reconstruct micro-environments at molecular, cellular, and tissue levels and flexibility for fabrication and formation of tissue co-cultures. Hence, with the help of the spatio-temporally regulated microbioreactor approach, cell co-culture can be carried out in an accessible, controlled arrangement. (Wu, Min-Hsien, et. al., 2010; Zhang, Zhuo, et. al., 2013; 3D Biomatrix Web Page; Xu, Feng, et al., 2011; Halldorsson, Skarphedinn, et al., 2015; van Duinen, Vincent, et al., 2015; Mehling, Matthias, et. al., 2014).

Various microfluidic based co-culture models of human tissues are presently being investigated for drug screening applications. In one such attempt, a multi-compartmental microfluidic co-culture model was developed to mimic airway mucosa. Airway epithelial cells (at an air–liquid interface to maintain polarity), lung fibroblasts and microvascular endothelial cells were co-cultured for five days. The three cellular layers were co-cultured in discrete compartments separated by porous Polytetrafluoroethylene (PTFE) and Polyethylene terephthalate (PET) membranes respectively. The designed model was proposed for use in drug screening as well as to study molecular mechanisms contributing to disease pathogenesis and remodeling. (Sellgren, Katelyn L., et al., 2014). This invention achieves cell growth in channels. However, in the present invention the cells are grown in the cell culture layer.

Microfluidic chip-based 3D co-culture model has also been used to test drug sensitivity for individualized treatment of lung cancer. The model involved co-culture of lung cancer cells and stromal cells, under continuous supplementation of medium, to assay anti-cancer drug efficacy in an *in-vivo* like tumor micro-environment. In addition, a drug gradient generator was integrated into this platform to collect information about responses of tumor cells to different concentrations of drugs. With this device, sensitive single or multidrug combination chemotherapy schemes at appropriate doses were compared and investigated effectively. Thus, this model was projected to assist researchers to study toxicities and side effects of anti-cancer drugs as well as the economic factors of a combination scheme before treatment (Xu, Zhiyun, et al., 2013).

Efforts have been made to design placenta-on-chip model by co-culturing human trophoblasts (JEG-3) and human umbilical vein endothelial cells (HUVECs), to mimic the placental barrier. The two cell types were cultured in different compartments separated by a vitrified collagen membrane (Lee, JiSoo, et al., 2015). However, the present invention cultures cells in a chamber layer.

A microfluidic platform has been developed to allow for long-term maintenance of full thickness human skin equivalents (HSE), comprising of epidermal and dermal compartments. This model exhibited physiological relevance by mimicking the blood residence times in human skin tissue and lead to the establishment of an air–epidermal interface that is essential for maturation and terminal differentiation of HSEs. Thus, these efforts put forward several attempts for developing microfluidic based *in-vitro* models for testing various pharmaceutical molecules (Abaci, Hasan Erbil, et al., "Pumpless microfluidic platform for drug testing on human skin equivalents." *Lab on a Chip* 15.3

(2015): 882-888).Abaci et al suggest culturing cells outside the microfluidic device followed by transferring them into the device. However, the present invention cultures cells insitu.

Accordingly, a person of ordinary skill in the art related to the state of art have recognized a long-felt need for in vitro system models that can mimic the in vivo systems in humans or animals.

The related art includes **United States published application No. 2009/0234332 A1**(M/s **The Charles Stark Draper Laboratory, Inc,**) describes a microfluidic system for developing artificial micro-vascular constructs for drug screening applications. In order to mimic the ability of the *in-vivo* blood vessels to stretch in response to several mechanical or biochemical stimuli, the device has reinforced the design of distensible walls aligning the cell culture channels. Two co-culture channels are used for culturing vascular and endothelial cell types separately. These channels are separated by distensible walls. Distensibility of walls has been proposed to be dependent upon thickness and elastic modulus of the material used for fabricating the wall. However, the present invention comprises of a single channel for the flow of materials.

United States published application No.2011/0250585 A1 (assigned to **M/s CHILDREN'S MEDICAL CENTER CORPORATION**,) describes a microfluidic based organ-mimicking device consisting of a central channel that is divided into two or more microchannels by an elastic porous membrane of optimum pore size to co-culture different cell types of a tissue. Each microchannel is supplied by different set of inlets and outlets and thus the medium delivery system. The patent claims that the device possesses potential to be applied to conduct drug bioavailability, clearance, efficacy, toxicity studies, etc. However, the present invention comprises of a single channel for the flow of materials.

United States published application No.2008/0261288 A1(by Gonda et al) describes a micro-organ device comprising of a microscale support, having one or more microfluidic channels for housing a micro-organ, particularly a micro-liver. The patent claims micro-organ printing by computer-aided tissue engineering system. It also teaches that the cells are cultured on a 3D scaffold to mimic the physiological state of the *in-vivo* organ. However, the present invention cultures cells and does not involve micro-organ printing, which is a more complex procedure involving sophisticated instrumentation.

United States published application No. 2005/0260745 A1 (assigned to **Massachusetts Institute of Technology**) describes a high-throughput multi-welled microbioreactor based system consisting of an

array of wells of bioreactors and medium reservoirs. The array includes pair of bioreactor and medium reservoir wells, wherein, each pair is fluidically isolated from the other sets of bioreactors and medium reservoirs. Each pair of bioreactor and reservoir wells is connected by fluidic channels, thereby, allowing re-circulation of cell culture medium. The system also consists of valves and pumps actuated in parallel via common control channels which supply re-circulating medium through the array of bioreactor and reservoir pairs.

United States published application No. 2008/0032380 A1 (assigned to **THE UNIVERSITY OF HOUSTON SYSTEM**), describes a microfluidic system involving culture of individual or multiple cell types by maintaining a single cell culture chamber comprising of multiple proliferation zones. The system consists of different zones for culturing similar or different cell types by applying the concepts of laminar flow of a fluid. Thus, these laminar flow based zones allow culture of single or multiple cell types (i.e. co-culture), without the need for physical barrier such as porous membrane in between each of the zones. Each zone is independently regulated by individual inlet and outlet flow systems. The patent claims for the applicability of the device for the development of artificial organ constructs, drug testing, etc. However, the present invention comprises of a single chamber or zone for culturing multiple cell types, supported by a porous membrane, having only a single inlet and outlet flow system, thus comprising of a simple design.

United States published application no. 2012/0135452 A1 (assigned to **Cornell University**) describes a microfluidic system for culturing multiple cell types in a gravity based media perfusion system. The device consists of a microchannel layer consisting of plurality of channels, a single/multiple cell culture chamber supported by a polymeric membrane, lower gasket and metal base. The device is claimed to provide more physiological relevance and applicability for drug screening applications. However the present invention consists of a single microchannel for providing media to the cells and includes a pump to enable accurate control over media flow.

Through detailed study of the existing findings, it is observed that these models, although achieving marginal success, possess few limitations. It is found that most of these microfluidic co-culture models focus on culturing different cell types, of a particular tissue of interest, in discrete compartments or contain multiple channels to supply cell culture medium for co-culture. Thus, they fail to replicate the tissue organization and barrier integrity, as observed in the *in-vivo* tissue. The importance of maintaining these conditions has been explained below.

Human body consists of various tissues and/or organs which perform coordinated functions in a perfused environment provided by a blood connective tissue. Moreover, every tissue possesses a specific cellular arrangement and morphology, which imparts it with a unique biological function. Cellular layers of the tissue are arranged in a tightly packed pattern rendering it a barrier-like structure that allows selective passage of nutrients, biochemical factors, gases, chemicals, etc. via passive or active mechanisms. This is referred to as a diffusional barrier, which limits the transport of various biochemical and functional moieties and/or chemical factors. Integrity of this barrier plays a vital role in the smooth functioning of various tissues and/or organs. For example, the human brain is guarded by the presence of blood-brain barrier achieved by the closely packed structure of brain endothelial cells and astrocytes. Apart from brain, epithelial cells of several tissues of human body also exhibit the barrier property. Retainment of this barrier function is extremely crucial to decipher the molecular and cellular events occurring in response to several stimuli and pathogenic conditions. Moreover, this would also indirectly pave way towards maintaining the physiological barrier and mimic permeability characteristics experienced by a drug molecule or any foreign agent or chemical undergoing transit through different tissues upon delivery and/or exposure. Thus, it is very essential that the *in-vitro* model should exactly replicate the *in-vivo* tissue microarchitecture and also mimic similar cellular arrangement and barrier integrity, as well as mimic similar permeability characteristics.

The presence of porous membrane between individual cell types under co-culture may lead to the involvement of an additional diffusional barrier, if these models are to be explored further for the drug screening and drug development applications. Moreover, their ability to mimic the *in-vivo* spatial organization of cell types of different lineages of a particular tissue would also be limited due to the presence of porous membrane, unless it is essential as observed in the case of barrier based models [for example, blood brain barrier (BBB) model]. The presence of additional barrier (usually a porous membrane) between the cellular layers, therefore, may lead to false positive or negative results with respect to drug pharmacokinetic studies.

The present invention, thus, provides a more realistic approach to overcome these limitations by developing a unique 3D co-culture model, which can be practically applied to several tissues, and can develop but is not limited to the development of skin, lung and retina.

Objective of the invention

The objectives of the present invention are

a) to design a microfluidic device for constructing a physiologically relevant *in-vitro* model mimicking *in-vivo* cellular architecture and conditions of human and mammalian tissues

b) to develop a perfusion based assembly to achieve continuous supply of nutrients and/or gases, thereby mimicking the *in-vivo* tissue microvasculature

c) to mimic the structure of diffusional barriers and cellular organization of the tissue as observed in *in-vivo* conditions, thus providing a realistic model to study the effects of pharmaceutical ingredients and/or pharmaceutical formulations and/or cosmetic ingredients and/or cosmetic products and/or excipients and actives with applications in healthcare and allied fields and/or their formulations

d) Another objective of the invention is to apply the developed platform to conduct pre-clinical studies, including but not restricted to pharmacokinetic, pharmacodynamic, efficacy and toxicity studies, and to assess molecular mechanisms contributing to disease pathogenesis, progression and remodeling, in order to alleviate several disease conditions.

Summary of the invention

A microfluidic device for the construction of media and scaffold guided three dimensional tissue or organ like cellular orientation, similar to in vivo conditions, comprising of

a. culture chamber layer;
b. porous membrane;
c. microchannel layer comprising a single channel; and
d. optionally baffle or wedge layer

A microfluidic device for preclinical or biomedical applications of pharmaceutical or cosmetic products comprising

a. culture chamber layer;
b. porous membrane;
c. microchannel layer comprising a single channel; and
d. optionally baffle or wedge layer

Description of the invention

The present invention provides a novel, simple *in vitro* model of mammalian including human tissues that will, accurately mimic the *in vivo* tissues. The proposed model serves as an *in-vitro* model for conducting preclinical as well as for disease or tissue specific molecular studies. It also provides to the

scientists and clinicians a scalable platform to provide artificial tissue constructs or equivalents for biomedical applications.

In accordance with the first embodiment of the present invention is a microfluidic device for the construction of media and scaffold guided three dimensional tissue or organ like cellular orientation, similar to in vivo conditions, comprising

a. culture chamber layer;
b. porous membrane;
c. microchannel layer comprising a single channel; and
d. optionally baffle or wedge layer.

The scaffold may be made from biopolymers like but not limited to collagen, chitosan, cellulose and the like.

The organ in the organ like cellular orientation may be selected from skin, lungs, retina and the like.

The layers are fabricated using polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like. The device involves sequential arrangement of the following layers in three dimensional manner.

a) **Upper cell culture chamber layer:**

The top polymeric layer is between 40-60 mm long, 15-25 mm wide and 2-6 mm thick. It consists of a single, circular cell culture chamber at the centre of diameter ranging from 6-10 mm. It is fabricated from polymer selected from polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like. The surface area of the cell culture chamber is in the range of 0.4-1 cm^2. This layer facilitates contact co-culture of individual cell types of mammalian including human tissues and obviates the limitations observed in conventional contactless co-culture approaches. Moreover, number of cell-culture chambers may be constructed in this layer depending on the number of tissues to be cultured on the device. Thus, the present embodiment will support multi-organ development by designing multiple cell-culture chambers. The design of the cell-culture chamber of this type offers flexibility to directly load the cell suspension of specific seeding density

as well as stains, enzymes, cell culture reagents, chemicals, pharmaceutical molecules and/or pharmaceutical formulations and/or cosmetic ingredients and/or cosmetic products and/or excipients and actives within applications in healthcare and allied fields and/or their formulations, directly into the cavity of the cell-culture chamber during culturing and while testing efficiency of the *in-vitro* model through characterization studies.

The culture is cultivated insitu and may be mono or contact co-culture.

b) **Porous membrane:**

A porous polymeric membrane of 8-12 microns in thickness and 0.1-5 microns with respect to pore size is attached using plasma treatment or a suitable adhesive at the lower side of the cell culture chamber (between upper cell culture chamber layer and middle channel layer). These porous membranes may be made of polymers selected from polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), Polycarbonate (PC), Mixed Cellulose Esters (MCE) and the like. The presence of porous membrane alters the surface chemistry of the cells to be cultured, provides support to the tissue and provides 3D environment for cell growth by coating with ECM and/or 3D scaffolds. The membrane also allows passage of nutrient media and gases flowing in the middle microchannel layer at a controlled flow rate by capillary action. The hydrophilic nature of the membrane and water absorption properties of the scaffold are also estimated to be the driving factors for enhancing the capillary forces for medium delivery.

c) **Middle microchannel layer:**

The middle layer, consisting of microchannel, is between 40-60 mm long, 15-25 mm wide and 2-6 mm thick. Height of microchannel ranges between 0.5-2 mm. The inlet and outlet openings of the microfluidic device are fabricated to range between 1-4 mm of thickness. It is fabricated from polymer selected from polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like. This layer will render passage of nutrient media and gases to the upper cell-culture chamber layer at a controlled flow rate. Inlet and outlet openings are fabricated in this layer. The device is attached by tubings made up of polyethylene or polymer of similar

chemistry, with or without connectors to the syringe pump. Syringe pump of a suitable configuration supplies culture medium at a controlled flow rate and concomitantly remove waste from the device. The flow rates of the medium are optimized in the range of few microliters per minute, depending on the need of perfusion for the tissue to be co-cultured in the device.

Bottom Layer

The device is supported at the bottom with a support comprising of glass or polymer of similar chemistry. The glass support is 70-80 mm long, 20-30 mm wide and 1-3 mm thick. This bottom layer provides a mechanical support to the device for ease of handling to facilitate culturing and characterization procedures.

d) **Lower Baffle/Wedge Layer:**

The dimensions of the lower layer, including positively protruding baffle or wedge will be fabricated to lie between 15-25 mm in width, 0.3-0.6 mm in thickness and 3-6 mm in length. Height of the baffle will be between 2-3 mm and thickness will be around 0.5-1.5 mm. Suitable number of baffles or wedges will be fabricated in this layer depending on requirement of flow rate. Baffles or wedges arising from the lower layer will be protruding in the middle microchannel layer, thereby, obstructing the medium flow. Due to the obstruction created in the channel layer located beneath the circular cell culture chamber area, the medium is expected to flow over the baffles or wedges, thereby, expediting its delivery to the cells through enhancement of the rate of capillary action.

The polymeric microfluidic devices are fabricated from metal molds, comprising of aluminium or other metals of suitable strength and operational flexibility. Molds are machined to be between 70-80 mm long and wide with thickness ranging from 6-8 mm. These metal molds are fabricated using machining methods, such as, Computer Numerical Control (CNC) machining or other mold fabrication techniques that are suitable to yield the device with desired resolution and dimensions. Polymeric solutions comprising selected from polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like are subject to the heat curing treatment at the temperatures ranging between 60-80°C. Time duration of the curing treatment for the fabrication of three polymeric layers will depend on the temperature utilized, such as, ranging from 4 h for 60°C to 2 h for 80°C .

The three polymeric layers are bonded together using plasma treatment or suitable adhesive which render the desired precision and resolution. The porous polymeric membrane, beneath the top polymeric layer, is attached to the lower side of cell-culture chamber using plasma treatment or suitable adhesive or other similar technique which provides requisite degree of resolution and accuracy.

The components of the microfluidic device have been specifically designed to provide a system that facilitates contact co-culture of mammalian including human cells and/or tissues in a single chamber or compartment. This geometry is particularly required to retain the integrity of diffusional barriers and cellular organization observed in the *in-vivo* tissue.

Thus, the device also possesses significant potential for scale up by fabricating and operating parallelized automation units and multi-welled assemblies. It is a novel platform to conduct high-throughput screening of pharmaceutical molecules and/or pharmaceutical formulations and/or cosmetic ingredients and/or cosmetic products and/or excipients and actives with applications in healthcare and allied fields and/or their formulations, thereby expediting process of their transition into market.

The microfluidic device of the present invention is used for preclinical or biomedical applications of pharmaceutical or cosmetic products.

According to another embodiment of the present invention is microfluidic device for preclinical or biomedical applications of pharmaceutical or cosmetic products comprising

a. culture chamber layer;
b. porous membrane;
c. microchannel layer comprising a single channel; and
d. optionally baffle or wedge layer.

The layers are fabricated using polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like. The device involves sequential arrangement of the following layers in three dimensional manner.

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The culture is cultivated insitu and may be mono or contact co-culture.

b) <u>Porous membrane:</u>

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The device is supported at the bottom with a support comprising of glass or polymer of similar chemistry. The glass support is 70-80 mm long, 20-30 mm wide and 1-3 mm thick. This bottom layer provides a mechanical support to the device for ease of handling to facilitate culturing and characterization procedures.

d) **Lower Baffle/Wedge Layer:**

The dimensions of the lower layer, including positively protruding baffle or wedge will be fabricated to lie between 15-25 mm in width, 0.3-0.6 mm in thickness and 3-6 mm in length. Height of the baffle will be between 2-3 mm and thickness will be around 0.5-1.5 mm. Suitable number of baffles or wedges will be fabricated in this layer depending on requirement of flow rate. Baffles or wedges arising from the lower layer will be protruding in the middle microchannel layer, thereby, obstructing the medium flow. Due to the obstruction created in the channel layer located beneath the circular cell culture chamber area, the medium is expected to flow over the baffles or wedges, thereby, expediting its delivery to the cells through enhancement of the rate of capillary action.

The polymeric microfluidic device are fabricated from the metal molds, comprising of aluminium or other metals of suitable strength and operational flexibility. Molds are machined to be between 70-80 mm long and wide with thickness ranging from 6-8 mm. These metal molds are fabricated using machining methods, such as, Computer Numerical Control (CNC) machining or other mold fabrication techniques that are suitable to yield the device with desired resolution and dimensions. Polymeric solutions comprising selected from polydimethylsiloxane (PDMS), Poly methyl methacrylate (PMMA), Glass (Silica), Polystyrene, Polycarbonate, Polyurethane, Cyclic Olefin Copolymer (COC), Mixed Cellulose esters, PDMS, PMMA, Polycarbonate, Polyester (PET), Poly vinylidene fluoride (PVDF), Poly tetrafluoroethylene (PTFE) and the like are subject to the heat curing treatment at the temperatures ranging between 60-80°C. Time duration of the curing treatment for the fabrication of three polymeric layers will depend on the temperature utilized, such as, ranging from 4 h for 60°C to 2 h for 80°C .

The three polymeric layers are bonded together using plasma treatment or suitable adhesive which render the desired precision and resolution. The porous polymeric membrane, beneath the top polymeric layer, is attached to the lower side of cell-culture chamber using plasma treatment or suitable adhesive or other similar technique which provides requisite degree of resolution and accuracy.

The components of the microfluidic device have been specifically designed to provide a system that facilitates contact co-culture of mammalian including human cells and/or tissues in a single chamber or compartment. This geometry is particularly required to retain the integrity of diffusional barriers and cellular organization observed in the *in-vivo* tissue.

Thus, the device also possesses significant potential for scale up by fabricating and operating parallelized automation units and multi-welled assemblies. It is a novel platform to conduct high-throughput screening of pharmaceutical molecules and/or pharmaceutical formulations and/or cosmetic ingredients and/or cosmetic products and/or excipients and actives with applications in healthcare and allied fields and/or their formulations, thereby expediting process of their transition into market.

Different mammalian tissues may be mimicked on such micro-organ devices for various applications, such as experimental pharmaceutical screening for efficacy, adsorption, distribution, metabolism, elimination, and toxicity. Thus, these devices may be used to assess the beneficial and detrimental effects of a novel drug after it passes through a given metabolic pathway. In addition, these devices may be used to evaluate the therapeutic benefits or toxicities of a drug compound or its formulations

or pharmaceutical or cosmetic excipients. Micro-organ devices as disclosed herein can address the need for in vitro micro-organ-like structures that substantially replicate in vivo structure and function.

Definition of terms

The meaning of certain terms as used herein are provided below

A microfluidic device as used herein refers to a set of micro-channels etched or molded into a material (glass, silicon or polymer such as PDMS, for Polydimethylsiloxane). The micro-channels are connected together in order to achieve the desired features.

"Scaffold" as used herein is a polymeric biomaterial either derived from natural source or prepared synthetically to form an in-vivo mimicking extracellular matrix structure thereby promoting cellular adhesion, differentiation, proliferation and tissue regeneration in a three dimensional orientation.

A "co-culture" as used herein can be defined as the growth of more than one distinct cell type in a combined culture. Contact co-culture involves direct contact between different cell types and populations, without the presence of additional diffusional barrier (viz. porous membrane) between them. It is also known as mixed co-culture.

"tissue or organ like cellular orientation" as used herein is defined as arrangement/organization in which cells comprising of a tissue/organ are patterned three-dimensionally to mimic the *in-vivo* architecture (i.e. anatomical layout), dynamics and physiological functionality (viz. barrier properties) of a tissue/organ.

"Channel" as used herein is defined as the hydraulic component of the microfluidic device fabricated/etched out of a specific geometry (viz. rectangular, circular, etc.) and dimension. It provides a gateway for fluid flow through it, under pressure.

"Baffle" as used herein is defined as a mechanical component or device specifically added to the obstruct/restrain/regulate the fluid flow.

"Wedge" as used herein is defined as a component possessing two principal faces which meet in a sharply acute angle. It is also used to regulate the fluid flow pattern (here).

Description of drawings

Figure 1 Front view of microfluidic device

Figure 2 Top view of microfluidic device

Figure 3 Front perspective view of microfluidic device

Figure 4: Phase contrast microscopical analysis of 3D HADF culture using microfluidic device on 48 h culture. Scale bar corresponds to 400µm (10X magnification)

Figure 5:3D Morphology of HADF cells (Phase contrast image) showing development of processes to establish contact with hydrogel matrix and adjacent cells. Scale bar corresponds to 100µm (40X magnification)

Figure 6: Calcein AM stained green fluorescent (live) cell population observed after 48 h culture. [Scale bar corresponds to 400µm (10X magnification)]

Figure 7: PI stained red fluorescent (dead) cell population observed after 48 h culture. [Scale bar corresponds to 400µm (10X magnification)]

Figure 8 : Z-stack confocal image observed after 48 h culture exhibiting 3D distribution of HADF cells [Scale bar corresponds to 200µm (10X magnification)]

The following examples illustrate preferred embodiments in accordance with the present invention without limiting the scope of the invention.

EXAMPLES

Example 1 : Culturing

The device was sterilized by UV irradiation prior to the initiation of culture. Human Primary Dermal Fibroblast (HADF) have been cultured in the microfluidic device under 3D dimensional conditions for the duration of 48 h. Commercial collagen based scaffold was used for conducting 3D culture of skin cells. HADF cells were mixed with the hydrogel based scaffold in the ratio, ranging from 1:1 to 1:2 to facilitate their entrapment within the matrix (in cold, at 4°C) and were seeded in the culture chamber at the seeding density of 25000-45000 cells per ml. Prior to the cell seeding, the device was subjected to fluid wetting by perfusing culture media (Dulbecco's Modified Eagle Medium with high-glucose comprising of 10 % Fetal Bovine Serum w/o 1% Antibiotic Antimycotic Solution) at the infusion and/or withdrawal flow rate of 50-100 µl/min using a programmable push pull syringe pump. K.D. Scientific Legato 272 Push-Pull Programmable Syringe Pump and Teflon tubing were used for conducting the experiments. Post-seeding, the chamber was covered with a glass cover slip and parafilm and maintained for culturing at the infusion and/or withdrawal flow rate of 0.5-1 µl/min using a programmable push pull syringe pump. The volume of the fluid in the chamber and the outlet was monitored throughout the culture operation.

Example 2 : Test method for testing culture

The device has been tested for 3D monoculture studies of Human Primary Dermal Fibroblast Cells. The 48h culture obtained from microfluidic device was processed and characterized by phase contrast (Figures 4 and 5), fluorescence and confocal microscopy. Live-Dead [Calcein AM-Propidium Iodide (PI)] staining was used for assessment of cell viability. Live cells emit green fluorescence (Figure 6) while dead cells exhibited fluorescence (Figure 7).

Calcein AM staining based confocal studies were carried out using laser ofexcitation wavelength of 488 nm and emission wavelength of 505-525 nm wavelength. On the other hand, PI staining based confocal studies were carried out using laser of Excitation wavelength of 561 nm and emission wavelength of 623-665 nm wavelength. 3D Z-stacking was conducted using confocal microscopy in order to assess 3D distribution of cells in the hydrogel matrix (Figure 8).

We claim

1. A microfluidic device for the construction of media and scaffold guided three dimensional tissue or organ like cellular orientation, similar to in vivo conditions, comprising
   a. culture chamber layer;
   b. porous membrane;
   c. microchannel layer comprising a single channel; and
   d. optionally baffle or wedge layer.
2. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein the scaffold is selected from collagen and the like.
3. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein the organ is selected from skin, lung, retina and the like.
4. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein the culture is mono or contact co-culture.
5. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein the culture is cultivated insitu.
6. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein culture chamber layer is selected from PDMS and the like.
7. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein the porous membrane is selected from PET, PTFE, PC, MCE and the like.
8. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 wherein microchannel layer is selected from PDMS and the like.
9. A microfluidic device for the preparation of media or scaffold guided 3D architecture of organ like orientation as claimed in claim 1 further wherein the device is used for preclinical or biomedical applications of pharmaceutical or cosmetic compounds or products.
10. A microfluidic device for preclinical or biomedical applications of pharmaceutical or cosmetic products comprising
    a. culture chamber layer;
    b. porous membrane;
    c. microchannel layer comprising a single channel; and
    d. optionally baffle or wedge layer.

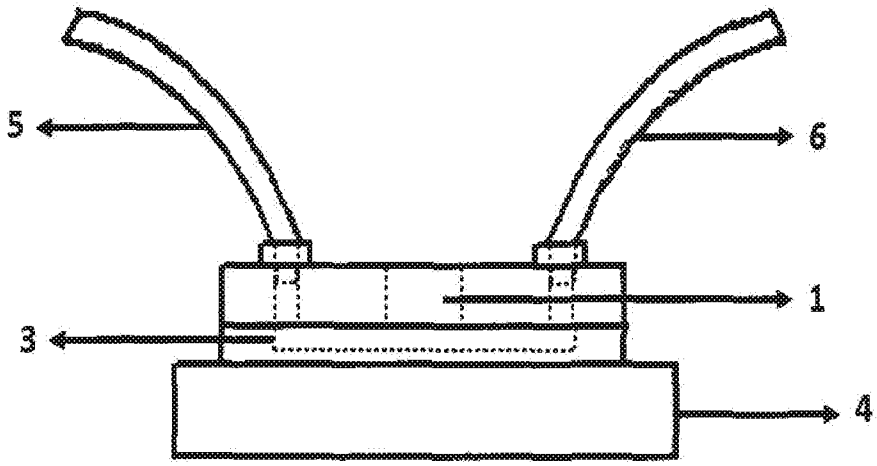

Figure 1

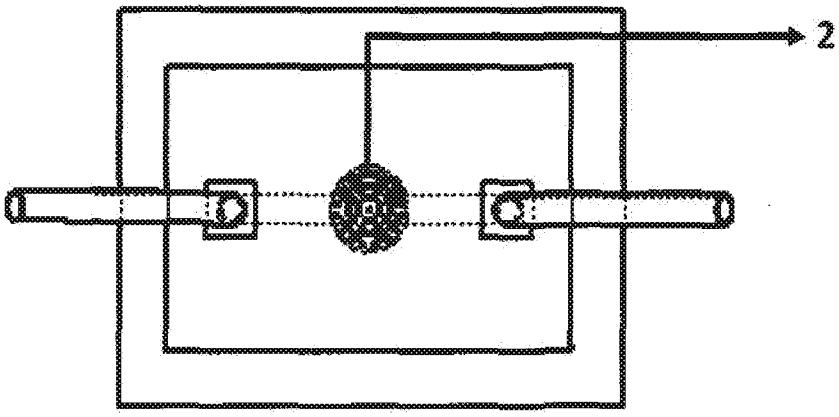

Figure 2

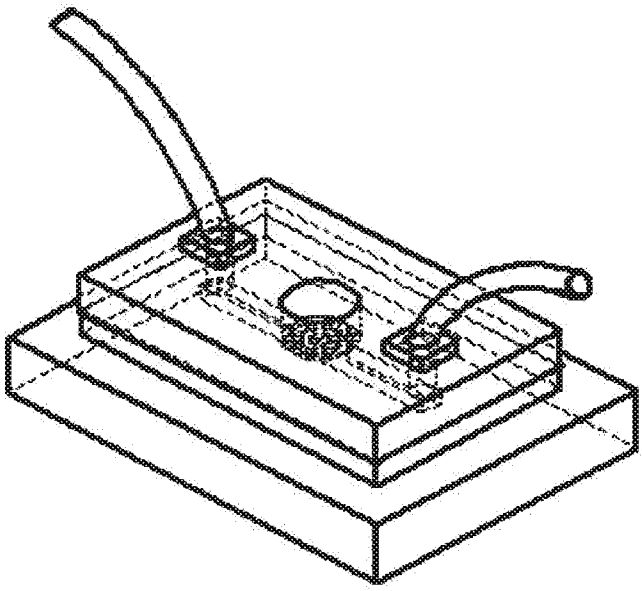

Figure 3

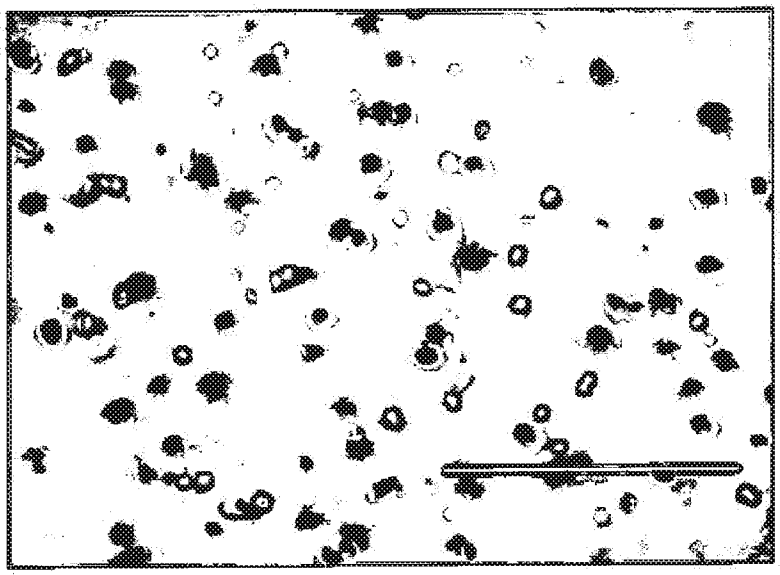

Figure 4

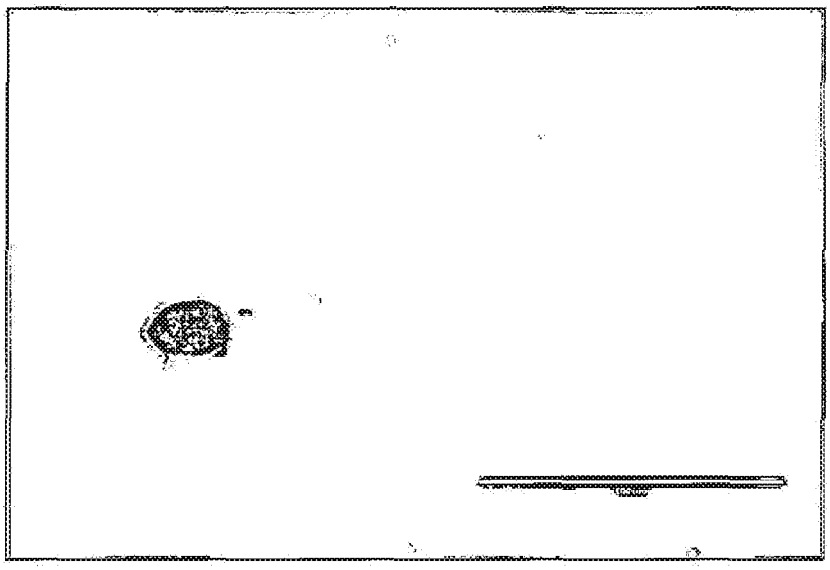

Figure 5

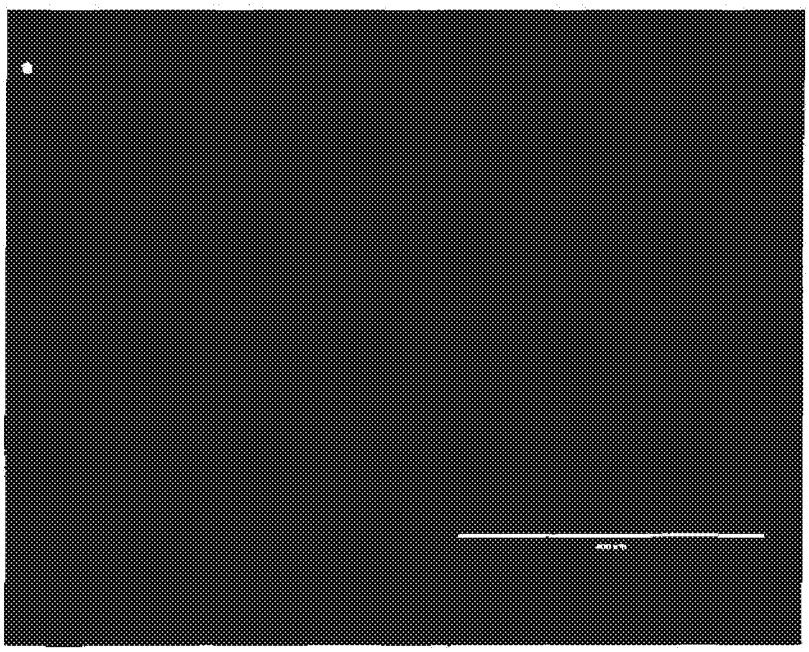

Figure 6

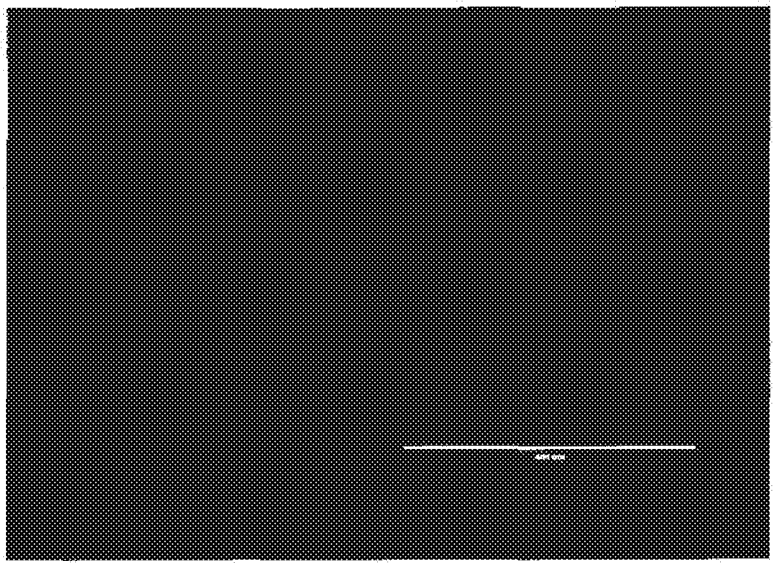

Figure 7

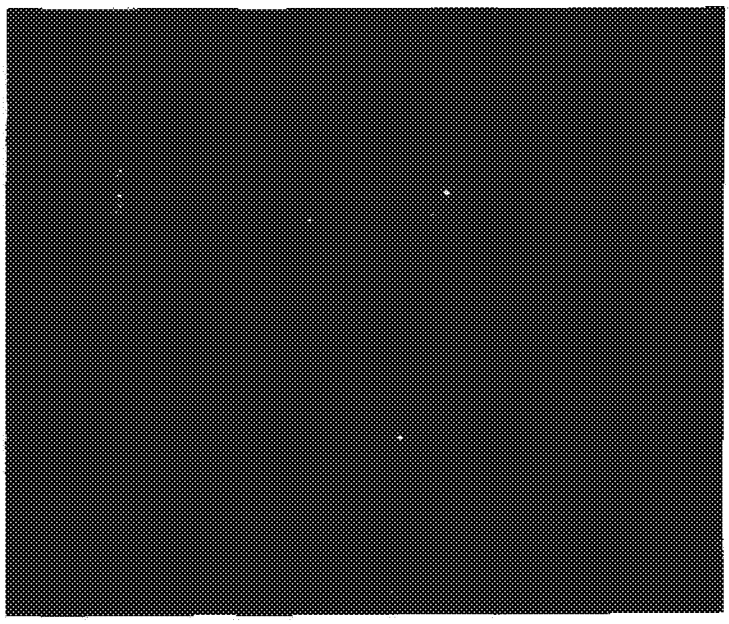

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT / IN 2017/000071

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12M 3/06*** (2006.01); ***C12M 1/12*** (2006.01); ***C12M 1/00*** (2006.01); ***C12M 3/00*** (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)

C12M

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, Fulltext

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003215941 A1 (CAMPBELL STEWART, KIM ENOCH, KIRK GREGORY L, OSTUNI EMANUELE, SCHUELLER OLIVIER, CASAGRANDE ROCCO, WANG EVELYN, SWEETNAM PAUL) 20 November 2003 (20.11.2003) paragraphs [0046][0095][0149][0187][0253]	1-10
X	US 2014142000 A1 (TUNG YI-CHUNG, PENG CHIEN-CHUNG, SHIH HSIU-CHEN, LIAO WEI-HAO) 22 May 2014 (22.05.2014) claims 1, 3, 4	1, 6-8, 10
X	WO 2004065618 A2 (THERMOGENIC IMAGING, HAFEMAN, DEAN, G) 05 August 2004 (05.08.2004) claims 1,4, 15-16	1, 6-8, 10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 May 2017 (30.05.2017)	02 June 2017 (02.06.2017)
Name and mailing address of the ISA/AT Austrian Patent Office Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 / 1 / 534 24-535	Authorized officer MOSSER R. Telephone No. +43 / 1 / 534 24-437

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT / IN 2017/000071

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			EP	A2	1490520	2004-12-29
			AU	A1	2003265228	2003-12-22
			CA	A1	2479072	2003-12-18
			WO	A2	03104439	2003-12-18
US	A1	2014142000	TW	A	201439537	2014-10-16
			US	A1	2014142000	2014-05-22
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			WO	A2	2004065618	2004-08-05