A method for fabricating a micro-organ device comprises providing a microscale support having one or more microfluidic channels and one or more micro-chambers for housing a micro-organ and printing a micro-organ on the microscale support using a cell suspension in a syringe controlled by a computer-aided tissue engineering system, wherein the cell suspension comprises cells suspended in a solution containing a material that functions as a three-dimensional scaffold. The printing is performed with the computer-aided tissue engineering system according to a particular pattern. The micro-organ device comprises at least one micro-chamber each housing a micro-organ and at least one microfluidic channel connected to the micro-chamber, wherein the micro-organ comprises cells arranged in a configuration that includes microscale spacing between portions of the cells to facilitate diffusion exchange between the cells and a medium supplied from the at least one microfluidic channel.
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**OTHER PUBLICATIONS**


* cited by examiner
FIG. 1

R = amine
hydroxyl
methacrylate
aldehyde
epoxy
mercaptan
olefin
fluoroalkyl

+ 2 CH₃OH

FIG. 2
FIG. 6

Drug Conversion by Micro-Organ

FIG. 9
FIG. 7

Micro-Organs
(Cells + Scaffold + Extracellular Matrix)

Fluid In 72a

Fluid Out 72b

Media Flow

Scaffold

70

72

75
micro-organ devices. In accordance with one embodiment, the method for fabricating includes U.S. Pat. No. 5,612,188 issued to Shuler et al., which is hereby incorporated by reference. This patent discloses a multi-compartmental cell culture system comprising culture chambers, sensors, and pumps, but is a system operating on a large scale, so the physiological parameters vary considerably from those found in an in vivo situation. The related art also includes U.S. Pat. No. 7,288,405 by Shuler et al., which is incorporated herein by reference. This reference discloses an in vitro microscale cell culture analog devices that permit cells to be maintained in vitro under conditions similar to those found in vivo. The microscale culture device comprises a fluidic network of channels segregated into discrete but interconnected chambers. The specific chamber geometry is designed to provide cellular interactions, liquid flow, and liquid residence parameters that correlate with those found for the corresponding cells, tissues, or organs in vivo. Fluidics are designed to accurately represent primary elements of the circulatory or lymphatic systems. In one embodiment, the components are integrated into a chip format. The design and validation of these geometries is based on a physiological-based pharmacokinetic (PBPK) model, a mathematical model that represents the body as interconnected compartments representing different tissues.

Similarly, U.S. Patent Application Publication No. 2003/0152909 A1 discloses micro-organ cultures which include isolated populations of cells having specific characteristics. These micro-organ cultures have the ability to be maintained in culture for relatively long periods of time, as well as the preservation of an organ micro-architecture which facilitates, for example, cell-cell and cell-matrix interactions analogous to those in the source organ.

These organ models of the related art demonstrate the advantages of in vitro systems for pharmaceutical or biological compounds testing. However, the related art approaches rely on directly placing the cells in the chamber or seeding the cells and allowing them to grow in the chamber. These approaches do not always provide reproducible organs. Therefore, there remains a need for in vitro organ devices, and methods for their preparation, which can mimic the in vivo organs or systems in humans and animals.

SUMMARY OF INVENTION

In one aspect, embodiments of the present invention relate to methods for fabricating a micro-organ device. In accordance with one embodiment, the method for fabricating includes providing a microscale support having one or more microfluidic channels and one or more micro-chambers for housing a micro-organ and printing a micro-organ on the microscale support using a cell suspension in a syringe controlled by a computer-aided tissue engineering system, wherein the cell suspension comprises cells suspended in a medium.

In another aspect, embodiments of the present invention relate to micro-organ devices. A micro-organ device in accordance with one embodiment includes at least one micro-chamber each housing a micro-organ; and at least one microfluidic channel connected to the micro-chamber, wherein the micro-organ comprises cells arranged in a configuration that facilitates diffusion exchange between the cells and a medium supplied from the at least one microfluidic channel.

Other aspects and advantages of the embodiments described herein will become apparent from the following
description and appended claims, taken in conjunction with the accompanying drawings, illustrating the principles of the embodiments by way of example only.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic series of processing steps illustrating a method for fabricating a microscale support or microchip for a micro-organ device in accordance with one embodiment of the invention.

FIG. 2 illustrates possible chemical modification reactions of silanol groups on a polydimethyl siloxane (PDMS) microchip.

FIG. 3 shows a schematic series of processing steps illustrating a computer-aided tissue engineering system that can be used to bioprint micro-organ devices in accordance with one embodiment of the invention.

FIGS. 4A-4C show three micro-organ devices each having different configurations of organ arrays and microfluidic channels in accordance with embodiments of the invention.

FIGS. 5A and 5B show perspective views along the top and side, respectively, of a section of a micro-organ, illustrating the substructures produced with bioprinting, in accordance with one embodiment.

FIG. 6 is a two-dimensional cross sectional view of a three-dimensional micro-organ in accordance with one embodiment of the invention.

FIG. 7 is a perspective view of a three-dimensional microorgan device in accordance with one embodiment of the invention.

FIG. 8 shows a schematic diagram of a system comprising two micro organs illustrating various microfluidic channels and interconnections in accordance with another embodiment.

FIG. 9 represents a chart of results from an activity assay using a liver micro-organ device in accordance with one embodiment.

FIGS. 10A-10E show examples of various configurations and arrays that may be produced for pharmacokinetic studies in accordance with another embodiment of the invention.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

Exemplary embodiments of the invention will now be described with reference to the accompanying figures. Like elements or components in the figures are denoted with the same reference characters for consistency.

Before beginning a detailed description of some exemplary embodiments of the invention, the meaning of certain terms as used herein will be given.

“Bioprint” or “bioprinting”, as used in this description, refers to a process of depositing biological materials, such as, for example, forming micro-organs using a computer-aided tissue engineering (CATE) system to print a micro-organ according to a particular design or pattern. These processes will be described in more detail below.

“Microscale” as used herein refers to dimensions no greater than 10 cm, preferably no greater than 1 cm.

“Microchip” as used herein refers to a microscale support having one or more microfluidic channels and one or more micro-chambers for housing micro-organs. A microchip typically has a dimension on the order of a few centimeters.

“Microfluidic channel” as used herein refers to a channel on a microchip. Such channels typically have depth and width that are less than a few millimeters, preferably less than 1 mm.

“Micro-chamber” as used herein refers to a structure (e.g., a well or indentation) on a microchip. A micro-chamber typically has a dimension of no greater than a few cm, preferably 1 cm or less. A micro-chamber is typically used for housing cells of a micro-organ.

“Micro-organ” as used herein refers to cells or tissue bioprinted on a microchip, typically in a micro-chamber on the microchip.

“Micro-organ device” or “MOD” as used herein refers to a device having at least one micro-organ on a support (such as a microchip).

Embodiments of the invention relate to methods for producing or fabricating micro-organ devices (MODs) using a bioprinting system for forming cells encapsulated in a structure and to the micro-organ devices thus produced. The micro-organ devices in accordance with the embodiments described herein comprise microscale three-dimensional (3D) tissue analogs in devices that include microfluidic channels and chambers.

In accordance with embodiments of the invention, 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. For example, the therapeutic or toxic effects of a drug on any cell type in vitro may be assessed in the presence of microencapsulated liver cells to take into account the liver-dependent metabolic modifications of the candidate drug. In addition, these devices may be used to evaluate the therapeutic benefits or toxicities of a drug compound. Micro-organ devices as disclosed herein can address the need for in vitro micro-organs that substantially replicate in vivo structure and function.

The micro-organ devices (MODs) in accordance with embodiments of the invention may be produced by direct bioprinting of specific cells, human or animal, to form micro-organisms in micro-chambers of microchips. Bioprinting processes are controllable and can produce reproducible micro-organs that also mimic the in vivo functions more closely. The process of bioprinting may use a technology known as “Computer Aided Tissue Engineering” or “CATE” for short, such as the technology developed at a laboratory located at Drexel University in Philadelphia, Pa. CATE is used in the design, simulation, and fabrication of cells/tissue to construct micro-organs. As will be discussed in more detail below, a CATE system integrates computer-aided design (CAD) technologies and modern design and manufacturing in bioengineering and biomedicine. The CATE system employs a biopolymer deposition system that is capable of extruding biopolymer solutions and living cells for freeform construction of three-dimensional tissue scaffolds. The deposition process is biocompatible and occurs at room temperature and low pressures to reduce damage to cells. The CATE system is capable of, simultaneously with scaffold construction, depositing controlled amounts of cells, growth factors, or other bioactive compounds with precise spatial position to form complex cell-seeded three-dimensional tissue constructs. An example of a CATE system is given in Khalil et al., “Multi-nozzle Deposition for Construction of 3D Biopolymer Tissue Scaffolds,” Rapid Prototyping Journal 2005, Vol. 11, No. 1: 9-17, which is hereby incorporated by reference. In accordance with other embodiments, the methods of fabricating a microorgan device may also comprise forming microchips that include microfluidic channels and micro chambers for housing micro-organs.
As shown in FIG. 1, a master 10 for making at least one microchip comprises a design pattern 12 on a silicon wafer 11. The master 10 may be fabricated using techniques that are adapted from lithographic techniques commonly used in integrated circuit (IC) chip manufacturing. Examples of using the lithographic techniques to fabricate tissue/organ devices can be found in U.S. Pat. No. 7,288,405, which is hereby incorporated by reference. The design pattern 12, for example, may include fluid communication channels or conduits and tissue chambers for forming a micro-organ device. A suitable polymer reagent is then poured onto the master 10 and allowed to cure, as shown in 13, to form a replica of the master 10. Any suitable polymer reagents may be used for making the replica. One suitable example includes a class of compounds generally referred to as polydimethylsiloxanes (PDMS). Other suitable materials may include rubber, polymers, plastic, elastomers, and silicone materials (such as Formulations RTV 108, RTV 615 or RTV 118 available from General Electric, New York, N.Y.).

As recognized by the skilled artisan, PDMS is a flexible elastomeric polymer that is an excellent material for fabrication of a microfluidic device. For instance, see McDonald et al., “Fabrication of microfluidic systems in poly(dimethylsiloxane),” Electrophoresis 2000, 21(1), pp. 27-40. Various PDMS are commercially available, including Sylgard® 184 from Dow Corning. Sylgard is a two-part resin system containing vinyl groups (part A) and hydroxiloxane groups (part B). Mixing the two resins under appropriate conditions (e.g., by heating at a temperature of 65°C or higher for 2 hours) leads to crosslinking (or curing), as illustrated below:

Once the polymer cures, the flexible replica 14 can be peeled off the master, leaving the master ready to produce another replica. The replica 14 has a pattern complementary to the design pattern 12. Once the replica 14 is peeled from the mold, it is ready for assembly to build a micro-organ device. The replica may be cut to proper size with a standard surgical blade, and access holes may be punched, if needed, for tubing and other conduits to provide access to the micro-organ device. For tubing inputs, a needle hole slightly smaller than the outer diameter of the tubing may be drilled so that adequate sealing can be achieved.

Another useful property of PDMS is that its surface can be chemically modified in order to obtain the interfacial properties of interest. See, Makamba et al., “Surface modification of poly(dimethylsiloxane) microchannels,” Electrophoresis 2003, 24(21), 3607-19. One suitable method to covalently functionalize PDMS is to expose it to an oxygen plasma, whereby the surface of Si—CH₃ groups along the PDMS backbone are transformed into Si—OH groups by the reactive oxygen species in the plasma. For example, the PDMS surface may be exposed to O₂ plasma (100 mW radiofrequency, 1% O₂) for 30 seconds to convert the surface methyl siloxane groups into silanol groups.

These silanol surfaces can then be transformed with other alkoxy silicones to yield many different chemistries, some of which are illustrated in FIG. 2, to facilitate further modification or reaction on the replica. The modified PDMS surface, which now has modified siloxane groups, can be reacted and bonded with another object 15 that contains silanol groups, such as a glass surface, another PDMS, or the like. In one embodiment, bonding of the PDMS microchip to a PDMS-coated glass slide acting as a substrate is accomplished by covering both contact surfaces with a reactive polymer (e.g., epoxide). Alternatively, the original PDMS replica 14 from the polymerization reaction shown above (which contains methyl siloxane groups) can be reacted directly with silanol groups from the other object 15. With either approach, a microchip comprising at least one microfluidic channel and at least one micro-chamber can be readily prepared and provided.

In accordance with embodiments of the invention, the cells or tissue of interest for mimicking organs may be fabricated or printed for placement into at least one micro-chamber of a fabricated microchip by a bioprinting system such as a computer-aided tissue engineering (CATE) system 30 of FIG. 3. System 30 enables computer-aided three-dimensional (3-D) printing of biopolymer scaffolds and cell-encapsulated scaffolds. The technique for fabrication employs solid freeform fabrication (SFF), which are computerized fabrication techniques that can rapidly produce highly complex three-dimensional objects using data from CAD systems and from computer medical imaging equipment such as Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans. The fabricated three-dimensional structures are built by reducing CAD designs of particular interest into a group of sliced two-dimensional layers, to where the prototyping material is deposited to build the structure in a layer-by-layer process.

System 30 comprises a computer 31 for controlling the placement and movement of one or more syringes 32. The computer 31 includes at least one central processing unit (CPU) for processing a set of program instructions and memory for storing at least one CATE program comprising a set of program instructions. The at least one CATE program is written so as to direct a path of movement and placement of a robotic arm to deposit alginate or a cell/alginate mixture from a syringe onto a substrate in accordance with a particular design or pattern. A CATE program typically includes computer-aided design/engineering/manufacturing (CAD/CAE/CAM) software. The CATE system 30 may comprise a precision deposition system, which includes the syringes 32 shown in FIG. 3, along with a robotic arm (not shown) for maneuvering the syringes as they “print” onto a particular surface. Cells for bioprinting are suspended in a solution containing extracellular matrix (e.g., collagens) and/or scaffold materials (e.g., alginate) and/or isopro-
be used to circulate the fluids in the microfluidic channels. At least one dimension of which is preferably no larger than 1 millimeter (mm), more preferably less than 500 µm. Such micro-organ would not be able to exchange efficiently with the interior of the micro-organ to efficiently exchange with the media in the microfluidic channels. In general, to ensure effective diffusion exchange, cells are preferably no more than 500 µm away from the microfluidic channels. In order to ensure efficient diffusion exchanges, a micro-organ in accordance with the embodiments may comprise substrates, at least one dimension of which is preferably no larger than 1 millimeter (mm), more preferably less than 500 µm. Such micro-organs may be prepared according to the structural embodiments of FIGS. 5A-8, using the CATE system shown in FIG. 3.

The microfluidic channels illustrated in the devices of FIGS. 4A-4C may be on the order of tens to hundreds of micrometers (µm) wide, while the micro-chambers that house the micro-organs may have a dimension as large as 1 cm or more. If the micro-organ were a solid mass of a millimeter (mm) or centimeter (cm) in size, the cells in the interior of the micro-organ would not be able to exchange efficiently with the media in the microfluidic channels. In general, to ensure effective diffusion exchange, cells are preferably no more than 500 µm away from the microfluidic channels. In order to ensure efficient diffusion exchanges, a micro-organ in accordance with the embodiments may comprise substrates, at least one dimension of which is preferably no larger than 1 millimeter (mm), more preferably less than 500 µm. Such micro-organs may be prepared according to the structural embodiments of FIGS. 5A-8, using the CATE system shown in FIG. 3.

Referring now to FIG. 5A, a perspective top view of one structural embodiment that may be bioprinted is shown as so to construct a three-dimensional tissue model of a microorgan. In particular, two substructures 51 and 52 of a micro-organ 50 are shown to have been fabricated (bioprinted) in an interdigitated configuration having microscale spacing between one another. The spacing between the two substructures 51 and 52 are designed to form the microfluidic channel 53 for the flow or circulation of media to nourish cells or tissue. FIG. 5B shows a side view of the substructures of FIG. 5A, further illustrating the 3-D structure of the tissue model comprising multiple layers or “struts” of scaffolding. Although only three layers of the substructures are shown in this illustration, a micro-organ may comprise more or less layers in other embodiments, as will be discussed with reference to FIGS. 6-8. In this particular example, each layer is about 200 µm thick, and the spacing between the substructures forming the channel 53 is about 20 µm. This particular microscale thickness and microscale spacing enables cells at the interior of the micro-organ to efficiently exchange with the media (by diffusion) in the micro-channels.

The interdigitated substructures and the multi-layer stacking may be bioprinted using the CATE system shown in FIG. 3. When bioprinting is used, cells of the micro-organ are suspended in a solution containing one or more extracellular...
matrix or scaffold materials. The solution is put in a syringe that is controlled by a computer for “printing” the micro-organ onto a surface, such as a microchip, according to a particular design. The printing process may be performed one layer at a time until the desired number of layers of the substructure is achieved.

The interdigitated configuration of the substructures 51 and 52 and the microscale spacing between them that forms the microfluidic channels 53 are also shown in a top view in FIG. 6. This top view shows a section of an entire micro-organ 60 comprising repeated layers (interdigitated folds) of substructures 51, 52, and the microfluidic channel 53 to achieve a particular overall dimension of interest (e.g., 1 cm).

The two-dimensional (2D) top view shown in FIG. 6 represents a layer (section) of a 3D micro-organ. FIG. 7 shows a perspective view of a 3D micro-organ 71 that comprises a plurality of the 2D sections shown in FIG. 6. FIG. 7 shows that the 3D micro-organ 71 is disposed in a micro-chamber of a micro-organ device (MOD) 70. The micro-organ device (MOD) 70 also comprises a microfluidic channel or conduit 72 with an inlet 72a and an outlet 72b. As noted above, the MOD 70 may be made of any suitable materials, such as PDMS. The MOD 70 is shown to be supported on a substrate and 75, which may be a silicon wafer, glass, glass coated with PDMS, or plastic and have a dimension on the order of 1 to several centimeters.

As shown in FIGS. 8B and 8C, a micro-organ device (MOD) of the invention may include more than one micro-organ, which may be operatively interconnected to one another by one or more microfluidic channels. FIG. 8 shows a system 80 in accordance with one embodiment of the invention that comprises two micro-organs 81, 82 connected by a network of microfluidic channels 83. The network of microfluidic channels 83 is shown to include various valves (denoted by the reference character V), reservoirs (R), pumps (P), and detectors (D).

In the example shown in FIG. 8, the chip footprint may be in the range of 1-5 cmx1-5 cm. The fluidic channels may be about 25-50 µm deep and about 100-350 µm wide. The fluidic channels in the electrophoresis chamber or section may be smaller, e.g., about 50 µm wide. The valves (V) may be controlled by pneumatic control lines, which serve to actuate the valves for controlling fluid flow and fluid pumping. The pneumatic control lines may be 10 µm deep and 200 µm wide, for example. Note that these dimensions are for illustration of this particular example. One of ordinary skill in the art, now having benefit of this description, would appreciate that a MOD in accordance with other embodiments may also be prepared in other sizes and dimensions.

The pumps shown in FIG. 8 may be peristaltic pumps or syringe pumps. The detector may be any suitable detector (optical, UV, fluorescence, IR, laser, etc.) for detecting or assessing drug conversion or metabolite analysis. The system shown in FIG. 8 also includes an electrophoresis separation and detection section, which may be used to analyze any metabolites or converted drugs.

The methods for bioprinting of cells in accordance with embodiments of the invention do not adversely impact the viability of the cells. In one example, using mouse hepatocytes (ATCC, CRL-2254) encapsulated in alginate hydrogels, it was found that the resultant micro-organ retained 79% viable cells. A micro-organ of liver cells thus prepared was found to produce a higher amount of urea than the same number of hepatocytes cultured on tissue culture plastic, suggesting that the cells in the MOD are healthier.

More importantly, micro-organ devices constructed in accordance with these methods are found to be able to perform functions that mimic, or are similar to, the in vivo functions of the corresponding organ. For example, FIG. 9 is a chart of results from an experiment concerning the conversion of 7-ethoxy-4-trifluoromethylcoumarin (EFC) to 7-hydroxy-4-trifluoromethylcoumarin (HFC) by a liver micro-organ device in accordance with one embodiment of the invention. EFC is converted to HFC by liver cytochromes P450 (P450s). Liver cytochrome P450 enzymes are involved in drug metabolism. EFC has weak or no fluorescence, while HFC has strong fluorescence. Therefore, this system provides a convenient assay for the drug metabolizing enzymes in the liver. Liver MODs in accordance with an exemplary embodiment are found to convert EFC to HFC at rates ranging from 1 to 10 picomoles/hr/10⁶ cells. Results shown in FIG. 9 indicate the MODs can have functions like those of its corresponding in vivo organs. Thus, MODs can be used to mimic such organs for the study of drug metabolism or other pharmacokinetic applications.

In particular, at least one embodiment of the micro-organ device (MOD) may be designed to mimic the complex in vivo microenvironment of human or animal cells and tissues, including proper inter-communications between different organs, by constructing a three-dimensional tissue model to be housed within a microchip that replicates the in vivo micro-organ structure, extra-cellular matrix composition and architecture, and the in vivo fluid flow. One or more micro-organs may be created by suspending human or animal mammalian cells in a specific soluble extra-cellular matrix (ECM) and scaffold and printing three-dimensional micro-organs with a computer aided tissue engineering (CATE) system. The micro-organs may be directly printed and arrayed as single or multi-organs on at least one microchip or microfabricated microfluidic device. The microchip houses the micro-organs to form a micro-organ device. Fluids are then circulated through the micro-organs to maintain viability and to deliver specific soluble factors and molecules. A system, such as the system 80 of FIG. 8, may be constructed to control and direct the perfusion of cell culture medium and specific soluble factors such as investigative drugs and chemicals using algorithms related to the law of mass conservation.

In one embodiment, system 80 may be constructed to control and direct perfusion by equations related to the conservation of mass. First, the rate of metabolism in the tissue analog (or MOD), represented by the variable D, is a product of the volume of in-flow to the tissue analog from which the drug would be entirely removed in unit time, represented by the variable CL, and the drug concentration entering the tissue analog, as represented by the variable C₁. This relation may be denoted by the equation:

\[ R = C₁ \times CL \]

The tissue analog of this embodiment may be represented as comprising the following additional set of variables:

- \( Q \) — circulating rate of perfusate
- \( C₂ \) — drug concentration exiting tissue analog
- \( R \) — constant rate of continuous infusion; and,
- \( D \) — total amount of drug in the medium.

Further, given that

\[ \frac{dC₁}{dt} = -QC₁ + QC₂ + R \]  \quad (1)

\[ \frac{dC₂}{dt} = -QC₂ + QC₁ - CLC₁ \]  \quad (2)