(19) Octrooicentrum Nederland

(11) **2011895**

(12)C **OCTROOI**

(21) Aanvraagnummer: **2011895**

(22) Aanvraag ingediend: **04.12.2013**

(51) Int.Cl.:
C12M 3/00 (2006.01) ***C12M 1/12*** (2006.01)
C12M 1/00 (2006.01)

(43) Aanvraag gepubliceerd:
-

(47) Octrooi verleend:
08.06.2015

(45) Octrooischrift uitgegeven:
17.06.2015

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(54) **Fluidic device and perfusion system for in vitro tissue reconstruction.**

(57) The present invention relates to a fluidic device for *in vitro* tissue reconstruction comprising an interior comprising at least one set of at least three separated channels, wherein the at least three separated channels are separated from one another at least partially by a separating material which material allows communication of the at least three channels with one another. The present invention also relates to the use of the fluidic device of the present invention for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells and neuronal cells, interstitial cells, products and/or metabolites from the fluidic device. The present invention further relates to the use of the fluidic device for coculturing, evaluating, sampling and/or harvesting of acellular, unicellular and/or multicellular organism and/or tissue, material, products and/or metabolites from the fluidic device other than the reconstructed tissue. The present invention further relates to a perfusion system comprising the fluidic device and to a method for *in vitro* tissue reconstruction and/or coculture using the fluidic device and/or perfusion system of the present invention, as well as to a hollow fibre and use of the hollow fibre for coculturing, evaluating, sampling and/or harvesting of tissue cells, blood cells, vascular cells, neuronal cells and/or interstitial cells, products and/or metabolites from the fluidic device of the present invention.

NL C 2011895

Fluidic device and perfusion system for *in vitro* tissue reconstruction

The present invention relates to a fluidic device and a perfusion system for *in vitro* tissue reconstruction. The present invention also relates to the use of the fluidic device of the present invention for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells and neuronal cells, interstitial cells, products and/or metabolites from the fluidic device. The present invention relates to the use of the fluidic device of the present invention for coculturing, evaluating, sampling and/or harvesting of acellular, unicellular and/or multicellular organism and/or tissue, material, products and/or metabolites from the fluidic device other than the reconstructed tissue. The present invention further relates to a method for *in vitro* tissue reconstruction and/or coculture using the fluidic device of the present invention, as well as to a hollow fibre and use of the hollow fibre for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells, neuronal cells, interstitial cells and/or products and/or metabolites from the fluidic device of the present invention.

Advances in medical genetics and human genetics have enabled a more detailed understanding of the impact of genetics in disease. Large collaborative research projects (e.g. the Human genome project) have laid the groundwork for the understanding of the roles of genes in normal human development and physiology, revealed single nucleotide polymorphisms (SNPs) that account for some of the genetic variability between individuals, and made possible the use of genome-wide association studies (GWAS) to examine genetic variation and risk for many common diseases.

The use of genetic information has played a major role in developing personalized medicine, i.e. the customization of healthcare – with medical decisions, practices, and/or products being tailored to the individual patient. Examples of personalized medicine can be found in, for example, the field of oncology, wherein personalized cancer management include the testing for disease-causing mutations in the breast cancer type 1 (BRCA1) and breast cancer type 2 (BRCA2) genes, which are implicated in hereditary breast-ovarian cancer syndromes.

Furthermore, personalized medicine can also be found in the field of organ transplantation. Transplantation medicine is one of the most challenging and complex

areas of modern medicine. Some of the key areas for medical management are the problems of transplant rejection, during which the body has an immune response to the transplanted organ, possibly leading to transplant failure and the need to immediately remove the organ from the recipient. When possible, transplant rejection can be reduced through serotyping to determine the most appropriate donor-recipient match and through the use of immunosuppressant drugs. The emerging field of regenerative medicine is allowing scientists and bioengineers to create organs to be re-grown from the patient's own cells (stem cells, or cells extracted from the failing organs).

Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself. Importantly, regenerative medicine has the potential to solve the problem of the shortage of organs available for donation compared to the number of patients that require life-saving organ transplantation. Depending on the source of cells, it can potentially solve the problem of organ transplant rejection if the organ's cells are derived from the patient's own tissue or cells. However, the current application of regenerative medicine is limited and the (re)construction of organs is still labour-intensive.

Also in drug development, e.g. drug discovery, the role of personalized medicine is of increasing importance. Drug development has been hampered because it relies on the use of animal models that are costly, labour-intensive, time-consuming and questionable ethically. Of even greater concern is that animal models often do not predict results obtained in humans, and this is a particular problem when addressing challenges relating to metabolism, transport and oral absorption of drugs and nutrients.

The present invention provides a fluidic device for *in vitro* tissue reconstruction. It is proposed that the *in vitro* tissue reconstructed by the fluidic device of the present invention closely mimics the *in vivo* tissue of a living multicellular organism. The present invention provides hereto a fluidic device for *in vitro* tissue reconstruction comprising:

- at least one set of separated channels, which set comprises at least one tissue channel, at least one humoral channel and at least one neural channel;
- the tissue channel being arranged for receiving living tissue cells;

- the humoral channel being arranged for receiving blood and/or vascular cells; and
- the neural channel being arranged for receiving neuronal cells,

wherein the at least one tissue channel, at least one humoral channel and at least one neural channel are separated from one another by a separating material which separating material allows communication of the at least three channels with one another.

The fluidic device of the present invention provides a simple and elegant cell coculture *in vitro* model wherein the *in vitro* reconstruction of human and/or animal tissue closely resembles the construction of human and/or animal tissue *in vivo*, e.g. in structure (morphology) and in function. The fluidic device of the present invention provides a system wherein cellular communication between the different cell cultures (including immune cells) is allowed via direct contact, i.e. juxtacrine signalling, communication over a short distance, i.e. paracrine signalling, and/or communication over a relatively longer distance, i.e. endocrine signalling by mimicking the juxtacrine, paracrine an/or endocrine signalling in the fluidic device, the present invention provides a cell coculture model which mimics the complex *in vivo* like structure and function of a tissue of a living multicellular organism. The fluidic device of the present invention allows scientists/bioengineers to evaluate the formed structure of the *in vitro* reconstructed tissue, e.g. via coculturing, sampling, harvesting or the like, and to evaluate the function of the *in vitro* reconstructed tissue, e.g. via genomics, transcriptomics, proteomics, metabolomics or the like. The fluidic device of the present invention further allows coculturing, evaluating, sampling and/or harvesting other than reconstructed tissue acellular, unicellular, multicellular organisms and/or tissue, material, products and/or metabolites, e.g. intestinal microbiota, biomedical materials or the like, from the fluidic device other than the reconstructed tissue. The human and/or animal models known in the art do not provide an *in vitro* model wherein both the structure and function of a reconstructed tissue as well as responses to coculture with a guest organism and/or material can be studied. In fact, none of the *in vitro* models known in the art provide a fluidic device wherein the reconstruction of human and/or animal tissue is regulated, coordinated and integrated by providing a neurohumoral regulation. However, the fluidic device of the present invention, comprising at least one set of separated channels, which set comprises at least one tissue channel, at least one humoral channel and at least one neural channel, provides an *in vitro* reconstruction of a human and/or animal

tissue wherein the (to be) reconstructed tissue is regulated by a neurohumoral regulation.

As used herein the "fluidic device" refers to a device of any size or orientation which comprises one or more sets of at least three channels and is suitable for the culture of living cells. A fluidic device can be capable of moving any amount of fluid within the fluid flow ranges described herein below, e.g. a fluidic device can be a microfluidic device or a device capable of moving larger volumes of fluid.

Furthermore, as used herein the term "communication" refers to the possibility to exchange cells, compounds, products and/or metabolites between each of the separated channels. Also, the term "communication" refers to possibility of, for example, neuronal cells to extend outside the neural channel by formation of neurites, e.g. an axon and/or a dendrite.

As used herein, the term "channel" refers to any capillary, channel, tube, or groove that is deposed within or upon a substrate. A channel can be a microchannel; i.e. a channel that is sized for passing through microvolumes of liquid. The channels of the fluidic device of the present invention may have any suitable form. In an embodiment of the present invention, the fluidic device comprises at least one set of separated channels, wherein the channels are substantially tubular, to form a tubular fluidic device, or substantially rectangular, to form a planar fluidic device. It is noted that the channels of the present invention may be a triangular prism, a pentagonal prism, a hexagonal prism, and the like. It is further noted that a combination of different forms may be used. In an embodiment of the present invention, the fluidic device comprises at least one set of separating channels having a substantially tubular form which set of separating channels is combined with channels with a substantially triangular prism form.

It is now proposed that by providing a fluidic device according to the present invention wherein the fluidic device comprises three separated channels, which three separated channels are in communication with one another, the *in vitro* reconstruction of living tissue, e.g. human and/or animal tissue, closely resembles the way living tissue occurs *in vivo*, i.e. in nature. By mimicking the *in vivo* method of reconstruction of living tissue, the *in vitro* reconstructed living tissue mimics the *in vivo* living tissue more

closely and more precisely than compared to *in vitro* methods of reconstruction of living tissue known so far. The essence of the present invention resides in the reconstruction of living tissue by the distinct characteristics of the three separated channels and the possibility to communicate with one another through the separating material separating the at least three separated channels. To allow the living tissue cells, blood and/or vascular cells and neuronal cells to communicate with one another, it is possible to create an *in vitro* cell coculture, which closely resembles the natural environment of the living tissue to be reconstructed.

Even further, by providing different separated channels having distinct functionality, it is possible to reconstruct living tissue *in vitro* based on living tissue cells, blood and/or vascular cells and neuronal cells extracted from the same unique multicellular living organism, e.g. human being. The fluidic device of the present invention therefore provides a method for the reconstruction of unique living tissue each time the fluidic device of the present invention is seeded with cell culture material. As a consequence, the living tissue constructed by the fluidic device of the present invention may closely resemble the natural tissue of a multicellular living organism and provide therefore an *in vitro* alternative method which empowers scientists/bioengineers to grow different types of living tissue and/or organs, e.g. skin, stomach, intestine, muscles, bone, adipose tissue or the like, as well as to support culture other than reconstructed tissue acellular, unicellular, multicellular organism, tissue and/or materials for scientific and industrial needs. It should be noted that the fluidic device of the present invention may construct any kind of living tissue, e.g. mammal tissue such as human and/or animal tissue.

Additionally, the fluidic device of the present invention empowers scientists/bioengineers to construct patient-unique tissue in order to select the most promising treatment therapy for a specific individual. It has to be understood that the fluidic device of the present invention further provides also a method to construct living tissues which can be used in the drug development to select the most promising drug candidates. Thus, the use of the fluidic device of the present invention for *in vitro* reconstruction of human tissues may reduce and/or replace the application of animal models in drug discovery. The coculture of living tissue cells, blood and/or vascular cells and neuronal cells provided by the fluidic device of the present invention empowers the scientists/bioengineers to design desired types of *in-vivo*-like living tissue

in vitro and therefore offers a more promising test-model of a desired multicellular organism drug compared to *in vivo* and/or *in vitro* models used nowadays.

The living tissue cells may comprise a wide variety of human and/or animal tissue cells. The tissue cells may be selected from the group consisting of primary cells, cultured cells, passaged cells, immortalized cells, transgenic cells, genetically modified cells, cancerous cells or cells from a multicellular organism with a cancer, cells from a multicellular organism with disease or disorder, stem cells, embryonic stem cells (ESCs), induced pluripotent stem cells (IPSCs), tissue-specific progenitor/stem cells. The tissue cells may be selected from the cells derived from tissue and/or organoid, i.e. a structure that resembles an organ, of a desired multicellular organism.

The blood and/or vascular cells may be selected from the group consisting of primary blood and/or endothelial cells, primary pericytes, cultured cells, passaged cells, immortalized cells, transgenic cells, genetically modified cells, cancerous cells or cells from a multicellular organism with a cancer, cells from a multicellular organism and/or organoids with disease or disorder, stem cells, ESCs, IPSCs, tissue-specific progenitor/stem cells, peripheral blood mononuclear cells (PBMC), plasmacytoid dendritic cells (PDC), myeloid dendritic cells (MDC), B cells, macrophages, monocytes, natural killer cells, NKT cells, CD4+ T cells, CD8+ T cells, granulocytes or precursors thereof. The blood and/or vascular cells may be derived from a multicellular organism.

The neuronal cells may be selected from the group consisting of primary cells, cells, cultured cells, passaged cells, immortalized cells, transgenic cells, genetically modified cells, cancerous cells or cells from a multicellular organism with a cancer, cells from a multicellular organism and/or organoids with disease or disorder, stem cells, ESCs, IPSCs, tissue-specific progenitor/stem cells, unipolar or pseudounipolar cells, bipolar cells and/or multipolar cells (e.g. Golgi I and Golgi II). The neuronal cells may further be selected from the group consisiting of basket cells, betz cells, lugaro cells, medium spiny neurons, purkinje cells, pyramidal cells, renshaw cells, unipolar brush cells, granule cells, anterior horn cells or spindle cells. The neural cells may also be derived from a desired multicellular organism.

The separating material may be made of an impermeable material which material comprises at least one area having a plurality of pores. By providing a fluidic device wherein the at least three channels are separated by a separating material made of a material comprising at least one area having a plurality of pores, the three channels are able to communicate with one another. The size of the pores may be chosen such that the communication is in one-way direction or in a two-way direction. The pattern of the pores between the different separated channels may be chosen such that different areas of the material where the separating material may be made of provide different functionality with regard to the permeability of the material. It is even possible to define the size of the pores in such way that the pores connecting the tissue channel and the humoral channel are different compared to the size of the pores connecting the humoral channel and neural channel and even further different compared to the size of the pores connecting the neural channel and the tissue channel.

The pore aperture in the material where the separating material may be made of separating the at least three channel from one another depends on the specific needs of the living tissue to be reconstructed. Preferably the pores of the area comprised by the separating material may be between about 0,5 μm and about 10 μm in diameter. Preferably, the pores of the material may be about 8 μm or about 1 μm in diameter. In case transmigration of cells across the material (e.g. chemotaxis and/or motility studies), is desired, pores of about 5 μm in diameter are particularly useful. As already described above, the pores of the material can be varied per area of the material. Furthermore, the pores of the material can be irregularly and/or regularly spaced. Even the distance between the pores can vary. Preferable the pores in the material may be 0,1 μm or further apart, more preferably 1 μm apart, 5 μm apart, 10 μm apart, 15 μm apart, 20 μm apart, 25 μm apart, 50 μm apart, 100 μm apart, 1000 μm apart or even further apart.

The area having a plurality of pores may be made of a permeable and/or semi-permeable material, e.g. a membrane and/or a matrix. As already explained above, the permeability of the permeable and/or semi-permeable material may be varied between the different channels. Also, the permeability of the permeable and/or semi-permeable material may be varied per area of the permeable and/or semi-permeable material itself.

The separating material may be formed by a permeable and/or semi-permeable material, e.g. the material as described above entirely consist of a permeable and/or semi-permeable material. Again, it should be noted that the permeability of the permeable and/or semi-permeable material and the pattern of the permeability of the permeable and/or semi-permeable material may be varied between the different channels.

The above defined permeable and/or semi-permeable material separating the at least three channels from one another may have the form of a permeable and/or semi-permeable matrix. Preferably the permeable and/or semi-permeable matrix may be located in such way that the matrix is in connection with the at least three channels. The use of such a matrix is particularly applicable in a fluidic device having a planar channel structure wherein the fluidic device is divided into at least three different channels wherein the separating material comprising the matrix separating the at least three channels having a T- or Y-shaped form. The matrix may be preferably located at the junction area of the separating material allowing the at least three channels to communicate with one another.

The separating material may be at least partially made of a biodegradable and/or non-biodegradable material. In other words, the material of the separating material comprising at least one area having a plurality of pores may be biodegradable or non-biodegradable. The biodegradability of the material can be varied between the different channels. By providing a fluidic device comprising at least three separated channels wherein the at least three separated channels are separated by a biodegradable material, the present invention therefore provides the possibility to design complex structures of biodegradable material in order to reconstruct complex living tissue, e.g. mammal organs. By providing a fluidic device wherein the material separating the at least three channels is made of a biodegradable material, the resulting reconstructed tissue may have a three-dimensional structure wherein separating material and/or the area having a plurality of pores (e.g. membrane and/or matrix), is no longer present.

The at least three channel structure of the fluidic device of the present invention may be designed by using an intelligent design unit, e.g. a computer, using a 3D printer to actual print the three-dimensional fluidic device comprising the at least three channels separated from one another by a separating material, e.g. a material comprising at least

one area having a plurality of pores. However, other methods such as etching, machining or micro-machining may be suitable as well. After seeding the living tissue cells, blood and/or vascular cells and neuronal cells to the corresponding channels, the living tissue can be reconstructed in a three-dimensional way. Such three-dimensional reconstruction of a complex living tissue empowers the scientist/bioengineer to reconstruct *in vitro* a patient specific complex tissue, e.g. an organ, such as skin or intestine reconstructed with patient specific tissue which may be used for organ transplantation.

Even further, the fluidic device of the present invention may be formed by a solid material comprising at least partially a semi-permeable and/or permeable material wherein at least one set of at least three separated channels is created, e.g. by providing boreholes into the solid material comprising at least partially a semi-permeable and/or permeable material.

In an embodiment of the present invention, the fluidic device comprises at least one set of at least three channels wherein each of the at least three channels define an inner surface enclosing the interior of the channel and an outer surface adjacent to the inner surface of the channel facing at least a part of the outer surface of the at least two other channels. In such embodiment, the at least three channels may be formed by using a material, e.g. the above described permeable and/or semi-permeable membrane, enclosing the respective channel which channel is physically separated from the at least two other channels. As a consequence, the materials enclosing the at least three physically separated channels may be different from one another. At least a part of the outer surfaces of the physically separated channels may be located at a minimal distance from one another. In a favourable embodiment of the present invention the minimal distance between the outer surfaces of the physically separated materials does not exceed 1000 µm, since by a minimal distance between the outer surfaces of greater than 1000 µm direct contact communication between the separated channels (e.g. juxtacrine signalling) is hindered. Preferably, the minimal distance between the outer surfaces of the physically separated channels may be in the range from 0 µm to about 500 µm. More preferably, the minimal distance between the outer surfaces of the physically separated channels may be in the range from about 5 µm to about 10 µm. In a further favourable embodiment of the present invention, at least a part of the outer surface of a

physically separated channels may comprise a surface which contacts with at least a part of the outer surfaces of the at least other two channels, i.e. a minimal distance between the outer surfaces of the physically separated channels of 0 µm.

In an embodiment of the present invention, the fluidic device comprises at least one interstitial space enclosed by the outer surfaces of the at least three channels. The interstitial space may also be formed naturally between the outer surfaces of the at least three channels. In a further embodiment, the at least one interstitial space is being arranged for receiving interstitial cells, products and/or metabolites, e.g. signalling molecules comprised in the interstitial fluid, forming an interstitial space.

The interstitial space may comprise an extracellular matrix (ECM), e.g. basement membranes and/or interstitial fluid produced by cells of the tissue channel, the humoral channel, the neural channel and/or the interstitial cells. In an embodiment of the present invention, the interstitial cells may be selected from the group consisting of resident and wandering primary cells of connective tissue, cells, cultured cells, passaged cells, immortalized cells, transgenic cells, genetically modified cells, cancerous cells or cells from a multicellular organism with a cancer, cells from a multicellular organism and/or organoids with disease or disorder, stem cells, ESCs, IPSCs, tissue-specific progenitor/stem cells, fibroblasts, fibrocytes, reticular cells, tendon cells, myofibroblasts, adipocytes, melanocytes, mast cells, macrophages. The cells of the connective tissue may be derived from a desired multicellular organism.

The products and/or metabolites may further comprise a water solvent comprising sugars, salts, fatty acids, amino acids, coenzymes, signalling molecules, hormones, neurotransmitters, mucus, unicellular, multicellular and/or acellular organisms, e.g. intestinal microbiota, as well as waste products and/or cellular metabolites from human, animal and/or guest organism, e.g. intestinal commensal and/or pathogen microbiota. The interstitial fluid may further comprise blood plasma without the plasma proteins and may also comprise some types of wandering cells, e.g. white blood cells.

In even a further embodiment of the present invention, the at least one interstitial space comprises at least one fluid channel wherein the fluid channel is in communication with the tissue, humoral and neural channel.

The interstitial space may be formed entirely of a plurality of fluid channels wherein each of the fluid channels is in communication with at least one set of the at least three channels. Favourably, the fluid channels may be arranged to receive interstitial cells, products and/or metabolites, e.g. the interstitial fluid channel. By providing an interstitial space comprising at least one fluid channel, the present invention empowers scientists/bioengineers to design more complex fluidic devices wherein the location and therefore the accessibility of interstitial cells, products and/or metabolites is controllable. In a further embodiment of the present invention, the fluid channel is made of a permeable and/or semi-permeable material, e.g. membrane. The fluid channel of the present invention may be made of a biodegradable or non-biodegradable material. The pore aperture, the porosity and/or molecular weight cut off (MWCO) of the material of the interstitial fluid channel depend on the size of the compounds desirable to separate from the interstitial space. By defining the permeability of the fluid channel, wherein the fluid channel optionally comprises products and/or metabolites, e.g. interstitial fluid, the access of living tissue cells, blood and/or vascular cells and neuronal cells can be controlled.

In a further embodiment of the present invention, the fluidic device may comprise two or more sets of separated channels wherein in each set the separated channels are in communication with one another and, optionally, the two or more sets of separated channels are in communication with one another. Since the fluidic device of the present invention is not restricted to one particular set of at least three channels, the reconstruction of complex living tissues, e.g. organs, is one of the possibilities provided by the fluidic device of the present invention. It is even possible to reconstruct patient specific healthy body tissue and patient specific body tissue affected with a certain disease in one single fluidic device. Such fluidic device may be useful in selecting the most optimal patient unique therapy wherein the affected tissue is cured and wherein the healthy body tissue of the patient is unaffected by the chosen treatment.

The fluidic device of the present invention as well as the at least one set of at least three channels may have any particular form, preferably a planar and/or tubular form. The fluidic device may be any pressure resistant capillary, channel, tube, groove, chamber, container, reservoir or the like. It is noted that a planar shaped fluidic device is preferred

to perform a dynamical (i.e. live) visual control of the coculture, e.g. by fluorescent microscopy, to evaluate tissue integrity and/or permeability, e.g. by measuring trans-epithelial electrical resistance, and/or morphology, e.g. by using hematoxylin and eosin stain or immunofluorescence. It is further noted that a tubular shaped fluidic device is preferred for sampling cells as well as acellular, unicellular, multicellular organisms, tissue and/or materials and/or products and/or metabolites from the fluidic device of the present invention.

In an embodiment, the inner and/or outer surface of one or more channels is at least partially coated with a layer of cells selected from living tissue cells, blood and/or vascular cells or neuronal cells. In a favourable embodiment, the tissue channel is at least partially coated with a layer of living tissue cells. In another favourable embodiment, the humoral channel is at least partially coated with a layer of blood and/or vascular cells preferably forming a capillary endothelium. Such capillary endothelium may be formed by coating the entire inner and/or outer surface of the humoral channel with a layer of blood and/or vascular cells or by the formation of a capillary endothelium by blood and/or vascular cells within the humoral channel itself. The capillary endothelium may be formed by coating the outer surface of the humoral channel made by a biodegradable material with blood and/or vascular cells. Finally, also the inner and/or outer surface of the neural channel may be at least partially coated with a layer of neuronal cells.

In an embodiment of the present invention, at least a part of the at least partially coated inner and/or outer surface of one of the channels is contiguous to at least a part of the inner and/or outer surface of the at least two other channels. In this context the term "contiguous" has to be understood that the inner and/or outer surfaces of the different channels share a common border, e.g. the separating materials optionally including the interstitial space enclosed by the outer surfaces of the channels.

In an even further embodiment of the present invention, at least a part of the at least partially coated inner and/or outer surface of the one or more channels may further comprise a layer of connective tissue. Preferably the connective tissue is located in between the inner and/or outer surface of at least one of the channels and the layer of cells selected from living tissue cells, blood and/or vascular cells and neuronal cells.

The layer of connective tissue may comprise ECM, interstitial cells, products and/or metabolites. The connective tissue may further be chosen such that the layer of connective tissue has adhesive properties, e.g. by using fibroblasts, to adhere cells selected from living tissue cells, blood and/or vascular cells and neuronal cells to the inner and/or outer surface of the channel and/or to the area comprising a plurality of pores, e.g. the above-described permeable and/or semi-permeable material, e.g. permeable and/or semi-permeable membrane.

Other adhesive materials may be used as well to adhere cells selected from living tissue cells, blood and/or vascular cells and neuronal cells to the inner and/or outer surface of one or more channels. Preferably the material used to adhere cells to the inner and/or outer surface of one or more channels is selected from a biocompatible material. The adhesive material is preferably applied to the inner and/or outer surface of the channel as a gel, solution, hydrogel, or other composition that will adhere to the inner and/or outer surface of the channel via or without binding to the material of which the surface of the channel is made of.

In an embodiment of the present invention, the adhesive material is chemically coupled to the inner and/or outer surface of the channel, e.g. via a covalently bond or cross-link. In another embodiment, the membrane comprised in the separating material is created (e.g. polymerized) with adhesive material embedded in the membrane. In even another embodiment, the adhesive material can be a molecule bound by a molecule on the surface of a living tissue cell. In even a further embodiment, the adhesive material can be a molecule which binds a molecule on the surface of the living tissue cell.

Preferably the adhesive coating material is selected from the group consisting of collagen, laminin, proteoglycan, vitronectin, fibronectin, fibrin, poly-D-lysine, elastin, hyaluronic acid, glycoasaminoglycans, integrin, polypeptides, oligonucleotides, DNA, polysaccharide, MATRIGEL™, extracellular matrix and combinations thereof.

In an embodiment of the present invention, the adhesive material may be obtained from a mammal or synthesized or obtained from a transgenic organism. Preferably, the adhesive material is mammalian, e.g. murine, primate or human in origin. Furthermore, the concentration of the adhesive material may vary. Preferably, the adhesive material is

present at a concentration in range from about 10 µg/mL to about 1000 µg/mL, more preferably present in an amount of 10 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 500 µg/mL, 1000 µg/mL or any value in between.

In a particular embodiment of the present invention, the separating material of the fluidic device separating the at least three channels may be coated with a mixture comprising collagen type I, preferably, the separating material may be coated with 400 µg/mL collagen type I. In another embodiment of the present invention, the separating material is coated with a mixture comprising 0,1 U/mL thrombin and 2 mg/mL fibrinogen optionally dissolved in a desired cell culture medium.

In a further embodiment of the present invention, the at least one of the channels, e.g. the tissue, humoral or neural channel, and/or the interstitial space of the fluidic device of the present invention comprises at least one hollow fibre for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells, neuronal cells, interstitial cells, products and/or metabolites from the fluidic device.

As used herein, the term "hollow fibre" refers to any capillary, channel, tube, or groove that is deposed within or upon a substrate. The hollow fibre can be a microchannel; i.e. a fibre that is sized for passing through microvolumes of liquid.

Preferably the at least one hollow fibre is embedded in at least one of the coatings formed on the inner surface of one or more channels. Favourably, the hollow fibre is made of a permeable and/or semi-permeable material, e.g. permeable and/or semi-permeable membrane. Even further, the hollow fibre is made of a biodegradable and/or non-biodegradable material. The porosity of hollow fibre material and/or MWCO depend on specific needs and the maximum molecular weight of the desired dissolved compound that will pass through the permeable and/or semi-permeable membrane into the permeate stream. Since the permeability of the hollow fibre may be varied per surface area of the hollow fibre, the scientists/bioengineers have the possibility to design the hollow fibre in such a way that any kind of components can be administered to a specific part of the fluidic device by using the hollow fibre. Consequently, the permeability of the hollow fibre may be chosen such that samples can be taken from the interstitial space or tissue, humoral or neural channels depending on the location of the

hollow fibre. The usage of hollow fibres located in one of the channels or embedded in the coatings as described above, allows the (dynamic) sampling extracellular fluids (e.g. interstitial fluid), tissue, humoral or neural cells to evaluate cellular characteristics like proteomics and metabolomics to provide a more complete picture of a living organism.

The separating material separating the at least three channels, fluid channel and/or hollow fibre of the present invention may have different thickness. Preferably the separating material of the fluidic device separating the at least three channels, fluid channel and/or hollow fibre is from 0,5 µm or greater in thickness, favourably 5 µm or greater in thickness, preferably 10 µm or greater in thickness, more preferably 20 µm or greater in thickness, 25 µm or greater in thickness, 30 µm or greater in thickness, 35 µm or greater in thickness or 40 µm or greater in thickness. Favourably, the separating material of the fluidic device separating the at least three channels, fluid channel and/or hollow fibre have a thickness in the range from about 10 µm to about 50 µm.

At least a part of the separating material of the fluidic device separating the at least three channels, fluid channel and/or hollow fibre is made of a biocompatible polymer wherein biocompatible polymer refers to materials which do not have toxic or injurious effects on biological functions. Biocompatible polymers may include natural, ECM derived compounds like collagen, laminin or the like or synthetic biodegradable or non-biodegradable polymers, e.g. poly(alpha esters) such as poly (lactate acid), poly(glycolic acid), polyorthoesters and poly anhydrides and their copolymers, polyglycolic acid and polyglactin, cellulose ether, cellulose, cellulosic ester, fluorinated polyethylene, phenolic, poly-4-methylpentene, polyacrylonitrile, polyamide, polyamideimide, polyacrylate, polybenzoxazole, polycarbonate, polycyanoarylether, polyester, polyestercarbonate, polyether, polyetheretherketone, polyetherimide, polyetherketone, poly ether sulf one, polyethylene, polyfluoroolefin, polyimide, polyolefin, polyoxadiazole, polyphenylene oxide, polyphenylene sulfide, polypropylene, polystyrene, polysulfide, polysulfone, polytetrafluoroethylene, polythioether, polytriazole, polyurethane, polyvinyl, polyvinylidene fluoride, regenerated cellulose, silicone, urea- formaldehyde, polyglactin, or copolymers or physical blends of these materials.

At least a part of the separating material of the fluidic device separating the at least three channels, fluid channel and/or hollow fibre may also be made of, for example, ceramic coatings on a metallic substrate. However, any type of coating material may be suitable. The coating may be made of different types of materials including metals, ceramics, polymers, hydrogels or a combination of any of these materials. Biocompatible materials may include, but are not limited to an oxide, a phosphate, a carbonate, a nitride or a carbonitride. The oxide may be selected from the group consisting of tantalum oxide, aluminum oxide, iridium oxide, zirconium oxide or titanium oxide.

The present invention further relates to the use of the fluidic device of the present invention for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells and neuronal cells, interstitial cells, products and/or metabolites from the fluidic device. The at least one set of separated channels, i.e. the tissue channel, humoral channel and neural channel, may be used to coculture, evaluate, sample and/or harvest cell material and/or fluids. The fluidic device therefore allows the scientist/bioengineer to coculture, evaluate, sample and/or harvest *in vitro* reconstructed tissue and to study possible relevant therapies for patient specific tissues.

In another aspect the present invention relates to a perfusion system, e.g. a bioreactor, comprising at least one fluidic device as described above, at least one first, at least one second and at least one third inlet port each inlet port being arranged for feeding medium to fluidic device and at least one first, at least one second and at least one third outlet port outlet port being arranged for discharging medium from the fluidic device, wherein the at least one first inlet and outlet port are connected to the at least one tissue channel, the at least one second inlet and outlet port are connected to the at least one humoral channel and the at least one third inlet and outlet port are connected to the at least one neural channel.

The term “port” refers to a portion of the perfusion system described herein which provides a means for fluid and/or cells to enter and/or exit the system and/or to enter and/or exit portions of the system. The port can be of any size and shape to accept and/or secure a connection with tubes, connections, or adaptors of a fluidic or

microfluidic system and allow passage of fluid and/or cells when the port is attached to a fluidic or microfluidic system.

The perfusions system of the present invention may further comprise at least fourth inlet port for feeding medium to the fluidic device and at least one fourth outlet port for discharging medium from the fluidic device, wherein the at least one fourth inlet and outlet port are connected to the at least one interstitial space of the fluidic device. Preferably, the fourth inlet and outlet port are arranged for feeding and discharging interstitial cells and/or fluid to the fluidic device of the present invention.

In case the fluidic device is provided with hollow fibres to evaluate and/or sample tissue cells, blood cells, vascular cells, neuronal cells, interstitial cells and/or products and/or metabolites from the fluidic device of the present invention, the fluidic device of the present invention may further comprise at least one fluid inlet port and at least one fluid outlet port connected to the hollow fibre of the fluidic device.

In a further embodiment, in case the fluidic device of the present invention, e.g. tubular shaped fluidic device, provides a fourth channel comprising one or more fluid channels, e.g. interstitial fluid channels, the fourth inlet and outlet port of the fluidic device may be connected with the one or more fluid channels. The fluidic device may further comprises at least one fifth inlet port for feeding medium to the fluidic device and at least one fifth outlet port for discharging medium from the fluidic device, wherein the at least one fifth inlet port and at least one fifth outlet port may be connected to the remaining external space of the fluidic device, wherein the external space is the space enclosed by the interior of the fluidic device and the outer surfaces of the separated channels (optionally in combination with interstitial, living tissue, humoral and/or neural cells separating the external space from the interstitial space).

In an even further embodiment, the perfusion system of the present invention comprises a fluidic device comprising two or more sets of separated channels and wherein the at least one first inlet and outlet port are connected to two or more living tissue channels, the at least one second inlet and outlet port are connected to two or more humoral channels and the at least one third inlet and outlet port are connected to two or more neural channels.

Additionally, the inlet ports arranged in the perfusion system of the present invention may further comprise one or more sample inlet ports allowing the scientist/bioengineer to administer any kind of component, e.g. cell material, microbial cells, pathogens, parasites, pharmaceutically active ingredients, signalling molecules, growth factors, hormones or the like to the fluidic device of the present invention. The fluidic device of the present invention may further comprise one or more sample outlet ports allowing the scientist/bioengineer to collect samples, e.g. cells, products and/or metabolites, products of coculture with other than reconstructed desired tissue acellular, unicellular and/or multicellular organism and/or tissue, material, products and/or metabolites or the like, from the fluids discharged from the channels of the fluidic device of the present invention.

Physical, chemical and/or biological stimuli, e.g. irradiation, light, gas, cell material, microbial cells, pathogens, parasitic and/or symbiotic organism, pharmaceutically active ingredients, signalling molecules, growth factors, hormones or the like, may be used to evaluate responses of a constructed living tissue to desired stimuli and/or to evaluate responses of applied stimuli to constructed tissue. The above-mentioned stimuli may be applied and/or administered by the scientist/bioengineer to the fluidic device of the present invention wherein the constructed and maintained living tissue and/or cocultured guest organism, tissue and/or material in the fluidic device are exposed to the desired stimuli for a predefined period of time. For example, to stimulate the natural reconstruction of intestinal epithelial cells, microbial cells may be maintained in the fluidic device of the present invention for at least 1 day.

The above-mentioned pharmaceutically active ingredients, signalling molecules, growth factors, hormones or the like may be selected from the group consisting of therapeutics, small molecules, nutriceuticals, drugs, probiotics, foods, vitamins, food supplements, commensal and pathogenic microflora, toxins and combinations thereof.

The biological stimuli may be acellular and/or cellular, unicellular and/or multicellular, aerobic and/or anaerobic and the fluidic device of the present invention may comprise a combination. Even further, to stimulate the natural growth of living tissue cells, e.g. gut,

intestinal microbiota are preferably supplied to the tissue channel of the fluidic device of the present invention.

It is noted that the fluidic device and/or perfusion system of the present invention allows the scientist/bioengineer to coculture the *in vitro* constructed tissue with another organism and/or tissue, wherein the other tissue is not necessarily constructed *in vitro*. Even further, the tissue, blood, vascular, and/or neuronal cells comprised in the tissue, humoral and neural channels may be cocultured with other organisms, tissues and/or materials. For example, coculturing intestinal epithelium and intestinal microbiota in the tissue channel may be used to study host microbe interactions and/or to culture difficult to culture intestinal microbiota. The blood and/or vascular cells in the humoral channel may be cocultured with the *Plasmodium malaria* and the neuronal cells may be cocultured with the poliovirus to study host pathogen interactions. Even further, connective tissue applied to the channels may be combined with other tissues and/or organisms as well. For example, the connective tissue may be combined with *Echinococcus*. In an even further aspect the fluidic device of the present invention allows to use the reconstructed tissue as a feeding and/or support tissue, e.g. to study *in utero* embryonic development. The fluidic device and/or perfusion system of the present invention further allow the scientist/bioengineer to coculture human and/or animal tissue with other than reconstructed tissue acellular, unicellular and multicellular organisms and/or tissue and/or material, e.g. biomedical polymers and/or donor tissues to study transplantation rejection.

In a further aspect the present invention relates to a perfusion system of the present invention further comprising at least one first, at least one second and at least one third reservoir coupled to the at least one first, at least one second and at least one third inlet ports of the fluidic device for feeding medium to the fluidic device. The reservoir may be selected from a pressure resistant reservoir or other container comprising a medium such as a fluid (e.g. water or tissue specific medium) or a gas (e.g. air, pressurized gas and/or other gas).

The reservoir can be a container comprising a volume of fluid such that the fluid can be caused to move from the reservoir and through the one or more channels of the fluidic device. The reservoir can be coupled to the one or more fluidic devices of the perfusion

system by any means of conducting a fluid, e.g. tubing, piping, channels, or the like. The fluidic device and/or the reservoir can comprise ports. The reservoir may also be a syringe connected to the fluidic device of the present invention. The use of a syringe allows the scientist/bioengineer to add and/or sample products and/or metabolites from the fluidic device, e.g. interstitial fluid, without a permanent flow of fluid through the respective compartment, e.g. separated channel, interstitial space or external space.

The medium which is caused to flow through the one or more channels, fluid channels and/or hollow fibres of the fluidic device described herein may be any medium appropriate for maintaining or culturing living tissue cells, blood and/or vascular cells, neuronal cells and/or interstitial cells. The medium flow through the different channels, fluid channels and/or hollow fibres may be substantially the same medium or may vary per part of the fluidic device of the present invention. In a preferred embodiment of the present invention, the medium flow through the different channels, fluid channels and/or hollow fibres is substantially different from one another. In case microbial cells are present in the fluidic device, the medium should be appropriate for maintaining or culturing microbial cells, preferably the medium should not contain antibiotics to which the microbial cells are susceptible. The medium may comprise cell culture medium, solutions, buffers, nutrients, tracer compounds, dyes, antimicrobials, or other compounds not toxic to the cells being cultured in the fluidic device described herein. Suitable media for culturing or maintaining living tissue cells, e.g. intestinal cells, intestinal epithelial cells, endothelial cells, immune cells, and/or connective tissue cells, and microbial cells are well known in the art. By way of non-limiting example, media suitable for maintaining or culturing living tissue cells, e.g. intestinal epithelial cells can include Advanced DMEM/F12 Medium (Invitrogen) containing BSA (Sigma) supplemented with EGF, R-spondin 1 and Noggin growth factors (Peprotech), penicillin, streptomycin (Gibco) and/or Normocin (Invivogen, San Diego, CA).

The at least one first, at least one second and at least one third reservoir may be coupled to the at least one first, at least one second and at least one third outlet ports respectively for receiving medium from the fluidic device. By connecting the outlet ports of the fluidic device with the at least three reservoirs a closed system can be created in order to reduce any negative influence from the surrounding environment. As already explained above, such closed system may be provided with one or more sample inlet and/or

sample outlet ports to allow the scientist/bioengineer to influence the system in a controllable way. In order to provide a constant flow of medium, the perfusion system of the present invention may further comprise at least one pump coupled to the at least one fluidic device and the at least one first, at least one second and/or at least one third reservoirs. It is noted that further ports, e.g. the fourth and fifth port, may be connected to a pump as well. Even further, as already mentioned above, the ports may be connected to a syringe.

The at least one pump may be any dynamic or displacement pump and may be selected from the group consisting of a syringe pump, a peristaltic pump, pulse-free pump, positive displacement pump and combinations thereof.

The flow of the medium through the fluidic device is capable to generate well-defined wall shear stress that affects cellular morphology and physiology, e.g. genomics, transcriptomics, proteomics and/or metabolomics. Biomechanical stimulation of physiological magnitude can modulate cellular phenotype via modulation of gene expression. As already explained above, the fluidic device of the present invention can be planar. The flow shear stress (τ) at the wall of the channels contained in a planar fluidic device is a function of flow rate and height of the channel. The shear stress on the cells is assumed approximately equal to the channel wall in case the cell height is approximately two orders of magnitude less than the channel. Equation 1 describes the relationship between the shear stress and the flow rate in a planar fluidic device.

$$\tau = 6Q\mu/(wh^2) \qquad (1)$$

wherein:

τ is the shear stress in dyne/cm^2;

Q is the flow rate in cm^3/s;

μ is the dynamic viscosity of the culture medium in g/cm·s;

w is the flow channel width in cm; and

h is the flow channel height in cm.

The channels contained in the fluidic device of the present invention can also have a tubular form. In a tubular shaped channel the wall shear stress in the circumferential direction on the inner surface of the channel wall/cells can be described by equation 2.

$$\tau = 4\mu Q/\pi r^3 \qquad (2)$$

wherein:

τ is the shear stress in dyne/cm^2;

μ is the dynamic viscosity of the culture medium in g/cm·s;

Q is the volume flow rate in cm^3/sec;

π is the known mathematical constant; and

r is the radius in cm.

The shear stress on the medium flowing through the fluidic device channels may be from 0 to 1000 dyne/cm^2. Preferably, the shear stress can be in the range from about 0,5 dyne/cm^2 to about 120 dyne/cm^2. The shear stress and/or the flow rate can be modulated to create a desired state and/or condition of the living tissue cells, such as intestinal epithelial cells, e.g. modelling "flush-out" of the luminal components of the intestine.

The shear stress may be about the same for the duration of the time during which living cells are cultured in the fluidic device. However, in an embodiment of the present invention, the shear stress may be increased and/or decreased during the time in which living cells are cultured in the fluidic device, e.g. the shear stress may be decreased for a time to allow newly added cells to attach to the membrane and/or pre-existing cells. Preferably, the shear stress may be varied in a regular, cyclic pattern to mimic desired tissue deformation, e.g. blood vessels pulsation. On the other hand, in another embodiment of the present invention, the shear stress can be varied in an irregular pattern, e.g. mimic intestinal motility. The shear stress of the medium flowing through the fluid channel on the cells presented in the flow channel can vary over time. In an embodiment of the present invention, the shear stress can vary over time from 0 to 1000 dyne/cm^2. In a particular embodiment of the present invention, the shear stress can vary over time from 0,5 dyne/cm^2 to 34 dyne/cm^2.

Different flow rates of the medium through the channels of the fluidic device may be applied to the perfusion system of the present invention. The flow rate may be varied between the different channels and may be varied in such way to mimic the *in vivo* flow rate of a flow through the desired living tissue. Even so, the flow rate of the medium can be adjusted to mimic the flow of a medium in case the living tissue is suffering from a disorder affecting the respective living tissue constructed in the *in vitro* system of the present invention, e.g. to mimic diarrhoea.

The flow rate may be varied over time. In an embodiment of the present invention, the medium flow rate may be about the same for the duration of the time during which living cells are cultured in the fluidic device of the present invention. In a particular embodiment, the medium flow rate can be increased and/or decreased during the time in which living cells are cultured in the fluidic device, e.g. the medium flow rate can be decreased for a time to allow newly added cells to attach to the membrane and/or pre-existing cells. Alternatively, the medium flow rate can be varied in a regular, cyclic pattern or in an irregular pattern.

The perfusion system of the present invention may further comprise units for monitoring and controlling several process parameters, including the pH value, temperature and the like. The perfusion system of the present invention may further comprise filters and/or an oxygenator.

In another aspect the present invention relates to a method for *in vitro* tissue reconstruction and/or coculture, comprising the following steps:

a) providing a perfusion system of the present invention;

b) providing living tissue cells, blood and/or vascular cells, and neuronal cells;

c) allowing medium to flow through the fluidic device;

d) closing the inlet ports and outlet ports of the fluidic device to stop the flow of medium once the fluidic device is filled with medium;

e) seeding the living tissue cells to the tissue channel of the fluidic device;

f) seeding the blood and/or vascular cells to the humoral channel of the fluidic device;

g) seeding the neuronal cells to the neural channel of the fluidic device; and

h) open the inlet ports and outlet ports of the fluidic device to allow medium to flow through the fluidic device.

The above-described method can be applied for any type of fluidic device. In an embodiment of the present invention, the method further comprises the step of providing a connective tissue and coating the inner and/or outer surface of the tissue channel, humoral channel and/or neural channel with the connective tissue before seeding the living tissue cells, the blood and/or vascular cells and/or neuronal cells to the respective channels.

The separating material of the fluidic device separating at least a part of the at least three different channels may be pre-coated with connective tissue before placing the separating material, e.g. permeable and/or semi-permeable membrane, into the fluidic device of the present invention. Even further, living tissue cells, the blood and/or vascular cells and/or neuronal cells may be seeded to the separating material separating at least a part of the at least three different channels before placing the separating material into the fluidic device of the present invention.

Alternatively the present invention relates to a method for *in vitro* tissue reconstruction and/or coculture, comprising the following steps:

a) providing at least three separating materials for forming at least three physically separated channels;

b) providing living tissue cells, blood and/or vascular cells, and neuronal cells;

c) seeding each of the living tissue cells, blood and/or vascular cells and neuronal cells onto the inner and/or outer surface of one of the at least three separating materials;

d) placing the seeded at least three separating materials into a fluidic device of to the present invention;

e) connecting the fluidic device to a perfusion system of the present invention; and

f) allowing medium to flow through the fluidic device.

The physically separated separating materials may be formed such that planar or tubular fluid channels are created. Again, connective tissue may be provided to the wall of the separating materials before seeding living tissue cells, blood and/or vascular cells and neuronal cells onto the material.

The separating material may be pre-coated with an adhesive, e.g. collagen type I, to enhance the cell adhesion to the separating material. The separating material may be selected from the group consisting of collagen, laminin, proteoglycan,, vitronectin, fibronectin, poly-D-lysine, elastin, hyaluronic acid, glycoasaminoglycans, integrin, polypeptides, oligonucleotides, DNA, polysaccharide, MATRIGEL™, extracellular matrix, and combinations thereof.

In an even further aspect the present invention relates to a hollow fibre for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells, neuronal cells, interstitial cells, products, products and/or metabolites from the fluidic device of the present invention, wherein the fibre is made of a permeable and/or semi-permeable material, e.g. permeable and/or semi-permeable membrane. The hollow fibres are in particular suitable to meet scientific and industrial needs to allow scientists/bioengineers to control, evaluate, sample and/or harvest any characteristic of the *in vitro* reconstructed tissue. Preferably, the membrane of the hollow fibre is made of regenerated hydrophilic and/or hydrophobic, coated and/or uncoated biocompatible material for a long-term cell culture system. The material of the membrane may be selected from the group consisting of cellulose, cellophane, polyethylene, silicone, carbon nanomembranes and combinations thereof.

In a final aspect the present invention relates to the use of the hollow fibre as described above for coculturing, evaluating, sampling and/or harvesting of living tissue cells, blood cells, vascular cells, neuronal cells, interstitial cells, products and/or metabolites.

The invention will be elucidated on the basis of non-limitative exemplary embodiments shown in the following figures, in which:

figure 1a shows a schematic view of a planar fluidic device for *in vitro* tissue reconstruction according to the present invention;
figure 1b shows an exploded view of a planar fluidic device for *in vitro* tissue reconstruction according to the present invention;
figure 2 shows a schematic view of a tubular fluidic device for *in vitro* tissue reconstruction according to the present invention;

figure 3 shows a schematic view of a perfusion system comprising the fluidic device for *in vitro* tissue reconstruction according to the present invention; and
figure 4 shows a schematic view of a further tubular fluidic device for *in vitro* tissue reconstruction according to the present invention.

Figure 1a shows a schematic view of a planar fluidic device 1. The planar fluidic device 1 comprises an interior 2 comprising a first channel, i.e. upper flow channel 3, a second channel, i.e. lower left flow channel 4, and a third channel, i.e. lower right flow channel 5. The three different channels 3, 4, 5 are separated from one another by T-shaped separating portion 6. The separating portion 6 may also have a different form than illustrated, e.g. Y-shaped, as long as the separating portion 6 separates the three different channels 3, 4, 5. Each of the channels 3, 4, 5 of figure 1a is enclosed by a part of the interior 2 and a part of the separating portion 6. It is noted that the channels 3, 4, 5 may be enclosed entirely by the separating portion 6 (see in this respect: figure 2). Even so, the separating portion 6 may be made of physically separated materials wherein the outer surfaces of the physically separated materials enclose a space (not shown) situated in between the different channels 3, 4, 5. The separating portion 6 further comprises a membrane 7 for culturing living tissue cells, blood and/or vascular cells and/or neuronal cells each seeded to a part of the membrane 7 facing the channels 3, 4, 5. The membrane 7 physically separates the three flow channels 3, 4, 5 from one another, but allows cells cultured on the membrane 7 to communicate with each other. The membrane 7 may be a matrix whereon and/or wherein the cells can be seeded. The membrane 7 is preferably provided with (a layer of) hollow fibres 15 placed on and/or into the membrane 7 for evaluating, sampling and/or harvesting cells and/or fluid from the interstitial space (see in this respect: figure 1b). Favourably, the membrane 7 and hollow fibres are assembled as an insert but may also be directly incorporated into the fluidic device. Optionally, the separating portion 6 may consist entirely of a semi-permeable and/or permeable membrane, e.g. the membrane 7 as illustrated. It is noted that the material of the separating portion 6 dividing the interior 2 of the fluidic device 1 into an upper part 8 and a lower part 9 may be different from the material of the separating portion 6 dividing the lower part 9 into a lower right part 10 and a lower left part 11. It is further noted that the arrangement of the channels 3, 4, 5 may be completely different from the arrangement of the channels 3, 4, 5 illustrated in figure 1a, as long as the three different channels 3, 4, 5 are physically separated by a

separating portion 6, which separating portion 6 comprises means, such as pores (not shown) or a membrane 7, allowing communication between each of the channels 3, 4, 5. The fluidic device 1 of figure 1a further comprises inlet ports 12a, 12b, 12c and outlet ports 13a, 13b, 13c to allow medium to flow through the different channels in a direction illustrated by arrows P_1, P_2. P_3. The fluidic device 1 of figure 1a further comprises an inlet port 12d and outlet port 13d connected to the hollow fibre (not shown) for evaluating, sampling and/or harvesting cells and/or fluid of the fluidic device. Optionally, each hollow fiber may be connected to a separate inlet and/or outlet port (not shown) to separate interstitial fluid and/or cells from different areas of living tissue. Figure 1a depicts a fluidic device 1 wherein the flow of medium in each channel 3, 4, 5 is parallel to one another (see: arrows P_1, P_2. P_3). However, the flow of medium in one channel may be in opposite direction compared to the direction of the flow of medium in another channel. Furthermore, the type flow of medium may differ between the different channels 3, 4, 5, e.g. the flow in one channel may be laminar where the flow in another channel may be turbulent.

Figure 1b shows an exploded view of the planar fluidic device 1. It is noted that the fluidic device 1 is in general preferably made from transparent material to allow a visual control of the *in vitro* model. Figure 1b shows the upper part 8 comprising the interior 2 and first channel 3. The first channel 3 is provided with an opening 14. Figure 1b further shows the lower part 9 comprising a lower right part 10 and a lower left part 11 separated by T-shape separating portion 6. Channels 4, 5 are enclosed by the interior 2 and separating portion 6. Separating portion 6 is provided with an opening 7a. Figure 1b further shows an insert with membrane 7 which fits the membrane 7 onto the opening 7a provided in separating portion 6. The fluidic device 1 is assembled by attaching membrane 7, whether or not seeded with cells, onto opening 7a and subsequently attaching upper part 8 to lower part 9.

Figure 2 shows a schematic view of a tubular fluidic device 20 comprising an interior 21 comprising a first channel, i.e. tubular shaped flow channel 22, a second channel, i.e. tubular shaped flow channel 23, and a third channel, i.e. tubular shaped flow channel 24. Each tubular shaped flow channel 22, 23, 24 is preferably formed by a membrane having a certain degree of permeability to allow communication of cells contained in each of the tubular shaped flow channel 22, 23, 24. In figure 2, the tubular shaped flow

channels 22, 23, 24 are located adjacent to each other to enclose an interstitial space 25 separated from external space 25a enclosed by the inner surface of the interior 21 and the outer surface of the tubular shaped flow channels 22, 23, 24. The interstitial space 25 may be arranged to receive interstitial fluid and/or interstitial cells. It is noted that the adjoining of flow channels 22, 23, 24 is not necessary to allow communication between the different channels 22, 23, 24. The different flow channels 22, 23, 24 may be placed at a distance from one another. The interstitial space 25 enclosed by the outer surfaces of the flow channels 22, 23, 24 may be presented by an interstitial fluid channel formed by the outer surfaces of the flow channels 22, 23, 24, which flow channels are in communication with the interstitial fluid channel. The use of such natural occurred interstitial channel is preferred to provide sampling interstitial fluid for separation and/or purification of desired compounds and/or products and/or metabolites using separation and/or purification technology, e.g. liquid chromatography. The external space 25a may comprise supernatant from the different cells seeded to each of the channels 22, 23, 24. It is noted that the supernatant from the different channels 22, 23, 24 may also be separated using separating portions 21a defining an external space 25a divided into different compartments enclosed by the outer surface of one of the flow channels 22, 23, 24 the inner surface of the interior 21 and the inner surface of separating portions 21a. The fluidic device 20 further comprises inlet ports 26a, 26b, 26c (not visible), 26d (not visible), 26e and outlet ports 27a, 27b, 27c, 27d, 27e, each of the inlet ports 26a, 26b, 26c, 26d, 26e and outlet ports 27a, 27b, 27c, 27d, 27e are connected to respectively one of the channels 22, 23, 24, the interstitial space 25 and the external space 25a of the fluidic device 20. It is noted that the inlet port 26d and outlet port 27d may be connected to a hollow fibre (not shown) which hollow fibre is in communication with each of the channels 22, 23, 24. In other words, the interstitial space 25 may include a plurality of hollow fibres wherein each of the hollow fibres is in close communication with the at least three channels 22, 23, 24.

It is further noted that both figures 1 and 2 depicts a schematic view of a fluidic device 1, 20 wherein one set consisting of at least three channels 3, 4, 5, 22, 23, 24, and at least one interstitial space 25 is illustrated. It should be understood that the cell fluidic device 1, 20 of figures 1 and 2 may comprise a plurality of sets consisting of at least three channels 3, 4, 5, 22, 23, 24, and at least one interstitial space 25. Also, the fluidic device

1, 20 of figures 1 and 2 may comprise more than one interior 2, 21 each of the interiors comprising at least one set of at least three channels 3, 4, 5, 22, 23, 24.

Figure 3 shows a schematic view of a perfusion system 40. The perfusion system 40 comprises at least one fluidic device 41 of the present invention. The perfusion system 40 may also comprise additional fluidic devices (not shown). The fluidic device 41 comprises inlet ports 42 and outlet ports 43. Each of the ports 42, 43 may be provided with sample inlet ports 44 and sample outlet ports 45 to allow the scientist/bioengineer to add desired components, e.g. cells, active agents, microorganisms or the like, to the fluidic device 41 and/or to collect samples from the fluidic device 41. The inlet ports 42 are connected to a pump 46. Each of the inlet ports 42 may be connected to separate pump heads 46a to allow the scientist/bioengineer to apply different type of flow of medium to the different flow channels and/or interstitial space and/or the external space of the fluidic device 41. The outlet ports 43 may be connected to a control unit 47 which control unit 47 is arranged to control the flow of medium through the perfusion system 40 and/or each of the channels, the interstitial space and the external space (not shown) enclosed in the fluidic device 41. Preferably, the control unit 47 is connected with a computer 48. The perfusion system 40 further comprises one or more reservoirs 49, e.g. a feeding and/or collecting reservoir of medium, connected with the inlet ports 42, via the heads 46a of the pump 46, and the outlet ports 43, via control unit 47. The reservoirs 49 may comprise different media, e.g. liquid medium or gaseous medium. It is noted that the closed perfusion system 40 as illustrated in figure 3 may also be arranged as an open perfusion system. In such open perfusion system, the outlet ports 43 are connected to a different (collecting) reservoir (not shown). Also combinations of both systems are possible. The pump 46 is preferable selected from the group consisting of pulse-free pumps, peristaltic pumps and combinations thereof to provide a desired flow of medium. The flow of medium may be in the direction as indicated by arrows P_{10}, P_{11}, P_{12}. However, the direction of flow of medium does not necessarily have to be in parallel to one another.

Figure 4 shows a schematic view of a further tubular fluidic device 50, i.e. a set of three separated channels around a hollow fibre-like structure. The fluidic device is made of a semi-permeable and/or permeable, biodegradable and/or non-biodegradable membrane 55, optionally provided with a semi-permeable, permeable and/or impermeable,

biodegradable and/or non-biodegradable outer surface 55a. The membrane 55 is provided with four channels: a tissue channel 51, a humoral channel 52, a neural channel 53 and an interstitial fluid channel 54. The membrane 55 allows communication between the different channels 51, 52, 53, 54. The membrane 55 may be made from a matrix of hollow multi-fibres. Even further, the interstitial fluid channel 54 may further comprise additional hollow fibres. It is noted that the different hollow fibres may need different inlet/outlet ports (not shown).

The invention will now be further illustrated with reference to the following example.

Example

Living tissue cells, connective tissue cells, vascular and/or blood cells and neuronal cells (e.g. derived from human and/or porcine) were purchased from cell banks or isolated from tissue samples using methods isolating living tissue cells as described in Sato et al. (*Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche*; Nature Letters, 459 (2009): pp. 262-266), isolating vascular and/or blood cells as described in Yamamoto et al. (*Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress*; Journal of Applied Physiology, 95 (2003): pp. 2081-2088) and isolating neuronal cells as described in Bondurand et al. (*Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures;* Development and disease, 130 (2003): pp. 6387-6400), which methods are herewith incorporated by reference.

Reference is made to figure 1b wherein the different components of the planar fluidic device are shown. The membrane and the outer surfaces of the hollow fibres of an insert were coated (on both sides) with a mix of collagen I and connective tissue cells, e.g. myofibroblasts. Living tissue cells were seeded onto the coated surface of the hollow fibres and the membrane facing the upper part of the fluidic device. The insert was incorporated into the opening provided in the separating portion. Subsequently, the upper part of the fluidic device was connected to the lower part of the fluidic device.

The inlet ports of the separated channels and hollow fibers were connected via a conduit with the pump of the perfusion system (see: figure 3). The outlet ports of the separated channels and hollow fibers were connected via a conduit with the control unit of the perfusion system. Both the pump and control unit were connected to medium reservoirs providing a medium to the fluidic device. Medium from the reservoirs was allowed to flow through the fluidic device.

Cell suspensions comprising blood and/or vascular or neuronal cells were prepared. The pump of the perfusion system was stopped and the inlet and outlet ports of the fluidic device were closed. Syringes comprising suspensions of blood and/or vascular and neuronal cells were connected with one of the sample inlet ports connected with the inlet port of the second or third channel, i.e. the inlet port of the lower right or lower left flow channel. The cells were loaded into the flow channels and excess of medium was removed from the flow channels of the fluidic device via the sample outlet ports using empty syringes. After removal of the syringes from the sample ports, the inlet and outlet ports were opened and the medium from the medium reservoirs was allowed to flow through the fluidic device. The system was placed into an incubator or climate room at 37°C.

The cell growth and differentiation were checked under a microscope via the transparent parts of the fluidic device. After the desired level of cell differentiation was reached, several stimuli, e.g. immune cells, pathogen, control compounds, test compounds or the like, were added to the system and/or collected from the system. The formed cell culture perfusion system could be used for scientific and industrial needs, e.g. testing therapies to the constructed mammal tissue.

Conclusies

1. Fluïduminrichting voor *in vitro* weefselreconstructie omvattende:
   - ten minste één stel gescheiden kanalen, welk stel ten minste één weefselkanaal omvat, ten minste één lichaamssapkanaal en ten minste één zenuwkanaal;
   - waarbij het weefselkanaal is ingericht voor het opnemen van levende weefselcellen;
   - waarbij het lichaamssapkanaal is ingericht voor het opnemen van bloed- en/of vaatcellen; en
   - waarbij het zenuwkanaal is ingericht voor het opnemen van zenuwcellen,

waarbij het ten minste ene weefselkanaal, ten minste ene lichaamssapkanaal en ten minste ene zenuwkanaal van elkaar zijn gescheiden door een scheidingsmateriaal, welk scheidingsmateriaal communicatie tussen de ten minste drie kanalen onderling mogelijk maakt.

2. Fluïduminrichting volgens conclusie 1, waarbij het scheidingsmateriaal is gemaakt van een ondoorlaatbaar materiaal, welk materiaal ten minste één gebied met een aantal poriën omvat.

3. Fluïduminrichting volgens conclusie 2, waarbij het gebied met een aantal poriën is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

4. Fluïduminrichting volgens conclusie 1, waarbij het scheidingsmateriaal is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

5. Fluïduminrichting volgens één van de voorgaande conclusies, waarbij het scheidingsmateriaal ten minste gedeeltelijk is gemaakt van een biologisch afbreekbaar en/of niet-biologisch afbreekbaar materiaal.

6. Fluïduminrichting volgens één van de voorgaande conclusies, waarbij elk van de ten minste drie kanalen een binnenste oppervlak bepaalt dat het inwendige van het kanaal omsluit en een buitenste oppervlak dat grenst aan het binnenste oppervlak van het kanaal en is gericht naar ten minste een deel van de buitenste oppervlakken van de ten minste twee andere kanalen.

7. Fluïduminrichting volgens conclusie 6, waarbij de fluïduminrichting ten minste één interstitiële ruimte omvat die wordt omsloten door de buitenste oppervlakken van de ten minste drie kanalen.

8. Fluïduminrichting volgens conclusie 7, waarbij de ten minste ene interstitiële ruimte is ingericht voor het opnemen van interstitiële cellen, producten en/of metabolieten.

9. Fluïduminrichting volgens conclusie 7 of 8, waarbij de ten minste ene interstitiële ruimte ten minste één fluïdumkanaal omvat en waarbij het fluïdumkanaal in communicatie met het weefsel-, lichaamssap- en zenuwkanaal verkeert.

10. Fluïduminrichting volgens conclusie 9, waarbij het fluïdumkanaal is ingericht voor het opnemen van interstitiële cellen, producten en/of metabolieten en/of het fluïdumkanaal is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

11. Fluïduminrichting volgens één van de voorgaande conclusies, waarbij de fluïduminrichting twee of meer stellen gescheiden kanalen omvat, waarbij in elk stel de gescheiden kanalen in communicatie met elkaar verkeren en, optioneel, de twee of meer stellen gescheiden kanalen in communicatie met elkaar verkeren.

12. Fluïduminrichting volgens één van de voorgaande conclusies, waarbij het binnenste en/of buitenste oppervlak van één of meer kanalen ten minste gedeeltelijk is bedekt met een laag cellen welke zijn gekozen uit levende weefselcellen, bloed- en/of vaatcellen of zenuwcellen.

13. Fluïduminrichting volgens conclusie 12, waarbij ten minste een deel van het ten minste gedeeltelijk bedekte binnenste en/of buitenste oppervlak van één van de kanalen grenst aan ten minste een deel van het binnenste en/of oppervlak van de ten minste twee andere kanalen.

14. Fluïduminrichting volgens conclusie 12 of 13, waarbij ten minste een deel van het ten minste gedeeltelijk bedekte binnenste en/of buitenste oppervlak van de één of meer kanalen verder een laag bindweefsel omvat.

15. Fluïduminrichting volgens conclusie 14, waarbij de laag bindweefsel is gelegen tussen het binnenste en/of buitenste oppervlak van ten minste één van de kanalen en de laag cellen is gekozen uit levende weefselcellen, bloed- en/of vaatcellen of zenuwcellen.

16. Fluïduminrichting volgens conclusie 14 of 15, waarbij de laag bindweefsel ECM, interstitiële cellen, producten en/of metabolieten omvat.

17. Fluïduminrichting volgens één van de voorgaande conclusies, waarbij ten minste één van de kanalen en/of de interstitiële ruimte verder ten minste één holle vezel omvat voor het beoordelen, bemonsteren en/of oogsten van levende weefselcellen, bloedcellen, vaatcellen, zenuwcellen, interstitiële cellen, producten en/of metabolieten uit de fluïduminrichting.

18. Fluïduminrichting volgens conclusie 17, waarbij de ten minste ene holle vezel is ingebed in ten minste één van de deklagen volgens de conclusies 12 tot en met 16.

19. Fluïduminrichting volgens conclusie 17 of 18, waarbij de holle vezel is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

20. Fluïduminrichting volgens één van de conclusies 17 tot en met 19, waarbij de holle vezel is gemaakt van een biologisch afbreekbaar en/of niet-biologisch afbreekbaar materiaal.

21. Gebruik van de fluïduminrichting volgens één van de conclusies 1 tot en met 20, voor het cocultiveren, beoordelen, bemonsteren en/of oogsten van levende weefselcellen, bloedcellen, vaatcellen en zenuwcellen, interstitiële cellen, producten en/of metabolieten uit de fluïduminrichting.

22. Gebruik van de fluïduminrichting volgens één van de conclusies 1 tot en met 20, voor het cocultiveren, beoordelen, bemonsteren en/of oogsten van acellulair,

unicellulair en/of multicellulair organisme en/of weefsel, materiaal, producten en/of metabolieten uit de fluïduminrichting anders dan het gereconstrueerde weefsel.

23. Doorstroomsysteem omvattende:

- ten minste één fluïduminrichting volgens één van de conclusies 1 tot en met 20;
- ten minste één eerste, ten minste één tweede en ten minste één derde inlaatopening waarbij elke inlaatopening is ingericht voor het toevoeren van medium naar de fluïduminrichting; en
- ten minste één eerste, ten minste één tweede en ten minste één derde uitlaatopening waarbij elke uitlaatopening is ingericht voor het afvoeren van medium uit de fluïduminrichting,

waarbij de ten minste ene eerste inlaat- en uitlaatopening zijn verbonden met het ten minste ene weefselkanaal, de ten minste ene tweede inlaat- en uitlaatopening zijn verbonden met het ten minste ene lichaamssapkanaal en de ten minste ene derde inlaat- en uitlaatopening zijn verbonden met het ten minste ene zenuwkanaal.

24. Doorstroomsysteem volgens conclusie 23, verder omvattende:

- ten minste één vierde inlaatopening voor het toevoeren van medium naar de fluïduminrichting; en
- ten minste één vierde uitlaatopening voor het afvoeren van medium uit de fluïduminrichting,

waarbij de ten minste ene vierde inlaat en uitlaat zijn verbonden met de ten minste ene interstitiële ruimte van de fluïduminrichting.

25. Doorstroomsysteem volgens conclusie 23 of 24, waarbij de fluïduminrichting verder ten minste één fluïduminlaatopening en ten minste één fluïdumuitlaatopening omvat die met de holle vezel van de fluïduminrichting zijn verbonden.

26. Doorstroomsysteem volgens één van de conclusies 23 tot en met 25, waarbij de fluïduminrichting twee of meer stellen gescheiden kanalen omvat en waarbij de ten minste ene eerste inlaat- en uitlaatopening zijn verbonden met twee of meer weefselkanalen, de ten minste ene tweede inlaat- en uitlaatopening zijn verbonden met twee of meer lichaamssapkanalen en de ten minste ene derde inlaat- en uitlaatopening zijn verbonden met twee of meer zenuwkanalen.

27. Doorstroomsysteem volgens één van de conclusies 23 tot en met 26, waarbij het doorstroomsysteem verder ten minste één eerste, ten minste één tweede en ten minste één derde reservoir omvat dat is gekoppeld met respectievelijk de ten minste ene eerste, ten minste ene tweede en ten minste ene derde inlaatopening voor het toevoeren van medium naar de fluïduminrichting.

28. Doorstroomsysteem volgens conclusie 27, waarbij het ten minste ene eerste, ten minste ene tweede en ten minste ene derde reservoir is gekoppeld met respectievelijk de ten minste ene eerste, ten minste ene tweede en ten minste ene derde uitlaatopening voor het opnemen van medium uit de fluïduminrichting.

29. Doorstroomsysteem volgens conclusie 27 of 28, waarbij het systeem verder ten minste één pomp omvat die is gekoppeld met de ten minste ene fluïduminrichting en ten minste één eerste, ten minste één tweede en/of ten minste één derde reservoir.

30. Werkwijze voor *in vitro* weefselreconstructie en/of cocultivering, omvattende de volgende stappen:

a) het verschaffen van een doorstroomsysteem volgens één van de conclusies 23 tot en met 29;

b) het verschaffen van levende weefselcellen, bloed- en/of vaatcellen, en zenuwcellen;

c) het laten stromen van medium door de fluïduminrichting;

d) het sluiten van de inlaatopeningen en uitlaatopeningen van de fluïduminrichting om het stromen van medium te stoppen, zodra de fluïduminrichting met medium is gevuld;

e) het uitzaaien van de levende weefselcellen in het weefselkanaal van de fluïduminrichting;

f) het uitzaaien van de bloed- en/of vaatcellen in het lichaamssapkanaal van de fluïduminrichting;

g) het uitzaaien van de zenuwcellen in het zenuwkanaal van de fluïduminrichting; en

h) het openen van de inlaatopeningen en uitlaatopeningen van de fluïduminrichting om medium door de fluïduminrichting te laten stromen.

31. Werkwijze volgens conclusie 30, waarbij de werkwijze verder de stap omvat van het verschaffen van een bindweefsel en het bedekken van het binnenste en/of buitenste oppervlak van het weefselkanaal, lichaamssapkanaal en/of zenuwkanaal met het bindweefsel vóór het uitzaaien van de levende weefselcellen, de bloed- en/of vaatcellen en/of zenuwcellen in de respectievelijke kanalen.

32. Werkwijze voor *in vitro* weefselreconstructie en/of cocultivering, omvattende de volgende stappen:

a) het verschaffen van ten minste drie scheidingsmaterialen voor het vormen van ten minste drie fysisch gescheiden kanalen;

b) het verschaffen van levende weefselcellen, bloed- en/of vaatcellen, en zenuwcellen;

c) het uitzaaien van elk van de levende weefselcellen, bloed- en/of vaatcellen en zenuwcellen op het binnenste en/of buitenste oppervlak van één van de ten minste drie scheidingsmaterialen;

d) het aanbrengen van de uitgezaaide ten minste drie scheidingsmaterialen in een fluïduminrichting volgens één van de conclusies 1 tot en met 20;

e) het verbinden van de fluïduminrichting met een doorstroomsysteem volgens één van de conclusies 23 tot en met 29; en

f) het laten stromen van medium door de fluïduminrichting.

33. Holle vezel voor het beoordelen, bemonsteren en/of oogsten van levende weefselcellen, bloedcellen, vaatcellen, zenuwcellen, interstitiële cellen, producten en/of metabolieten uit de fluïduminrichting volgens één van de conclusies 1 tot en met 20, waarbij de vezel is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

34. Holle vezel volgens conclusie 33, waarbij het membraan is gemaakt van hydrofiel en/of hydrofoob, bedekt en/of onbedekt biover enigbaar materiaal.

35. Holle vezel volgens conclusie 33 of 34, waarbij het materiaal van de holle vezel is gekozen uit de groep bestaande uit cellulose, cellofaan, polyethyleen, silicone, koolstof nanomembranen en combinaties daarvan.

36. Gebruik van de holle vezel volgens één van de conclusies 33 tot en met 35 voor het cocultiveren, beoordelen, bemonsteren en/of oogsten van levende weefselcellen, bloedcellen, vaatcellen, zenuwcellen, interstitiële cellen, producten en/of metabolieten.

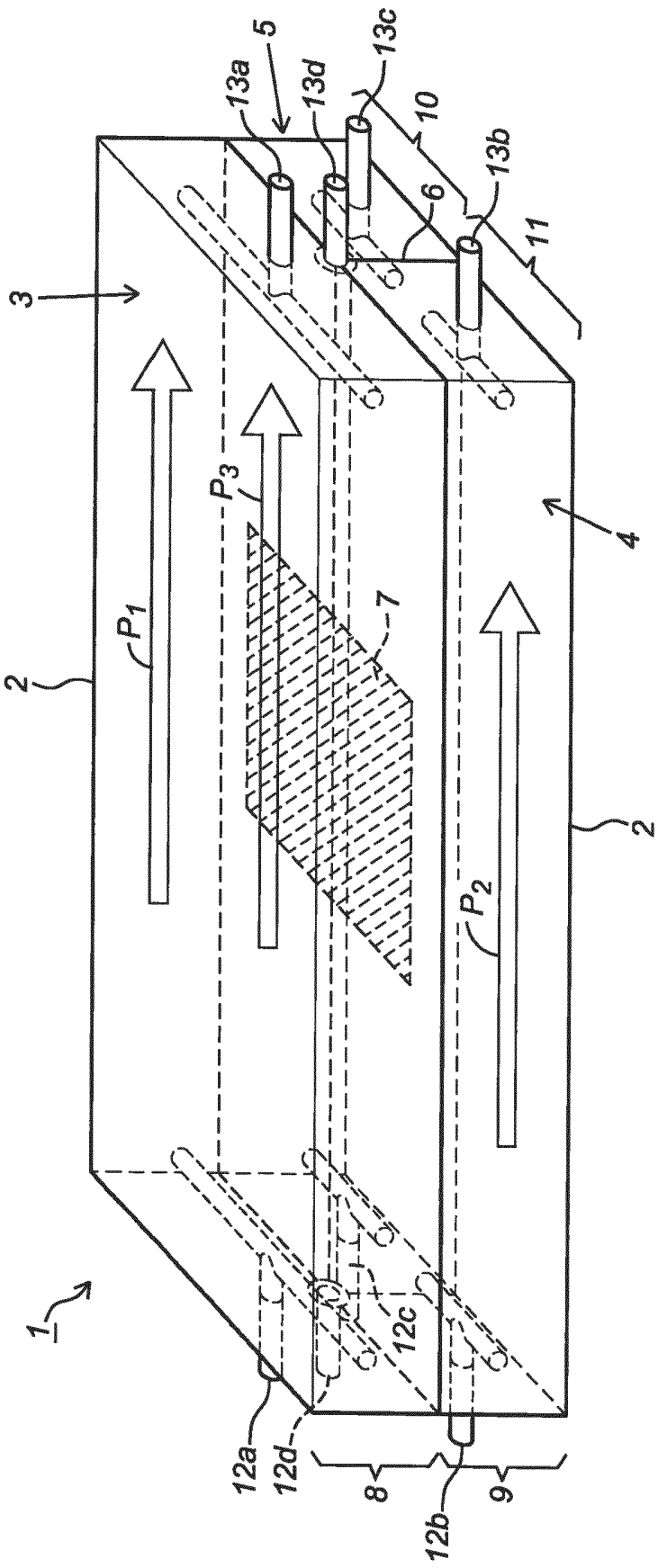

Fig. 1a

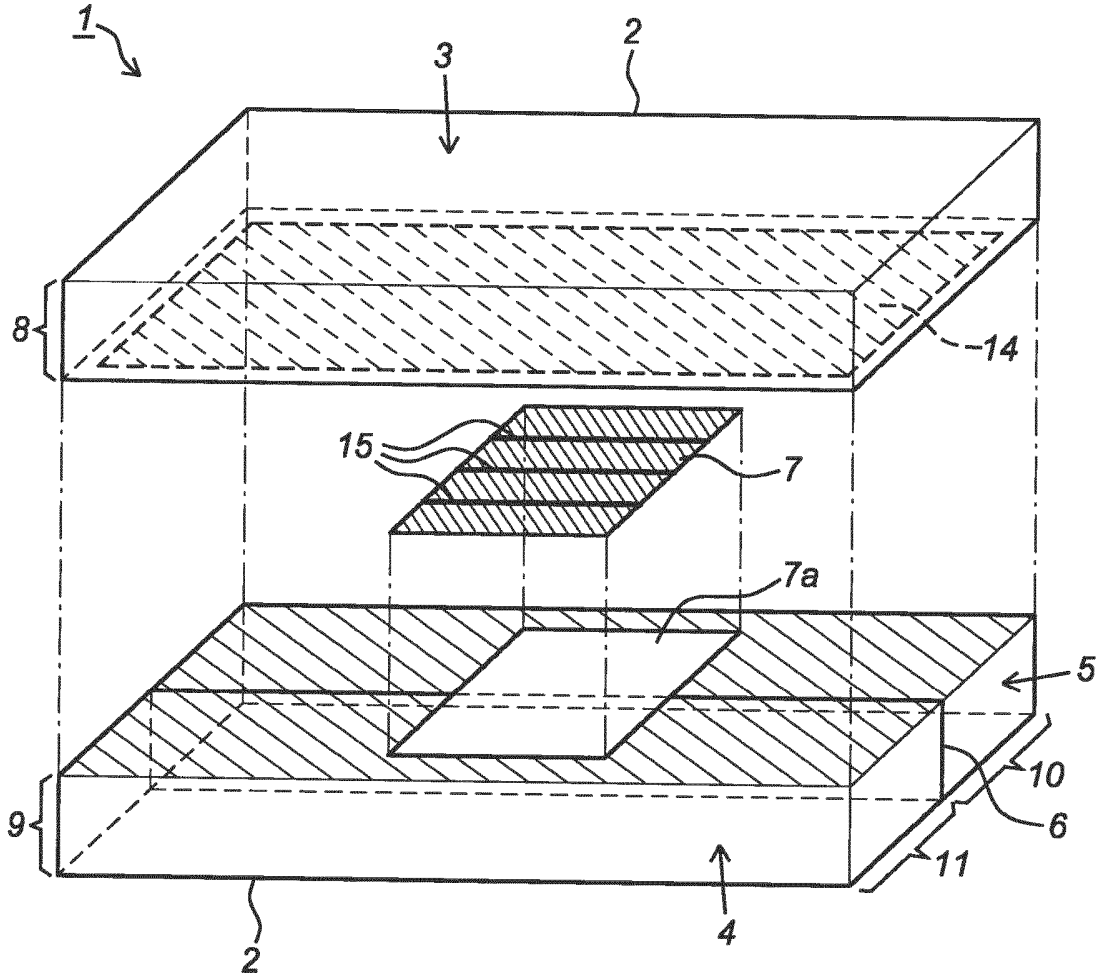

Fig. 1b

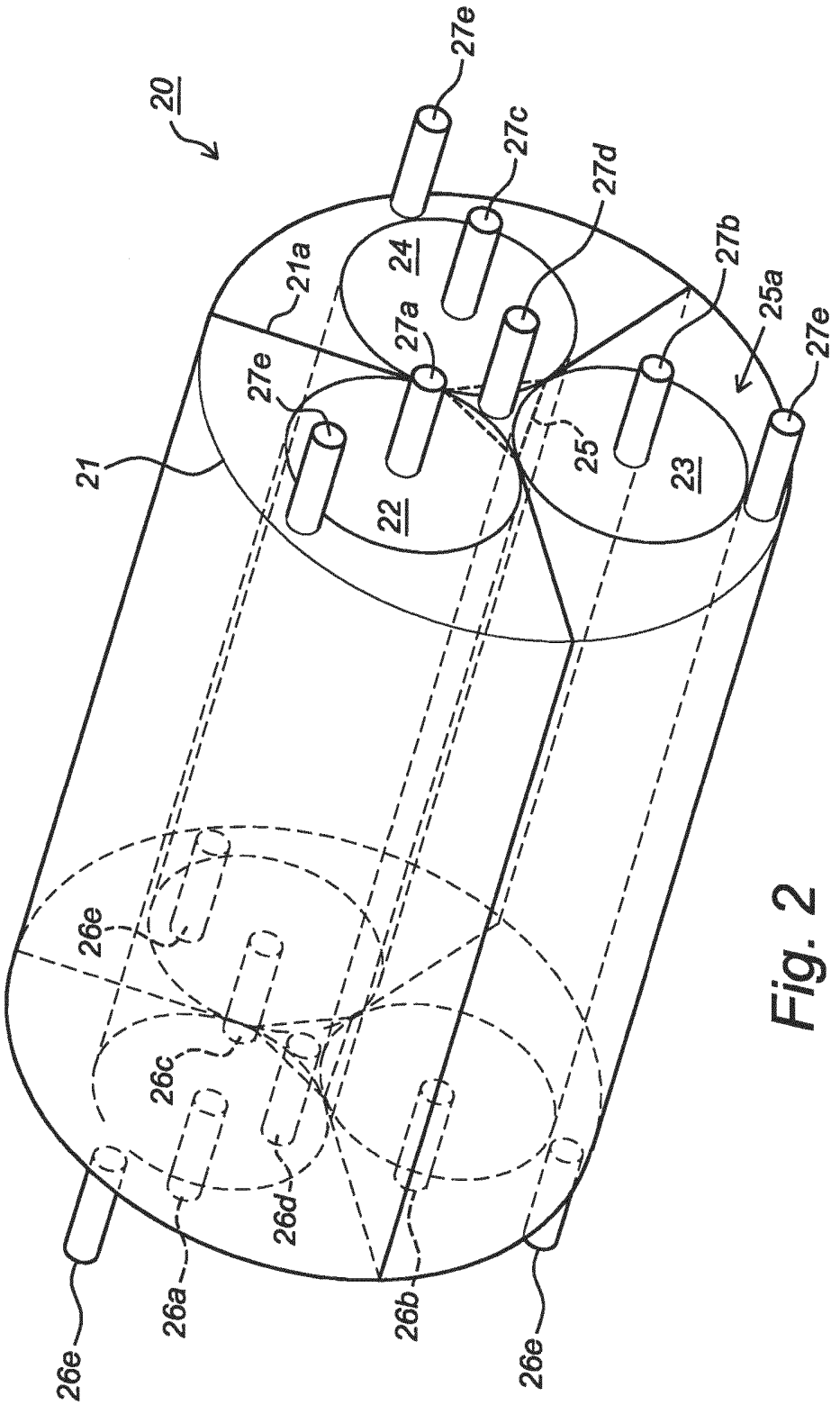

Fig. 2

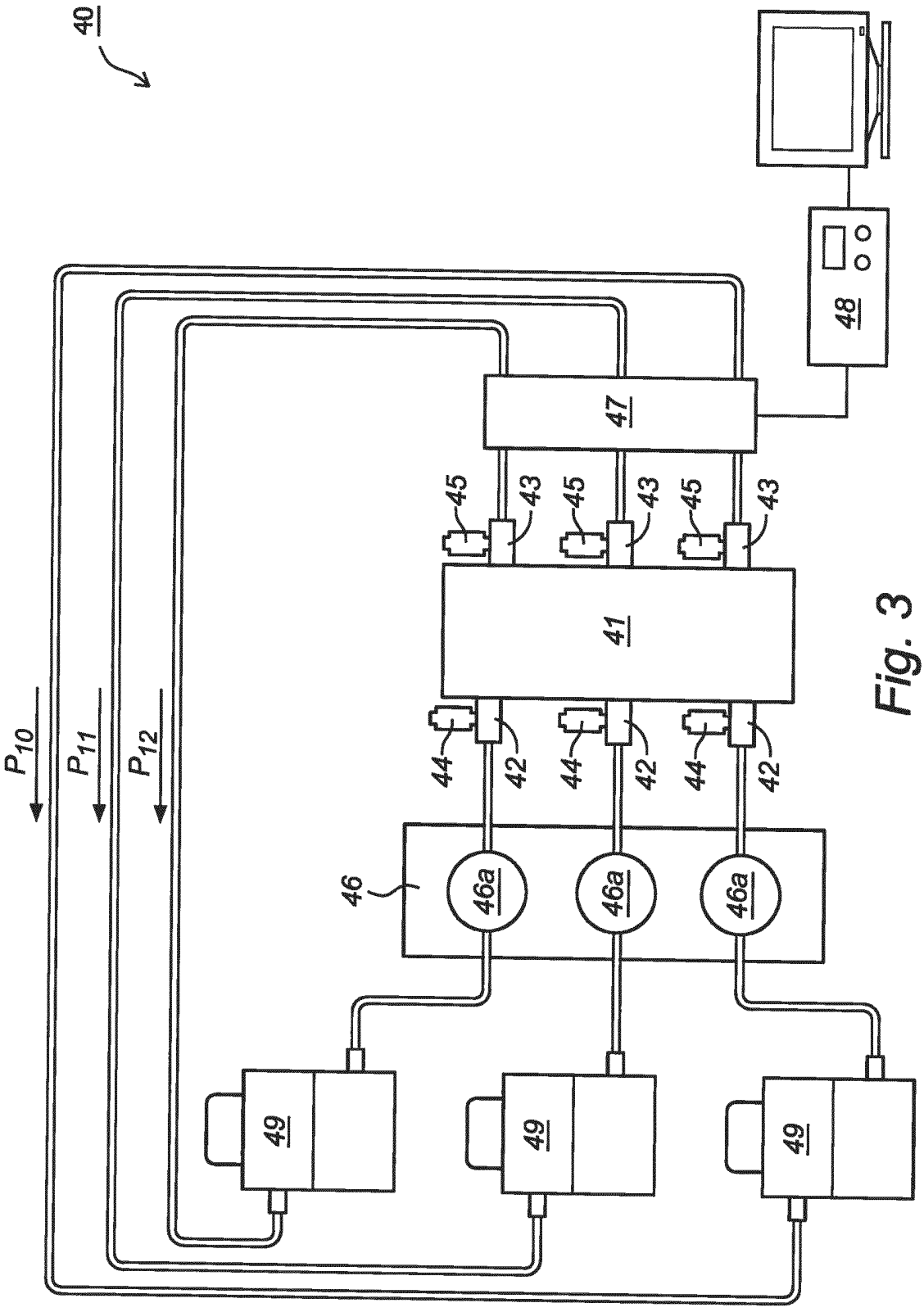

Fig. 3

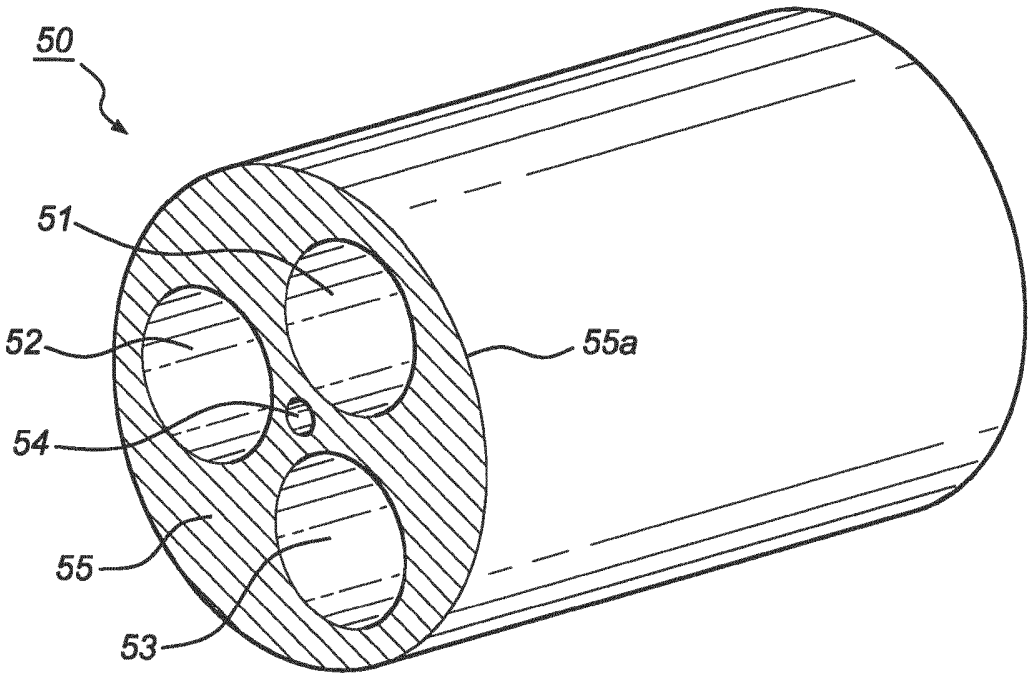

Fig. 4

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	1.1078.001 NL
Nederlands aanvraag nr. **2011895**	Indieningsdatum **04-12-2013**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Ponomarenko**	
Datum van het verzoek voor een onderzoek van internationaal type **12-04-2014**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN 61775**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

C12M3/00 **C12M1/12** **C12M1/00**

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**C12M**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐ **GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐ **GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2011895

A. CLASSIFICATIE VAN HET ONDERWERP
INV. C12M3/00 C12M1/12 C12M1/00
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
C12M

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X Y	US 2011/082563 A1 (CHAREST JOSEPH [US] ET AL) 7 april 2011 (2011-04-07) * alineas [0001], [0004] - [0007], [0009], [0010], [0021] * * figuren 1C,4A-4D * -----	1-17, 21-34 18,19, 33-36
X Y	WO 2013/086502 A1 (HARVARD COLLEGE [US]) 13 juni 2013 (2013-06-13) * alineas [0006] - [0009], [0033], [0034], [0036], [0050], [0053], [0055], [0056] - [0057], [0059], [0064], [0069], [0070], [0078], [0081], [0088] * * figuren 2,8,9,11 * -----	1-17, 21-34 18,19, 33-36
	-/--	

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
31 juli 2014	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Böhm, Ingo

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ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2011895

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
Y	WO 03/022985 A2 (ISIS INNOVATION [GB]; TRIFFITT JAMES TOMLINSON [GB]; XIA ZHIDAO [GB];) 20 maart 2003 (2003-03-20) * bladzijde 2 - bladzijde 5; figuur 13 *	18,19, 33-36
A	WO 2010/009307 A2 (CHILDRENS MEDICAL CENTER [US]; INGBER DONALD E [US]; HUH DONGEUN [US]) 21 januari 2010 (2010-01-21) * alinea [0046]; figuur 2C *	1,23,30, 32,33
A	EP 1 367 119 A2 (TOYO BOSEKI [JP]; FUNATSU KAZUMORI [JP]) 3 december 2003 (2003-12-03) * alineas [0001], [0023], [0029], [0052]; figuur 3 *	1,23,30, 32,33
A	US 2012/308531 A1 (PINXTEREN JOZEF ALBERT MARTHA [BE] ET AL) 6 december 2012 (2012-12-06) * alineas [0010] - [0021]; figuren 1-3 *	17-19, 33-36

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek: NL 2011895

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
US 2011082563 A1	07-04-2011	AU 2010303620 A1	19-04-2012
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		JP 5415538 B2	12-02-2014
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		JP 2014073125 A	24-04-2014
		KR 20110044226 A	28-04-2011
		US 2011250585 A1	13-10-2011
		US 2014093905 A1	03-04-2014
		US 2014093906 A1	03-04-2014
		US 2014147880 A1	29-05-2014
		WO 2010009307 A2	21-01-2010
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WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN61775	04.12.2013		NL2011895

International Patent Classification (IPC)
INV. C12M3/00 C12M1/12 C12M1/00

Applicant
Ponomarenko

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

	Examiner
	Böhm, Ingo

Application number

WRITTEN OPINION

NL2011895

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	18-20, 35, 36
	No:	Claims	1-17, 21-34
Inventive step	Yes:	Claims	20
	No:	Claims	1-19, 21-36
Industrial applicability	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 US 2011/082563 A1 (CHAREST JOSEPH [US] ET AL) 7 april 2011 (2011-04-07)

D2 WO 2013/086502 A1 (HARVARD COLLEGE [US]) 13 juni 2013 (2013-06-13)

i) independent claims:

- claim 1:

Fluïduminrichting voor in vitro weefselreconstructie omvattende:
- ten minste één stel gescheiden kanalen, welk stel ten minste één weefselkanaal omvat, ten minste één lichaamssapkanaal en ten minste één zenuwkanaal;
- waarbij het weefselkanaal is ingericht voor het opnemen van levende weefselcellen;
- waarbij het lichaamssapkanaal is ingericht voor het opnemen van bloed- en/of vaatcellen; en
- waarbij het zenuwkanaal is ingericht voor het opnemen van zenuwcellen, waarbij het ten minste ene weefselkanaal, ten minste ene lichaamssapkanaal en ten minste ene zenuwkanaal van elkaar zijn gescheiden door een scheidingsmateriaal, welk scheidingsmateriaal communicatie tussen de ten minste drie kanalen onderling mogelijk maakt.

- claim 23:

Doorstroomsysteem omvattende:
- ten minste één fluïduminrichting volgens één van de conclusies 1 tot en met 20;
- ten minste één eerste, ten minste één tweede en ten minste één derde inlaatopening waarbij elke inlaatopening is ingericht voor het toevoeren van medium naar de fluïduminrichting; en
- ten minste één eerste, ten minste één tweede en ten minste één derde uitlaatopening waarbij elke uitlaatopening is ingericht voor het afvoeren van medium uit de fluïduminrichting,waarbij de ten minste ene eerste inlaat- en uitlaatopening zijn verbonden met het ten minste ene weefselkanaal, de ten minste ene tweede inlaat- en uitlaatopening zijn verbonden met het ten minste ene lichaamssapkanaal en de ten minste ene derde inlaaten uitlaatopening zijn verbonden met het ten minste ene zenuwkanaal.

- claim 30:

Werkwijze voor in vitro weefselreconstructie en/of cocultivering, omvattende de volgende stappen:
a) het verschaffen van een doorstroomsysteem volgens één van de conclusies 23 tot en met 29;
b) het verschaffen van levende weefselcellen, bloed- en/of vaatcellen, en zenuwcellen;
c) het laten stromen van medium door de fluïduminrichting;
d) het sluiten van de inlaatopeningen en uitlaatopeningen van de fluïduminrichting om het stromen van medium te stoppen, zodra de fluïduminrichting met medium is gevuld;
e) het uitzaaien van de levende weefselcellen in het weefselkanaal van de fluïduminrichting;
f) het uitzaaien van de bloed- en/of vaatcellen in het lichaamssapkanaal van de fluïduminrichting;
g) het uitzaaien van de zenuwcellen in het zenuwkanaal van de fluïduminrichting; en
h) het openen van de inlaatopeningen en uitlaatopeningen van de fluïduminrichting om medium door de fluïduminrichting te laten stromen.

- claim 32:

Werkwijze voor in vitro weefselreconstructie en/of cocultivering, omvattende de volgende stappen:
a) het verschaffen van ten minste drie scheidingsmaterialen voor het vormen van ten minste drie fysisch gescheiden kanalen;
b) het verschaffen van levende weefselcellen, bloed- en/of vaatcellen, en zenuwcellen;
c) het uitzaaien van elk van de levende weefselcellen, bloed- en/of vaatcellen en zenuwcellen op het binnenste en/of buitenste oppervlak van één van de ten minste drie scheidingsmaterialen;
d) het aanbrengen van de uitgezaaide ten minste drie scheidingsmaterialen in een fluïduminrichting volgens één van de conclusies 1 tot en met 20;
e) het verbinden van de fluïduminrichting met een doorstroomsysteem volgens één van de conclusies 23 tot en met 29; en
f) het laten stromen van medium door de fluïduminrichting.

- claim 33:

Holle vezel voor het beoordelen, bemonsteren en/of oogsten van levende weefselcellen, bloedcellen, vaatcellen, zenuwcellen, interstitiële cellen, producten en/of metabolieten uit de fluïduminrichting volgens één van de conclusies 1 tot en met 20, waarbij de vezel is gemaakt van een doorlaatbaar en/of halfdoorlaatbaar materiaal.

I) Novelty

Independent claims 1,23,33 claim an apparatus.

The applicant's attention is drawn to the fact that an apparatus as claimed in independent claims 1,23 and 33 is understood as being "suitable for".

The claims stand on their own and they are read in the light of the description. If a claim is unclear, the claim is read in its broadest sense.

Independent claims 1,23 and 33 claim:

- a fluidic device for in vitro tissue reconstruction having three channels suitable for tissue, humoral and neural cells

- a perfusion apparatus comprising said fluidic device

- a hollow fiber apparatus for monitoring and measuring suitable for living tissue, blood fat, neral, interstitial cells and products as well as metabolites from the fluidic device

The term "for" is an intended use term. The manner in which a claimed apparatus is intended to be employed does not differentiate the claimed apparatus from a prior art apparatus satisfying the claimed structural limitations.

A claimed channel is always considered to be suitable for all the claimed kind of cells if the channel is not especially adapted to be NOT suitable for a kind of cells, for example only surface seeding cells cannot be cultured within a channel being modified with surface repellent coatings in order to avoid cell adhesion.

Claims 1, 14-17,21-23,36 do not meet the criteria for clarity in that the matter for which protection is sought is not defined. The claim attempts to define the subject-matter in terms of the result to be achieved. Such a definition is only allowable under the conditions that it appears not being possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

A tissue channel, a humoral channel or a neural channel being prepared to receive the specific cells, such as tissue cells, humoral cells as well as neural cell respectively is not clearly defined how this result may be achieved.

1.

D1 discloses a system and a method for culturing cells in a multi-layer microfluidic cell culture devices having **multiple intercommunicating channels**.

D1 provides a microfluidic multi-channel bioreactor device for culturing cells in an environment that controls multiple chemical, biological, and biophysical parameters, thereby facilitating, for example, better simulation of in vivo conditions. Such bioreactor systems may, for example, include two polymer layers separated by a **permeable or semi-permeable membrane**. Each layer defines one or **more microchannels**, which are, in operation, filled with a fluid such as, e.g., buffer solution, cell culture medium, blood, or urine. Fluid flow may be induced in a channel, e.g., by applying a pressure difference between the channel inlet and outlet.

Channels in one layer may "**communicate**" with one or more channels in the other layer through the **membrane**. Communication refers to any type of interaction between the channels, whether chemical, physical (e.g., thermal, mechanical, or fluid-mechanical) or biological in nature. For example, communication may involve fluid communication, i.e., the transport of fluid or components thereof between the channels, or a mechanical interaction, such as, i.e., the conferment of pressure in one channel onto the other channel.

Channels within the same layer may also communicate with each other through the membrane and a channel in the other layer that overlaps geometrically with both of them. Geometric overlap of channels herein connotes that projections of the channels into a plane parallel to the membrane (or locally parallel to the membrane in cases where the layers and membrane are not flat) overlap, even though the channels do not occupy the same physical space. (see also fig. 4A-4D)

The degree of communication between channels in one layer depends, among other factors, on the distance between the channels and on the height and width of their cross-sections. Thus, by varying a subset or all of these parameters along the length (i.e., along the longest dimension or axis) of the channels, the level of communication can be controlled as a function of the position along the channels. Such control over the communication between the channels, in turn, facilitates control over fluid-mechanical and chemical parameters along the channels via the injection of fluids and the control of flow rates and pressures at the input and output ports. For example, if two fluids of different composition are injected into neighboring channels at the inlet, the two fluids may mix due to chemical communication, i.e., mass transfer, between the channels and result in a third, mixed composition at the outlets. Similarly, different pressures may be applied at the ports (inlets and/or outlets) of two channels, and result in profiles of fluid-mechanical parameters along the channel length that are determined (at least in part) by the geometries of the individual channels and the level of mechanical communication therebetween.

Microfluidic devices as described above may be advantageously employed for the culturing of cells. The channels may be populated with cells of multiple types, which may be placed at distinct locations within the channels. The relative placement and

shape of the channels, the cell location inside the channels, and operational parameters such as fluid compositions and pressures applied to the ports collectively afford an unprecedented level of control over the microenvironment of the cultured cells and the administration of chemical, biological, mechanical, and biophysical signals to the cells. With control over these parameters, the bioreactor may be utilized to influence cell function and to facilitate culture of multiple cell types at distinct locations within the bioreactor structure.

In one aspect, the invention of D1 provides a microfluidic bioreactor device that includes at least one polymer layer defining at least **three microchannels** therein.

A membrane separates the first and second channels from the third channel at geometrically overlapping portions **while permitting communication** (e.g., fluid communication or mechanical communication) between the overlapping portions of the microchannels. The first and second channels may communicate with each other via the third channel. In certain embodiments, the device includes two polymer layers separated by the membrane, one of the layers defining the first and second channels and the other layer defining the third channel. At least one geometric parameter of at least one of the microchannels varies along a length of the channel within the overlapping portion(s).

The polymer layers include or essentially consist of a biopolymer. The membrane, or a portion thereof, may be **semi-permeable**, and may be formed by a **porous** or semi-bulk-permeable material. In certain embodiments, the membrane includes or consists essentially of fleece, micromolded polydimethylsiloxane (PDMS) or other silicone polymer, polyethersulfone, an electrospun material, or a tracked-etched membrane.

The disclosure of D1 is considered to be novelty destroying for the subject-matter of claims 1-17,21-32.

2.

D2 discloses a microengineered organ chips or organ-on-a- chip device. Organ chips (also known as "organ-on-a-chip device") are microfluidic devices that are configured to mimic at least one physiological function and/or response of organs of interest, e.g., from a mammal (e.g., a human), other animal or organism, an insect, or a plant.

An organ chip or organ-on-a-chip device can be configured to represent a functional microenvironment of an organ {e.g., a functional unit or section of an organ, and/or a tissue-capillary interface).

A flexible porous membrane can expand and contract to mimic the movement of an alveolar wall during lung breathing, by controlling the pressure gradient induced in the microfluidic device.

Living human cells can be cultured in organ chips described herein to mimic at least one physiological function and/or response of the corresponding human organs.

A plurality of (e.g., 2 or more) organ chips representing various organs can be assembled or connected (e.g., fluidically connected) together to form an in vitro

microphysiological system that mimics at least one physiological function and/or response of one or more systems in vivo, e.g., including, but not limited to, a circulatory system, a respiratory system, an excretory system, a nervous system, a gastrointestinal system, or any combinations thereof.

The organ chips can be used, individually or connected together (e.g., fluidically connected), for various applications where simulation of a physiological condition is desirable, e.g., drug screening, pharmacokinetics/ pharmacodynamics studies, engineered scaffolds for **tissue/organ repair** or replacement, development of a disease model, and/or personalized therapeutic treatment.

The term "fluidically connected" refers to two or more organ chips connected in an appropriate manner such that a fluid or a least a portion of a fluid (e.g., any flowable material or medium, e.g., but not limited to, liquid, gas, suspension, aerosols, cell culture medium, and/or biological fluid) can directly or indirectly pass or flow from one organ chip to another organ chip. In some embodiments, two or more organ chips can be fluidically connected together, for example, using one or more fluid-transfer connecting means (e.g. , adaptors, tubing, splitters, valves, pumps and/or channels) between the two or more organ chips. For example, two or more organ chips can be fluidically connected by connecting an outlet of one organ chip to an inlet of another organ chip using tubing, a conduit, a channel, piping or any combinations thereof.In some embodiments, two or more organ chips can be fluidically connected by, e.g., at least one pumping device and/or at least one valve device.

Organ chips generally comprise a base substrate and at least one channel disposed therein. The **number and dimension of channels** in an organ chip can vary depending on the design, dimension and/or function of the organ chip. In an organ chip can comprise **a plurality of channels** (e.g., at least two, **at least three**, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten or more channels). One of skill in the art will readily be able to design and determine optimum number and/or dimension of channels required to achieve a certain application. For example, if assessment of reproducibility and/or comparison of at least two experimental conditions are desirable, an organ chip can be constructed to comprise at least two, at **least three**, at least four, at least five identical channels.

This can provide for a number of read-outs per chip, e.g., allowing assessment of reproducibility and/or for validation and implementation of the technology. For example, each channel can run a different condition (e.g., culturing normal (healthy)

cells vs. diseased cells in different channels, or applying different dosages of the same drug to different channels, or applying different drugs at the same dosage to different channels). In some embodiments, an organ chip can comprise at least two parallel (e.g., 2, **3**, 4, 5, 6, 7, 8, 9, 10, or more) **channels**.

The organ chips can be sized to a specific need, e.g. , for high throughput drug screening, or scaffolding, e.g., **for tissue repair** and/or replacement. In some embodiments, the organ chips can be implantable, and thus they can be sized to suit a target implantation site.

At least one channel of the organ chips can comprise one or more **membranes**, e.g., at least 1, at least 2, at **least 3 or more membranes to separate the channel into sub-channels.** The membrane can be rigid or at least partially flexible.

In some embodiments, the membrane can be non-porous or at least partially porous. In some embodiments, the pore size of the membrane can be large enough to allow cells pass through it. In some embodiments, the pore size of the membrane can be too small for cells to pass through, but large enough for nutrient or fluid molecules to pass through or permeate.

The membrane can be non-coated or coated with extracellular matrix molecules (ECM) to facilitate cell adhesion (e.g., but not limited to, fibronectin, collagen, Matrigel, laminin, vitronectin, and/or any combinations thereof), other proteins such as growth factors or ligands.

The surface of the membrane can be modified and/or activated, e.g., with any art-recognized polymer surface modification techniques such that bioactive molecules, e.g., ECM molecules, carbohydrates, proteins such as growth factors or ligands, can be covalently or non-covalently attached to or coated on it.

The membrane can be seeded with or without cells, where cells are seeded on the membrane, cells can be seeded on one side or both sides of the membrane. The membrane can be seeded with the same cells. Both sides of the membrane can be seeded with **different cells**, e.g. comprising vascular endothelial cells and an Interstitial channel (comprising organ- specific parenchymal cells).

The membrane can be seeded with at least one layer of cells, including, at least 2 layers of cells or more. Each layer of cells can be the same or different.

The organ chips can comprise a plurality of ports. (see claim 23) The organ chips can comprise at least one inlet port for introducing culture medium, nutrients or test agents such as drugs into the organ chips, and at least one outlet port for a fluid to exit.

At least one port can be connected to a pump or a syringe, e.g., via a tubing, to facilitate the fluid transfer through the channel and/or to apply a pressure to the channel. In some embodiments, at least one port can be connected to at least one electrical component.

The organ chips can be designed to have a common shape and have positioned inlets and outlets for delivery of fluids to the Microvascular channels lined by microvascular endothelium and Interstitial fluid channels lined by organ-specific parenchymal cells (e.g., but not limited to, alveolar epithelium, heart muscle, hepatocytes).

Different kinds of organ chips described herein can comprise additional cell types, e.g. immune cells, stromal cells, smooth muscle cells, **neurons**, **lymphatic cells, adipose cells** are disclosed in D2.

An in vitro microphysiological system can comprise at least two different organ chips using one or both of the first and second organ chip designs described in D2. The first organ chip design is based a microfluidic device comprising: a body comprising a central channel therein, and an **least partially porous** and at least partially flexible first **membrane** positioned within the central channel and along a plane, wherein the first membrane is configured to separate the central channel to form two sub-channels, wherein one side of the first membrane is seeded with vascular endothelial cells, and the other side of the first membrane is seeded with at least one type of organ- specific parenchymal cells.

The disclosure of D2 is considered to be novelty destroying for the subject-matter of claims1-17,21-32.

II) Inventive step

Problem addressed in the application

The **problem** to be solved may be regarded as the provision of a fluidic device for in-vitro tissue reconstruction as well as the provision of a method for in-vitro co-culturing and tissue reconstructing and the use of said device (see descr. p.2, lines 26-33)

The **solution** is claimed in independent claims 1,23.30,32 and 33 with respect to the fluidic device, a hollow fiber apparatus comprising said fluidic device, their use and a method of tissue reconstruction.

Closest prior art: D1

D1 discloses a microfluidic bioreactor device for culturing cell comprising intercommunicating microchannels defined in two polymer layers separated by membrane.

The microfluidic bioreactor device of D1comprises at least one polymer layer defining **first, second and third microchannels**; and a membrane separating the first and second channels from the third channel at geometrically overlapping portions, the **membrane permitting communication between the overlapping portions of the microchannels**.

Technical difference between the subject-matter of claim 18-20, 33-36 and D1/D2:

- the hollow fiber surrounds said fluidic device (claims 18-19)

- the hollow fiber is made of biological degradable/non-degradable material (claim 20)

Evaluation of the solution of claims 1,23,30,32 and 33:

The subject-matter which has been already destroyed by novelty cannot considered as involving an inventive step, because there is no contribution over the prior art.

Dependent claims 2-19,21,22,24-29,31

Dependent claims 2-19,21,22,24-29,31 do not contain any features which, in combination with the features of any claim to which they refer, meet the criteria in respect of novelty and/or inventive step.

The claims are related to obvious alternatives known in the state of the art:

- in claim 2/3/4 it says that the separating material is provided with a number of pores and being permeable/semi-permeable:

D1 and D2 disclose a porous and permeable/semi-permeable membrane.

- in claim 5, the membrane is degradable or not, "tertium-non-datur" !

- in claim 6 the outer surface of a channel has to be oriented to the outer surface of the outer channels: this feature is not clear and the figures of D1 and D2 disclose three channels in close contact

- claim 7-17: D1 and D2 disclose at least a further channel for interstitial material such as interstitial cells

- claims 21-33:

D1 and D2 disclose the use of said fluidic device for coculturing of different cells and the arrangement within the fluidic devices a few inlet and outlet ports for medium, drugs and waste material, as well as couple to reservoir for fluid supply (see D1: paragr. 4,6, figures; D2: paragr. 64,65, fig.8,11)

- claims 18,19,34-35:

Hollow fiber bioreactors are known in the art as well as made of hydrophilic or hydrophobic material for cell coculture.

The subject-matter of claim 20 with respect to the hollow fiber being made of biological degradable material is not disclosed in the cited prior art and not obvious for the skilled person in the art.

Therefore the subject-matter of dependent claim 20 can be considered as novel vis-à-vis D1 and D2.