

HUMAN-ON-CHIP OPERATING SYSTEM

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/293,422 filed December 23, 2021, the entire contents of which are incorporated by reference herein.

BACKGROUND

[2] Developing a successful pharmaceutical drug is expensive and time consuming. Additionally, many drugs fail in late stages of the development process, causing significant sunk costs. One contributing reason for drugs failing in late developmental stages is that pharmaceutical companies typically test drugs on cells grown on cell culture platforms that do not sufficiently well replicate human tissue. The 3D organ-on-chip technology emerged to create better resembling tissue models solving for the structural and 3D tissue assembly problem. However, there persists the problem of operating system systems in a way that replicates the mechanics and the fluid dynamics of operating system advanced 3D models, while feeding, maintaining and growing the tissues, and real-time measuring & analyzing the aimed parameters in operating system advanced systems.

[3] Current cell culture experiments often do not accurately mimic physiological conditions as they use simplistic approaches, which do not reproduce the entire system, but rather only a few aspects. Multiple factors go hand-in-hand in developing & operating 3D advanced cell culture models. Operating system factors span a wide range of design parameters, including the level of complexity of the model, the connection mechanism between the different organ models, the pumping, seeding, and sampling mechanisms, and also the measurement, sensing & imaging mechanisms, followed by the analysis and prediction techniques. A holistic system spanning all of the later factors is needed to get precise drug prediction from preclinical testing.

[4] One main enduring issue facing the current tissue culture semi-automated operating systems, is the need for large volumes, tens of milliliters, of materials that are wasted just to seed and use only few microliters of cells and biological materials. This limits the culturing and modeling of tissues using expensive or scarce materials such as biopsies.

[5] Another important aspect for the operation of a complex 3D culture model are the analytical read-outs which limit high-throughput analysis as well as real time data collection. Furthermore, often accessing cell and/or media samples for analysis at different time-points is challenging in the current organ-chips. Media is defined as any fluid containing any of the

following: therapeutic agents, growth factors, nutrients, chemicals, cells in suspension and/or any other molecule, biomolecule or substance flowing through the channels.

[6] Typical complex 3D cell culture platforms are incapable of real-time imaging or measurement of biological parameters of separate tissues connected into a human-on-chip model, while operating and flowing media into the biochip. One of the limitations is in the real estate of the design that prevents the placement of one or more analytical devices in proximity to the chips. For example, to be able to model the metastasis of cancer from one tissue to another through 3D vasculature, one cannot image both tissues at the same time while pumping the fluids through the blood vessel. Similarly, much of the valuable data generated is in most cases restricted to few data points rather than continuous measurements.

[7] One essential aspect of the typical chip designs, and the connectivity method used between the organs, is the inability to set up complex experiments having multi-organs while preserving accurate sensing and communication. These systems lack the communication and sensing that is found in the human body, also the ability to set up any experiment by a chip pattern.

[8] One main limitation these systems face while sampling and inserting the media is the need for high volumes of fluids to compensate for the volume of the tubes in the system. The usage of ultra-thin tubes or micro-channels prevents the need for having a dilution factor, which is used in other systems to move the fluids. However, such low-diameter tubes maintain high capillary forces and a higher surface tension inside the tubes, where a low volume of fluid can be transferred to a further destination. Also, not having a dilution factor ensures having a sample that is highly concentrated with the molecules we need to detect, thus more accurate measurements and results.

[9] One of the essential aspects of a cell culture experiment is the ability to accurately measure and read physiologically relevant data. Microscopy, spectroscopy, and FTIR are some of the widely used techniques to perform quantitative measurements on biological materials, which indicate cell viability, functionality, and metabolism. Most human-on-chip operating systems currently found in the market depend on external measuring devices to perform such assays. These tests will require the operator to halt the experiment and transfer it to the measuring device. This causes missing valuable observations and data in between the set time-points. Performing such manipulation will increase the number of variables, which may cause an unplanned effect on the experiment results. There is a dire need for implementing multiplexed real-time measurements on running cell-culture experiments. For instance, implementing an onboard triple measuring system that consists of microscopy, spectroscopy,

and ATR-FTIR with tight integration with the operating system will provide more valuable information and a better representation of the experiment while eliminating any variables that might form as a result of the culture relocation. The importance of having multiple measurement techniques performing various readings simultaneously, while being connected to the same data reader, is that it allows for obtaining a multiplexed analysis and conclusions impossible otherwise.

[10] **SUMMARY**

[11] The present disclosure provides a new and innovative biochip operating system that acts as a cell culture platform that models and operates on multiple human organs simultaneously. The operating system contains and operates on a human-on-chip plate, which is an array of multiple biochips fluidly connected together via ducts modeling multiple organs of the human body. Cells can be inserted into the biochip and grow into 3-dimensional tissues to be used in drug testing. Each biochip may contain one or multiple ultra-thin porous plastic cylindrical shaped ducts. Each duct can be accessed from the inside or from the area surrounding the ducts through microfluidic channels.

[12] The present disclosure provides, thanks to the biochips, a means to replicate a ductal organoid microenvironment by growing the ductal cells on the inner walls of each duct and growing the surrounding tissue from the outside by seeding cells and delivering media components through a gel from the other side surrounding the ducts. Each biochip may be utilized to replicate ductal or non-ductal tissues including, but not limited to: pancreas, renal, hepatic, breast, brain, lung, vasculature, prostate, fallopian tubes, testicular, and lymph ducts. The human-on-chip plate is an array of biochips each growing and modeling a specific tissue and fluidly connected to the other biochips through one or more ducts of each biochip. The biochip ducts are separated by a valving system that allows for the connection between different biochips. These biochips can be connected in series, in parallel or in a combination. Furthermore, the valving system allows for these connections to be altered at any point. The chips may also be connected to a sampling chamber, the ATR-FTIR or a waste chamber.

[13] The present disclosure provides a biochip operating system is a semi or fully automated device that operates on the human-on-chip plate to pump and control the flow of fluids containing cell growth media, chemicals, and cells into each of the biochip's channels. The human operating system also contains sensing and measurement devices to measure and control different biological parameters of the tissues grown inside the biochips. The actuating of the fluid valves and pumps, and the sensors and images feeds into a computer software that automates the cell culture and measurement process. The biochip operating system could be

used by, but not limited to, researchers, pharmaceutical companies and clinical physicians to test their drugs or other components on full human models made of cells from biopsies, primary cells, stem cells and/or cell-lines. This system could perform, control, and measure full cell culture experiments of a wide range of applications.

[14] The present disclosure provides a system containing devices and methods to pump and control the flow of very small volumes (as low as 0.1 μ l) of fluids containing cells, media, therapeutic agents, chemicals, reagents and other biological components into each channel inside every individual biochip. The disclosure allows operating precise, repeatable, and reproducible biological experiments on tissue models of very high resemblance.

[15] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a biochip operating system includes at least one biological system on chip plate, at least one valving means, at least one actuation means for actuating the valving means, at least one fluidic handling means, at least one analytical device; and, a control system for controlling the pumping means, actuation means and valving means, and the analytical device.

[16] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the analytical device may inspect biochips utilizing at least one of microscope images or spectral measurements.

[17] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the analytical device may inspect media in the fluidic handling means or fluidic pathways.

[18] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the operating system facilitates, semi-automates or automates at least one aspect of experimental set up including at least one of cleaning, sterilizing or preparing the system; priming the system with a biocompatible fluid; populating the chips with the correct cell types in the correct locations as required for the desired experiment; selectably connecting the chips in the correct arrangement for the desired experiment; and/or metering the correct amount of media or other compounds into the system.

[19] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the operating system facilitates, semi-automates or automates at least one aspect of experimental execution including at least one of circulating the media between chips as required for the experiment; controlling flow rates of

fluidic movements; extracting samples, possibly at specific times or time intervals; inspecting using analytical means at specific times or time intervals and/or isolating and fixing biochips for later analysis.

[20] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the operating system further comprises a motion stage to move the biological system on chip plate relative to the analytical device and/or actuation means.

[21] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a biochip for growing multiductal tissue includes a chassis, a plurality of internal regions, wherein the chassis contains the plurality of the internal regions, wherein at least one of the internal regions is in permeable or semipermeable communication with at least one other internal region and a plurality of fluidic media.

[22] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a plurality of internal regions are tubular ducts.

[23] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, at least two tubular ducts are arranged with an inlet and outlet to permit through flow of the first fluidic medium.

[24] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, at least two tubular ducts are arranged with an inlet and outlet distinct from those of a first duct to allow the through flow of the second fluidic medium that can be distinct from the first fluidic medium.

[25] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, at least one region is a stromal compartment external to the ducts but inside the biochip chassis.

[26] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the plurality of fluidic media are the same media.

[27] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the plurality of fluidic media are different media.

[28] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the plurality of fluidic media is combination of fluidic media types.

[29] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a biochip fluidic control includes at least one human-on-chip containing at least one organ-on-chip system, wherein the at least one organ-on-chip system containing at least one ductal scaffold interfacing at least one surrounding compartment, at least one valving system controlling the flow to each compartment, at least one micro-pumping mechanism that can pump and control the flow to at least one compartment, at least one actuator controlling the valves on the chip and leading to the chip, at least one actuator controlling the fluid pumping to the chip compartments, wherein the at least one valving system could open or close and shift the flow pathways to every compartment.

[30] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism may be at least one of pneumatically, mechanically, electrically, and fluidically actuated.

[31] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects the at least one biochip to the operating system.

[32] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects the at least two biochips in human-on-chip connection plate.

[33] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism controls the at least two biochips in human-on-chip connection plate.

[34] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism controls the at least one inlet and outlet biochip.

[35] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects and controls the connection between the at least one duct to another duct.

[36] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects the at least one stroma to another stroma.

[37] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects and controls the controls the connection between at least one duct to at least one stroma.

[38] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism connects the at least one biochip to at least one measuring device.

[39] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism contains at least one bistable valve.

[40] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism contains at least one open close valve.

[41] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism contains at least one bistable and open close valves.

[42] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one bistable valve maintains a state position by a beam, magnet, or any drilled geometrical shape.

[43] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein,

[44] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one open close valve maintains a state position by a drilled geometrical shape.

[45] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism controls the media insertion in at least one biochip.

[46] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism controls the cell insertion in at least one biochip.

[47] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the at least one valving mechanism maintains a cell culture by media insertion.

[48] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a human-on-chip plate including at least one 3D culture biochip, at least one micro-valve, at least one microfluidic channel, at least one inlet or outlet plate port, wherein the outlet ports from one chip may be connected to another chip by routing the valves, wherein multiple chips may be connected in parallel, in series or in combination and one or more chips in the human-on-chip plate can be bypassed, wherein each fluid connection may be altered independently and at any time, wherein the at least one valving system connecting or separating different compartments of and between the organ-on-chip system, wherein at least one inlet and one outlet ports to access and block the microchannels, wherein the at least one valving system could allow for sampling, changing the model flow map, and introduce or reduce at least one fluidic chamber, wherein the valves, which may be controlled in a bistable position by the actuators and the sensors, all positioned at the channel ports of the chips wherein, the valves are positioned as immediately adjacent to the chips and may be controlled in a bistable position.

[49] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the human-on-chip plate includes at least one organ-on-chip system including at least one ductal scaffold interfacing at least one surrounding compartment, wherein, at least one organ-on-chip system containing at least one ductal scaffold interfacing at least one surrounding compartment.

[50] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the plate may be rapidly connected from the bottom or the side, wherein fluids will be exchanged.

[51] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the human-on-chip plate is interchangeable, wherein ports on the human-on-chip will align with fitting in the biochip, while the valves are fixed or removable.

[52] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the human-on-chip plate includes interconnected built-in chips.

[53] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the valves are fixed or removable.

[54] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the human-on-chip allows for the fluid to be accessed from either the top or the side.

[55] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, a multiplex logic micro-valve system includes a plurality of micro-valves, at least one fluidic pathway including at least one channel, an inlet and outlet port, at least one bistable mechanism, at least one actuating mechanism, at least one structure which encloses the other elements of the micro-valve, wherein the fluid pathway has no dead volumes, wherein multiplexing between the plurality of valves reduces the amount of actuating inputs and wherein the valves may control the fluid within a channel so that the flow can be either hydrostatic or in motion.

[56] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, at least one logic multiplex micro-valve is used to control fluid in the systems.

[57] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the valves may direct a sample to at least one sampling port.

[58] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the bistable mechanism may be a bistable beam on a flexible tube valve.

[59] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the fluidic pathway may connect at least one biochip to a plurality of components within a human-on-chip system.

[60] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the bistable valves have two stable positions.

[61] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, at least one channel contains a balloon that is pneumatically actuated.

[62] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the system may only require power when switching between two states, and once actuated, the bistable beam will remain in position.

[63] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the micro-valve may be a bistable magnetic drilled-piston valve.

[64] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the bistable mechanism may contain magnetic channels.

[65] In another aspect of the present disclosure, which may be used in combination with any other aspect or combination of aspects listed herein, the bistable beam of the micro-valve may be either solenoidly or pneumatically actuated.

[66] In summary, the human operating system is a self-enclosed system where multiple cell types can be grown and connected with each other and experiments can be automatically run within the system. The aim of the system is to be used for tissue modeling and drug testing, for the better prediction of the effect of diseases, biological parameters and drugs before testing on humans. This allows for standardization of cell culture conditions to ensure physiological conditions to an unprecedented level. It also ensures real-time and end-point experiments to be carried out within the system. The presently disclosed system allows for an all-in-one approach which ensures standardization, reproducibility, accuracy, speed and reduced costs.

BRIEF DESCRIPTION OF THE FIGURES

[67] Some example apparatus embodiments of the present disclosure, and example procedures for making and using one or more example embodiments, are described in detail

herein and by way of example, with reference to the accompanying drawings (which are not necessarily drawn to scale with regard to any internal or external structures shown) and in which like reference characters designate like elements throughout the several views, and in which:

[68] Fig. 1 illustrates a schematic view of two ducts (labeled I and IV) surrounded by one stroma (III) and encompassed in a chassis (II), according to an example of the present disclosure.

[69] Fig. 2A illustrates a schematic view of an embodiment of the 2-ducts 1-stroma biochip, according to an example of the present disclosure.

[70] Fig. 2B illustrates a schematic top view of an embodiment of the 2-ducts 1-stroma biochip, according to an example of the present disclosure.

[71] Fig. 2C illustrates a schematic view of the manufacturing and assembly process of the 2-ducts 1-stroma chip, according to an example of the present disclosure.

[72] Fig. 3A illustrates a schematic view of another embodiment of a 2-ducts 1-stroma chip with three parallel channels, according to an example of the present disclosure.

[73] Fig. 3B illustrates an exploded view of a 2-ducts 1-stroma chip with three parallel channels, according to an example of the present disclosure.

[74] Fig. 3C illustrates a schematic view of the manufacturing and assembly process of an embodiment of a 2-ducts 1-stroma chip with three parallel channels, according to an example of the present disclosure.

[75] Fig. 4A illustrates a schematic view of another embodiment of a 2-ducts 1-stroma chip with three parallel channels with a stroma feeding channel, according to an example of the present disclosure.

[76] Fig. 4B illustrates a detailed schematic top and bottom view of a 2-ducts 1-stroma chip with three parallel channels with a stroma-feeding channel, according to an example of the present disclosure.

[77] Fig. 4C illustrates an exploded view of the embodiment of a 2-ducts 1-stroma chip with three parallel channels with a stroma feeding channel, according to an example of the present disclosure.

[78] Fig. 4D illustrates a schematic view of the manufacturing and assembly process of the 2-ducts 1-stroma chip with three parallel channels with a stroma feeding channel, according to an example of the present disclosure.

[79] Fig. 5A illustrates a schematic view of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure.

[80] Fig. 5B illustrates a schematic top view of another embodiment of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure.

[81] Fig. 5C illustrates an exploded view of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure.

[82] Fig. 5D illustrates a schematic view of the manufacturing and assembly process of the 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure.

[83] Fig. 6A illustrates a schematic view of a 2-duct 1-stroma chip with multiple stroma per duct, according to an example of the present disclosure.

[84] Fig. 6B illustrates an exploded view of a 2-duct 1-stroma chip with multiple stroma per duct, according to an example of the present disclosure.

[85] Fig. 6C illustrates a schematic view of the manufacturing and assembly process of the 2-ducts 1-stroma chip with multiple stroma per duct, according to an example of the present disclosure.

[86] Fig. 7A illustrates a schematic view of an embodiment of a human-on-biochip plate, according to an example of the present disclosure.

[87] Fig. 7B illustrates a schematic view of the individual biochip component of the human-on-chip plate, according to an example of the present disclosure.

[88] Fig. 7C illustrates an exploded view of the human-on-chip plate, according to an example of the present disclosure.

[89] Fig. 8A illustrates a schematic view of an embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is cylindrical rotational mechanism, according to an example of the present disclosure.

[90] Fig. 8B illustrates a schematic view of an entity of an embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is cylindrical rotational mechanism, according to an example of the present disclosure.

[91] Fig. 8C illustrates an exploded view of an embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is cylindrical rotational mechanism, according to an example of the present disclosure.

[92] Fig. 9A illustrates a schematic view of an embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (isometric view), according to an example of the present disclosure.

[93] Fig. 9B illustrates a schematic view of another embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (top view), according to an example of the present disclosure.

[94] Fig. 9C illustrates a schematic view of another embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (top view), according to an example of the present disclosure.

[95] Fig. 9D illustrates a schematic view of an embodiment of the lower chassis features of another embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (top view), according to an example of the present disclosure.

[96] Fig. 10A illustrates a schematic view of an embodiment of the top view of an embodiment of the HOC plate where OOCs are removable from the HOC plate and the fluid is accessed from the side, according to an example of the present disclosure.

[97] Fig. 10B illustrates a schematic view of an embodiment of the exploded view of an embodiment of the HOC plate where OOCs are removable from the HOC plate and the fluid is accessed from the side, according to an example of the present disclosure.

[98] Fig. 10C illustrates a schematic view of an embodiment of the in-plate channeling of an embodiment of the HOC plate where OOCs are removable from the HOC plate and the fluid is accessed from the side, according to an example of the present disclosure.

[99] Fig. 11 illustrates a schematic view of an embodiment of the top view of an embodiment of the HOC plate where OOCs are removable from the HOC plate and the fluid and cells are sampled on spot, according to an example of the present disclosure.

[100] Fig. 12 illustrates a schematic view of an embodiment of the top view of an embodiment of the HOC plate where OOCs are removable from the HOC plate and the fluid and cells are

sampled through channels, chambers and valves, according to an example of the present disclosure.

[101] Fig. 13 illustrates a schematic view of an embodiment of the in-plate channeling of an embodiment of the HOC plate where two duct chips are connected to a 1-duct biochip, according to an example of the present disclosure.

[102] Fig. 14 illustrates a schematic diagram which contains pressure sensors at each chip inlet and outlet fluid ports to manipulate the pressure inside the chips' channels. This allows for the fluid to controllably perfuse from the ductal channel to the stromal one or vice versa, according to an example of the present disclosure.

[103] Fig. 15A illustrates a perspective view of the hydraulic valving mechanism, connecting individual biochips, according to an example of the present disclosure.

[104] Fig. 15B illustrates a perspective view of the cylindrical roll valving mechanism, connecting individual biochips, according to an example of the present disclosure.

[105] Fig. 15C illustrates a perspective view of the flexible membrane valving mechanism connecting individual biochips, according to an example of the present disclosure.

[106] Fig. 15D illustrates a perspective view of the chip connection micro-valve assembly process, according to an example of the present disclosure.

[107] Fig. 16A illustrates a perspective view of the directional change flow micro-valve plate actuator, according to an example of the present disclosure.

[108] Fig. 16B illustrates a detailed view of the directional change flow micro-valve plate actuator, according to an example of the present disclosure.

[109] Fig. 16C illustrates a perspective view of the solenoidal magnet open-close micro-valve, according to an example of the present disclosure.

[110] Fig. 16D illustrates a detailed view of the solenoidal magnet open-close micro-valve, according to an example of the present disclosure.

[111] Fig. 16E illustrates a perspective view of the solenoidal magnet open-close micro-valve plate, according to an example of the present disclosure.

[112] Fig. 16F illustrates a detailed view of the solenoidal magnet inlet-outlet micro-valve actuator plate, according to an example of the present disclosure.

[113] Fig. 17A illustrates a perspective view of the pneumatic actuated open-close membrane micro-valve that is normally closed, according to an example of the present disclosure.

[114] Fig. 17B illustrates an exploded view of the pneumatic actuated open-close membrane micro-valve that is normally closed, according to an example of the present disclosure.

[115] Fig. 17C illustrates a perspective view of an additional embodiment of the pneumatic actuated open-close membrane micro-valve that is normally opened, according to an example of the present disclosure.

[116] Fig. 17D illustrates an exploded view, an additional embodiment of the pneumatic actuated open-close membrane micro-valve that is normally opened, according to an example of the present disclosure.

[117] Fig. 18A illustrates a perspective view of the bistable beam pneumatically actuated on a flexible tube micro-valve, according to an example of the present disclosure.

[118] Fig. 18B illustrates an exploded view of the bistable beam pneumatically actuated on a flexible tube micro-valve, according to an example of the present disclosure.

[119] Fig. 19A illustrates a perspective view of the bistable magnetic drilled-piston micro-valve, according to an example of the present disclosure.

[120] Fig. 19B illustrates an exploded view of an embodiment of a bistable magnetic drilled-piston micro-valve, according to an example of the present disclosure.

[121] Fig. 20A illustrates a perspective view of the bistable mechanically actuated flexible tube micro-valve, according to an example of the present disclosure.

[122] Fig. 20B illustrates a top view of an embodiment of a bistable mechanically actuated flexible tube micro-valve in which one bistable beam is in the close position, according to an example of the present disclosure.

[123] Fig. 20C illustrates a cross sectional view of the bistable mechanically actuated flexible tube micro-valve in the open position, according to an example of the present disclosure.

[124] Fig. 20D illustrates a cross sectional view of the bistable mechanically actuated flexible tube micro-valve in the close position, according to an example of the present disclosure.

[125] Fig. 21A illustrates a perspective view of the mechanically actuated ball open-close micro-valve, according to an example of the present disclosure.

[126] Fig. 21B illustrates a close perspective view of an embodiment of a mechanically actuated ball open-close micro-valve (the chip is moving and the screwdriver is moving), according to an example of the present disclosure.

[127] Fig. 21C illustrates a detailed view of the socket engaging with the micro-valve to switch if in the open or close position, according to an example of the present disclosure.

[128] Fig. 21D illustrates a cross section of the detailed view of the mechanically actuated ball open-close micro-valve, according to an example of the present disclosure.

[129] Fig. 22A illustrates a cross section view of the permanently sealed open-close micro-valve in its close position, according to an example of the present disclosure.

[130] Fig. 22B illustrates a side view of the permanently sealed open-close micro-valve in its pre-set close position where a pin is tightly inserted into the channel blocking it, according to an example of the present disclosure.

[131] Fig. 22C illustrates a cross section view of the permanently sealed open-close micro-valve in its pre-set open position, according to an example of the present disclosure.

[132] Fig. 22D illustrates a side view of the permanently sealed open-close micro-valve in its open position where the pin is not tightly inserted into the channel, according to an example of the present disclosure.

[133] Fig. 23A illustrates a perspective view of an embodiment of the assembled operating system, according to an example of the present disclosure.

[134] Fig. 23B illustrates a perspective view of an embodiment of the assembled operating system main components, according to an example of the present disclosure.

[135] Fig. 23C illustrates a perspective of the top view of an embodiment of the assembled operating system fluidic components, according to an example of the present disclosure.

[136] Fig. 23D illustrates a perspective view of an embodiment of the assembled operating system with the mixing chamber drawer open, according to an example of the present disclosure.

[137] Fig. 23E illustrates an exploded view of an embodiment of the operating system, according to an example of the present disclosure

[138] Fig. 23F illustrates a side view of an embodiment of the assembled operating system pressure driven pumping for the micro chambers, according to an example of the present disclosure.

[139] Fig. 23G illustrates a bottom perspective view of an embodiment of the assembled operating system pressure driven pumping for the micro chambers, according to an example of the present disclosure.

[140] Fig. 23H illustrates a bottom perspective view of an embodiment of the assembled operating system pressure driven pumping for the micro chambers, according to an example of the present disclosure.

[141] Fig. 23I illustrates a bottom perspective view of an embodiment of the assembled operating system pressure driven pumping for the micro chambers, according to an example of the present disclosure.

[142] Fig. 23J illustrates a bottom perspective view of an embodiment of the assembled operating system pressure driven pumping for the micro chambers, according to an example of the present disclosure.

[143] Fig. 23K illustrates a perspective view of an embodiment of the assembled operating system flow diagram, according to an example of the present disclosure.

[144] Fig. 23L illustrates a flow diagram of a 1 to 72 distributor that connects the mixing chamber with the media chambers connected to the human-on-chip plate, according to an example of the present disclosure.

[145] Fig. 24 illustrates a flow diagram of another embodiment of the assembled operating system, according to an example of the present disclosure.

[146] Fig. 25A illustrates a perspective view of another embodiment of the operating system cover, according to an example of the present disclosure.

[147] Fig. 25B illustrates an exploded view of the operating system cover, according to an example of the present disclosure.

[148] Fig. 25C illustrates a perspective view of the human-chip insertion process inside the operating system, according to an example of the present disclosure.

[149] Fig. 25D illustrates a perspective view of the spectroscopy probe and sensing plates insertion into the operating system, according to an example of the present disclosure.

[150] Fig. 25E illustrates an exploded view of all the operating system components, according to an example of the present disclosure.

[151] Fig. 25F illustrates a perspective view of the fluidic control part of the operating system, according to an example of the present disclosure.

[152] Fig. 26A illustrates a perspective view of an embodiment of the pneumatic valve actuated operating system, according to an example of the present disclosure.

[153] Fig. 26B illustrates an exploded view of the pneumatic valve actuated operating system, according to an example of the present disclosure.

[154] Fig. 26C illustrates a top view of the pneumatic valve actuated operating system, according to an example of the present disclosure.

[155] Fig. 27A illustrates an embodiment of the assembly of media chambers of another embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (isometric view)

[156] Fig. 27B illustrates an exploded assembly of media chambers of another embodiment of the human-on-chip where the individual chip entities are incorporated as 1 part within the chassis and the valving mechanism connecting individual chip components is flexible membrane mechanism actuated pneumatically (isometric view)

- [157] Fig. 27C illustrates a perspective view of the media chamber plate, according to an example of the present disclosure.
- [158] Fig. 27D illustrates an exploded view of the media chamber plate, according to an example of the present disclosure.
- [159] Fig. 27E illustrates a perspective view of the piston plate, according to an example of the present disclosure.
- [160] Fig. 27F illustrates a perspective view of an additional embodiment of the piston plate, according to an example of the present disclosure.
- [161] Fig. 27G illustrates a side view of an embodiment of the piston plate, according to an example of the present disclosure.
- [162] Fig. 27H illustrates a detailed view of a single piston of Figs. 27E-27G, according to an example of the present disclosure.
- [163] Fig. 27I illustrates a top perspective view of the piston actuator plate, according to an example of the present disclosure.
- [164] Fig. 27J illustrates a bottom perspective view of the piston actuator plate, according to an example of the present disclosure.
- [165] Fig. 27K illustrates a perspective view of the fluid sourcing media plate, according to an example of the present disclosure.
- [166] Fig. 28A illustrates a flow diagram of the processes of the human-on-chip operating system, according to an example of the present disclosure.
- [167] Fig. 28B illustrates a flow diagram of the media chamber filling process of the human-on-chip operating system, according to an example of the present disclosure.
- [168] Fig. 28C illustrates a flow diagram of the media chamber emptying process of the human-on-chip operating system, according to an example of the present disclosure.
- [169] Fig. 28D illustrates a flow diagram of the media chamber seeding process of the human-on-chip operating system, according to an example of the present disclosure.
- [170] Fig. 28E illustrates a flow diagram of the media sample collecting process of the human-on-chip operating system, according to an example of the present disclosure.
- [171] Fig. 28F illustrates a flow diagram of the cell sample collecting process of the human-on-chip operating system, according to an example of the present disclosure.
- [172] Fig. 29A illustrates a flow diagram of the fluid control in the triple measurement mechanism, according to an example of the present disclosure.
- [173] Fig. 29B illustrates a logic control diagram of the fluid control for FTIR sampling, according to an example of the present disclosure.

[174] Fig. 30A illustrates a flow diagram to pump a sample from the media chamber to a biochip, according to an example of the present disclosure.

[175] Fig. 30B illustrates a flow diagram to pump a sample from the biochip to a measurement device, according to an example of the present disclosure.

[176] Fig. 31A illustrates a flow diagram of pump gel into the stroma channel, according to an example of the present disclosure.

[177] Fig. 31B illustrates a flow diagram of pump gel out of the stroma channel, according to an example of the present disclosure.

[178] Fig. 32A illustrates a schematic view of the fully assembled embodiment of the mixing chamber, according to an example of the present disclosure.

[179] Fig. 32B illustrates a schematic view of the embodiment of the cell sourcing mixing chamber, according to an example of the present disclosure.

[180] Fig. 32C illustrates a schematic view of the embodiment of the media sourcing mixing chamber, according to an example of the present disclosure.

[181] Fig. 32D illustrates a schematic view of the Cell chamber and media chamber side by side, according to an example of the present disclosure.

[182] Fig. 32E illustrates a flow diagram of an embodiment of the bubble filter and pressure sensors of the mixing chamber, according to an example of the present disclosure.

[183] Fig. 33A illustrates a flow diagram of an embodiment of the mixing chamber where all 36 tubes leave the mixing system, according to an example of the present disclosure.

[184] Fig. 33B illustrates a perspective view of an embodiment of the mixing chamber where all 36 tubes feed into only three tubes leaving the mixing system, according to an example of the present disclosure.

[185] Fig. 33C illustrates a flow diagram of an embodiment of the mixing chamber where all the 36 gas tubes are connected to the cell chamber, according to an example of the present disclosure.

[186] Fig. 33D illustrates a flow diagram of an embodiment of the mixing chamber where all the three pressure valves are connected to the cell chambers, according to an example of the present disclosure.

[187] Fig. 33E illustrates a flow diagram of an embodiment of the mixing chamber where each row of the cell chambers is connected to the 1/12 way valve that can switch between them and send a sample through a flow sensor, air filter, and a pressure gauge connected to a quick connect that is linking the mixing chamber with the human-on-chip plate, according to an example of the present disclosure.

[188] Fig. 33F illustrates a flow diagram of a single cell chamber entity where its connected to the pressure and gas from one side and to the 1/12 way valve from the other which interim is connected to a sensor, air filter, and a pressure gauge connected to a quick connect that is linking the mixing chamber with the human-on-chip plate, according to an example of the present disclosure.

[189] Fig. 33G illustrates a flow diagram of an embodiment of the mixing chamber where all the 12 tubes are connected to the media chamber, according to an example of the present disclosure.

[190] Fig. 33H illustrates a flow diagram of an embodiment of the mixing chamber where all the three pressure valves are connected to the media chambers, according to an example of the present disclosure.

[191] Fig. 33I illustrates a flow diagram of an embodiment of the mixing chamber where each row of the media chambers are connected to the 1/4 way valve that can switch between them and send a sample through a flow sensor, air filter, and a pressure gauge connected to a quick connect that's linking the mixing chamber with the human-on-chip plate, according to an example of the present disclosure.

[192] Fig. 33J illustrates a flow diagram of a single media chamber entity where it is connected to the pressure and gas from one side and to the 1/4 way valve from the other which interim is connected to a sensor, air filter, and a pressure gauge connected to a quick connect that is linking the mixing chamber with the human-on-chip plate, according to an example of the present disclosure.

[193] Fig. 34A illustrates a flow diagram of an embodiment of the mixing chamber where all the containers have access to the O₂, CO₂ and N₂ bottles, O₂, and CO₂ sensors that are located under the cell chambers, according to an example of the present disclosure.

[194] Fig. 34B illustrates a diagram of an embodiment of the O₂ and CO₂ sensors at the level of the tissues grown in the chips, according to an example of the present disclosure.

[195] Fig. 35A illustrates an exploded view of the heat exchange plate passing temperature-controlled fluid in contact with the Human-on-chip, according to an example of the present disclosure.

[196] Fig. 35B illustrates a schematic view of the heat exchange generator chamber that heats and cools the fluid coming to the heat exchange plate, according to an example of the present disclosure.

[197] Fig. 36A illustrates a flow diagram of an embodiment of the machine starting mechanism getting the fluid to run into the system while ensuring that there is no channel

blocked or any valve, actuator or sensor damaged, according to an example of the present disclosure.

[198] Fig. 36B illustrates a flow diagram of an embodiment of the machine starting mechanism getting the temperature, O₂ and CO₂ levels to the aimed valve while flushing the system pre-cell seeding, actuator or sensor damaged, according to an example of the present disclosure.

[199] Fig. 36C illustrates a flow diagram of an embodiment of the machine starting mechanism using pressure sensors and with the assistance of the microscopy computer vision to make sure there is no air bubble in the system prior to cell seeding (pressuring all the liquids, imaging, light contrast, fluorescence), according to an example of the present disclosure.

[200] Fig. 36D illustrates a flow diagram of an embodiment of the machine starting using thermal imaging to detect and view fluid flow, un-wetted surfaces and air bubbles. (Flash thermography), according to an example of the present disclosure.

[201] Fig. 36E illustrates a flow diagram of an embodiment of the machine starting and running mechanism showing the temperature, O₂, CO₂, and pressure sensors in addition to the fluid channeling, CO₂ and O₂ mixing, air bubble traps, and heating plates controlling all the parameters of the system, according to an example of the present disclosure.

[202] Fig. 37A illustrates a perspective view of the embodiment of the microscopy and spectroscopy mechanism where there is more than one lens, according to an example of the present disclosure.

[203] Fig. 37B illustrates a perspective view of the embodiment of the microscopy and spectroscopy mechanism where there is one lens moving in an x-y plane, according to an example of the present disclosure.

[204] Fig. 37C illustrates a perspective view of the embodiment of the FTIR reading mechanism where there are multiple sensors, according to an example of the present disclosure.

[205] Fig. 37D illustrates a perspective view of the embodiment of the FTIR reading mechanism where there is only one sensor, and the fluid is being sampled and measured with a time gap calculated, according to an example of the present disclosure.

[206] Fig. 37E illustrates a perspective view of the detailed sampling mechanism of an embodiment of the FTIR reading mechanism where there is only one sensor, and the fluid is being sampled and measured with a time gap calculated, according to an example of the present disclosure.

[207] Fig. 37F illustrates a perspective view of the embodiment of the FTIR reading mechanism where there is a flow cell built in the chip, according to an example of the present disclosure.

[208] Fig. 38A illustrates a top view of possible biochip arrangements for human-on-chip modeling, according to an example of the present disclosure.

[209] Fig. 38B illustrates a prospective view of possible biochip arrangements for human-on-chip modeling, according to an example of the present disclosure.

[210] Fig. 38C illustrates a top view of an additional embodiment of possible biochip arrangements for human-on-chip modeling, according to an example of the present disclosure.

[211] Fig. 38D illustrates a perspective view of an additional embodiment of possible biochip arrangements cell tissue modeling, according to an example of the present disclosure. In brief, the ductal channel containing endothelial or epithelial cells (II) connects one biochip (I) to at least another one (III).

[212] Fig. 39A illustrates a cross-sectional view of the embodiment of the Tubular architecture of a tissue in our 2-duct chips, according to an example of the present disclosure. Where I. is the Biochip's stomal channel; II. is the extracellular matrix, III. are endothelial and/or epithelial cells which may also contain cancer cells, IV. is ductal channel with media V. is stromal channel with cells which may contain cancer cells.

[213] Fig. 39B illustrates a perspective view of the embodiment of multiple cultured tissues in our chips connected with a common duct/vessel, according to an example of the present disclosure.

[214] Fig. 39C illustrates a schematic view of the embodiments of an organ-on-chip grown in the human-on-chip, according to an example of the present disclosure. The cells may be primary cells, cell lines, iPSCs, patient-derived cells or biopsies and it may be used for precision medicine analysis or for a more general analysis. I. is the Biochip's stomal channel; II. is the extracellular matrix, III. is the tissue or tumor cells of interest, IV. are immune cells, V. are endothelial and/or epithelial cells and VI. is the biochip's ductal channel.

[215] Fig. 39D illustrates a schematic view of the embodiment of a Liquid-air-Interface Lung-on-chip grown in the Human-on-chip, according to an example of the present disclosure. Where, I. is the Biochip's stomal channel; II. is the extracellular matrix, III. represents endothelial cells, IV. is the biochip's ductal channel containing air, V. represents the lung tissue, VI. are respiratory and VII. are astrocytes.

[216] Fig. 39E illustrates a schematic of an embodiment of a glioblastoma model and the blood brain barrier in the biochip, according to an example of the present disclosure, where I.

is the Biochip's stomal channel; II. is the extracellular matrix, III. represents glioblastoma cells with immune cell infiltration, IV. are immune cells, V. are endothelial cells and pericytes, VI. is the biochip's ductal channel and VI. are astrocytes.

DETAILED DESCRIPTION OF EXAMPLE EMBODIMENTS

[217] The present disclosure provides five aspects that enable a system of precise and repeatable semi-automatic precise and predictive experimentations. The provided experimentation enables the precise creation and maintenance of tissue models, drug testing, real-time sampling & measurement, and software assisted control and data analysis. The provided system enables more precise prediction of a drug's toxicity, efficacy and dosage, and is a powerful tool for personalized and precise drug prognosis.

[218] The first aspect of the present disclosure provides a biomimetic tissue culture platform that enables the fundamental aspects of a biomimetic model. The provided biochip embeds a number of cylindrical porous ductal scaffolds forming different compartments in the biochip.

[219] The second aspect of the present disclosure provides a hybrid human-on-chip tissue culture platform for a human model, wherein it can be used by itself and with the aid of the operating system.

[220] The third aspect of the present disclosure provides a semi-automated fluidic control system of valving and pumping, wherein, very small amounts of volumes could be manipulated and controlled in any way moving samples across all of the regions of the system, and also into and out of the system.

[221] The fourth aspect of the present disclosure provides a semi-automated sample collection and real-time measurement add-ons, wherein, the configuration of the system allows to interface, microscopy, spectroscopy, Fourier Transform Infrared (FTIR), and other measurement devices to measure every sample while the experiments are going and pumping into and out of the system.

[222] The fifth aspect of the present disclosure provides software to automate and control the operating system, wherein, the software contains different commands for different experimental setups. Additionally, the software is connected to the measurement devices and allows for execution of the data acquisition that acts on the system's measurement devices and the fluid control mechanism.

[223] MULTI-DUCT BIOCHIP

[224] In an embodiment, the biochip contains at least two ducts and one stroma, the ducts being formed from ultrathin porous membranes that are cylindrical with no need to curve it. In an additional embodiment, the ducts could be made of gore flexible tubing, or it could be of

any materials and the pores are created by laser gunning or by extruding. In an embodiment, the biochip may contain multiple ducts and multiple stromas. Additionally, a solution different from the solution being passed through the plurality of ducts may be inserted in the stroma. In an additional embodiment, known materials such as stents may be utilized in the multiple biochips.

[225] In an embodiment, separate membranes that are hydrophobic and hydrophilic could be utilized to create the multiple ducts. In an additional embodiment, one of the multiple ducts could be formed from a single membrane with different regions treated to be hydrophobic or hydrophilic by plasma or UV treatment or through coating. Depending on the specific requirements of the user of the system, the hydrophobic and hydrophilic properties of the multiple ducts may be modulated according to known treatment techniques.

[226] In an embodiment, the biochip may contain a plurality of cavities to allow for multiple organs to be cultured on a single multi-duct biochip.

[227] In an embodiment, biochips with a single duct may also be utilized by the presently disclosed system. For example, in an embodiment, the biochip utilized may be the biochip described in PCT App. No. PCT/QA2021/050016, filed June 25, 2021, entitled DUCT ORGANOID-ON-CHIP, assigned to the assignee of the present invention and is hereby incorporated by reference in its entirety, which describes a biomimetic tissue culture platform or biochip that enables the fundamental aspects of a biomimetic model.

[228] Fig. 1 illustrates a schematic view of two ducts surrounded by one stroma, according to an example of the present disclosure.

[229] Fig. 2A illustrates a perspective view of a 2-duct, 1-stroma biochip, according to an example of the present disclosure. In an embodiment, the chassis of the biochip is constructed of a material that does not obstruct optical or spectroscopic access to the multiple ducts.

[230] Fig. 2B illustrates a top view of an additional embodiment of a 2-duct, 1-stroma biochip, according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber and air filtering areas for each duct which may allow for air bubbles to be released out of the biochip. In some examples, the air filtering area may also allow air to enter the biochip which allows the cells being cultured to have access to air. In some examples, the biochip may include on each duct an inlet and outlet, and for the stroma channel an inlet and outlet. The biochip may also include multiple segments of duct surrounded by plastic. In an example, the biochip includes a cover glass on the uppermost portion of the biochip to cover at least part of the top surface of the upper chassis of the biochip. The upper cover glass may be a thin coverslip glass made of a

material that is brittle, transparent, and has low autofluorescence such as glass or polymer. The upper chassis and the lower chassis contain features such that when placed in direct contact with each other, internal space is formed to accommodate porous ductal scaffolds made of the membranes. These features may be the result of engraving in the inner layers of the chassis. In another example, the upper chassis and the lower chassis contains features forming the microfluidic channels leading to the external compartment of the ductal scaffolds and could be engraved on the outer surfaces of the chassis, and covered with another chassis part or a thin coverslip creating the full channel on the lower chassis to accommodate the multiple porous membrane structures.

[231] In an embodiment, the upper chassis and the lower chassis contain features that hold the cylindrical ductal scaffolds in position giving access to the internal and the external compartments of each of the ductal scaffolds. The chassis and forming the microfluidic channels leading to the internal compartment of the ductal scaffold could extend beyond the inlet and outlet holes of the channel and later be plugged using plugs post-assembly of the biochip. A bottom cover glass, similar to the upper cover glass may be provided to cover the bottom-most portion of the lower chassis. In an example, coverslip glass forming the top & bottom layers of the chip is bonded to the chassis using a glass-polymeric glue. In an example, the porous membrane structure is formed by curving porous membranes into a 180° rotation, forming a cylindrical structure and bonding the access material of the membranes that were not curved into the cylindrical structure. For example, two pre-bonded cylindrical hydrophobic ultrathin porous membranes on each duct can be used for filtering. These components may combine to form the multiple porous membrane ductal structures surrounded by the same stroma.

[232] Fig. 2C illustrates a flow diagram of the manufacturing and assembly process of the 2-duct, 1-stroma biochip, according to an example of the present disclosure. Where curving and applying tension on the membrane is done simultaneously while bonding, porous membranes are curved in a cylindrical manner, approximately in a 180° orientation. In an example, the biochip configured according to this method may include, air filtering area, a duct surrounded by stroma, a duct inlet, a duct outlet, a stroma channel inlet, and a stroma channel outlet. Fig. 2C illustrates the steps and processes, according to this method, to execute the bonding process of the biochip components where the ductal scaffold is curved, tensioned, and bound to the chassis at its extremities in one step. For example, the method includes the on-chassis locating and the curving process of the hydrophobic and hydrophilic membranes over a rod and bonding it at its extremities to the chassis using glue, heat, or chemically. After locating the membranes

on the chassis, curving them over the two rods that will form each of the two ducts, applying tension on the membranes, and binding its extremities to the chassis, assemble the pin into the outlet stroma hole of the lower chassis. Then, sandwich everything between the two chassis, then binding them using chemical aided heat-press bonding. Finally, the method may involve the rods, and the pin removal process, plugging the extremities of both ductal channels beyond the inlet and outlet holes, and assembling the coverslip glasses on both surfaces of the biochip.

[233] Fig.3A illustrates a perspective view of an embodiment of a 2-duct, 1-stroma biochip, according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber having a stroma feeding channel, and the top ducts can be used for gel insertion into the stroma chamber.

[234] Fig.3B illustrates a perspective view of an additional embodiment of a 2-duct, 1-stroma biochip, with a stroma feeding channel according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber having a stroma feeding channel, where the stroma feeding channel is a perforated tube that enables direct feeding to the stroma, and the wide pores in the tube increase the diffusion rate between the ducts and the stroma.

[235] Fig.3C illustrates a flow diagram of the manufacturing and assembly process of a 2-duct, 1-stroma biochip with three parallel channels according to an example of the present disclosure. The upper chassis and the lower chassis contain three parallel channels that hold two cylindrical ductal scaffolds and a perforated tube, and one stroma inlet in position giving access to the internal and the external compartments of each of the ductal scaffolds. In an embodiment, a perforated tube is glued at its extremities in the middle channel, and the ducts being formed from ultrathin porous membranes that are curved in a cylindrical manner around a rod, and bonding it at its extremities to the chassis using glue, heat, or chemically, and assemble the pins into the outlet stroma hole of the lower chassis. Then, sandwich everything between the two chassis, then binding them using chemical aided heat-press bonding. Finally, the method may involve the rods, and the pin removal process, plugging the extremities of both ductal channels beyond the inlet and outlet holes, and assembling the coverslip glasses on both surfaces of the biochip.

[236] Fig.4A illustrates another perspective view of a 2-duct, 1-stroma biochip, with three parallel channels, according to an example of the present disclosure. In an embodiment, two ducts with porous membranes are surrounded by one stroma, in which the upper middle ducts are connected to the stroma chamber, where these two ducts can be used for feeding.

[237] Fig.4B illustrates a perspective view of a 2-duct, 1-stroma biochip, with three parallel channels, according to an example of the present disclosure. In an embodiment, two ducts with porous membranes are surrounded with one stroma, in which two types of endothelial cells can be cultured in ducts, and one type of epithelial cells can be cultured in a stroma.

[238] Fig.4C illustrates a perspective view of an additional embodiment of a 2-duct, 1-stroma biochip, with three parallel channels with a stroma feeding channel according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber having a stroma feeding channel, where the stroma feeding channel is a perforated tube that enables direct feeding to the stroma, and the wide pores in the tube increase the diffusion rate between the ducts and the stroma.

[239] Fig.4D illustrates a flow diagram of the manufacturing and assembly process of a 2-duct, 1-stroma biochip, with three parallel channels, according to an example of the present disclosure. The upper chassis and the lower chassis contain three parallel channels that hold two cylindrical ductal scaffolds and one stroma inlet in position giving access to the internal and the external compartments of each of the ductal scaffolds. In an embodiment, the ducts being formed from ultrathin porous membranes that are curved in a cylindrical manner around a rod, and bonding it at its extremities to the chassis using glue, heat, or chemically, and assemble the pins into the outlet stroma hole of the lower chassis. Then, sandwich everything between the two chassis, then binding them using chemical aided heat-press bonding. Finally, the method may involve the rods, and the pin removal process, plugging the extremities of both ductal channels beyond the inlet and outlet holes, and assembling the coverslip glasses on both surfaces of the biochip.

[240] Fig. 5A illustrates a perspective view of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber having different stroma inlets to allow for different types of cell organs to be cultured in the same duct on a 2-ducts 1-stroma biochip.

[241] Fig. 5B illustrates a top view of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure. In an embodiment, the biochip may include two porous cylindrical ductal channels surrounded by the same stroma chamber having different stroma inlets, where each inlet can be used to inject the gel into the stroma that it is connected to it.

[242] Fig. 5C illustrates a perspective view of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure. In an embodiment, the biochip may

include two porous cylindrical ductal channels surrounded by the same stroma chamber having different stroma inlets, where the stroma chamber has a perforated barrier that splits into two separate stromas.

[243] Fig.5D illustrates a flow diagram of the manufacturing and assembly process of a 2-ducts 1-stroma chip with different stroma inlets, according to an example of the present disclosure. The upper chassis and the lower chassis contain three parallel channels that hold two cylindrical ductal scaffolds and two different stroma inlets for each stroma chamber in position giving access to the internal and the external compartments of each of the ductal scaffolds. In an embodiment, the stroma chamber is split into two separate stromas by a perforated barrier that is glued at its extremities in the middle channel. The ducts are formed from ultrathin porous membranes that are curved in a cylindrical manner around a rod, and bonding it at its extremities to the chassis using glue, heat, or chemically, and assemble the pins into the outlet stroma hole of the lower chassis. Then, sandwich everything between the two chassis, then binding them using chemical aided heat-press bonding. Finally, the method may involve the rods, and the pin removal process, plugging the extremities of both ductal channels beyond the inlet and outlet holes, and assembling the coverslip glasses on both surfaces of the biochip.

[244] Fig. 6A illustrates a perspective view of a multiple stroma per duct biochip, with three stromas and two ducts, according to an example of the present disclosure. In an embodiment, the biochip may contain several cavities to allow for multiple types of cell organs to be cultured in the same duct on a single multiple stroma per duct biochip.

[245] Fig. 6B illustrates a perspective view of a multiple stroma per duct biochip, with three stromas and two ducts, according to an example of the present disclosure. In an embodiment, the biochip may contain several cavities to allow for the same type of cell organs to be cultured in the same duct on a single multiple stroma per duct biochip.

[246] Fig.6C illustrates a flow diagram of the manufacturing and assembly process of a multiple stroma per duct biochip, with three stromas and two ducts, according to an example of the present disclosure. The upper chassis and the lower chassis contain three parallel channels that hold two cylindrical ductal scaffolds and one stroma inlet for each stroma chamber in position giving access to the internal and the external compartments of each of the ductal scaffolds. In an embodiment, the ducts being formed from ultrathin porous membranes that are curved in a cylindrical manner around a rod, and bonding it at its extremities to the chassis using glue, heat, or chemically, and assemble the pins into the outlet stroma hole of the lower chassis. Then, sandwich everything between the two chassis, then binding them using

chemical aided heat-press bonding. Finally, the method may involve the rods, and the pin removal process, plugging the extremities of both ductal channels beyond the inlet and outlet holes, and assembling the coverslip glasses on both surfaces of the biochip.

[247] **THE HUMAN-ON-CHIP PLATE**

[248] In an embodiment, the human-on-chip plate contains cylindrical cross-sectional ducts surrounded by stromas so that a blood vessel duct could pass through the stromas of different tissues that also contain epithelial ducts passing through its individual stromas.

[249] In an additional embodiment, the biochip plate uses valving between the individual biochips and components so that the valves could be opened or closed manually or automatically through the operating system. In an embodiment, the present disclosure is a hybrid-controlled organ-on-chip.

[250] The human-on-chip plate could be created by puzzling different individual chips together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate. The human-on-chip plate could also incorporate different chips in the same chassis, not separate components with valves that could be assembled within the biochip while assembling the other components. The valves connecting the different biochip tissue growth channels could be hydraulic piston mechanism, valve, hydraulic rolling, or a pneumatically actuated membrane piston valve, or a simple membrane deformation valve. Any valve type could be incorporated in this system.

[251] The human-on-chip plate could also include normally closed open/close valves and waste removal channels incorporated within the biochip for its potential usage when assembled within the operating system. The cover glasses covering the individual chip units could be made of one or more glass covering the surface channels of each entity, or only one glass could be used to cover the full surface of the biochip instead. The interconnection between the different biochip entities could be through the duct openings straight along their axis, or it can just go through the inlet/outlet holes to the surrounding biochips. The interface of the biochip with the operating system could be through the same inlet/outlet holes used in manual use, or through different holes that are separate from the system by normally closed valves that are actuated by the operating system.

[252] In an embodiment, the presently disclosed system includes means for rotating the human-on-chip plate about a vertical and/or horizontal axis to ensure homogenous distribution of reagents within a solution, and the homogeneous distribution and attachment of cells post seeding in the channels.

[253] In an embodiment, the human-on-chip plate allows for individual biochips to be connected to each other to form a series of interconnected biochips. Additionally, in an embodiment, specific biochips can be selectively disconnected from the remaining interconnected biochips to create multiple biochip circuits. As discussed later in the present disclosure, modulation of fluid control mechanism allows for specific valves to be either opened or closed to facilitate numerous combinations of interconnected biochip circuits. The present disclosure allows for a plurality of protocols to be performed in parallel within a single human-on-chip plate because of the modular nature of the human-on-chip plate allows for dynamically routing solutions to specific biochips. Dynamic routing of solutions in the presently disclosed system allows for specific biochips to be isolated from the remaining biochips to allow for precision medicine protocols to be performed.

[254] In an embodiment, the human on plate fluidically connects multiple chips to each other and to other components of the operating system. These components may be, but not limited to, any other chip, the waste chamber or the sampling chamber. In an embodiment, the connections can be altered at any time.

[255] Fig. 7A illustrates a perspective view of the human-on-chip plate and the individual chips respectively, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate.

[256] Fig. 7B illustrates a perspective view of an individual chip found in the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate, and the fluid is pumped through the duct from the top of the human-on-chip plate and from a duct to another through a cylindrical rotational valving mechanism.

[257] Fig. 7C illustrates an exploded view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate, and the fluid is pumped through the duct from the top of the human-on-chip plate and from a duct to another through a cylindrical rotational valving mechanism.

[258] Figures 8A and 9A illustrate a perspective view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individual biochip entities are incorporated as one human-on-chip plate where a cylindrical rotational mechanism connects the chassis and the valving mechanism to connect the individual chip components.

[259] Fig. 8B and 9B illustrate a detailed view of the valving mechanism connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individual biochip entities are incorporated as an integrated human-on-chip. Specifically, a cylindrical rotational mechanism connects the chassis and the valving mechanism connecting individual biochip components.

[260] Fig. 8C and 9C illustrate an exploded view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individual biochip entities are incorporated as one component within the chassis and the valving mechanism and connected through a cylindrical rotational mechanism.

[261] Fig. 10A illustrates a detailed view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate. The biochip is made up of several plate layers stacked on top of each other, consisting of a heat plate, chip holding plate, chip adhering plate, duct 1 fluid channeling plate, stroma fluid channeling tube, duct 2 fluid channeling plate, Row actuating plane, column 1 actuating plate, column 2 actuating plate, and two chip actuating plates.

[262] Fig. 10B illustrates a schematic view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the heating plate contains a fluidic channel that passes in the inlet and the outlet of the top of the chip, and it fits into the operating system, wherein we can pump any liquid of any temperature to this heat plate, in order to control, increase, decrease or maintain, the temperature of the cell culture.

[263] Fig. 10C illustrates an exploded view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the row actuating plate is a valve actuating channel plate that is connected to the first bistable beam of each valve on the same row (3 Rows, 3 signals). The column 1 actuating plate is a valve actuating channel plate that is connected to the second bistable beam of each valve on the first two columns (2 columns, 2 signals). The column 2 actuating plate is a valve actuating channel plate that is connected to the second bistable beam of each valve on the second two columns (2 columns, 2 signals). The

two chips actuating plates are valves actuating channel plates that are connected to the third bistable beam of each valve in each chip (2 ducts and 1 stroma, 3 signals). In some cases, the bistable valve could open two bistable beams with each other. For example, in the case of opening the valve connecting duct 1 in chip 1 located in row 1 and column 1, to duct 1 in chip 2 located in row 1 and column 2, the signal would be (1,1,1), where this signal will open the first pneumatic tube in the first, second and fourth layer.

[264] Fig. 11A illustrates a perspective view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate, and the fluid is sampled from a specific sampling spot inside each biochip in the human-on-chip plate, where a pipet like mechanism can be used, or implemented in the system in order to take samples.

[265] Fig. 12A illustrates a perspective view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individually removable biochips are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate, and the sample is taken on spot through the channels, chambers and valves, where the cell samples can be either sampled on spot using a pipet like mechanism, pumped through a valving mechanism to a sampling chamber, or to the media chamber.

[266] In an embodiment, the individual biochip entities are incorporated as one human-on-chip plate where a cylindrical rotational mechanism connects the chassis and the valving mechanism to connect the individual chip components, and the fluid is pumped through the duct from the top of the human-on-chip plate and from a duct to another through a valving system.

[267] Fig. 13A illustrates a perspective view of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the individual biochip entities are incorporated as one human-on-chip plate where a cylindrical rotational mechanism connects the chassis and the valving mechanism to connect the individual chip components, and the fluid is pumped through the side ducts of the human-on-chip plate and from a duct to another through a valving system.

[268] In an embodiment, the individual biochip entities are incorporated as an integrated human-on-chip. Specifically, a cylindrical rotational mechanism connects the chassis and the

valving mechanism connecting individual biochip components, and the fluid is pumped through the side ducts of the human-on-chip plate and from a duct to another through a valving system.

[269] In an embodiment, the individual biochip entities are incorporated as one component within the chassis and the valving mechanism and connected through cylindrical rotational mechanism, and the fluid is pumped through the side ducts of the human-on-chip plate and from a duct to another through a valving system.

[270] In an embodiment, the individual biochip entities are incorporated as one human-on-chip plate where a cylindrical rotational mechanism connects the chassis and the valving mechanism to connect the individual chip components, and the fluid is sampled from a specific area inside each biochip in the human-on-chip plate.

[271] In an additional embodiment, the human-on-chip plate, can have biochips, individually removable biochips that are puzzled together through interconnecting different chips through its ducts to inlet/outlet valves on a biochips and valve holder plate, or the individual biochip entities are incorporated as one human-on-chip plate where a cylindrical rotational mechanism connects the chassis and the valving mechanism to connect the individual chip components. In an embodiment, a two-duct chip can be connected to a one-duct chip.

[272] Fig. 14A illustrates a schematic diagram which contains pressure sensors at each chip inlet and outlet fluid ports to manipulate the pressure inside the chips' channels. In an additional embodiment, fluid is controllably perfused from the ductal channel to the stromal one or vice versa, thanks to the pressure sensors at each chip.

[273] In an additional embodiment, a biological system-on-chip plate includes a plurality of ducted biochips, fluidic channeling means in fluidic communication with at least one biochip. The fluidic channeling means includes plurality of fluidic pathways and selectable connection means providing the ability to selectably connect or disconnect fluidic communication therethrough. In an additional embodiment, the ducted biochips have inlet and outlet ports. In an embodiment, the ports of at least two biochips are fluidically connected to at least one fluidic pathway. In an embodiment, the fluidic channeling means enables transfer or circulation of media through ducts or stromal regions of multiple biochips. The selectable connection means enable arrangement of biochips in series or parallel as required for a particular experiment or application. In an additional embodiment, at least one selectable connection means is configured to enable a permanent selection process to either connect (e.g. a frangible seal that can be ruptured) or disconnect (e.g. a thermally bondable material that can be heat-sealed)

fluidic communication therethrough. In an embodiment, at least one selectable connection means includes valving means that provides reversible connection and disconnection. The valving means enable isolation of a biochip during an experiment.

[274] In an embodiment, the valving means enable access to remove media samples during or after an experiment. The valving means enable introduction of media before or during and experiment. Additionally, the valving means enable control of where different media, especially cell types, are deposited during experimental set up introduction of media before or during and experiment. In an embodiment, the valving means is stable in both the connected and disconnected state. Additionally, several other components can be utilized such as pinch valve with cam (friction held, or over-center profile), rotary valve (cylinder or ball valve with friction), snap-through bistable lever on pinch valve, self-stabilized piloting pneumatic valve.

[275] In an additional embodiment, the biological system on chip plate includes a number of valving means actuated by a number of actuator(s), wherein the number of actuator(s) required to actuate the valving means is fewer than the number of valving means. In an embodiment, the biochip is a multi-duct chip. In an embodiment, the biochips are organ-on-chip biological simulations. In an additional embodiment, a first biochip simulating a first organ type is connected to a second biochip simulating a second organ type. In an embodiment, the biological system on chip plate simulates multiple organs of an organism. The biological system on chip plate simulates sufficient organs to gain insight into the impact of experimental compounds on multiple organs within the organism. In an embodiment, the organism is human. In an additional embodiment, the biological system on chip plate is configured to engage with external actuation and pumping means to enable a lower cost or even disposable interchangeable biological system on chip plate.

[276] **THE MICRO-VALVING MECHANISM**

[277] Fig. 15 A-C, illustrates a perspective view of different types of mechanical micro-valves. In one embodiment of the mechanical micro-valve in Fig. 15A, two pistons move in opposite directions to open and close two ports located at close proximity and required to be of an opposite (on-off) form at all times. The hydraulic pistons are connected with a constant volume of incompressible fluid where one piston moves in one direction, the fluid acts as a pushing or pulling force on the other piston, moving it in the opposite direction.

[278] Fig. 15B illustrates a perspective view of a rolling cylinder mechanical micro-valve, where drilled cylinders containing the inlet and out ports rotate around its axis to either align

the holes towards its intended location and connect the fluids to a microfluidic channel in the outside of the system to fluids getting into the chips.

[279] Fig. 15C illustrates a perspective view of a pneumatically actuated drilled piston micro-valve, where drilled pistons connecting the ends of a microfluidic channel are pneumatically actuated to move a piston and clog the channel.

[280] Fig. 16 A-F illustrates multiple embodiments of an electrically actuated solenoid bistable micro-valve. This micro-valve contains a beam with a metal end located between two magnets where electric signals trigger the magnets force direction moving the beam between two locations. The other side of the beam contains a hole that when aligned with the microfluidic port opening of the microchannel it is connected to, the channel would be open, and if the holes are not aligned, the channel would be closed.

[281] Fig. 16 A-C illustrates two embodiments of the electrically actuated solenoid bistable micro-valve, wherein the micro-valves are at open positions since the drilled beams are both in their extended positions.

[282] Fig. 16 B-D illustrates two embodiments of the electrically actuated solenoid bistable micro-valve, wherein the internal components of the micro-valve are shown and wherein one beam is at its minimum extended position, blocking the microfluidic channel, and the other beam is at its maximum position, and the hole drilled on the beam is aligned to the microfluidic channel holes it is acting upon, opening the flow in the microfluidic channel.

[283] Fig. 16 E-F illustrates the assembly of the electrically actuated solenoid bistable micro-valve on the human on-chip plate. Wherein, the plate in Fig. 16 E shows the microfluidic channeling the micro-valves intend to block or open. In addition, Fig F shows the top view of the human-on-chip plate having the micro-valves and its electric circuiting.

[284] Fig. 17 A-D illustrates different embodiments of the pneumatically actuated drilled piston micro-valve. Fig. 17A shows the perspective view of a normally closed micro-valve wherein the drilled piston hole is not aligned with the hole connecting with the microfluidic channel.

[285] Fig. 17 B shows an exploded view of a normally closed micro-valve wherein the drilled piston hole is not aligned with the hole connecting with the microfluidic channel.

[286] Fig. 17 C shows the perspective view of a normally open micro-valve wherein the drilled piston hole is aligned with the hole connecting with the microfluidic channel.

[287] Fig. 17 D shows an exploded view of a normally open micro-valve wherein the drilled piston hole is aligned with the hole connecting with the microfluidic channel.

[288] Fig. 18A illustrates a perspective view of the bistable beam on a flexible tube micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is bistable, where the micro-valves have two stable positions. The bottom balloons channel is pneumatically actuated, where when the air flows in the channel, it opens all the channels in a micro-valve, and the upper set of balloons, that are singularly pneumatically actuated, each one closes the channel that is below it.

[289] Fig. 18B illustrates an exploded view of the bistable beam on a flexible tube micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is bistable, where the micro-valves have two stable positions. They only require power when switching between two states, and once actuated, the bistable beam will stay in one of those two bistable positions. The bottom balloons channel is pneumatically actuated, where when the air flows in the channel, it opens all the channels in a micro-valve, and the upper set of balloons, that are singularly pneumatically actuated, each one closes the channel that is below it by squishing the tube to block the flow.

[290] Fig. 19A illustrates a perspective view of the bistable magnetic drilled-piston micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is bistable, where the micro-valves have two stable positions. The bottom and upper channels are magnetic, that make the micro-valve bistable, and the drilled-piston is pneumatically actuated, which controls the state of the micro-valve.

[291] Fig. 19B illustrates an exploded view of the bistable magnetic drilled-piston micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is bistable, where the micro-valves have two stable positions. The bottom and upper channels are magnetic, that make the micro-valve bistable by attaching the drilled-piston into the surface of the channel, wherein the drilled piston can be replaced by a ball micro-valve. The drilled-piston is pneumatically actuated, which controls the state of the

micro-valve to be opened or closed, where the drilled piston is any piston with a hole, and the hole can be concentric with the duct at the open position in order to pass a fluid.

[292] Figures 20A, 20B, 20C, and 20D illustrate different perspective views of the bistable mechanically actuated flexible tube micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is bistable, where the micro-valves have two stable positions. The bistable beam is mechanically actuated, where when closed, it squishes the flexible tube in order to block any fluid to pass. The design can be solenoidly or pneumatically actuated in order to move the main beam, that actuates the bistable beam, and the bistable beam can be replaced by a bistable magnetic piston.

[293] Figures 21A, 21B, 21C, and 21D illustrate different perspective views of the mechanically actuated pinched ball open-close micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is either open or close, where a micro-valve having a ball with a grooved hole can be rotated to either pass a fluid, or block it. The ball-micro-valve is mechanically actuated by a motor controlled by a system which can be actuated by a robot that moves in a 2D or 3D plane that opens and closes the micro-valves, or in a standalone system that can preset the micro-valves configuration in order to mimic an organ in the human body.

[294] Figures 22A, 22B, 22C, and 22D illustrate different perspective views of the permanent seal open-close micro-valve connecting the individual biochip components of the human-on-chip plate, according to an example of the present disclosure. In an embodiment, the valving mechanism connecting individual biochip components is either open or close, where the permanent seal is preset once to either have an opened or closed micro-valve, where a pin is permanently inserted into the channel, and either blocking or opening the channel depending on the shape of the pin.

[295] **THE OPERATING SYSTEM COMPONENT ASSEMBLY**

[296] Fig23 A-K illustrates a perspective view of an embodiment of the assembled operating system. In an embodiment, the operating system consists of multiple interconnecting subsystems. The subsystems are the mixing chamber, the fluidic control operating system, the human-on-chip plate, the individual biochip component, the machine control system, and the analytical device system.

[297] Fig 23G shows on one side of the device, the ports that connects the operating system with the tubes that goes out of the chip into the waste chamber. It also shows the ports that connects the operating system with the pneumatic air signal inlet that would actuate the valves that controls the flow to the waste chamber. In addition to the fluid tubings that enters the temperature controlled liquid to the system to heat it, and the pneumatic ports to actuate the valves controlling the flow of the heat-controlled fluid distributing heat homogeneously across the system.

[298] Fig 23F also shows on the backside of the device two ports that connects to the pneumatic control device. One port contains 11 tube fittings which aims to pneumatically control the 33 valves in the chip and another port contains 16 pneumatic tube fittings which aims to control the 72 valves of the 72 media micro-chambers that will pump into the 72 different channels in the human-on-chip plate. On the same side, the device has 72 fluidic ports that connect the fluid coming from outside the system to the media micro-chambers. Those fluids pass from the mixing chambers into the valve distributor and then into those ports,

[299] Fig 23G shows the side that contains four electric plugs controlling the Temperature, pressure/flow, O₂ and CO₂ sensors respectively.

[300] Fig 23H shows the human-on-chip plate connected to the fluidic operating system, which Fig 23I shows the ports in the fluidic operating system device that connects the biological fluids port to each channel of the human-on-chip plate, also the pneumatic air control ports that connects with the valves inside the human-on-chip plate and the heated fluid ports that connects heats the human-on-chip. Fig 23J shows the three main components in the system, which are the fluidic operating system device, the media chambers connecting to it and the human-on-chip plate.

[301] In an embodiment, the fluid consisting of cells or biological or chemical components, we call media, is first inserted manually by the user into the mixing chambers. After being in the mixing chamber, the media is pumped through a pressure sensor and an air bubble filter and a 1-to-12 way valve into the tubings of the fluidic operating system.

[302] The 1-to-12 way valve allows for the media to pass from a predefined mixing chamber to the operating system before being distributed by the valving system, which is the first sub-system in the fluidic operating system. The distribution valves consist of a combination of valves: one 1-12 way valve connected to 12 other 1-6 way valves creating a 1-72 routing pathway. The media then reaches a micro-chamber located at close proximity with the human-on-chip. The micro-chamber is interfaced with a pumping mechanism where the fluid inside

the micro-chamber is to be precisely pumped either to the channel in a chip entity of the human-on-chip or to the waste chamber.

[303] In an embodiment, the microfluidic pumping mechanism controls the precise amount of fluid which can be as little as 0.1µl which is being pumped to the micro-chamber as well as from the microchamber to the chips.

[304] In an embodiment, each channel of a chip has an inlet and an outlet port and the pumping control mechanism can control the pressure, flow and flow direction.

[305] In an embodiment, at least one analytical device can be used for real-time measurement of chemical, biological, or physical parameters

[306] In an embodiment, the operating system contains pressure sensors at each chip inlet and outlet fluid ports to manipulate the pressure inside the chips' channels. This allows for the fluid to controllably perfuse from the ductal channel to the stromal one or vice versa.

[307] In an embodiment of the operating system, at least one valving system could open or close and shift the flow pathways to every compartment, while the experiments are running.

[308] In an embodiment of the operating system, the ductal scaffolds of a plurality of organ-on-chip on a human-on-chip plate are connected together allowing for a continuous flow.

[309] In an embodiment of the operating system, the ductal scaffolds of a plurality of organ-on-chip on a human-on-chip plate are connected together only for a controllable amount of time.

[310] In an embodiment of the operating system, the plurality of organ-on-chips on a human-on-chip plate are isolated from one another.

[311] In an embodiment of the operating system, a microfluidic automated flow control mechanism allows for the addition and removal of fluid in the ductal scaffold of an organ-on chip.

[312] In an embodiment of the operating system a microfluidic, automated flow control mechanism allows for the addition and removal of fluid in the ductal scaffold of a plurality of organ-on chips on a human-on-chip plate.

[313] In an embodiment of the operating system, a microfluidic automated flow control mechanism allows for the addition and removal of fluid to the external compartment surrounding the ductal scaffold of an organ-on chip.

[314] In an embodiment of the operating system the pumping control mechanism can transfer fluid being pumped to a waste chamber.

[315] In an embodiment of the operating system the pumping control mechanism can transfer fluid being pumped to a sample chamber.

[316] In an embodiment of the operating system the pumping control mechanism can transfer fluid being pumped to a different biochip.

[317] In an embodiment of the operating system wherein the pumping control mechanism can transfer fluid being pumped for sampling.

[318] In an embodiment of the operating system wherein an electro-mechanically controlled robotic arm that is embedded and interlinked to the operating system, can operate in at least one dimension to perform different operations.

[319] In an embodiment of the operating system an electro-mechanically controlled robotic arm that is embedded and interlinked to the operating system, can situate at least a built in microscope, spectroscope or monitoring sensor over the human-on-chip.

[320] In an embodiment of the operating system wherein an electro-mechanically controlled robotic arm that is embedded and interlinked to the operating system, can have at least one built-in pipet that can take samples from at least one compartment of the sample sourcing chamber.

[321] In an embodiment of the operating system wherein an electro-mechanically controlled robotic arm that is embedded and interlinked to the operating system, can have at least one socket head that can engage and actuate at least one type of a mechanically actuated valve.

[322] **THE OPERATING SYSTEM FLUIDIC CONTROL MECHANISM**

[323] In an embodiment, a fluidic control mechanism allows the usage and manipulation of microliters of fluid to be used in the biochip cell culture, without losing milliliters of fluids in the process of reaching the biochips channels as lost volumes in the tubings as shown in figures 23F, 23G and 23H. The close proximity to the biochip channels, the valving mechanism and the small individual media chamber for every inlet and outlet hole in the system plays an

important role for this system. In an embodiment, the piston pumping mechanism acts to insert the fluid into the individual media chamber and pump it to the biochips.

[324] Figures 25, 26, 27 and 28 show several designs of the fluidic control mechanism of the operating system is based on interfacing the biochip's channels with the channels of the machine. The machine would control the movement of the valves incorporated within the biochip, mainly the inlet/outlet holes valves, in a mechanism that depends on the valve type. In an embodiment, if the valve is mechanical, the valve could be actuated through micro linear actuators in the system. Additionally, if the valves are normally opened/closed valves, they could be actuated mechanically or pneumatically through actuators incorporated inside the system or through an actuator interface in the system that is actuated from outside the system.

[325] Figures 28 show the interface of the operating system with the biochip, the first level of the fluidic control beyond the inlet/outlet holes and the separate compartment interface opening/closing, are three in series valves. The first valve is an open/close valve that opens/closes the biochip channels to the operating system. The second valve is a waste channels direction shift valve, that flips the direction of the flow to the second open close valve or to the waste microchannels that are either incorporated inside the human-on-chip if this valve is on the biochip, or on a layered plate interfaced with the biochip is this valve is rather in that plate. The third valve is the second open/close valve, which separates the waste valve and the first open close valve connecting to the biochip channels, from the media chambers plate utilizing the operating system Three in series valves and waste micro-channeling could be incorporated into the biochip itself in the cases where the valves used are cheap, such as in the case of membrane deformation valves that are actuated pneumatically from outside the system. Or operating system valves could be incorporated on separate plates assembled on top of the biochip, mainly in cases where the valves are either expensive or are actuated on from the same place of the valve, such as the case of solenoid magnet actuators or squiggle motor actuators.

[326] At the interface with the chip holes, we may add a plate that could create different routing for the different ducts and microfluidic channels. This chip plate may be fundamental in cases where the valves are not included in the design of the human-on-chip or in the case where this routing plate is used to connect different individual chips creating a new human-on-chip assembly.

[327] At the interface of the three in-series valve is interfaced to the individual holes media chamber plate. Operating system chambers could also be incorporated within the biochip above its surface in the case where the three in-series valves are also incorporated within the biochip, or could be incorporated within a separate plate, which is the preferred situation, that is

interfaced with the biochip surface or with the in-series valves plate surface. Each individual media chamber in this plate preferably feeds into a single inlet/outlet hole, could fit a few hundreds of microliters of fluid, and is actuated by a piston incorporated within a plate from above.

[328] At the interface with the media chamber plate, is a piston plate that contains individually actuated pistons for every media chamber. Each piston in the piston plate is made of two subcomponents. The lower component of the piston has a bottom surface cross-section with the same size and shape of the media chamber it operates. In addition, the piston contains a channel across its length that goes to the upper component of the piston. The upper component of the piston contains on its lower surface an interface with the lower piston component. This interface is open to a channel that connects the channel of the lower piston component open at the media chamber, to the side of the upper component of the piston where a tubing interface is located that takes the media from the media chamber through the piston towards the top valving plate. The full piston plate is assembled by inserting the lower piston components into the plate containing the holes for the pistons from below, and the upper piston components to their respective pistons from the other side. The top surface of the upper piston components contains a pressure plugging interface feature that is interfaced with a respective piston actuator contained in the piston actuator plate.

[329] At the interface with the piston plate, is a piston actuator plate that contains an actuator for each piston. The actuators could be directly connected to the piston, or through a connector that reaches the actuator located outside the system. The actuators included in the operating system interfaced with the piston plate as shown in figures 27I and 27J, are better to be of small size, dissipates minimal heat, and moves a small and controllable step, a perfect example for this are the squiggle motors. Other motors could be used in the system, such as solenoidal magnetic actuators or small stepper motors. For the actuators that are located outside the operating system, any actuator type could be used, as long as the interface is prone to minimal errors. So the pneumatic or hydraulic actuators could be used to actuate the pistons from outside the system, as long as the tubings connecting operating system external actuators to the system are thermally controlled sealed and the compressibility of the gasses is taken into account.

[330] The tubes connected to the pistons that feed into the small media chambers, pass to the top open/close valving plate that is located at a minimal distance from the piston plate above it or beside it. The open/close valve plate contains valves that connect and separate each tube that goes from the small media chamber through the piston to the media source plate. Each individual open/close valve could be any valve of small volume or size, it could be an on spot

actuated solenoid magnetically actuated valve, or any other valve type electronically actuated from its position. It could also be a membrane or a piston-based valve that is actuated pneumatically or hydraulically through tubings that reach actuators outside the system.

[331] The tube leaving the top valve plate goes to the fluid source plate, which is a plate where the mechanism for the fluid insertion is connected. Individual syringes could fit into the inlets of the fluid insertion plate that could be used for some types of experiments. Also, a media sourcing chambers plate mechanism could also be connected to the media source plate. This media sourcing chamber plate has its own valving and pumping system, which is external to the operating system but could be controlled by the same software. The main external chambers are connected to a valving plate that has a mesh of micro-valves, which could open, mix and flow media from any of the media chambers on the external plate to any inlet of the source plate in the operating system. From the other side of the fluid source plate inside the operating system, three way valves may be incorporated in the system to act as a pressure relief exit, and also to remove any air bubble from the system that may result from the interfacing while connecting the tubings or the syringes to the operating system.

[332] The fluids, cells, and other biological components leaving the system could be sampled and aliquoted at any designed time-lapses, by pumping and opening & closing their respective valves. Also, the tubes coming out of the system are interfaced with various measurement techniques, such as Fourier-Transform Infrared (FTIR) Spectroscopy devices, O₂ & CO₂ sensing devices, and pH sensing devices placed along the tubes coming out of the system.

[333] In an embodiment, the human-on-chip plate includes a manifold that ensures the internal space of the system remains at specific environmental conditions that can be modulated for specific testing protocols. Additionally, heat dissipaters or heat generators may be utilized to mitigate undesirable temperature conditions.

[334] In an embodiment, the human-on-chip plate includes a fluid control system which is able to deliver and/or collect the necessary reagents, at precise times and channels of one or more biochips within the operating system, required to carry out analytical assays as shown in figures 29A and 29B. These assays may be measured with the inbuilt microscope, spectrometer and/or FTIR, or with any other appropriate analytical device either inbuilt or not.

[335] In an embodiment, the human-on-chip plate includes a fluid control system which is able to deliver and/or collect liquid samples from any channel in any biochip within the operating system. The liquid samples collected or delivered may contain any required chemical or biological material, which includes but is not limited to cells, enzymes, drugs, growth

factors, nutrients, etc. The volume of the sample delivered to each channel can be between 0.5 and 25 μl .

[336] In an embodiment, the human-on-chip plate includes a fluid control system which is able to pump liquid samples from any channel in any biochip within the operating system. These may be pumped either from the top inlet holes or from the side. The fluids delivered to the plurality of the channels within each biochip and/or across different biochips within the operating system may be the same or different. Gasses may also be pumped in one or a plurality of the channels instead of a fluid. Liquids or gasses may also be pumped at different flow rates. Liquids being delivered may contain chemicals, drugs, polymers, biological materials, cells, growth factors, nutrients, or any other substance.

[337] In an embodiment, the human-on-chip plate includes a fluid control system which is able to deliver a polymerizing substance which may contain cells or other biological or chemical substances to any or multiple channels (stromal, and/or ductal) of one or more biochips within the operating system. This control system will be coupled with temperature controllers to ensure that the temperature is maintained such that the polymerizing substance is in its liquid state until it is delivered to the desired duct. Once it has been delivered, the temperature will be raised to ensure gelification.

[338] Figures 30A and 30B show the flow diagram in order to pump a sample from the media chamber to a biochip and from a biochip to a measurement device such as ATR FTIR respectively.

[339] **MIXING CHAMBERS**

[340] Figures 32A, 32B, 32C and 32D illustrate several schematic views of the mixing chamber and its parts, according to an example of the present disclosure. In an embodiment, the fluid consisting of cells or biological or chemical components, we call media, is first inserted manually by the user into the mixing chambers, where gasses are bubbled and a heating element heats the fluids. The system then automatically distributes the media to either one or more biochips, the waste chamber or any other component of the operating system. O₂, CO₂ and temperature sensors are used to maintain physical parameters at the levels set by the user.

[341] In an embodiment, the pumping mechanism is pressure driven and is actuated pneumatically through air tubes multiplexed from a pressure control system.

[342] In another embodiment, the pumping system mechanism is driven by a syringe pump where fluid is pushed through the syringe through the system.

[343] In another embodiment, the pumping mechanism is peristaltic pump driven where the fluid in the containers are sucked and pumped into the system.

[344] Figures 33 illustrate several flow diagram cases of the embodiment of the mixing chamber, in an example of the present disclosure. In an embodiment, after being in the mixing chamber, the media is pumped into the fluidic control operating system where the fluid while still in the mixing chamber, passes through a pressure sensor and an air bubble filter and a 1-to-12 way valve that takes fluids from one of the containers of the mixing chamber into the tubings of the fluidic operating system.

[345] **SENSING MECHANISM AND CONTROL FOR THE CO₂, O₂ AND TEMPERATURE**

[346] Figures 34A illustrate a flow diagram of an embodiment of the mixing chamber where all the containers have access to the O₂, CO₂ and N₂ bottles and O₂ and CO₂ sensors, in an example of the present disclosure. While the media is in the containers of the mixing chambers, gasses are bubbled and a heating element heats the fluids. O₂, CO₂ and temperature sensors are used to maintain physical parameters at the levels set by the user.

[347] In an embodiment, the O₂, CO₂ and temperature control system also applies and measures at the level of the cells and media in the chip entities and not just in the mixing chamber as shown in figure 34B. This gives insight on the metabolic activity of the cells and gives a more precise control of those physical parameters.

[348] In an embodiment, the O₂, and CO₂ are pumped directly into the containers of the mixing chamber, in a closed loop control system, where optical or biochemical sensors measure the ratio of the diffused O₂ and CO₂ in the fluid.

[349] In an embodiment, the temperature is induced through a heating element and is measured using a heat sensor, where the temperature is controlled through a feedback control system as shown in figures 35A and 35B.

[350] **SOLUTION INSERTION**

[351] In an embodiment, to fill the media chamber, as illustrated in FIG 28B, a user of the system performs two steps repeatedly until the media chamber is filled. In the first step, close the valve below the media chamber to block the flow to the biochip or to the waste channels, and open the valve above the media chamber before the media source plate, to insert the fluid from the source plate. After opening the valves, pull the piston up, to pump the fluid from the source plate towards the media chamber. Since the volume of the media chamber is small (few 100s microliters), the volume of the tubings reaching the media chambers from the source plate is larger, and thus the piston will reach the top most position before the media reaches the media chambers in the piping. When the piston reaches its top-most position, open the valve below the media chamber and the waste channel valve and close the valve going to the biochip

channels and the top valve before the media source plate. After opening and closing the respective valves, push down the piston to remove the excess air from the media chamber. When the piston reaches its bottom-most position, repeat step one and then step two until the media fills the pipes and reaches and fills the media chamber. In an embodiment, the solutions may be cell culture media.

[352] **SOLUTION REPLACEMENT**

[353] To empty the media chamber, as illustrated in FIG 28C, the user of the system should perform two steps repeatedly until the media chamber is empty. In the first step, close the valve below the media chamber to block the flow to the biochip or to the waste channels, and open the valve above the media chamber before the media source plate, to push the fluid into the source plate. After opening the valves, push the piston down, to pump the fluid from the media chamber towards the source plate. Since the volume of the media chamber is small (few 100s microliters), the volume of the tubing reaching the media chambers from the source plate is larger, and thus the piston will reach the bottom most position before the media reaches the source plate in the piping. When the piston reaches its bottom-most position, open the valve below the media chamber and the waste channel valve and close the valve going to the biochip channels and the top valve before the media source plate. After opening and closing the respective valves, pull up the piston to fill the media chamber with additional air. When the piston reaches its top-most position, repeat step one and then step two until the media empties the pipes and reaches the source plate. In an embodiment, the solutions may be cell culture media.

[354] **CELL INSERTION**

[355] To seed cells into the biochip channels, described in FIG 28D, the user should first insert the media containing cells into the media chamber in the same way described in FIG 28B, and the method to fill the media chambers. Then the user should open the valve below the media chamber on the two sides of the waste valve to give access of the flow to the biochip, and close the valve of the waste channels to block the flow towards the waste channels. Then the user should close the valve above the media chamber before the media source plate, to block the flow from and to the source plate. After opening the valves, the user should open the outlet waste valve to push excess air or media outside the system to replace them with the cell-filled media, and then push the piston down, to pump the fluid from the media chamber towards the biochip channel. From the outlet, the user can open the channel between the different biochips if needed to seed the same cells into the different biochips so that the outlet waste channel opened would be the last biochip one needs the cells to reach. After the cells reach

their intended location, close all valves and stop pumping, and wait until the cells attach. After the cells precipitate on the ducts bottom surface and attach, flip the full operating system, and then open the same valve and do the same steps, and then pump cells into the biochip ducts, and wait for the cells to attach now on the top surface of the ducts. After the cells precipitate on the ducts top surface and attach, flip the full operating system to its initial form, and continue the experiments.

[356] In an embodiment, cells may be inserted into the biochip by any available channel, including but not limited to the side channel. Cells may be inserted suspended in an appropriate liquid or in an appropriate polymer which will solidify.

[357] In an embodiment, skin tissue may be cultured into the plurality of biochips to allow human-on-chip operating system to perform protocols related to, but not limited to, assessment of skin health and physiology.

[358] In an embodiment, cells, media or any other substance may be inserted to a specific location within any channel of a biochip or any biochip within an operating system. Cells may be, also but not exclusively, sampled by delivering an appropriate enzyme or other reagent which will cause cellular detachment at specific locations. Once the cells are detached, the fluid control system will collect the suspended cells.

[359] **MEDIA SAMPLING**

[360] In an embodiment, to collect the media sample from the biochip channels, described in FIG 28E, a user of the system should perform two steps repeatedly until the media chamber is filled with the intended sample quantity. In the first step, for both the inlet and outlet fluid channels, open the valve below the media chamber on the two sides of the waste valve to give access of the flow from and to the biochip, and close the valve of the waste channels to block the flow towards the waste channels. Then one should close the valve above the media chamber before the media source plate, to block the flow from and to the source plate. After opening and closing the intended valves, push the piston controlling the inlet fluid down, and at the same rate and volume, pull up the piston controlling the outlet piston, to collect the intended sample volume in the outlet media chamber. After collecting the intended sample in the media chamber, initiate step two which is transferring the collected sample up the system, by emptying the outlet media chamber. The emptying process is as described before in FIG 28C.

[361] In an embodiment, to collect the media sample from any of the biochip channels within the operating system. In some instances, the media or any other liquid substance will be collected from the side channel.

[362] **CELL SAMPLING**

[363] In one embodiment of the operating system, the same cells or tissue types can be grown across different chips with a common duct(s). Each biochip thus represents sub-samples of the same organ. Each individual biochip can then be removed independently, at different time points, from the circulating system and taken out of the system for downstream analysis. This allows for the sampling at different time points.

[364] In one embodiment of the operating system, the same cells or tissue types can be grown across different biochips with a common duct(s). At time of drug dosing, the biochips can be isolated from each other. Each individual biochip can then be sampled or removed independently for downstream analysis.

[365] To collect the cell sample from the biochip channels, described in FIG 28F, one should perform four steps until the media chamber is filled with the intended sample quantity. In the first step, the biochips were previously connected together along their ducts making them one continuous tissue sample, thus each biochip taken at different time lapses, is a sample of the same tissue. Thus, in the first step this interface is needed. In the second step, one should close the valve between the different biochip entities, to make each biochip duct a sample to be collected at a specific time-point. At this phase, block the intended valves and pump, as described before, to replace the media with trypsin in the media chamber of the inlet of the biochip where the cell sample is aimed to be taken. In the third step, one should pump trypsin into the intended media channels as described before, and collect the sample of cells into the outlet media chamber in the same manner the media sample is collected. In step four, after collecting the intended sample in the media chamber, transfer the collected sample up the system, by emptying the outlet media chamber. The emptying process is as described before in FIG 28C.

[366] In an embodiment, cells, media or any other substance may be sampled from a specific location within any channel of a biochip or any biochip within a operating system. Cells may be, also but not exclusively, sampled by delivering an appropriate enzyme or other reagent which will cause cellular detachment at specific locations. Once the cells are detached the fluid control system will collect the suspended cells. Sampling may occur from the inlet and outlet holes of the biochip. In some instances, the cell sampling may occur from the side channels of the operating system.

[367] INSERTING MULTIPLE REAGENTS SEQUENTIALLY

[368] In one embodiment of the operating system, we can add reagents sequentially. To fill the media chamber is filled with the first reagent as described previously, and this is then inserted into the stromal chamber of the biochip. The media chamber is then emptied, as previously described, and filled with a different reagent which can then be inserted in the stromal chamber. This can be repeated as many times as necessary. The time between the different reagents being added to the stromal chamber is controllable and programmable. They can be added one after the other immediately or after a set interval of time.

[369] In an embodiment, inserting reagents sequentially is essential, not only for dosing with multiple chemicals but also when running assay including but not limited to in chip immunohistochemistry and cell health assays.

[370] TUBULAR ARCHITECTURE AND BARRIER FUNCTIONS OF DUCTAL TISSUES

[371] Fig. 39E shows an embodiment, the system is used as a blood-brain barrier model for drug screening and permeation studies. Here the duct would be populated by brain microvascular endothelial central nervous system cells interfaced by tissue composed of pericytes, astrocytes and neurons. This would then be used to perform a drug permeability test and/or toxicity and efficacy analysis on the neural tissue.

[372] CULTURING OF MULTIPLE TISSUE IN DIFFERENT CHIPS CONNECTED BY A COMMON DUCT/VESSEL

[373] Figure 39B illustrates a perspective view of the embodiment of multiple cultured tissues in our chips connected with a common duct/vessel. In one embodiment of the operating system, it can be used to study the microbiota-gut-brain axis. Here, the stromal chamber of one biochip contains intestinal epithelium cells layered with intestinal mucus and patient derived microbiome. The media in the stromal chamber is hypoxic. The duct, which connects various biochips, is seeded with vascular endothelial cells and the media flowing through it is fully oxygenated. The other biochip contains cultured neurons, astrocytes, and microglia embedded in a hydrogel matrix. Effects of different microbiota on the brain biochip can be assessed by microscopy or sampling of the neural cells and running ex-system protein and gene expression assays.

[374] Fig. 39B is a perspective view of the embodiment of multiple cultured tissues in our chips connected with a common duct/vessel. In an embodiment of the system which may be configured such that each biochip contains a different tissue and organ system but these are connected in parallel and or series by common ducts or ducts which emulate the vascular and/or

lymphatic systems. Some biochips may have multiple ducts which may be interconnected with one or more of the other biochips. Taken together this would be a human-on-a-chip and is valuable to screen for off-target and/or systemic effects of drugs and permeability of a drug through different anatomical barriers. It would also give a more accurate indication of the drug's half-life in-vivo.

[375] In an embodiment, the system may be configured to simultaneously assess hepato- and nephro-toxicity in drug screenings. As both the kidney and the liver are responsible for the clearing and metabolism of xenobiotics, it is essential to assess drug efficacy in the presence of these two tissues. In this embodiment, one or more biochips contain a renal-epithelium and another (or multiple) biochips hepatic cells, as described elsewhere herein. The two tissues are connected by a duct lined with endothelial cells. The drugs are dissolved within the duct and cytotoxicity, proliferation and apoptosis assays can be carried out on the two biochips using spectroscopic assays. The system may also be configured to include one or more tissues in separate biochips, which are the targeted organ(s) of the tested drug.

[376] Fig 39A illustrates a cross-sectional view of the embodiment of the Tubular architecture of a tissue in our 2-duct chips. In an embodiment, the system provides for at least two different tissues to be grown either in direct contact (one within the duct surrounded by a second tissue) or in contact via a duct or ducts (vascular or lymphatic vessel or both). This allows for tissue-tissue interaction studies.

[377] An embodiment of the system whereby a biochip plate can contain different independent biochips each with a specific tissue. The plate can also simultaneously contain another set of biochips which form a human-on-chip which are interconnected by one or more ducts to other chips.

[378] **LIQUID-AIR-INTERFACE LUNG-ON-A-CHIP**

[379] In an embodiment, as shown in figure 39C, the system may be used in a model for testing gas exchange in the lungs to study lung physiology, disease, and the effect of drug candidates. The human epithelial and lung endothelial cells are cultured within the stromal gel in the external compartment containing one or two ducts. One duct has human small airway epithelial cells grown attached to the inner walls of the ductal scaffold. Air is pumped through the lumen of the duct. If a second duct is present, it may contain vascular cells, epithelial cells and pericytes to recapitulate capillaries. This duct has constant flow of red blood cells at 5–0.03 cm/s a constant pressure of 30 mmHg. Equally, in an embodiment, air could be pumped through the stromal channel of the chip and cells can be grown in one or multiple ducts present in the chip.

[380] IMMUNE SYSTEM MODEL

[381] In an embodiment, as shown in figure 39D, the system provides for components of the circulating immune system, such as but not limited to T-cells, natural killer cells and/or B-cells, to be added to individual or multiple chips within the system. This allows for tissue (or tumor)-immune system interaction studies as well as immunotherapy efficacy studies.

[382] In an embodiment, the system provides for components engineered immune components such as, but not limited to, CAR-T cells can be added to individual or multiple chips within the system. This allows for the system to be used in assessing efficacy and toxicity of immunotherapies.

[383] In one embodiment of the operating system, is the modeling of the immune system. This can include one or more of the following organs being mimicked on one or more chips within the system: spleen, bone marrow, and/or lymph-nodes. These can be used either as standalone organs or connected to other organs to study immune-system interactions with other components.

[384] BIOPSY CULTURE FOR PRECISION & PERSONALIZED MEDICINE DRUG PROGNOSIS

[385] In an embodiment, the system may be used for precision medicine, where a biopsy or primary cells from a patient can be grown in 3D space to recapitulate the physiological/disease architecture. Drugs can be then administered to the system, according to the processes and methods disclosed herein, and specific toxicity and efficacy testing can be carried out to quantify the effectiveness of the drug or combination of drugs.

[386] In an embodiment primary cells from different patients can be grown on different biochips independent of each other.

[387] In an embodiment, biochips containing different cells from the same patient can be connected to each other to form a human-on-plate.

[388] MICROSCOPY MEASUREMENT INTERFACE

[389] In another embodiment of the operating system, the system is able to perform real-time live-cell imaging as well as end-point imaging for the visualization of, but not limited to, cells, cell components and or tissue morphology. These can aid in the assessment of, among other things, cell proliferation, migration and/or death. The integrated system software then analyzes the images delivering quantitative data.

[390] In one embodiment of the operating system, it performs tissue culture imaging using fluorescent and bright-field microscopy in time-lapse. In an additional embodiment of the operating system, the system is able to fix and complete all the steps to perform

immunohistochemistry and/or immunofluorescence staining, subsequent automated imaging using fluorescent and/or bright-field microscopy followed by high throughput image analysis.

[391] In another embodiment of the operating system, it can be used for testing and screening of drug and pharmaceutical ingredients.

[392] **SPECTROSCOPY MEASUREMENT INTERFACE**

[393] In one embodiment of the operating system, high throughput absorbance and colorimetric assays could be performed to assess the physiological state of the tissue. Some but not all the specific parameters which could be measured include cell health, viability, enzyme function or cytochrome activity. In another embodiment of the operating system, high throughput fluorometric assays could be used to assess cell metabolism, viability, health, cell death, ADME toxicology or other parameters. In another embodiment of the operating system, high throughput luminescence assays could be performed to assess cell health, metabolism, viability, cell proliferation, ADME toxicology, luciferase activity, inflammation, oxidative stress, or apoptosis and/or any other assay which uses this technology.

[394] In another embodiment of the operating system, a combination of fluorescence, colorimetry, absorbance and luminescence spectroscopy allows for single or multiplex assays which assess cell function and processes such as, but not limited to, basic indicators of cell health (cell proliferation, cell viability and cytotoxicity, specific mechanism of cell death, such as apoptosis or necrosis) and/or cellular processes (e.g., metabolism, inflammation, cytochrome p450 activity, oxidative stress, or autophagy). In brief, in these assays a reagent is added to the biochip which interacts with a cellular component of interest and emits luminescence, fluorescence or alters the turbidity of the media which is recorded by the in-system spectrophotometer.

[395] **FOURIER TRANSFORM INFRARED SPECTROSCOPY MEASUREMENT INTERFACE**

[396] In an embodiment of the operating system, an ATR-FTIR will be used as a measuring technique for cell expression and functionality by delivering a media sample of a minute quantity from the biochip through a set of valves and tubes that operate in a software controlled sequence at multiple time points.

[397] In another embodiment of the operating system, the ATR-FTIR will perform tests investigating the introduced drug efficacy or toxicity, that include but not limited to, element footprint, amide I, amide II, and molecular structure.

[398] In another embodiment of the operating system, ATR-FTIR which can detect at least one sample of at least 2.3 micrometers in thickness, ATR-FTIR system contains at least one

ATR crystal with a certain reflective index. The ATR crystal might have a reflective index that ranges from 1.38 to 4.2. In an additional embodiment, the presently disclosed system may utilize the use of sample aperture signal noise reduction or/and a software base signal noise reduction by implementing mapping mode in reference to the pre-created FTIR database.

[399] In another embodiment of the operating system, Automated ATR cover with sensors to detect if the sample is situated properly in its place. An ATR crystal cleaning system consists of valves, tubes, and buffer solution controlled through software. After the cleaning is done, a test is performed by checking the reflective index and cross-referencing it with the actual index. If the reflective index is equal to the actual reflective index this means that the ATR crystal is clean. If not another cycle of cleaning will be performed by the operating system.

[400] ATR-FTIR measures the cell reaction to the drug by providing quantitative data on drug uptake, metabolism, and cell expression. A set of selective data from the ATR-FTIR will be stored in the operating system and then cross-referenced with the other measuring techniques to further validate the obtained results.

[401] It should be understood that various changes and modifications to the example embodiments described herein will be apparent to operating system skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present subject matter and without diminishing its intended advantages. It is therefore intended that such changes and modifications be covered by the appended claims. Also, it should be appreciated that the features of the dependent claims may be embodied in the systems, methods, and apparatus of each of the independent claims.

[402] Many modifications to and other embodiments of the disclosure set forth herein will come to mind to one skilled in the art to which this disclosure pertains, once having the benefit of the teachings in the foregoing descriptions and associated drawings. Therefore, it is understood that the disclosure is not limited to the specific embodiments disclosed, and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for the purpose of limitation.

CLAIMS

What is claimed:

1. A biochip operating system, the operating system comprising:
at least one biological system on chip plate;
at least one valving means;
at least one actuation means for actuating the valving means;
at least one fluidic handling means;
at least one analytical device; and
a control system for controlling the pumping means, actuation means and valving means,
and the analytical device.
2. The biochip operating system of claim 1, wherein the analytical device may inspect biochips utilizing at least one of microscope images or spectral measurements.
3. The biochip operating system of claim 1, wherein the analytical device may inspect media in the fluidic handling means or fluidic pathways.
4. The biochip operating system of claim 1, wherein the operating system facilitates, semi-automates or automates at least one aspect of experimental set up including at least one of cleaning, sterilizing or preparing the system; priming the system with a biocompatible fluid; populating the chips with the correct cell types in the correct locations as required for the desired experiment; selectably connecting the chips in the correct arrangement for the desired experiment; and/or metering the correct amount of media or other compounds into the system.
5. The biochip operating system of claim 1, wherein the operating system facilitates, semi-automates or automates at least one aspect of experimental execution including at least one of circulating the media between chips as required for the experiment; controlling flow rates of fluidic movements; extracting samples, possibly at specific times or time intervals;

inspecting using analytical means at specific times or time intervals and/or isolating and fixing biochips for later analysis.

6. The biochip operating system of claim 1, wherein the operating system further comprises a motion stage to move the biological system on chip plate relative to the analytical device and/or actuation means.

7. A biochip for growing multiductal tissue, the biochip comprising:

a chassis;

a plurality of internal regions;

wherein the chassis contains the plurality of the internal regions,

wherein at least one of the internal regions is in permeable or semipermeable communication with at least one other internal region; and

a plurality of fluidic media.

8. The biochip of claim 7, wherein a plurality of internal regions are tubular ducts.

9. The biochip of claim 7, wherein at least two tubular ducts are arranged with an inlet and outlet to permit through flow of the first fluidic medium.

10. The biochip of claim 7, wherein at least two tubular ducts are arranged with an inlet and outlet distinct from those of a first duct to allow the through flow of the second fluidic medium that can be distinct from the first fluidic medium.

11. The biochip of claim 7, wherein at least one region is a stromal compartment external to the ducts but inside the biochip chassis.

12. The biochip of claim 7, wherein the plurality of fluidic media are the same media.

13. The biochip of claim 7, wherein the plurality of fluidic media are different media.

14. The biochip of claim 7, wherein the plurality of fluidic media is combination of fluidic media types.

15. A biochip fluidic control system, the fluidic control system comprising:

at least one human-on-chip containing at least one organ-on-chip system;

wherein the at least one organ-on-chip system containing at least one ductal scaffold interfacing at least one surrounding compartment;

at least one valving system controlling the flow to each compartment;

at least one micro-pumping mechanism that can pump and control the flow to at least one compartment;

at least one actuator controlling the valves on the chip and leading to the chip;

at least one actuator controlling the fluid pumping to the chip compartments;

wherein the at least one valving system could open or close and shift the flow pathways to every compartment.

16. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism may be at least one of pneumatically, mechanically, electrically, and fluidically actuated.

17. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects the at least one biochip to the operating system.

18. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects the at least two biochips in human-on-chip connection plate.

19. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism controls the at least two biochips in human-on-chip connection plate.

20. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism controls the at least one inlet and outlet biochip.

21. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects and controls the connection between the at least one duct to another duct.

22. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects the at least one stroma to another stroma.

23. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects and controls the controls the connection between at least one duct to at least one stroma.

24. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism connects the at least one biochip to at least one measuring device.

25. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism contains at least one bistable valve.

26. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism contains at least one open close valve.

27. The biochip fluidic control system of claim 15, wherein the at least one valving mechanism contains at least one bistable and open close valves.

28. The biochip fluidic control system of claim 15, wherein the at least one bistable valve maintains a state position by a beam, magnet, or any drilled geometrical shape.

29. The biochip fluidic control systems of claim 15, wherein the at least one open close valve maintains a state position by a drilled geometrical shape.

30. The biochip fluidic control systems of claim 15, wherein the at least one valving mechanism controls the media insertion in at least one biochip.

31. The biochip fluidic control systems of claim 15, wherein the at least one valving mechanism controls the cell insertion in at least one biochip.

32. The biochip fluidic control systems of claim 15, wherein the at least one valving mechanism maintains a cell culture by media insertion.

33. A human-on-chip plate, the human-on-chip plate comprising:

at least one 3D culture biochip;

at least one micro-valve;

at least one microfluidic channel;

at least one inlet or outlet plate port;

wherein the outlet ports from one chip may be connected to another chip by routing the valves,

wherein multiple chips may be connected in parallel, in series or in combination and one or more chips in the human-on-chip plate may be bypassed,

wherein each fluid connection may be altered independently and at any time,

wherein the at least one valving system connecting or separating different compartments of and between the organ-on-chip system,

wherein at least one inlet and one outlet ports to access and block the microchannels;

wherein the at least one valving system may allow for sampling, changing the model flow map, and/or introducing or reducing at least one fluidic chamber,

wherein the valves, which may be controlled in a bistable position by the actuators and the sensors and positioned at the channel ports of the chips,

wherein the valves are positioned immediately adjacent to the biochips and may be controlled in a bistable position.

34. The human-on-chip plate of claim 33, further comprising at least one organ-on-chip system including at least one ductal scaffold interfacing at least one surrounding compartment;

wherein, at least one organ-on-chip system containing at least one ductal scaffold interfacing at least one surrounding compartment.

35. The human-on-chip of claim 33, wherein the plate may be rapidly connected from the bottom or the side, wherein fluids will be exchanged.

36. The human-on-chip plate of claim 33, wherein the human-on-chip plate is interchangeable, wherein ports on the human-on-chip will align with fitting in the biochip, while the valves are fixed or removable.

37. The human-on-chip of claim 33, wherein the human-on-chip plate includes interconnected built-in chips.

38. The human-on-chip of claim 33, wherein the valves are fixed or removable.

39. The human-on-chip plate of claim 33, wherein the human-on-chip allows for the fluid to be accessed from either the top or the side.

40. A multiplex logic micro-valve system, the micro-valve system comprising:
a plurality of micro-valves;
at least one fluidic pathway including at least one channel, an inlet and outlet port;
at least one bistable mechanism;
at least one actuating mechanism;
at least one structure which encloses the other elements of the micro-valve
wherein the fluid pathway has no dead volumes,
wherein multiplexing between the plurality of valves reduces the amount of actuating inputs,
wherein the valves may control the fluid within a channel so that the flow can be either hydrostatic or in motion.

41. The micro-valve system of claim 40, wherein at least one logic multiplex micro-valve is used to control fluid in the systems.

42. The micro-valve system of claim 40, wherein the valves may direct a sample to at least one sampling port.

43. The micro-valve system of claim 40, wherein the bistable mechanism may be a bistable beam on a flexible tube valve.

44. The micro-valve system of claim 40, wherein the fluidic pathway may connect at least one biochip to a plurality of components within a human-on-chip system.

45. The micro-valve system of claim 40, wherein the bistable valves have two stable positions.

46. The micro-valve system of claim 40, wherein at least one channel contains a balloon that is pneumatically actuated.

47. The micro-valve system of claim 40, wherein the system may only require power when switching between two states, and once actuated, the bistable beam will remain in position.

48. The micro-valve system of claim 40, wherein the micro-valve may be a bistable magnetic drilled-piston valve.

49. The micro-valve of claim 40, wherein the bistable mechanism may contain magnetic channels.

50. The micro-valve system of claim 40, wherein the bistable beam of the micro-valve may be mechanically actuated and when closed, squishes the flexible tube.

51. The micro-valve system of claim 40, wherein the bistable beam of the micro-valve may be either solenoidly or pneumatically actuated.