

ADDITIVE MANUFACTURE OF COMPLEX IMPLANTABLE LIVING DEVICES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to United States Provisional Patent Application No. 62/676,602, filed on May 25, 2018, entitled “Additive Manufacture of Complex Implantable Living Devices,” the entire contents of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present disclosure relates to implantable cell scaffold devices. More particularly, the present disclosure relates to implantable cell scaffold devices formed by additive manufacturing.

Description of Related Art

[0003] Generally, an implant is a device configured to restore, maintain, improve, or a combination thereof, a biological structure or mechanism. Typical implant devices are disposed *in vivo*. However, such devices can also be disposed *in vitro* for various uses including pharmaceutical testing and biological system analysis.

[0004] According to the Organ Procurement and Transportation Network (accessed December 2017), from 2005 to 2015 a number of patients on an organ donation waiting list increased from 90,500 to 122,000 total patients. Simultaneously, a total number of received organ donors each year held constant at 15,000 donors. Of these 15,000 received donors, approximately 5,600 donors were living persons while the remaining 9,400 donors were deceased. Since 1998, the total number of deceased donors has increased due to improved organ harvest and transportation protocols for the deceased donor. However, the number of patients on the organ donation

waiting list remains greater than the number of organ donors in the United States and throughout the world. Thus, to satisfy the needs of all current and future patients requiring a transplant, whole organ fabrication is required.

[0005] Such approaches to whole organ fabrication include engineered tissue(s). Engineered tissues have been designed in labs, and include engineered skin, cartilage, and vascular grafts. Despite these advances in the field of engineered tissues, whole organ fabrication has yet to be realized as there are fundamental limitations to overcome when scaling from engineered tissues to whole organ fabrication.

[0006] Implant and tissue manufacturing methods fail to recapitulate the geometry, complexity, and life span of human tissues. A major limitation in engineering whole organs arises from various mass transport mechanisms and biological properties within an organ. In organ systems, such as aerobic respiration and waste disposal systems, diffusion is a leading mass transport mechanism. For instance, regarding aerobic respiration, most cells are within 100 microns (μm) to 200 μm from a capillary lumen. An oxygen diffusion distance limit, which is a minimum distance between a cell and an oxygen source, is dependent upon a rate of cellular oxygen consumption and a diffusion rate of oxygen through a given tissue. Additionally, a given cellular concentration in a tissue ($[Cell]$) and a diffusion distance to a center of a tissue (d) hold an inverse square relation ($[Cell] \propto 1/d^2$). When a characteristic dimension of an engineered tissue construct, such as a characteristic length of a blood vessel, is scaled by a factor of n , the theoretical limit of cellular density is decreased by a factor of n^2 . Thus, an intrinsic vascular network is required to increase sizes of engineered tissues while maintaining required minimum oxygen diffusion distances.

[0007] Conventional engineered tissue designs contain two-dimensional microchannel arrays. The two-dimensional arrays can theoretically be applied to whole organs, however, these organs are inherently three-dimensional and their respective vascular organization must reflect this. When cells, including hepatocytes, are developed in a three-dimensional scaffold and disposed in a body near a capillary bed, only cells in close proximity to a blood vessel will thrive. As time elapses, new blood vessels may grow into the implanted cells, however, cells that are far from existing blood vessels will not flourish without immediate blood supply.

[0008] Thus, prior to the present disclosure there existed a need for a diverse cell scaffolding

device capable of growing complex cells in three dimensions.

[0009] The information disclosed in this Background of the Invention is only for enhancement of understanding of the general background of the invention and should not be taken as an acknowledgement or any form of suggestion that this information forms the prior art already known to a person skilled in the art.

BRIEF SUMMARY

[0010] Advantageously, the cell scaffold devices detailed in the present disclosure address the shortcomings in the prior art detailed above.

[0010A] According to an aspect of the present disclosure, there is provided a cell-scaffold device comprising:

- a plurality of branching channel networks comprising

- a first branching channel network,

- a second branching channel network, and

- a third branching channel network,

- wherein the first branching channel network comprises a plurality of first channels formed by at least three generations of branching, the plurality of first channels comprising a first-generation first channel and a plurality of final-generation first channels;

- the second branching channel network comprises a plurality of second channels formed by branching and recursive recombination, the plurality of second channels comprising an inlet second channel, an outlet second channel and a plurality of intermediate second channels branched from the inlet second channel and recursively recombined to the outlet second channel, wherein one or more second channels in the plurality of second channels are located in a first layer and one or more other second channels in the plurality of second channels are located in a second layer that is different than the first layer;

- the third branching channel network comprises a plurality of third channels formed by at least three generations of branching, the plurality of third channels comprising a first-generation third channel and a plurality of final-generation third channels; and

the plurality of final-generation first channels of the first branching channel network is disposed adjacent to the first layer of the second branching channel network, the plurality of final-generation third channels of the third branching channel network is disposed adjacent to the second layer of the second branching channel network, the plurality of final-generation first channels of the first branching channel network and the plurality of final-generation third channels of the third branching channel network are connected with each other by a plurality of bypass channels, and each of the plurality of bypass channels bypasses the second branching channel network, thereby producing a compact three-dimensional structure with interwoven channels.

[0011] Various aspects of the present disclosure are directed to providing a cell-scaffold device having a fractal structure utilized for growing tissue. The device includes at least one channel network. The channel network includes an inlet and a plurality of channels. The plurality of channels includes a parent channel having a first end portion in communication with the inlet and a second end portion in communication with a first bifurcation. The first bifurcation forming two child channels. Each child channel has a first end portion in communication with an end portion of the first bifurcation and a second end portion in communication with a second bifurcation. The second bifurcation forming two grand-child channels from each child channel. Each grand-child channel has a first end portion in communication with an end portion of the second bifurcation and a second end portion. The second end portion of the grand-child channel forms either an outlet or a third child channel in communication with the grand-child channel. Each forming of grand-child channels defines a generation of the fractal structure.

[0012] In some embodiments, the device is formed by an additive manufacturing method. The manufacturing method is selected from the group consisting of binder jetting, material extrusion, material jetting, polyjet, powder bed, sheet lamination, VAT photopolymerization, or a combination thereof. In some embodiments, the device is formed by injection molding. Further, in some embodiments the device is formed by layered fabrication. In specific embodiments, the layered fabrication method is selective laser sintering or stereolithography.

[0013] In some embodiments, the device is formed as a positive mold such that the channels of the device are formed by a printing material of a particular manufacturing device. In another

embodiment, the device is formed as a negative mold such that gaps in-between the channels of the device are formed by a printing material of a particular manufacturing device.

[0014] In some embodiments, the channel network is formed from a resorbable or biodegradable material. In some embodiments, the channel network is formed from a non-resorbable material. Furthermore, in some embodiments, the channel network is formed from a non-resorbable material, a biodegradable material, a resorbable material, or a combination thereof.

[0015] In some embodiments, the channel network is formed from an optically transparent or translucent material. In some embodiments, the channel network is formed from a synthetic polymer, a natural polymer, or a combination thereof. In some embodiments, the channel network is formed from a material selected from the group consisting of poly-dimethyl-siloxane (PDMS), poly-glycerol-sebacate (PGS), polylactic acid (PLA), poly-L-lactic acid (PLLA), poly-D-lactic acid (PDLA), polyglycolide, polyglycolic acid (PGA), polylactide-co-glycolide (PLGA), polydioxanone, polygluconate, polylactic acid-polyethylene oxide copolymers, modified cellulose, collagen, polyhydroxybutyrate, polyhydroxypriopionic acid, polyphosphoester, poly(alpha-hydroxy acid), polycaprolactone, polycarbonates, polyamides, polyanhydrides, polyamino acids, polyorthoesters, polyacetals, polycyanoacrylates, degradable urethanes, aliphatic polyesters, polyacrylates, polymethacrylate, acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, polyvinyl imidazole, chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, Teflon®, nylon silicon, and shape memory materials, such as poly(styrene-block-butadiene), polynorbornene, hydrogels, metallic alloys, and oligo(ε-caprolactone)diol as switching segment/oligo(p-dioxanone)diol as physical crosslink.

[0016] In some embodiments, a diameter of a smallest channel of the channel network is predetermined. Each preceding channel after the smallest channel has a diameter increased by a first predetermined factor relative to a diameter of an immediately subsequent channel.

[0017] In some embodiments, a diameter of the inlet of the channel network is predetermined. Each subsequent channel after the inlet has a diameter reduced by a first predetermined factor relative to a diameter of an immediately preceding channel.

[0018] In some embodiments, the first predetermined factor is defined by Murray's Law, $D_o^3 =$

$\sum_{i=1}^n D_i^3$, where D_o is a diameter of the parent channel, n is a number of child channels, and D_i is a diameter of an i^{th} child channel. In some embodiments, the first predetermined factor is a constant less than or equal to 1 including but not limited to $2^{-1/3}$ or 0.5.

[0019] In some embodiments, a length of the parent channel of the channel network is predetermined. A length of each subsequent channel is reduced by a second predetermined factor relative to a length of an immediately preceding channel. In some embodiments, the second predetermined factor is a constant less than or equal to 1 including but not limited to 0.5.

[0020] In some embodiments, the diameter of the smallest channel is in a range of from 5 microns (μm) to 650 μm .

[0021] In some embodiments, a ratio of a diameter to a length of a channel is fixed along the length of the channel. In some embodiments, the ratio of the diameter to the length is in a range of from 1:4 to 1:25.

[0022] In some embodiments, the device has an overall length in a range of from 0.1 centimeters (cm) to 30 cm. In some embodiments, a cross-section of the device is square or rectangular. In some embodiments, each channel has a uniform aspect ratio of 1:1 including, but not limited, to a circular cross-section or a square cross-section. Furthermore, in some embodiments a channel network includes at least a first cross-section of a first aspect ratio and a second cross-section of a second aspect ratio. In some embodiments, the cross-section of the device is a cross-section of a platonic solid.

[0023] In some embodiments, a gross morphology of the device has an implantable shape, in which the implantable shape externally resembles a member to be replaced by the device.

[0024] In some embodiments, a wall thickness of each surface of a channel is the same thickness. In some embodiments, the wall thickness of a channel is in a range of from 5 μm to 10,000 μm . Additionally, in some embodiments, a distance between a surface of a channel and a nearest adjacent channel surface is in a range of from 10 μm to 4 cm.

[0025] In some embodiments, each channel and each bifurcation exhibits laminar flow therein.

[0026] In some embodiments, a connection between each channel and its corresponding bifurcation includes a linear ramp, a smooth concave ramp, a smooth convex ramp, a step, a plurality of steps, a reducer, or a combination thereof. In some embodiments, the step intersects

a diameter of an immediately subsequent channel at a front end portion of the connection, a rear end portion of the connection, or an intermediate portion of the connection. In some embodiments, the connection is configured to include a surface of a channel in a channel network that is flush with a coplanar surface of each channel of the channel network.

[0027] In some embodiments, each bifurcation includes a circular jog, a semi-circle, a C-shape, a T-shape, a U-shape, or a V-shape. In some embodiments, each bifurcation includes at least one chamfered or filleted edge portion. In some embodiments, each bifurcation includes a protrusion protruding towards an immediately preceding channel. In some embodiments, the protrusion includes a semi-circle, a V-shape, a wedge, a concave wedge, or a convex wedge. In some embodiments, the protrusion is an indentation. In some embodiments, each bifurcation branches orthogonal to an immediately preceding channel. In other embodiments, each bifurcation branches at an angle in a range of from 75 degrees (°) to 105° relative to an immediately preceding channel.

[0028] In some embodiments, the channel network is recursively formed such that outlets of a first layer and outlets of a second layer combine to form a paired layer.

[0029] In some embodiments, the at least one channel network includes a first channel network and a second channel network. The first and second channel networks are linked through communicating outlets, which forms a first paired layer.

[0030] In some embodiments, the first channel network is formed of a material different than a material of the second channel network. In another embodiment, the first channel network is formed of the same material as the second channel network.

[0031] In some embodiments, the first channel network is stacked onto the second channel network, such that the outlets of the first channel network and the outlets of the second channel network are in communication. This communication allows material to flow between the channel networks. In another embodiment, the first channel network is stacked onto the second channel network such that the outlets of the first channel network and the outlets of the second channel network are not in communication, preventing material from flowing between the channel networks.

[0032] In some embodiments, the first channel network is embedded internally in the second

channel network. In such embodiments, the first channel network is either centered or offset inside the second channel network. In another embodiment, the second channel network is embedded internally in the first channel network. In such embodiments, the second channel network is either centered or offset inside the first channel network.

[0033] In some embodiments, the first channel is oriented 0° , 90° , or 180° to the second channel. The 0° orientation is defined as an inlet of the first channel network and an inlet of the second channel network existing on a same side of the device. The 90° orientation is defined as the inlet of the first channel network existing on an adjacent side of the device to the inlet of the second channel network. Furthermore, the 180° orientation is defined as the inlet of the first channel network and the inlet of the second channel network existing on opposing sides of the device.

[0034] In some embodiments, the communication is defined as either a direct flow of material from the first channel network to the second channel network or an indirect flow of material from the first channel network to the second channel network by an exchange mechanism. In other embodiments, the communication is defined as either a direct flow of material from the second channel network to the first channel network or an indirect flow of material from the second channel network to the first channel network by an exchange mechanism.

[0035] In some embodiments, the exchange mechanism is mediated by a membrane or a plurality of pores. In some embodiments, the exchange mechanism has a thickness in a range of from $10\ \mu\text{m}$ to $5,000\ \mu\text{m}$. In some embodiments, the exchange mechanism is formed on at least a portion of at least a first surface of at least one channel of a member selected from the first channel network, the second channel network, and a combination thereof. In some embodiments, the exchange mechanism is on each side of either the first channel network or the second channel network, but not both channel networks.

[0036] In some embodiments, an exchange mechanism includes a material having a non-zero solubility to a solution. In some embodiments, the exchange mechanism includes a polymer having a high permeability for a solution. In some embodiments, the exchange mechanism includes polydimethylsiloxane.

[0037] In some embodiments, a side channel extends from at least a first surface of the first channel network to at least a first surface of the second channel network. In some embodiments,

the side channel is formed and the exchange mechanism is disposed interposing between the side channel and the channel networks.

[0038] In some embodiments, the membrane is selected from the group consisting of a Track-etch membrane and a fibrous membrane.

[0039] In some embodiments, a parameter of the exchange mechanism is configured to selectively allow predetermined compounds, particles, cells, or a combination thereof to pass through the exchange membrane while retaining other material. In some embodiments, the parameter includes a pore diameter, a pores per unit area of the exchange mechanism, a pore depth, a porosity, a pore proximity, a membrane thickness, or a combination thereof.

[0040] In some embodiments, the pores per unit area of the exchange mechanism is in a range of from 1 pore per square micron (μm^2) to 10 pores per μm^2 .

[0041] In some embodiments, the porosity of the exchange mechanism is in a range of from 5 % to 95 %.

[0042] In some embodiments, a distance from a center of a first pore to a center of an adjacent pore is in a range of from 5 μm to 100 μm .

[0043] In some embodiments, a diameter of each pore is in a range of from 5 μm to 100 μm .

[0044] In some embodiments, the depth of each pore is in a range of from 10 μm to 5,000 μm .

[0045] In some embodiments, a wall thickness of a channel and a thickness of the exchange mechanism are the same value.

[0046] In some embodiments, the device includes a third channel network and a fourth channel network. The third channel network and the fourth channel network are linked through communicating outlets, which forms a second paired layer.

[0047] In some embodiments, the first paired layer and the second paired layer communicate with each other through an exchange mechanism disposed interposing between the paired layers. According, the paired layers combine to form a stackable unit, or a stack.

[0048] In some embodiments, the first paired layers of each stack are in communication with each other by an exchange mechanism and the second paired layers of each stack are in communication with each other by the exchange mechanism, forming a stacked first layer and a

stacked second layer. In some embodiments, the stack is a member of a plurality of stacks.

[0049] In some embodiments, the first paired layer is a distribution layer and the second paired layer is a collection layer.

[0050] In some embodiments, the inlets and the outlets of the first paired layers combine to form a first master inlet and a first master outlet respectively. Further, the inlets and the outlets of the second paired layers combine to form a second master inlet and a second master outlet respectively.

[0051] In some embodiments, the device is disposed in a closed compartment. The closed compartment permits only the master inlets and the master outlets to be externally accessible.

[0052] In some embodiments, the first channel network is configured as a hepatobiliary network and the second channel network is configured as a portal venous network. Furthermore, in some embodiments, the first paired layer is a hepatobiliary layer and the second paired layer is a portal venous layer.

[0053] In some embodiments, a plurality of cells populate an exterior of the device in a naturally occurring manner. In another embodiment, the cells populate an exterior of the device in a preassembled manner. In yet another embodiment, the cells are bio-printed concurrently, sequentially, or a combination thereof during manufacturing of the device. In some embodiments, the cells are disposed on the device simultaneous to manufacture of the device. In another embodiment, the cells are disposed surrounding the device after manufacture of the device. In yet another embodiment, the cells develop around the device after manufacture of the device. In various embodiments, the cells are nourished by an exchange of medium through an exchange mechanism of the device. In some embodiments, the cells are derived from, or develop into, a complex tissue. In some embodiments, the cells encapsulate the device in three dimensions.

[0054] In some embodiments, the device is encapsulated in a hydrogel. This encapsulation in hydrogel promotes development of the cells within the device. In some embodiments, the hydrogel includes induced stem cells including totipotent cells (iTC), pluripotent cells (iPSC), progenitor cells (iMSC), or combinations thereof. In some embodiments, a first type of induced stem cell is initially included in the hydrogel and at least one other induced stem cell type is post-

populated in the hydrogel.

[0055] In some embodiments, the device is consumed, deteriorates, dissolves, resorbs, or a combination thereof after a predetermined time period. In another embodiment, the device is consumed, deteriorates, dissolves, resorbs, or a combination thereof after the cells have reached a predetermined maturity or cell density. In yet another embodiment, the device is consumed, deteriorates, dissolves, resorbs, or a combination thereof due to a photochemical reaction. In some embodiments, the predetermined time period is related to or describable by a chemical equation or equilibrium. In some embodiments, after the device is consumed, deteriorates, dissolves, or resorbed the cells are seeded into a resulting structure.

[0056] The cell scaffold device of the present invention has other features and advantages that will be apparent from, or are set forth in more detail in, the accompanying drawings, which are incorporated herein, and the following Detailed Description, which together serve to explain certain principles of exemplary embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] **FIG. 1** illustrates a schematic view of an exemplary single layer of a scaffold device in accordance with embodiments of the present disclosure;

[0058] **FIG. 2** illustrates an isometric schematic view of the scaffold device of **FIG. 1**;

[0059] **FIG. 3** illustrates an isometric schematic view of an exemplary scaffold device in accordance with embodiments of the present disclosure;;

[0060] **FIG. 4A** and **FIG. 4B** are illustrations of exemplary connection types between channels in accordance with embodiments of the present disclosure;

[0061] **FIG. 4C**, **FIG. 4D**, and **FIG. 4E** are illustrations of exemplary channel cross-sections in accordance with embodiments of the present disclosure;

[0062] **FIG. 5A**, **FIG. 5B**, **FIG. 5C**, **FIG. 5D**, **FIG. 5E**, **FIG. 5F**, **FIG. 5G**, **FIG. 5H**, **FIG. 5I**, **FIG. 5J**, and **FIG. 5K** are illustrations of exemplary bifurcation types in accordance with embodiments of the present disclosure;

[0063] **FIG. 5L** and **FIG. 5M** illustrate exemplary plots for determining a maximum number

of bifurcations before a channel intersection occurs in accordance with embodiments of the present disclosure;

[0064] **FIG. 5N** illustrates an exemplary plot for determining an area coverage efficiency by branching angle and length reduction in accordance with embodiments of the present disclosure;

[0065] **FIG. 6A, FIG. 6B, FIG. 6C, and FIG. 6D** are illustrations of an exemplary dimensionless shear rate analysis in accordance with embodiments of the present disclosure;

[0066] **FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E, FIG. 7F, and FIG. 7G** are illustrations of an exemplary dimensionless velocity, streamline, pressure, and shear rate analysis in accordance with embodiments of the present disclosure;

[0067] **FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D, FIG. 8E, and FIG. 8F** are illustrations of an exemplary dimensionless velocity, streamline, pressure, and shear rate analysis at a Reynolds number equal to 100 in accordance with embodiments of the present disclosure;

[0068] **FIG. 9A, FIG. 9B, and FIG. 9C** are illustrations of exemplary parametric iso-surfaces of dimensionless shear rate in terms of two inner curve weights and a dimensionless length of a smooth connector in accordance with embodiments of the present disclosure;

[0069] **FIG. 10** illustrates an exemplary layout of a portion of a first layer and a portion of a second layer of a scaffold device utilizing the bifurcations of **FIG. 5A** in accordance with embodiments of the present disclosure;

[0070] **FIG. 11** illustrates a flow chart of an exemplary design process for generating a scaffold device in accordance with embodiments of the present disclosure, in which optional steps or embodiments are indicated by dashed boxes;

[0071] **FIG. 12, FIG. 13, and FIG. 14**, collectively illustrate an exemplary process for generating a single channel network layer scaffold device in accordance with embodiments of the present disclosure;

[0072] **FIG. 15** illustrates an exemplary recursion of the process of **FIG. 12, FIG. 13, and FIG. 14** to form a paired channel network layer in accordance with embodiments of the present disclosure;

[0073] **FIG. 16** illustrates a side schematic view of the paired channel network layer of **FIG.**

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[0074] **FIG. 17** illustrates an isometric schematic view of an exemplary dual paired channel network layer in accordance with embodiments of the present disclosure;

[0075] **FIG. 18** illustrates a side schematic view of the dual paired channel network layer of **FIG. 17**;

[0076] **FIG. 19** illustrates an isometric schematic view of an exemplary stacked dual paired channel network layers in accordance with embodiments of the present disclosure;

[0077] **FIG. 20** illustrates an isometric schematic view of an exemplary stacked dual paired channel network layers in accordance with embodiments of the present disclosure;

[0078] **FIG. 21** illustrates a side schematic view the stacked dual paired channel network layers of **FIG. 20**;

[0079] **FIG. 22, FIG. 23, FIG. 24, FIG. 25, FIG. 26, FIG. 27, and FIG. 28** collectively illustrate an exemplary process for generating a stacked dual paired channel network layers scaffold device including master inlets and master outlets in accordance with embodiments of the present disclosure;

[0080] **FIG. 29, FIG. 30, FIG. 31, FIG. 32, and FIG. 33** collectively illustrate an exemplary process for generating a stacked bypass channel network layer scaffold device in accordance with embodiments of the present disclosure;

[0081] **FIG. 34** illustrates an isometric schematic view of the stacked bypass channel network layer scaffold device of **FIG. 33**;

[0082] **FIG. 35** illustrates an isometric schematic view of an exemplary stacked bypass channel network layers scaffold device including master inlets and master outlets in accordance with embodiments of the present disclosure;

[0083] **FIG. 36** illustrates an isometric schematic view of an exemplary stacked bypass channel network layer scaffold device including an exchange mechanism in accordance with embodiments of the present disclosure;

[0084] **FIG. 37** illustrates an exemplary exchange mechanism in accordance with embodiments of the present disclosure;

[0085] **FIG. 38** illustrates an exemplary exchange mechanism in accordance with embodiments of the present disclosure;

[0086] **FIG. 39** illustrates an exemplary exchange mechanism and side channels in accordance with embodiments of the present disclosure;

[0087] **FIG. 40A** and **FIG. 40B** illustrate exemplary layouts of a first channel network, a second channel network, and an exchange mechanism in accordance with embodiments of the present disclosure;

[0088] **FIG. 41** illustrates an exemplary scaffold device in accordance with embodiments of the present disclosure;

[0089] **FIG. 42** illustrates another view of the scaffold device of **FIG. 41**;

[0090] **FIG. 43** illustrates an exemplary vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[0091] **FIG. 44** illustrates an exemplary vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[0092] **FIG. 45** illustrates an exemplary vascularized heart muscle patch in accordance with embodiments of the present disclosure;

[0093] **FIG. 46** illustrates an exemplary cross-section of multiple channels including a vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[0094] **FIG. 47** illustrates an exemplary complex tissue construct including multiple units for implantation in an arm in accordance with embodiments of the present disclosure;

[0095] **FIG. 48A** and **FIG. 48B** illustrate an exemplary two generation vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[0096] **FIG. 49A**, **FIG. 49B**, and **FIG. 49C** collectively illustrate an exemplary process for generating a four tissue hierarchical design in accordance with embodiments of the present disclosure;

[0097] **FIG. 50A**, **FIG. 50B**, **FIG. 50C**, and **FIG. 50D** illustrate exemplary arrays and stacking configurations of a scaffold device in accordance with embodiments of the present

disclosure;

[0098] **FIG. 51** illustrates an exemplary four by four scaffold device in accordance with embodiments of the present disclosure;

[0099] **FIG. 52** illustrates an exemplary two generation vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[00100] **FIG. 53** illustrates an exemplary four generation vascularized tissue layer with drainage in accordance with embodiments of the present disclosure;

[00101] **FIG. 54** illustrates an exemplary scaffold device including a first scaffold device and a second scaffold device in accordance with embodiments of the present disclosure;;

[00102] **FIG. 55A, FIG. 55B, FIG. 55C, and FIG. 55D** illustrate an exemplary scaffold device in accordance with embodiments of the present disclosure;

[00103] **FIG. 56A, FIG. 56B, and FIG. 56C** illustrate an exemplary scaffold device in accordance with embodiments of the present disclosure;

[00104] **FIG. 57A, FIG. 57B, FIG. 58A, FIG. 58B, FIG. 59A, FIG. 59B, FIG. 60A, FIG. 60B, FIG. 61A, FIG. 61B, FIG. 62A, FIG. 62B, FIG. 63A, FIG. 63B, FIG. 64A, FIG. 64B, FIG. 65A, and FIG. 65B** collectively illustrate a progressive cross-section splicing of the scaffold device of **FIG. 56A, FIG. 56B, and FIG. 56C**;

[00105] **FIG. 66, FIG. 67, FIG. 68, FIG. 69, FIG. 70, and FIG. 71** illustrate an exemplary scaffold device including a lattice structure in accordance with embodiments of the present disclosure; and

[00106] **FIG. 72** illustrates the scaffold device of **FIG. 66** without the lattice structure.

[00107] It should be understood that the appended drawings are not necessarily to scale, presenting a somewhat simplified representation of various features illustrative of the basic principles of the invention. The specific design features of the present invention as disclosed herein, including, for example, specific dimensions, orientations, locations, and shapes will be determined in part by the particular intended application and use environment.

[00108] In the figures, reference numbers refer to the same or equivalent parts of the present invention throughout the several figures of the drawing. Furthermore, in the figures, arrows

depict a flow of material unless otherwise stated.

DETAILED DESCRIPTION

[00109] Reference will now be made in detail to various embodiments of the present invention(s), examples of which are illustrated in the accompanying drawings and described below. While the invention(s) will be described in conjunction with exemplary embodiments, it will be understood that the present description is not intended to limit the invention(s) to those exemplary embodiments. On the contrary, the invention(s) is/are intended to cover not only the exemplary embodiments, but also various alternatives, modifications, equivalents and other embodiments, which may be included within the spirit and scope of the invention as defined by the appended claims.

[00110] In the interest of clarity, not all of the routine features of the implementations described herein are shown and described. It will be appreciated that, in the development of any such actual implementation, numerous implementation-specific decisions are made in order to achieve the specific goals of a design, such as compliance with use case- and business-related constraints, and that these specific goals will vary from one implementation to another and from one design to another. Moreover, it will be appreciated that such a design effort might be complex and time-consuming, but nevertheless be a routine undertaking of engineering for those of ordinary skill in the art having the benefit of the present disclosure.

[00111] It will also be understood that, although the terms first, second, *etc.* may be used herein to describe various elements, these elements should not be limited by these terms. These terms are only used to distinguish one element from another. For example, a first channel network could be termed a second channel network, and, similarly, a second channel network could be termed a first channel network, without departing from the scope of the present disclosure. The first channel network and the second channel network are both channel networks, but they are not the same channel network.

[00112] Additionally, it will be understood that, though the terms inlet and outlet may be used herein to describe various elements, these elements should not be limited by these terms. For example, an inlet could be termed an outlet, and similarly an outlet could be termed an inlet,

without departing from the scope of the present disclosure. The inlet and the outlet are both points of drawing flow, but they are not the same points of drawing flow.

[00113] By “biodegradeable,” as used herein, is meant materials that are bioresorbable and/or degrade and/or break down by mechanical degradation (*e.g.*, dissolve, resorb, *etc.*) upon interaction with a physiological environment into components that are metabolizable or excretable, over a period of time from minutes to three years, preferably less than one year, while maintaining the requisite structural integrity.

[00114] By “exchange mechanism,” as used herein, is meant a material or structure configured to substantially allow or inhibit a flow of material from a first element to a second element including fenestrated walls, permeable membranes, permeable walls, porous walls, porous membranes, perforations, and the like.

[00115] By “diameter,” as used herein, is meant to be inclusive of equivalent characteristic lengths including hydraulic diameters of non-circular structures.

[00116] By “flush,” as used herein, is meant as a surface of a first element and a coplanar surface of a second element to have a distance, or level, separating the first element and the second element to be within a tolerance of 0 μm , within a tolerance of 5 μm , within a tolerance of 10 μm , within a tolerance of 20 μm , or within a tolerance of 100 μm .

[00117] By “direct flow,” as used herein, is meant as a transfer or a flow of at least one substance or material from a first element to at least a second element.

[00118] By “indirect flow,” as used herein, is meant as an exchange or flow of at least one substance or material from a first element to at least a second element which is mediated by an exchange mechanism.

[00119] By “generation,” as used herein, is meant a complete series of a child channel and grand-child channel generation. A “generation” thus means a T-shaped branch.

[00120] By “natural manner,” as used herein, is meant a process or development as found in a nature.

[00121] By “polymer,” as used herein, is meant to include polymers and monomers that can be polymerized or adhered to form an integral unit. The polymer can be non-biodegradable or biodegradable, typically via hydrolysis or enzymatic cleavage.

[00122] By “subsequent channel,” as used herein, is meant, for a given channel, a channel which material flows therefrom. Accordingly, by “preceding channel,” as used herein, is meant, for the given channel, a channel which material flows thereto.

[00123] By “rigid,” as used herein, is meant a material that is stiff and does not deform easily. By “elastomeric,” as used herein, is meant a material or a composite material that is not rigid as defined herein.

[00124] By “ i^{th} ” or “ i ,” as used herein, is meant a generic element in a series of elements for each integer i in the set $\{1, \dots, i, \dots, k\}$, where k is a positive integer greater than or equal to two.

[00125] Furthermore, the term “channel” or “vessel” or other similar terms, as used herein, is inclusive of equivalent structures in general including a capillary, a conduit, a cylinder, a duct, a hose, a passage, a pipe, a pipeline, a siphon, a tube, and the like.

[00126] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, useful methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[00127] Throughout the specification, channels of the present disclosure will be reference X00-Y-Z {C,G,I,O, or P}. Hereinafter, the X00 series (100, 200, ..., $i00$, ..., $k00$) refers to a specific channel network of the device. Hereinafter, the Y series (1, 2, ..., i , ..., k) refers to a level or layer of a channel network of the device. Hereinafter, the Z {C, G, I, O, or P} series {I, 1P, 1C, 1G, 2C, 2G, 3C, ..., iC , iC , ..., kC , kG , kO , O} refers to a generation and channel type of a level or layer. “Z” refers to the generation of the channel. “C” refers to a child channel. “G” refers to a grand-child channel. “I” refers to an inlet. “O” refers to an outlet. Furthermore, “P” refers to a parent channel.

[00128] Embodiments of the present disclosure are described in the context of cell scaffold devices. In some embodiments, the device has a volume of at least 1 cubic centimeter (cm^3), 10 cm^3 , 100 cm^3 , 1,000 cm^3 , 5,000 cm^3 , 27,000 cm^3 , or 125,000 cm^3 , and of various shapes including but not limited to squares and rectangles. In some embodiments, a gross morphology

of the device is an implantable shape. Implantable shapes externally resemble organ and/or members to be replicated. Furthermore, in some embodiments the device has an overall length (*e.g.*, from a first side surface of the device to an opposing side surface of the device) in a range of from 0.1 cm to 1 meter (m), from 0.1 cm to 75 cm, from 0.1 cm to 50 cm, from 0.1 cm to 40 cm, or from 0.1 cm to 30 cm.

[00129] Devices of the present disclosure can be utilized for a variety of applications including *in vivo* implants such as a liver implant, a lung implant, a heart implant, and the like, or used *in vitro* to test various pharmaceuticals or biological system functions. For instance, in a liver implant embodiment, a first channel network can be a portal venous network that distributes blood flow across the device to enable oxygen transport and cell nourishment in various networks, while a second channel network can be a hepatobiliary network that provides an outflow of bile. In general, a second channel network can be configured for system specific outflows such as an outflow of air in a lung implant, an outflow of blood in a heart implant, or the like. Additionally, in some embodiments a first channel network can be configured to distribute fluid or material and a second channel network can be configured to collect fluid or material distributed by the first channel network. Accordingly, in some embodiments the devices of the present disclosure mimic physiological systems *in vivo* (*e.g.*, a liver, a heart, a bone, a kidney, a lung, *etc.*) to provide a controlled and physiological relevant cell culture within the device. Furthermore, in some embodiments the devices of the present disclosure mimic physiological systems *in vitro*, such as an “organ-on-a-chip,” to provide a mechanism for testing pharmacokinetics of pharmaceutical compositions, pharmaceutical composition clearance, pharmaceutical composition sensitivity, pharmaceutical composition toxicity, or a combination thereof.

[00130] The devices of the present disclosure can include various materials. In many cases, the device is formed from a resorbable or biodegradable material. In some embodiments, the resorbable or biodegradable material is consumed, deteriorates, dissolves, erodes, resorbs, or a combination thereof after a predetermined time period. In such embodiments, the predetermined time is typically a function of cell population maturity, cell density, a photocatalyst reaction, a chemical reaction or equilibrium, or a combination thereof. However, the present disclosure is not limited thereto. Manufacturing the device from resorbable or biodegradable materials is particularly useful *in vivo*, which forms a semi-permanent shell of the device. As the device

degrades, fresh blood vessels can form from the resulting structure without requiring additional modification to the device (*e.g.*, additional surgery to a subject with the device implanted *in vivo*). In some embodiments, the channels of the device are formed with a predetermined cross-section. However, as the device is worn away the cross-section transforms. In general, such transformations include a rounding of internal and/or external edge portions (*e.g.*, corners) of the channels. The rounding is further induced by cells newly formed at locations where channel walls previously were.

[00131] The device of the present disclosure is not limited to resorbable or biodegradable materials. For instance, in some embodiments the device includes a non-resorbable material, or a combination of resorbable, biodegradable, and non-resorbable materials. As such, the device can be formed of synthetic polymers, natural polymers, or a combination thereof. Suitable materials for the device include poly-dimethyl-siloxane (PDMS), poly-glycerol-sebacate (PGS), polylactic acid (PLA), poly-L-lactic acid (PLLA), poly-D-lactic acid (PDLA), polyglycolide, polyglycolic acid (PGA), polylactide-co-glycolide (PLGA), polydioxanone, polygluconate, polylactic acid-polyethylene oxide copolymers, modified cellulose, collagen, polyhydroxybutyrate, polyhydroxypriopionic acid, polyphosphoester, poly(alpha-hydroxy acid), polycaprolactone, polycarbonates, polyamides, polyanhydrides, polyamino acids, polyorthoesters, polyacetals, polycyanoacrylates, degradable urethanes, aliphatic polyesters, polyacrylates, polymethacrylate, acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, polyvinyl imidazole, chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, Teflon®, nylon, silicon, and shape memory materials including poly(styrene-block-butadiene), polynorbornene, hydrogels, metallic alloys, and oligo(ϵ -caprolactone)diol as switching segment/oligo(p-dioxanone)diol as physical crosslink. In some embodiments, the device includes glass filled nylon, thermoplastic polyurethane, acrylonitrile butadiene, and/or polycarbonate acrylonitrile butadiene. Furthermore, in some embodiments the device includes medical polyurethane (MPU), such as MPU 100. Other suitable polymers can be obtained by reference to The Polymer Handbook, 3rd edition (Wiley, N.Y., 1989), which is hereby incorporated by reference. In some embodiments, a combination of these polymers is used forming the device. In some embodiments, at least a portion of the device is formed from a porous material. The porous material allows nutrients, wastes, and other particles (*e.g.*, pharmaceutical compositions) to

exchange across the channel networks of the device. Furthermore, in some embodiments, (*e.g.*, *in vitro* embodiments) at least a portion of the device is formed from an optically transparent or translucent material (*e.g.*, a hybrid polymer including an epoxy acrylic polymer). An optically transparent or translucent material allows for external observation of a flow of material (*e.g.*, medium) and cell viability within the device. Furthermore, in some embodiments, the scaffold device is formed from a rigid material, an elastomeric material, or a combination thereof. For instance, in some embodiments a membrane of the device is formed from an elastomeric material while the channel networks are formed from a rigid material.

[00132] Additionally, in some embodiments the devices of the present disclosure are fabricated or manufactured through an additive manufacturing method. These additive manufacturing methods include binder jetting methods, material extrusion methods, material jetting methods, polyjet methods, powder bed methods, sheet lamination methods, VAT photopolymerization methods, injection molding methods, layered fabrication methods such as selective laser sintering and stereolithography, or a combination thereof. In some embodiments, the device is formed as a positive mold, meaning the walls of the channels themselves, shown as black solid lines in the appended figures, are produced by a given manufacturing device. In some embodiments, the device is formed as a negative mold where the gaps in between the plurality of channels, shown as white voids in the appended figures, are produced by a given manufacturing device. Throughout the description of the devices of the present invention, exemplary embodiments of negative molds will be described for clarity. However, the present disclosure is not limited thereto.

[00133] In general, a scaffold device of the present disclosure includes at least one channel network. Each channel network includes an inlet and a plurality of subsequent channels. The plurality of channels is formed in a series of branching (*e.g.*, bifurcating) channels, with each branch producing a channel of a smaller size. In some embodiments, each bifurcation produces a channel of a same size or a similar size (*e.g.*, within a range of $\pm 2\%$ a size of a preceeding channel, $\pm 5\%$, $\pm 10\%$, or $\pm 15\%$). In some embodiments, once the series of branching channels have achieved a predetermined size (*e.g.*, a predetermined smallest size channel), the plurality of channels recombine in a recursive manner to form an outlet. In some embodiments, the devices includes more than one channel network (*e.g.*, a first channel network and a second channel network) that are in fluidic communication with one another. In some embodiments, branching

of the channels occurs in a linear tree. In some embodiments, branching occurs in a radial tree.

[00134] Channels of the present invention can be formed in a variety of shapes and corresponding cross-sections including, but not limited to, a circular cross-section, a rectangular cross-section, or a corresponding cross-section of a platonic solid. In some embodiments, an aspect ratio of a cross-section of each channel in a channel network is uniform (*e.g.*, a uniform aspect ratio of 1:1). In some embodiments, an aspect ratio of a cross-section of each channel in a channel network is uniform except at a portion of a connector. In some embodiments, the cross-section of each channel is determined by a minimum thickness of a wall of the channel and a desired rigidity (*e.g.*, bending stiffness) of the channels. The wall thickness of the channels is configured to balance various design requirements for flexural rigidity and mass transport across the walls of the channels. For instance, in some embodiments a stiffness of the walls of the channels is modified by altering a cross-section of the channels. Furthermore, in some embodiments each channel network is designed (*e.g.*, a desired thickness, a number of generations, *etc.*) to account for a desired dead volume between the channels of the device. In some embodiments, a wall thickness (*e.g.*, thickness t of **FIG. 4E**) of each surface of a channel is the same thickness. In some embodiments, a wall thickness of each surface of a channel is in a range of from 5 μm to 10 millimeters (mm), from 5 μm to 1,000 μm , from 5 μm to 500 μm , or from 10 μm to 500 μm . Furthermore, in some embodiments a distance between a surface of a channel and a nearest adjacent channel surface (*e.g.*, a void, a dead volume) is in a range of from 5 μm to 5 cm, from 5 μm to 4 cm, from 10 μm to 4 cm, from 10 μm to 1 cm, from 10 μm to 1 cm, or from 10 μm to 1,000 μm .

[00135] Referring now to **FIG. 1** and **FIG. 2**, there is depicted an exemplary channel network in accordance with various embodiments of the present disclosure. As shown, the device **10** includes inlet **100-1-I** which in communication with parent channel **100-1-1P**. Accordingly, parent channels **100-1-1P** bifurcates (*e.g.*, bifurcation **30**) into child channels **100-1-1C**, which in turn each bifurcate into grand-child channels **100-1-1G**. Each grand-child channel ends a generation. Thus immediately subsequent channels of a grand-child channel are child channels which define a new generation. Accordingly, grand-child channels **100-1-1G** bifurcate into second generation child channels **100-1-2C**. The series of bifurcations can repeat until k^{th} grand-child channels **100-1-kG** are formed. Once a final desired channel generation is acquired, the final channels form outlets **100-1-O**.

[00136] **FIG. 1** and **FIG. 2** depict an embodiment of the device including three generations of channels. However, there can exist k total generations in another embodiment of the present disclosure. As such, **FIG. 3** depicts an embodiment of a channel network including four generations of channels. In some embodiments, a generation is interrupted such that the child channel is the end of a generation. Furthermore, in another embodiment, the inlet bifurcates leading to the parent channel can be integral with the inlet.

[00137] In some embodiments, medium (*e.g.*, a fluid) flowing within the channels of the device exhibit laminar flow. Exhibiting laminar flow is a vital function of some embodiments of the present disclosure, since laminar flow reduces a risk of choked flow and material blockage in the channels. Further, ensuring laminar flow also maintains an optimal distribution of cells and molecules (*e.g.*, nutrients, waste material, *etc.*) within the channels of the device. Thus, in some embodiments a connection exists prior to each bifurcation to ease the flow of fluid or material (*e.g.*, prevent turbulence) and maintain physiological relevant levels of shear rate generated by the flow of fluid or material through the channels. Typically, a sharp edge portion within a channel and/or a discrete transition between varying channel diameters (*e.g.*, an immediate transition from a first diameter to a second diameter as exemplified by connector **40** of **FIG. 4B**) forms localized regions of high shear rate or low shear rate. In regions that experience high shear rate, platelet activation occurs. Sharp edges at an inner side portion of a bifurcation or connection will experience a high shear rates since the flow therein forms singularities. Similarly, in regions that experience low shear rate stasis occurs leading to thrombus formation. Another consideration in designing a connection between channels of the present disclosure is dissipating flow recirculation. A sharp step (*e.g.*, connector **40** of **FIG. 4B**) or a smooth, albeit steep, ramp (*e.g.*, connector **40** of **FIG. 4A**) will induce recirculation, preventing distribution of cells and molecules at this connection.

[00138] In addition to mitigating high shear rates, the connections also allow a surface of a channel in a channel network to be flush with a coplanar surface of another channel in the same channel network. In some embodiments, the connections are configured to allow each channel in a channel network to have a surface (*e.g.*, an exterior wall of the channel) flush with a single coplanar surface. This flush layout allows the device to maintain a compact configuration and allow for a thickness of an exchange mechanism to have a higher degree of variability and control. Additionally, the connections aid in the dissipation of flow recirculation while allowing

reducing a distance between channel networks of the device, which increases a potential maximum cell density within the device.

[00139] **FIG. 4A** and **FIG. 4B** illustrate various types of connections **40**. The connection of **FIG. 4A** is a ramped connection. In an exemplary embodiment, the ramp is a linear slope; however, the ramp can also include a smooth concave ramp, a smooth convex ramp, or a reducer. **FIG. 4B** illustrates a stepped embodiment of connector **40**. In the present embodiment, connector **40** intersects a diameter or a side portion of a subsequent channel at a rear end portion thereof. The connector can also intersect a front end portion or an intermediate portion of the subsequent channel. In addition to the singular step described and shown, the connections can include a plurality of steps.

[00140] **FIG. 4C** through **FIG. 4E** illustrate exemplary channel cross-sections in accordance with embodiments of the present disclosure. Each cross-section illustrated in **FIG. 4C** through **FIG. 4E** has the same minimum wall thickness but different bending stiffnesses.

[00141] In accordance with the connections, the bifurcations of the present disclosure limit shear rate in the device while optimizing internal cross-section, volume, and packing efficiency of the channels as well as mass flow rates, pressures, and viscous drag forces experienced by medium within the channels. To maximize a number of plausible generations within a device before channels intersect and enable a higher ration of channel area to planned area of the channel network, an orthogonal bifurcation angle of approximately 90° was determined to be optimal since. However, the present disclosure is not limited thereto. For instance, in some embodiments the bifurcation angle is in a range of from 70° to 105° .

[00142] Referring briefly to **FIG. 5L** and **FIG. 5M**, exemplary plots for determining a maximum number of generations (*e.g.*, bifurcations or branches) as a function of branching angle and reduction of length of subsequent channels for a planar channel network, in which a ratio of a length to a diameter of a parent channel is 10. **FIG. 5L** illustrates a plot in which a length of a subsequent channel is reduced by $1/\sqrt{2}$ each channel, while **FIG. 5M** illustrates a plot in which a length of a subsequent channel is reduced by $1/2$ after each grand-child channel. Both plots yield a maximum number of generates at an angle of 90° . Further, as illustrated by the plots, in some embodiments if a branch angle is either smaller than 75° or larger than 105° , few (*e.g.*, less than or equal to five) generations occur in two-dimensions before an intersection occurs. Referring to

FIG. 5N, an exemplary plot is illustrated for determining a covering area efficiency (*e.g.*, maximizing channel surface area in a fixed two-dimensional area). In **FIG. 5N**, a ratio of a projected area of the channel network (*e.g.*, projected area **500**) to an area of a smallest bounding rectangle (*e.g.*, shell **550**) is calculated for a fixed number of generations in which a ratio of a length to a diameter of a parent channel is 10. As illustrated by **FIG. 5N**, as the branching angle increases from 65° to 90°, the area coverage metric also improves. Below an angle of 65°, the channel surface area may increase, but devices include only a very small number (*e.g.*, less than or equal to 5) of generations before channel intersection occurs. Above 90°, diminishing returns occur for improving the channel surface area since the areas of both the channels and the shell are the same, but a maximum number of allowed generations diminishes. Thus, a branching angle of 90° optimizes filling shell **550**. Furthermore, a branching angle of 90° with symmetric branching evenly distributes fluid across the area efficiently and incorporates channel diameters that obey Murray's Law.

[00143] Furthermore, in some embodiments a length of a parent channel of a channel network is predetermined. Accordingly, a length of each subsequent channel (*e.g.*, a child channel after bifurcation of the parent channel) is reduced by a predetermined factor relative to a length of an immediately preceding channel (*e.g.*, the length of the parent channel). In some embodiments, this predetermined factor of length reduction is a constant less than or equal to 1 (*e.g.*, 0.5). Furthermore, in some embodiments a ratio of a diameter to a length of a channel is fixed along the length of the channel. In some embodiments, the ratio of the diameter to the length is in a range of from 1:4 to 1:25 (*e.g.*, 1:10).

[00144] Embodiments of the present disclosure include a wide variety of bifurcation shapes, including the shapes depicted in **FIG. 5A** through **FIG. 5K**. In some embodiments, the bifurcations are circular jogs (*e.g.*, bifurcation **30** of **FIG. 5A** and **FIG. 10**). In such embodiments, bifurcation **30** provides a spatial region for a different channel to bypass a channel network without intersection, such as to supply fluid to a lower layer (*e.g.*, a layer at a lower elevation) or channel network without intersecting an intermediate layer or channel network. For instance, referring briefly to **FIG. 34**, in some embodiments a first channel network includes outlets that bypass a second channel network and are in communication with corresponding outlets of a third channel network (*e.g.*, outlets **100-1-20** are in communication with outlets **300-**

1-2O). Additional bifurcation shapes include a semi-circular shape, a C-shape, a T-shape, a U-shape, a V-shape, a convex wedge shape, a concave wedge shape, a chamfer shape, a fillet shape, or a Bézier curve shape. In some embodiments, the bifurcations include a protrusion (*e.g.*, a protrusion at an internal surface of the bifurcation), an indentation, or similarly formed shapes that are configured to prevent occurrence of jet impinging in a flow. In **FIG. 5E** through **FIG. 5H**, a length of a connector is fixed while two weighted curve values of a cubic Bézier curve are varied. Typically, the Bézier curve includes four parameters. However, two of these four parameters are selected in some embodiments of the present disclosure in order to ensure continuous curvature while enabling the other two parameters to be chosen freely and/or optimized accordingly. In the present analysis of **FIG. 5E** through **FIG. 5H**, weight values range from 0 to 1 in increments of 0.1. However, in some embodiments these weight values are reduced to finer increments (*e.g.*, 0 to 1 in increments of .001) in order to produce more accurate and precise results. Specifically, **FIG. 5E** depicts weight values of $wt_{1,2} = 0.2$, **FIG. 5F** depicts weight values of $wt_1 = 1$ and $wt_2 = 0.2$, **FIG. 5G** depicts weight values of $wt_1 = 0.2$ and $wt_2 = 1$, and **FIG. 5H** depicts weight values of $wt_{1,2} = 1$. In **FIG. 5I** through **FIG. 5K**, a length of the connector is varied while the weights are fixed (*e.g.*, $wt_{1,2} = 1$). Specifically, **FIG. 5I** depicts a dimensionless length of a smooth ramp connector as 0.2, **FIG. 5J** depicts a dimensionless length of a smooth ramp connector as 1, and **FIG. 5K** depicts a dimensionless length of a smooth ramp connector to be 0.5.

[00145] As previously described, minimizing increases in shear rate as well as a volume of low-velocity regions (*e.g.*, zero velocity regions such as a stagnant region) at bifurcations within the device is highly preferred in embodiments of the present disclosure. **FIG. 6A** through **FIG. 6D** illustrate an analysis of dimensionless shear rates generated within various bifurcation designs. **FIG. 6A** and **FIG. 6B** illustrate a bifurcation (*e.g.*, bifurcation **30**) and a connector (*e.g.*, connector **40**) with sharp edge portions and corners. This design causes local maximums in shear rate, represented by brighter or white regions. Further, a stagnant region (*e.g.*, stagnant region **35**), representing a low velocity, is formed at a rear end portion of the bifurcation as well as along edge portions of the channel. In the present embodiment, a predicted maximum dimensionless shear rate is 43.2, while a predicted dimensionless shear rate along a center of the walls of the channel is 9.61. **FIG. 6C** illustrates an embodiment of a bifurcation including circular fillets designed to mitigate generated increases in shear rate. Smoothing the connector

with circular fillets reduces the maximum generated dimensionless shear rate in comparison to a sharp-edged connector. However, even with a substantially large radius fillet, as shown in **FIG. 6C**, discontinuities in curvatures between straight portions of the channel (*i.e.*, zero curvature) and the fillet (*i.e.*, non-zero curvature) results in local maximums of shear rate. **FIG. 6D** illustrates a maximum dimensionless shear rate on a variety of channel boundaries for a bifurcation and a connector including a circular fillet at Reynolds number of 1 ($Re = 1$). As depicted in **FIG. 6D**, maximum shear rates at the connector and the bifurcation are approximately 15-20% above those of the straight channel. Thus, in order to minimize the increases in shear rate at connectors and bifurcations, the shapes of the bifurcations are preferred to have no sharp edge portions, have continuous curvature, and have a smooth ramp with a gentle slope. In some embodiments, if the shapes of the bifurcations are configured according to cubic Bézier curves the curvature varies continuously from zero along an upstream straight section to positive along the curve and back to zero at a downstream straight section. In some embodiments, a smoothed step function ramp of a length (*e.g.*, a predetermined length) leads from a first channel to a narrower second channel (*e.g.*, from a parent channel to a child channel, from the child channel to a grandchild channel, *etc.*).

[00146] Furthermore, as previously described, ensuring each channel and each bifurcation exhibits laminar flow within is highly preferred. If analyzing flow conditions in a channel and subsequent bifurcation, a variety of flow physics and boundary condition assumptions must be made. These assumptions are associated with a desired laminar flow having varying ratios of inertial effects and viscous effects. Furthermore, to ensure analysis results are applicable to a wide variety of connectors, bifurcations, channel dimensions (*e.g.*, length, width, height), flow rates, and fluid types (*e.g.*, a culture media, blood, collagen, water, *etc.*), this analysis is conducted in dimensionless variables. Specifically, all spatial coordinates are scaled by an inlet diameter d , all velocity components are scaled by an average velocity at the inlet $U = (\text{flow rate}) / d^2$, a pressure is scaled by $\text{density} \times U^2$, and scaling for time is d / U . Accordingly, the average dimensionless velocity at an inlet is 1 in the dimensionless variables. The analysis presented herein is in the steady-state, and thusly does not explicitly involve time. The dimensionless Navier-Stokes equations governing fluid flow in connectors and bifurcations depend on the Reynolds number, $Re = (U \times d) / \nu$, where ν is a kinematic viscosity of a fluid and d includes dimensionless geometrical parameters such as curve and connector shapes as well as channel

aspect ratios. To ensure analysis results are applicable to a wide variety of flow rates, channel sizes, and fluids, the present disclosure considers the Reynolds number (Re) in a range of from $Re = 0.1$ to approximately 2300, and the analysis presented herein includes Re in a range of from 0.1 through 100. For instance, **FIG. 7A** through **FIG. 7G** includes a Re of 1, while **FIG. 8A** through **FIG. 8F** include a Re of 100. A symmetry condition is imposed at a symmetry plane of $y = 0$, an exit pressure is specified as 0 at an outlet, and the no-slip condition is imposed at the walls of the channels. In the following analysis, channels have a square cross-section (*i.e.*, an aspect ratio of 1). Additionally, inner curved boundaries defined by the Bézier curve include two predetermined weighting parameters to maintain continuous curvature at side portions of the channels. The inner curved boundaries defined by the Bézier curve also includes two configurable weighting parameters $w_{1,2}$ that are in a range of from 0.01 to 1. For instance, **FIG. 7A** through **FIG. 8F**, the two configurable weighting parameters are $w_{1,2} = 1$. Additionally, the exemplary smooth ramp connector has dimensionless length in a range of 0.01 to 1. In **FIG. 7A** through **FIG. 7F** and **FIG. 8A** through **FIG. 8E**, the exemplary smooth ramp connector has a dimensionless length of 1. In **FIG. 7G** and **FIG. 8E**, the exemplary smooth ramp connector has a dimensionless length of 0.1.

[00147] Referring to **FIG. 7A** through **FIG. 7E**, an analysis of the previously described embodiments is conducted. In the figures, a flow of medium from inlet (I) to outlet (O). Referring to **FIG. 7A**, dimensionless velocity magnitude is illustrated in the right symmetric half of connector 40 and bifurcation 30. As previously described, the dimensionless average velocity is 1 at the inlet. Velocities are higher (*e.g.*, a number further from zero) at a center of the channel and 0 at the walls. Similarly, stagnant regions 35 exist at rear end portions of the bifurcation. Referring to **FIG. 7B** and **FIG. 7D**, streamlines are illustrated to visualize a flow of fluid. In the figure, a color of the streamline (*e.g.*, a level of darkness) indicates dimensionless velocity magnitude. No recirculation is observed in these streamlines. However, a stagnant region still exists in the rear end portions of the bifurcation. Referring to **FIG. 7D**, dimensionless pressure contours are illustrated. Contours that are evenly spaced indicate a gradual change in a flow profile across the bifurcation. However, an exception is a large (*e.g.*, a greater distance between adjacent contours) contour spacing near stagnant region 35. In the present embodiment, a laminar flow boundary condition is imposed at an outlet (*i.e.*, where an exit pressure is 0). Referring to **FIG. 7E** and **FIG. 7F**, dimensionless shear rate, which is equal to dimensionless

shear stress, is illustrated. In the present embodiment, a maximum shear rate is at a center of the walls, which correlates to a dimensionless shear rate of approximately 9.7, and 0 at the corners or edge portions (*e.g.*, a region where the walls intersect). In the present embodiment, the maximum shear rate occurs at connector **40**. However, in some embodiments, the maximum shear rate can occur downstream of connector 40 (*e.g.*, at the bifurcation). Referring to **FIG. 7G**, an embodiment including a steep ramp (*e.g.*, a dimensionless length of connector **40** is 0.1), the maximum shear rate occurs at the connector. No recirculation is observed in the present embodiment, as well as embodiments analyzed with $Re = 0.1$, $Re = 1$, and $Re = 10$.

[00148] Biologically relevant ranges for Reynolds numbers, where $Re = (U \times d) / \nu = 4Q / (\pi \times d \times \nu)$ if an average inlet velocity is $U = Q / ((\pi \times d^2) / 4)$, were determined for a bio-reactor as well as a mouse liver. Human liver equivalents may also be determined. In determining, an approximate blood flow rate entering a liver is Q , and a diameter of a cylindrical inlet channel is d in meters (m).

Subject	d (m)	Q (m ³ /s)	U (m/s)	Blood at 37°C		Culture Media at 37°C	
				ν (m ² /s)	Re	ν (m ² /s)	Re
Bio-reactor	1.00E-03	5.00E-08	6.37E-02	3.30E-06	1.93E+01	7.80E-07	8.16E+01
Mouse liver	6.40E-04	5.00E-08	1.55E-01	3.30E-06	3.01E+01	7.80E-07	1.28E+02

Table 1: Estimated Ranges of Biologically Relevant Reynolds Numbers

[00149] In some embodiments, recirculation can be observed when the connectors are substantially steep (*e.g.*, a dimensionless length of a connector is approximately 0) or a high Reynolds number (*e.g.*, Re is greater than 100). In these embodiments, the maximum shear rate occurs where a flow jet forms downstream of the connector, which in turn impacts the rear end portion of the bifurcation. These jet formations and impacts change the qualitative nature of a flow. Thus, as previously described, protrusions and/or indentations may be formed in and/or on the bifurcations. The protrusions and/or indentations direct a flow of medium and inhibit jet formation, reduce impacts against materials (*e.g.*, cells) and channel walls at the bifurcation, and reduce a maximum shear rate.

[00150] Referring to **FIG. 8A** through **FIG. 8F**, the aforementioned analysis was re-conducted. However, in conducting the analysis only the parameter $Re = 100$ was altered. In the present

embodiment, a total dimensionless pressure drop across the bifurcation is approximately a hundred times lower in comparison to the previous embodiment of $Re = 1$. This hundred times pressure drop can occur for a plurality of reasons. For instance, when the Re is increased from 1 to 100 by reducing the viscosity by a hundred times, a flow resistance would similarly decrease by a hundred times which is consistent with a hundred times decrease in the dimensionless pressure. When the Re is increased from 1 to 100 by increasing the inflow velocity a hundred time, the physical pressure increases by a hundred times. One skilled in the art would recognized this pressure drop is only predictive, and actual pressure drops may be greater or lower in various embodiments. Furthermore, this pressure drop enables material flow from a relatively high-pressure region to a relatively low-pressure region. Referring to **FIG. 8A** and **FIG. 8B**, streamlines illustrate a jet forming at (*e.g.*, over) the connector and impacting a rear end portion of the internal surface (*e.g.*, wall) of the bifurcation, indicative of a higher Reynolds number laminar flow. This jet and impact are also illustrated in **FIG. 8C** and **FIG. 8D** from a plot of velocity magnitude in which higher velocities are illustrated in a lighter shade compared to lower velocities. Referring to **FIG. 8E**, a low density of pressure contours at the smooth curved portion indicate that a high velocity jet forms at (*e.g.*, over) the connector and relatively maintains an initial trajectory. Accordingly, the jet does not expand or traverse with a curved portion of the channel. Furthermore, a high density of pressure contours at a rear end portion of the bifurcation indicate jet impact (*e.g.*, a flow impacts the rear end portion of the bifurcation). Referring to **FIG. 8F**, an x -component of the dimensionless velocity at a bottom end portion of a connector having a dimensionless length of 0.1 is illustrated to observe recirculation. In **FIG. 8F**, arrows depict a direction of the velocity, a white lines illustrates a streamline, and a solid grey line illustrates a velocity of 0.

[00151] **FIG. 9A** through **FIG. 9C** illustrate a broad parameter (*e.g.*, design space) for varying a ramped connector length and curve weights. Lengths and curve weights are optimized to minimize the additional shear rate caused by a flow division and re-direction. Similarly, to obey Murray's law, a diameter of a parent channel is larger than that of the children channels. Further, a smoothed ramp at a bottom end portion of the connection forms a transition region between the different channel diameters. The length of the ramp or, alternatively, the slope is parameterized and optimized to avoid, or reduce, a jump in shear rate. Iso-surfaces illustrated in these three-dimensional parameter domain bound shear rates within approximately 0.2 dimensionless units

of a maximum shear rate in a straight portion of a channel (*e.g.*, 9.61) at the same Reynolds number. Furthermore, the iso-surfaces illustrate a minimum shear rate increase beyond that observed in a straight portion of a channel. Referring to **FIG. 7F** and **FIG. 7G**, the previously predicted maximum shear rates of 11.8 and 18.1, respectively, are outside an optimal region illustrated by **FIG. 9A**. As previously described, in order to form a smoother (*e.g.*, less coarse, finer grain) iso-surface increments of the weights and the ramp length should be further refined (*e.g.*, from increments of 0.2 to 0.00001).

[00152] **FIG. 11** illustrates a flow chart of an exemplary design process for generating a scaffold device in accordance with embodiments of the present disclosure. In the flow chart, the preferred parts of the design process are in solid line boxes whereas optional variants of the processes, or optional equipment used by the processes, are in dashed line boxed. As such, **FIG. 11** illustrates processes for designing and fabricating a scaffold device.

[00153] In designing and engineering a scaffold device of the present disclosure, there are various core parameters that a designer manipulates and optimizes. Initially, a design for a scaffold device should consider an overall size, or active area (*e.g.*, shell **550**), of the scaffold device. In some embodiments, the device is formed in a square shape to optimize packing efficiency of the channels and cell density within the device. Accordingly, a length of the device is a distance between endpoints of corresponding channels on opposite side portions of the device. In some embodiments, a diameter of a smallest channel (*e.g.*, final generation) is an essential parameter of the device as well as a total number of generations to include within the device. In some embodiments, the diameter of the smallest channel is restricted by an effective fabrication device resolution, a material of the device, or the like. One skilled in the art will recognize that as manufacturing technologies improve so will the resolution of components of the present disclosure. In some embodiments, a diameter of a smallest channel is in a range of from 5 μm to 2 mm, from 5 μm to 1 mm, from 5 μm to 750 μm , from 5 μm to 650 μm , from 10 μm to 650 μm , from 10 μm to 500 μm , or from 100 μm to 300 μm . In some embodiments, a design for a scaffold device considers a minimum wall thickness and a ratio of diameter growth or reduction within the device. Since some embodiments of the present disclosure rely on material diffusion through the walls of the channels, or similarly a membrane, ensuring a proper wall thickness for molecular diffusivity is preferred. In some embodiments a minimum wall thickness of each channel in the one or more channel networks is in a range of from 5 μm to 500

μm, from 5 μm to 400 μm, from 10 μm to 500 μm, from 10 μm to 400 μm, or from 10 μm to 300 μm.

[00154] In some embodiments, and the exemplary embodiments described hereinafter, the device is formed as a negative mold. As such, the minimum wall thickness occurs near an inlet and end generation channel or outlet. In other embodiments, (*e.g.*, the device is formed as a positive mold) the minimum wall thickness occurs at the smallest diameter channel. Murray's Law has proven an essential tool in optimizing a diameter of branching channels. For instance, each preceding channel before the smallest channel has a diameter that is increased by a predetermined factor derived from Murray's Law, which is based on biological observation of a ratio of inflow and outflow diameters. Murray's Law determines that $D_o^3 = \sum_{i=1}^n D_i^3$, where D_o is a diameter of a parent channel, n is a number of child channels, and D_i is a diameter of an i^{th} child channel. Thus, in some embodiments, the diameter of the child channel is reduced by a factor of $2^{-1/3}$. However, the present disclosure is not limited thereto. For instance, in some embodiments the diameter of the child channel is reduced by a factor in a range of from of numbers less than 1 but greater than zero (*e.g.*, 0.5).

[00155] In some embodiments, obeying Murray's law for a first channel network but not a second channel network. For instance, in some embodiments providing or simulating a liver device, obeying Murray's law is required for a portal venous (PV) channel network that has a flow of blood, but not for a hepatobiliary (HB) channel network that has a flow of bile. In some embodiments, having each channel in the HB network is disposed at the same height (*e.g.*, flush) for all generations, while also maintaining a width equal to that of an adjacent PV channel (*e.g.*, a device of **FIG. 47** through **FIG. 53**) **(1002)**.

[00156] In some embodiments, an exchange mechanism is disposed between adjacent layers and/or channels of the device. In considering the exchange mechanism, a design for a scaffold device should determine a porosity or a pore density as a fraction of an exchange mechanism area as well as a thickness of the exchange mechanism. In some embodiments, approximating a shape of the pores as a square shape while conducting design calculations is appropriate to simplify optimizing the device **(1004)**.

[00157] Cells of various organs often differentiate in size, cell count, and functional capacity for the organ. In some embodiments, such as designing the device as a liver implant, hepatocyte

cells are a primary cell. As described above regarding a shape of the pores, in some embodiments approximating a shape of a cell as a cube while conducting design calculations is appropriate to simplify the system. Thus, in some embodiments, a design for a scaffold device considers a characteristic length of a cell, a count of cells per gram of a living target organ, and a fraction of the target organs capacity needed to function. For instance, a liver implant can produce only 30% of a capacity of a living liver yet still thrive *in vivo* (1006).

[00158] Once the above parameters have been determined, a non-transitory computer readable storage medium including instruction for execution by one or more processes to perform a device fabrication regimen derives a plurality of intermediate quantities that are useful to a design architect (1008, 1010).

[00159] In some embodiments, the device is mathematically modeled (*e.g.*, modeled through a computer-aided design tool such as Autodesk®, nTopology®, Creo®, SolidWorks®, *etc.*, modeled through a mathematical software tool such as MATLAB®, Mathematica®, *etc.*, modeled through a physics simulation software tool such as COMSOL Multiphysics®, ANSYS Fluent®, *etc.*, or modeled through a spreadsheet and/or a combination of the aforementioned computer software tools) to verify a number of end parameters (*e.g.*, outlet pressure, outlet flow rate, *etc.*) and outputs of the device. In some embodiments, an important verification is an actual minimum wall thickness of the device, as the present value must be greater than or equal to an input value of the design. If such a value is determined to be less than the input value of the design, this indicates that the geometry of the device is incorrect. To remedy such a problem a design can reduce the diameter of the smallest channel or reduce the number of generations in the device (1012 and 1014).

[00160] Referring to FIG. 11 through FIG. 27, an exemplary embodiment for fabricating a device according to the present invention will now be described. In the present exemplary embodiment, an inlet of each parent channel is formed as a portion of a master inlet (*e.g.*, inlet 70-IA FIG. 35). Once the above parameters have been determined, verified, and input, fabrication of device 10 may commence. From an active endpoint of a parent channel (*e.g.*, parent channel 100-1-1P), child channels (*e.g.*, child channels 100-1-1C) branch off in plane and perpendicular to the parent channel with a diameter reduced by the first predetermined ratio. Branching (*e.g.*, bifurcating) is recursively repeated for each child channel (*e.g.*, child channel

100-1-2C), and subsequently repeated until a specified number of generations is achieved. Once the specified number of generations is achieved (*e.g.*, grand-child channel **100-1-2G**), the smallest diameter channels are redirected (*e.g.*, reoriented) to form a plurality of outlets (*e.g.*, outlets **100-1-2O**). **FIG. 14** depicts completed channel network layer **100-1**. In some embodiments, the completed network layer delivers a fluid or substance to cover a planar area.

[00161] In some embodiments, including the present embodiment, the fluid or substance (*e.g.*, medium) is output to a single outlet. Therefore, a similarly designed (*e.g.*, looking) layer (*e.g.*, layer **100-2**) of branching channels is formed to collect fluid and output to a single channel (*e.g.*, outlet **100-2-O** of **FIG. 15** and **FIG. 16**, *etc.*). Once the recursive branching completes second level **100-2**, the outlets of the first layer and the second layer are combined (*e.g.*, coupled together), allowing fluidic communication between the layers. This fluidic communication forms a paired channel network layer (PCNL) **100**.

[00162] In some embodiments, a plurality of PCNLs is stacked (*e.g.*, stacked in a vertical orientation) to form a dual paired network. **FIG. 17** through **FIG. 21** depict various embodiments of device **10** including various numbers of stacked PCNLs. In the present embodiments, a maximum of eight PCNLs are illustrated. However, in some embodiments there exists any number k of PCNLs (*e.g.*, PCNL {100, 200, $i00$, ..., $k00$ }) with k being an integer greater than or equal to 1.

[00163] As described above, in some embodiments, combining the inlets and the outlets of the device into at least one master inlet and at least one master outlet is advantageous to allow for a simple system for the supply and collection of medium. Some such embodiments are depicted in **FIG. 22** through **FIG. 28**. By way of example, **FIG. 22** through **FIG. 28** demonstrate a process for forming a first master inlet (*e.g.*, master inlet **70-IA**), a second master inlet (*e.g.*, master inlet **70-IB**), a first master outlet (*e.g.*, master outlet **70-OA**), and a second master outlet (*e.g.*, master outlet **70-OB**) of device **10**. In some embodiments, the forming of the master inlet and/or the master outlet utilizes the bifurcating as described in the present disclosure. Shapes, layouts, configurations, and orientations are not restricted by the exemplary embodiments and may be fabricated according to an end goal of a design for a scaffold device.

[00164] In some embodiments, a first layer circumvents (*e.g.*, bypasses) a second layer of a channel network. In some embodiments, the first channel network circumvents the second

channel network. In such embodiments, the first channel network is formed with a single level (*e.g.*, layer) whose outlets are in communication with a single level of a third channel network. The present embodiment is known as a bypass embodiment, as the first channel network bypasses the second channel network to communicate with the third channel network.

[00165] FIG. 29 through FIG. 35 depict another exemplary embodiment of the scaffold device, in particular a bypass embodiment. As shown in FIG. 29, second channel network layer 200 is formed in the same recursive manner as shown in FIG. 12 through FIG. 16. However, first channel network layer 100 is only partially formed, as shown in FIG. 29 with the first layer omitting reoriented outlets. FIG. 34 depicts an additional stack of the first and second channel networks, forming third channel network 300 and fourth channel network 400. Final generations (*e.g.*, smallest diameter channels) of first channel network 100-1-2G and final generations of third channel network 300-1-2G are connected while bypassing second channel network 200, as shown in FIG. 31 through FIG. 34. In accordance with the previous exemplary embodiment, the inlets and the outlets of each channel network combine to form master inlets 70-I1 and 70-I2 as well as master outlets 70-O and 70-O.

[00166] In some embodiments, an exchange mechanism is disposed interposing between adjacent channel networks. FIG. 36 depicts an embodiment of the present disclosure including exchange mechanism 20 disposed interposing between networks 100 and 200 as well as 300 and 400. The exchange mechanism is configured to selectively allow a flow of material from the second channel network to the first channel network and/or from the first channel network to the second channel network. In the present embodiment, exchange mechanism 20 is a membrane. Exemplary membranes include but are not limited to a track-etch membrane, a fibrous membrane, and a membrane formed through additive manufacturing (*e.g.*, a membrane formed through three-dimensional printing). Track-etch membranes typically include cylindrical through-holes in a dense polymer matrix. These track-etch membranes are typically made by ion-etching. Fibrous membranes are made by a variety of deposition techniques of polymeric fibers. While these fibrous membranes do not have a well-defined pore topology, production methods have been sufficiently refined so that fibrous membranes have specific molecular weight cut-offs. In some embodiments, track-etch type membranes are preferred, as they limit the fluid motion in one direction. In some embodiments, a membrane forms through additive manufacturing (*e.g.*, 3D printing). This includes forming (*e.g.*, printing) the membrane

simultaneously to one or more channels of the scaffold device (*e.g.*, as a portion of a monolithic device). In some embodiments, a motion of a medium (*e.g.*, flow) is in a vertical direction (*e.g.*, opposite a force of gravity). Fibrous membranes permit fluid motion both laterally and vertically. Moreover, a membrane formed through additive manufacturing (*e.g.*, 3D printing) is configurable to control flow paths within and/or surrounding the device. In some embodiments, the exchange mechanism has a thickness in a range of from 5 μm to 10,000 μm , from 5 μm to 5,000 μm , from 10 μm to 5,000 μm , or from 10 μm to 4,000 μm . In some embodiments, the exchange mechanism includes a plurality of pores (*e.g.*, exchange mechanism **20** including pores **25** of **FIG. 38** through **FIG. 40**). In some embodiments, a pore size of the membrane is smaller than a diameter of a cell. Accordingly, cells will not be able to pass through the membrane (*i.e.* a low permeability for animal cells), while low molecular weight nutrients and fluids can pass through (*i.e.* a high permeability for nutrients), providing adequate cell-to-cell signaling. Cell sizes vary, and in general are in a range of microns. For example, a red blood cell has a diameter of approximately 8 μm . Preferably, the average membrane pore size is on a submicron-scale to ensure effective screening of the cells. In some embodiments, a permeability of the membrane is determined by a number of parameters including a property of the membrane (*e.g.*, a pore size and/or a porosity), an interaction and/or an affinity between the membrane and a material, a size of a cell species, a concentration gradient of a material, an elasticity of a material, and/or a combination thereof. In some embodiments, a distance from a center of a first pore to a center of an adjacent pore is in a range of from 5 μm to 150 μm , from 5 μm to 100 μm , or from 5 μm to 50 μm . In some embodiments, a diameter of each pore is in a range of from 5 μm to 150 μm , from 5 μm to 100 μm , or from 5 μm to 50 μm . Furthermore, In some embodiments, the depth of each pore is in a range of from 5 μm to 5,000 μm , from 10 μm to 5,000 μm , from 10 μm to 4,000 μm , or from 10 μm to 1,000 μm . In some embodiments, a pore has a rectangular shape (*e.g.*, a rectangular opening and/or cross-section), a square shape, a cylindrical shape, a conical shape, a cup shape, an hourglass, or the like. In some embodiments, an exchange mechanism includes a material with a non-zero solubility to a predetermined solution or chemical. In some embodiments, the exchange mechanism includes a polymer with a high permeability for a predetermined solution or chemical. In some embodiments, the exchange mechanism includes polydimethylsiloxane, which has a high permeability to fluids such as oxygen and carbon dioxide.

[00167] In some embodiments, to facilitate additional flow and/or exchange of material between channels, at least one side channel is formed in at least one channel network of the device. In some embodiments, the side channel extends from at least a first surface (*e.g.*, a first internal surface) of the first channel network to at least a first surface of a second channel network. In some embodiments, the exchange mechanism is further disposed interposing between the side channel each of the channel networks. By way of example, **FIG. 40** depicts side channels **60** with exchange mechanism **20** including pores **25** disposed interposing the side channels and first layer **100** and second layer **200**. Sizes and shapes of the side channels and the pores are not limited to the sizes and shapes depicted in the present exemplary embodiment. In some embodiments, the exchange mechanism is disposed on a portion of all sides of either the first channel network or the second channel network, but never both channel networks. **FIG. 40A** and **FIG. 40B** depict such an embodiment. In some embodiments, the second channel network is embedded internally in the first channel network, or similarly the first channel network can be embedded internally in the second channel network. In some embodiments, the internally embedded channel is disposed in a different position and/or orientations, including but not limited to a centered orientation of **FIG. 40A** or an offset orientation of **FIG. 40B**. Furthermore, in some embodiments a flow of material in the internally embedded channel opposes a direction of flow in the other channel (*e.g.*, as indicated by the arrows in **FIG. 40A**). However, the present disclosure is not limited thereto.

[00168] **FIG. 41** and **FIG. 42** depict a bypass embodiment of the present invention where the first channel network and the third channel network are not in fluidic communication.

[00169] **FIG. 43** illustrates an exemplary schematic of a vascularized tissue layer with drainage embodiment of the present disclosure. The vascularized tissue layer supplies nutrients and oxygen to tissues of interest through an exchange mechanism (*e.g.*, a fenestrated or a porous membrane). Nutrients and oxygen are exchanged to and from channel **91** lined with cells of interest **94** and tissue of interest **92** including drainage **93** with outflow. In some embodiments of the present disclosure (*e.g.*, those that are configured for livers), nutrients and oxygen are exchanged to and from channel **91** lined with endothelial cells **94** and hepatic cells **92** including drainage **93** with outflow of bile.

[00170] **FIG. 44** illustrates a schematic of a vascularized tissue layer with drainage embodiment

that is similar to the embodiment depicted by **FIG. 43**. However, in the present embodiment, drainage **93** outflow is connected to channel **94** configured for outflow. In some embodiments, this configuration is utilized for a heart muscle tissue patch to strengthen, for instance, the left ventricle of the heart, as shown in **FIG. 45**.

[00171] Referring to **FIG. 46** and **FIG. 47**, an embodiment of the present disclosure is depicted including a plurality of layers with drainage. In the present embodiment, nutrients and oxygen are exchanged to and from a first channel layer **91A** lined with first cells of interest **94A** and first tissue of interest **92A** as well as separately being exchanged to and from a second channel layer **91B** lined with second cells of interest **94B** and second tissue of interest **92B**.

[00172] Referring to **FIG. 48A**, a two generation vascularized tissue layer embodiment of the present disclosure is illustrated. This embodiment is configured to allow nutrients, oxygen, and/or drugs to be supplied to the tissue layer from the other vascular layer. Return of waste and metabolites from tissue layer **92** to vascular layer **91** is provided by the previously described pressure drop, or gradient. Similarly, referring to **FIG. 48B**, in some embodiments the vascular layer include a distributor network mirrored to a collector network (*e.g.*, distributor network **95** mirrored to collector network **96**). In some embodiments, there is a tissue layer disposed between the distributor and collector networks. This configuration allows new blood vessels to grow and nourish additional portions of the tissue layer, allowing cells of interest to further proliferate throughout the device.

[00173] **FIG. 49A** through **FIG. 49C** collectively illustrate an exemplary progression from a single unit one generation vascularized tissue layer device embodiment to a four unit two generation vascularized tissue layer device embodiment. The hierarchical nature of the present disclosure allows for symmetry and recursion to iteratively build upon larger tissue constructs from simple micro tissue unit devices (*e.g.*, device **10** includes micro devices **10-A**, **10-B**, **10-C**, ..., **10-i**). In some embodiments, a scaffold device includes a plurality of microscale unit devices coupled together. For instance, as shown in **FIG. 49C** and **FIG. 50A** through **FIG. 50D** a variety of arrays and stacks are plausible in a design of a scaffold device of the present disclosure. For instance, the embodiments shown in **FIG. 50A** and **FIG. 50B** depict larger tissue constructs including arrays of connected units. **FIG. 50C** and **FIG. 50D** depict larger tissue constructs including stacks of connected units.

[00174] **FIG. 51, FIG. 52, and FIG. 53** illustrate a unit, a four-unit network, and four stacks of four-unit networks, respectively. In some embodiments, the inflows and outflows of each stack are connected via a master inflow and a master outflow, respectively.

[00175] **FIG. 54** depicts an exemplary embodiment of a scaffold device including a first scaffold device and a second scaffold device with shared master inlet **70-I1** and shared master outlets **70-O1** and **70-O2**. Such an embodiment can be utilized in a liver device (*e.g.*, a liver implant) which includes two lobes. In addition to the embodiment including a plurality of scaffolding networks, **FIG. 54** illustrates various configurations of the channel networks with variations in number of generations, variations in network configuration (*e.g.*, bypass device **10-B** and stacked PCLN **10-A**), and the like. In some embodiments, a parent channel of a first channel network layer is in communication with a parent channel of a second channel network. In some embodiments, this communication is facilitated by a bypass channel (*e.g.*, bypass channel 93 of **FIG. 49C** and/or 100-1-10 of **FIG. 52**).

[00176] **FIG. 55A through FIG. 55D** depict an exemplary embodiment of a scaffold device encased in a closed structure (*e.g.*, shell **550**). In some embodiments, the shell is formed in a rectangular (*e.g.*, shell **550** of **FIG. 55A** and **FIG. 55B**). In some embodiments, the shell is formed to minimize free space (*e.g.*, voids) within the device (*e.g.*, shell **550** of **FIG. 55A** and **FIG. 55B**). Furthermore, in some embodiments the shell includes rounded edge portions, which allows for easier insertion if the scaffold device is implanted in a subject. In some embodiments, device **10** is manufactured in a single fabrication process (*e.g.*, as a monolithic device) such that internal channels and components are only accessible through master inlets **70-I1** and **70-I2** as well as master outlets **70-O1** and **70-O2**. However, the present disclosure is not limited thereto. For instance, in some embodiments a scaffold device is manufactured in a plurality of steps. For instance, in some embodiments, a first step manufactures a first channel network and a second step manufactures a second channel network. Likewise, in some embodiments a first step manufactures a first channel network and a second channel network, while a second step manufactures a shell of the device.

[00177] Similar to the device depicted in **FIG. 55**, **FIG. 56** depicts an exemplary embodiment of a scaffold device encased in a liver structure (*e.g.*, shell **550** formed in the shape of a liver). Accordingly, **FIG. 57 through FIG. 65** illustrate a progressive slicing of a cross-section of the

device depicted in **FIG. 56**.

[00178] In the present embodiment, the outlet is located on an opposite side of the device to a corresponding inlet. However, the present disclosure is not limited thereto. In some embodiments, an outlet is disposed above (*e.g.*, a higher elevation), below (*e.g.*, a lower elevation), or lateral to (*e.g.*, even with) an inlet. In some embodiments, an outlet is disposed on a face of the device other than a face including an inlet. Additionally, in some embodiments, layers and stacks are reoriented, or flipped, such that corresponding channels in adjacent layers align and have a fixed vertical distance there between. In some embodiments, the present flipped configuration facilitates adding the exchange mechanism between the layers.

[00179] In some embodiments, a first channel network (*e.g.*, a portal venous network) includes an inlet and an outlet, and a second channel network (*e.g.*, a hepatobiliary network) includes an outlet but lacks an inlet. In some embodiments, a second channel network (*e.g.*, a lung airway network) allows an inflow and an outflow of medium through a single port (designated as an outlet herein), but does not allow through flow.

[00180] In some embodiments, a lattice structure provides mechanical support to one or more channel networks of a scaffold device. For instance, in some embodiments the lattice structure interposes between various portions of a first channel network (*e.g.*, a portal venous network) and a second channel network (*e.g.*, a hepatobiliary network), to provide structural integrity to the channel networks. Further, the lattice structure allows an external medium to flow around the channel networks and transport material to and/or from the channel networks. Accordingly, in some embodiments a design of a scaffold device considers a number of bifurcations, a thickness of a channel wall, and a size of the lattice structure (*e.g.*, a thickness) to provide desired structural integrity to the device while also providing external medium flow and material transport. Furthermore, in some embodiments the lattice structure is formed in an array of rectangles, with each rectangle being at least partially intercepted by a channel of the device. In some embodiments, the lattice structure varies spatially depending on a geometry of the device. For instance, in some embodiments a density of beams of the lattice structure and a diameter of the lattice structure vary according to a desired material transport and structural integrity (*e.g.*, as a channel diameter decreases a density of a lattice structure increases, as a channel diameter decreases both a density and a diameter of a lattice structure decrease, *etc.*).

[00181] Referring to **FIG. 66** through **FIG. 71**, scaffold device **10** is illustrated including first channel network **100** (*e.g.*, portal venous network), second channel network **200** (*e.g.*, hepatobiliary network), membrane **20** interposing between the first channel network and the second channel network, and lattice structure **660** providing structural integrity to the device. For visual clarity, in **FIG. 66** through **FIG. 72**, first channel network **100** and membrane **20** are unshaded, second channel network **200** is shaded a light gray, and lattice structure **660** is shaded a dark gray. Both first channel network **100** and second channel network **200** includes two generations of channels (*e.g.*, channel **100-1-2G**). Furthermore, first channel network **100** provides nutrients and waste removal for second channel network **200**, which in turn cultures cells while collecting and draining (*e.g.*, outflowing) waste. Membrane **20** provides oxygen, nutrient, and waste exchange between first channel network **100** and second channel network **200**. Referring to **FIG. 72**, scaffold device **10** of **FIG. 66** through **FIG. 71** illustrated without lattice structure **660**.

[00182] Cells can be incorporated into and/or onto the device of the present disclosure in various manners and methods. For instance, in some embodiments cells populate an exterior of the device in a naturally occurring manner (*e.g.*, suspended in a solution that encompasses the device). In some embodiments, cells are bio-printed concurrently with the fabrication of the device. In some embodiments, the device is encapsulated and/or submerged in hydrogel or encapsulated therein. In some embodiments, the cells are encapsulated in at least two dimensions (*e.g.*, three dimensions) within the device. In some embodiments, the cells are encapsulated within a hydrogel that is introduced (*e.g.*, flows within) the device. An exemplary hydrogel includes totipotent cells (iTC), pluripotent cells (iPSC), progenitor cells (iMSC), or combinations thereof. Additionally, in some embodiments thin films of collagen are disposed on the device including in and/or on the exchange mechanism of the device. In some embodiments, a dense suspension of cells is seeded into at least one channel network and at least one channel network inlet is blocked once seeding is completed, allowing only an outflow of medium. In such embodiments, the dense suspension of cells is nourished by a flow of medium through at least a second channel network.

[00183] The scaffold device of the present invention is exceptionally diverse in design freedom, applicability, functionality, and size. Additionally, devices of the present disclosure can grow complex cells and tissues in three dimensions for a prolonged duration. As disclosed herein, a

scaffold device of the present invention includes an inlet and a plurality of channels. The plurality of channels branch in series to form at least one channel network. Furthermore, the channel network(s) replicate various natural physiological systems and internal material flow conditions while also optimizing cell density within the device.

[00184] For convenience in explanation and accurate definition in the appended claims, the terms “upper,” “lower,” “up,” “down,” “upwards,” “downwards,” “inner,” “outer,” “inside,” “outside,” “inwardly,” “outwardly,” “interior,” “exterior,” “front,” “rear,” “back,” “forwards,” “backwards,” “above,” and “over” are used to describe features of the exemplary embodiments with reference to the positions of such features as displayed in the figures.

[00185] As used in the description of the implementations and the appended claims, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[00186] The foregoing descriptions of specific exemplary embodiments of the present invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and many modifications and variations are possible in light of the above teachings. The exemplified embodiments were chosen and described in order to explain certain principles of the invention and their practical application, to thereby enable others skilled in the art to make and utilize various exemplary embodiments of the present invention, as well as various alternatives and modifications thereof. It is intended that the scope of the invention be defined by the Claims appended hereto and their equivalents.

WHAT IS CLAIMED IS:

1. A cell-scaffold device comprising:

a plurality of branching channel networks comprising

a first branching channel network,

a second branching channel network, and

a third branching channel network,

wherein the first branching channel network comprises a plurality of first channels formed by at least three generations of branching, the plurality of first channels comprising a first-generation first channel and a plurality of final-generation first channels;

the second branching channel network comprises a plurality of second channels formed by branching and recursive recombination, the plurality of second channels comprising an inlet second channel, an outlet second channel and a plurality of intermediate second channels branched from the inlet second channel and recursively recombined to the outlet second channel, wherein one or more second channels in the plurality of second channels are located in a first layer and one or more other second channels in the plurality of second channels are located in a second layer that is different than the first layer;

the third branching channel network comprises a plurality of third channels formed by at least three generations of branching, the plurality of third channels comprising a first-generation third channel and a plurality of final-generation third channels; and

the plurality of final-generation first channels of the first branching channel network is disposed adjacent to the first layer of the second branching channel network, the plurality of final-generation third channels of the third branching channel network is disposed adjacent to the second layer of the second branching channel network, the plurality of final-generation first channels of the first branching channel network and the plurality of final-generation third channels of the third branching channel network are connected with each other by a plurality of bypass channels, and each of the plurality of bypass channels bypasses the second branching channel network, thereby producing a compact three-dimensional structure with interwoven channels.

2. The cell-scaffold device of claim 1, wherein each respective branching channel network is formed from a non-resorbable material, a biodegradable material, a resorbable material, or a combination thereof.
3. The cell-scaffold device of claim 1, wherein diameters of channels in the plurality of first, second, or third channels formed by branching obey Murray's Law.
4. The cell-scaffold device according to any one of claims 1-3, wherein for a respective channel in the plurality of first, second, or third channels, a ratio of a diameter to a length of the respective channel is fixed along the respective channel.
5. The cell-scaffold device of claim according to any one of claims 1-3, wherein each respective branching channel network is configured to exhibit laminar flow of a medium located therein.
6. The cell-scaffold device of claim 1, further comprising an exchange mechanism interposing between a respective branching channel network and an adjacent corresponding branching channel network in the plurality of branching channel networks.
7. The cell scaffold device of claim 1, wherein a film of collagen is disposed on the cell scaffold device.
8. The cell scaffold device of claim 1, wherein the plurality of branching channel networks comprises an even number of branching channel networks greater than or equal to four.
9. The cell scaffold device of claim 1, wherein each channel of a respective branching channel network in the plurality of branching channel networks comprises a cross-sectional aspect ratio of one.
10. The cell scaffold device of claim 1, wherein a diameter of the inlet second channel is predetermined.

11. The cell scaffold device of claim 1, further comprising a polyhedron exterior surface configured to encompass the plurality of branching channel networks.

12. The cell scaffold device of claim 1, wherein one or more first channels of the first branching channel network are disposed adjacent to the one or more second channels located in the first layer of the second branching channel network to facilitate fluid communication between the first and second branching channel networks.

13. The cell scaffold device of claim 1, further comprising:

- a first master inlet, wherein one of the first-generation first channel and the first-generation third channel is connected to the first master inlet;

- a first master outlet, wherein the other of the first-generation first channel and the first-generation third channel is connected to the first master outlet;

- a second master inlet, wherein the inlet second channel is connected to the second master inlet; and

- a second master outlet, wherein the outlet second channel is connected to the second master outlet.

14. The cell scaffold device of claim 1, wherein each of the first, second and third channels is a pipe-like channel with a wall surrounding a hollow interior.

15. The cell scaffold device of claim 1, wherein a diameter of a smallest channel in the plurality of first, second, or third channels is predetermined.