(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2018 (27.12.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/236901 A1

(51) International Patent Classification:
C12Q 1/68 (2018.01)

(21) International Application Number:
PCT/US2018/038325

(22) International Filing Date:
19 June 2018 (19.06.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10-2017-0077641 19 June 2017 (19.06.2017) KR

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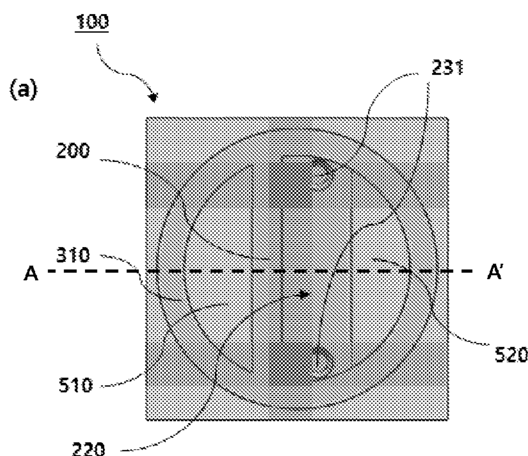
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title:** MICROFLUIDIC DEVICE HAVING PARTIALLY ENCLOSED MICROFLUIDIC CHANNEL AND USE THEREOF

FIG. 1



(57) **Abstract:** A microfluidic device in which microfluidic channels are embedded in a culture medium chamber and have open sides. The microfluidic device is patterned with a fluid moved along a hydrophilic surface due to capillary force, and the fluid may be rapidly and uniformly patterned along an inner corner path and a microfluidic channel. In the microfluidic device, the microfluidic channel is connected to facilitate fluid flow with a culture medium through open sides thereof and openings, and thus may provide a cell culture environment in which high gas saturation is maintained. In addition, several microfluidic devices formed on one common substrate are described. Such microfluidic devices may be manufactured of a hydrophilic engineering plastic by injection or molding.

WO 2018/236901 A1

MICROFLUIDIC DEVICE HAVING PARTIALLY ENCLOSED MICROFLUIDIC CHANNEL AND USE THEREOF

TECHNICAL FIELD

[0001] This application relates to microfluidic devices and methods for using the same. In particular, this application relates to methods for culturing cells or tissues using such microfluidic devices and methods for processing cells or tissues using such microfluidic devices, including co-culturing cells or tissue using a microfluidic device.

BACKGROUND

[0002] Microfluidic devices having various structures of a microfluidic channel, chamber or reservoir have been used in various fields such as three-dimensional cell co-culture. By using a microfluidic device, cells constituting a specific tissue or organ are cultured *in vitro* for the study and research of the function, characteristics, and epidemiological and physiological cell responses of the tissue or organ, and also used to replace an animal test in new drug development.

[0003] However, conventional microfluidic devices for cell culture require a microfluidic channel that defines at least four sides of a volume in which cells are cultured. Thus, the spatial activity of cells is reduced or restricted. In addition, such a conventional microfluidic device requires a separate culture medium channel through which cell culture medium is provided. In conventional microfluidic devices, the cell culture medium may not be provided evenly to cells in the microfluidic channel.

SUMMARY

[0004] Accordingly, there is a need for microfluidic devices for cell culture that address the above-discussed challenges and restrictions. The microfluidic devices described in this application address the above-discussed challenges and restrictions. In addition, the microfluidic devices described in this application address additional challenges and restrictions associated with conventional microfluidic devices, some of which are described below.

[0005] In accordance with some embodiments, a microfluidic device has a microfluidic channel which is embedded in a chamber and open at both ends.

[0006] In accordance with some embodiments, a method for patterning a fluid includes using a microfluidic device described herein.

[0007] In accordance with some embodiments, a method for culturing cells, tissue, or both cells and tissue, or co-culturing cells and tissue includes using a microfluidic device described herein.

[0008] In accordance with some embodiments, a microfluidic device having a microfluidic channel in which at least a surface in contact with a fluid is formed of a hydrophilic material includes: a substrate having a top surface and a bottom surface; outer walls which are attached (or coupled or bonded) to (or integrated with) the top surface of the substrate such that one or more cavities with an open top are formed and which include an inner surface facing the cavity and an outer surface opposite to the inner surface; inner corner paths formed by the boundary at which the inner surface of the outer wall meets the top surface of the substrate; and a microfluidic channel module including a top surface, a bottom surface and both ends, disposed in one or more cavities to cross the inside of the cavity by bonding the both ends to different positions on the inner surface of the outer wall. In the microfluidic device, a part or all of the bottom surface of the channel module is spaced apart from the top surface of the substrate to form one or more microfluidic channels connected with the inner corner path so as to be able to move a fluid by capillary force between the bottom surface of the microfluidic channel module and the top surface of the substrate. In some embodiments, a microfluidic device has a microfluidic channel which is embedded in a chamber and open at both ends, in which when an inner corner forming one closed curve has a circular, polygonal or atypical shape, and a path of the inner corner has a polygonal or atypical shape, a radius (R) value of the inner corner is 0.05 mm or more.

[0009] In some embodiments, in the microfluidic device, a part or all of the top surface of the microfluidic channel module extends upward such that a partition dividing the cavity is formed.

[0010] In some embodiments, the inner corner path forms one or more closed curves.

[0011] In some embodiments, when the closed curve has a polygonal shape, the microfluidic device includes a fillet on the inner surface of the outer wall such that a radius (R) value of a corresponding inner corner path is 0.05 mm or more. In some embodiments, the microfluidic device has a closed curve defining an inner corner path having a polygonal shape, and the microfluidic device includes a fillet of the inner surface of the outer wall, where a radius (R) value of a corresponding inner corner path is 0.05 mm or more.

[0012] In some embodiments, the microfluidic channel module includes two or more microfluidic channels sequentially increased in height.

[0013] In some embodiments, one or more recesses are formed, parallel to the microfluidic channel, on the bottom surface of the microfluidic channel module.

[0014] In some embodiments, two or more recesses are formed, parallel to the microfluidic channel, on the bottom surface of the microfluidic channel module, and sequentially increased in depth.

[0015] In some embodiments, the microfluidic channel module includes one or more openings passing from the top surface thereof to the recess such that a cavity formed on the top surface and the recess are connected to facilitate fluid flow.

[0016] In some embodiments, the microfluidic device includes a culture medium inlet for injecting a culture medium into the upper portion of a recess and a culture medium outlet for discharging the culture medium.

[0017] In some embodiments, the microfluidic device has a well shape.

[0018] In some embodiments, in the microfluidic device, two or more cavities are formed by an outer wall and a substrate, and the cavities are connected each other to facilitate fluid flow.

[0019] In some embodiments, a fluid patterning method includes using a microfluidic device described herein, which includes applying a patterning fluid to one position on an inner corner path; and performing patterning of the patterning fluid along the inner corner path and a microfluidic channel by capillary force.

[0020] In some embodiments, a microfluidic chip has a multi-well microfluidic device in which two or more microfluidic devices are formed on a common substrate and connected such that selective fluid flow is facilitated. In this case, there may be 4, 8, 16, 24, 48, 96 or 256 microfluidic device wells formed on one common substrate if necessary, but the embodiments are not limited thereto.

[0021] In some embodiments, a three-dimensional co-culture method for one or more among cells and tissue includes patterning a fluid including one or more among cells and tissue and providing a culture medium through a cavity using a microfluidic device described herein.

[0022] In accordance with some embodiments, a device includes a substrate having a top surface and a bottom surface opposite to the top surface; and one or more beams. A respective beam of the one or more beams has a bottom surface facing the top surface of the substrate and a top surface opposite to the bottom surface of the respective beam facing away from the top surface of the substrate. The respective beam is positioned adjacent to the substrate. At least a portion of the respective beam is spaced apart from the top surface of the substrate to

define one or more microfluidic channels to enable movement of a fluid by capillary force between the bottom surface of the respective beam and the top surface of the substrate along the one or more microfluidic channels.

[0023] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes flowing a liquid between the bottom surface of the respective beam of any device described herein and the top surface of the substrate of the device.

[0024] In accordance with some embodiments, a method includes, while a first liquid remains between the first linear portion of any device described herein as having a vertical divider defining a first chamber and the substrate, a second liquid remains between the second linear portion of the device and the substrate, and a third liquid remains between the third linear portion of the device and the substrate, providing a fourth liquid to the first chamber.

[0025] The "microfluidic channel" used herein refers to a path of fluid flow. In some cases, a fluid path defines a space in which cells or tissue is cultured and which is open at both sides to be connected with another flow path or chamber so as to allow exchange of a culture medium and a fluid between adjacent fluids or chambers.

[0026] The "cavity," "chamber" or "reservoir" refers to a space for containing a culture medium to culture cells or tissue. The "inner corner path" used herein refers to a path of fluid flow by capillary force as a boundary at which the inner surface of an outer wall meets a substrate.

[0027] The "culture medium channel" refers to a channel or flow path for providing a culture medium to cells from a chamber or reservoir containing a culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] For a better understanding of the various described embodiments, reference should be made to the Description of Embodiments below, in conjunction with the following drawings in which like reference numerals refer to corresponding parts throughout the figures.

[0029] FIG. 1 depicts (a) a plan view and (b) a side view of a microfluidic device in accordance with some embodiments.

[0030] FIG. 2 is a cross-sectional view of the microfluidic device of FIG. 1.

[0031] FIG. 3 is a schematic diagram illustrating a fluid patterning process using the microfluidic device in accordance with some embodiments.

[0032] FIG. 4 illustrates (a) a microfluidic device including a step-like microfluidic channel in accordance with some embodiments and fluid patterning using the same and (b) a

microfluidic device having a structure in which adjacent microfluidic channels have different heights in accordance with some embodiments and fluid patterning using the same.

[0033] FIG. 5 illustrates four well-type microfluidic devices formed on a common substrate in accordance with some embodiments. FIG. 5 shows (a) the microfluidic devices connected to selectively move a fluid from a microfluidic device to another microfluidic device, (b) the microfluidic devices having a common supply path, and (c) the microfluidic devices having separate supply paths.

[0034] FIG. 6 schematically illustrates the structure of a stopper of the microfluidic device and a process of stopper sealing in accordance with some embodiments.

[0035] FIG. 7 is a schematic diagram illustrating that patterning areas and thicknesses are adjusted by adjusting the amount of a fluid applied to a microfluidic device in accordance with some embodiments.

[0036] FIG. 8 is a set of diagrams comparing (a) a microfluidic device according to the conventional art and (b) a microfluidic device having an open-type microfluidic channel embedded in a chamber in accordance with some embodiments.

[0037] FIG. 9 shows the direction of fluid flow allowed by an open-type microfluidic channel after two chambers divided by a partition are filled with a culture medium at different heights.

[0038] FIG. 10 shows various types of inner corner paths in accordance with some embodiments.

[0039] FIG. 11 shows an angiogenesis experiment performed using a device described herein.

[0040] FIG. 12 shows example time-lapse fluorescence images of angiogenesis obtained using a device described herein.

[0041] FIG. 13 shows example perfusability test images obtained using a device described herein.

[0042] FIG. 14 shows example lymphangiovasculogenesis obtained using a device described herein.

[0043] FIG. 15 illustrates a microfluidic device in accordance with some embodiments.

[0044] FIG. 16 shows example angiogenesis images obtained using a device described herein.

[0045] FIG. 17 shows example vasculogenesis images obtained using a device described herein.

[0046] FIG. 18 shows images confirming formation of vascular system using a device described herein.

[0047] FIG. 19 shows reproducibility of vascular system formed using a device described herein.

[0048] FIG. 20 shows uniformity of vascular system formed using a device described herein.

[0049] FIG. 21 shows images showing cell assays using a device described herein.

[0050] FIG. 22 shows images obtained by real-time monitoring of cytotoxic activity of NK-92 cells using a device described herein.

[0051] FIG. 23 shows migration and cytotoxic activity of NK-92 cells, as monitored using a device described herein.

[0052] FIG. 24 shows migration and cytotoxic activity of NK-92 cells against various concentrations of HeLa cells, as monitored using a device described herein.

[0053] FIG. 25 shows a vascularized tumor spheroid formed by using a device described herein.

[0054] FIG. 26 illustrates a method of forming a vascularized tumor spheroid using a described herein in accordance with some embodiments.

[0055] FIG. 27 shows an image of a tumor spheroid formed by using a device described herein.

[0056] FIG. 28 shows tumor spheroids that have been formed by using a device described herein.

[0057] FIG. 29 shows an effect of a tyrosine kinase inhibitor on a tumor spheroid, as monitored using a device described herein.

[0058] FIG. 30 shows a structure of a device for high-throughput experiment.

[0059] FIG. 31 shows reconstruction of a three-dimensional neural network in the PMMB.

[0060] FIG. 32 shows reconstruction of a three-dimensional BBB with coculture of CNS neuron-astrocyte-HUVEC in the PMMB.

[0061] FIG. 33 shows reconstruction of a three-dimensional formation of myelin sheaths in the PMMB.

DETAILED DESCRIPTION

[0062] Reference will now be made to embodiments, examples of which are illustrated in the accompanying drawings. In the following description, numerous specific details are set forth in order to provide an understanding of the various described embodiments. However, it will be apparent to one of ordinary skill in the art that the various described embodiments

may be practiced without these specific details. In other instances, well-known methods, procedures, components, circuits, and networks have not been described in detail so as not to unnecessarily obscure aspects of the embodiments.

[0063] FIG. 1 depicts (a) a plan view and (b) a side view of a microfluidic device 100 in accordance with some embodiments. FIG. 1 shows line AA' represents a view from which the cross-section shown in FIG. 2 is taken.

[0064] FIGS. 1 and 2, the microfluidic device 100 includes a substrate 320, outer wall(s) 310, a cavity including chambers 510 and 520, and a microfluidic channel module 220, a partition 200, a microfluidic channel module 220 with one or more openings 231.

[0065] In some embodiments, the microfluidic device 100 is manufactured to overall have a hydrophilic surface on components. Alternatively, the microfluidic device 100 may be manufactured to have a hydrophilic surface at least in contact with a fluid.

[0066] The substrate 320 includes a top surface and a bottom surface, and preferably has a flat plate shape as shown in the illustrated examples.

[0067] The outer wall 310 includes an inner surface facing a cavity to be described below and an outer surface opposite to the inner surface. In some embodiments, the outer wall has a cylindrical shape with an open top and bottom. However, the shape of the outer wall is not limited to the cylindrical shape with an open top and bottom, and the outer wall may have various shapes such as a hollow polygonal column or elliptical cylindrical shape. In some embodiments, the outer wall 310 has a shape in which a plurality of cylindrical shapes is connected and their sides are open to each other as shown in FIG. 5(a). In some embodiments, the outer wall 310 is manufactured in a shape such as a cylinder block including a plurality of cylinders as shown in FIG. 5(b) or 5(c).

[0068] In some embodiments, the outer walls 310 are attached, coupled, or bonded to (or integrated with) the top surface of the substrate 320, thereby forming a cavity with an open top with the substrate 320. There is no limit in the number of cavities formed with the outer walls 310 and the substrate 320. For example, as described with respect to FIGS. 1 and 2, one cavity may be formed, or as described with respect to FIG. 5, four cavities may be formed, or if necessary, 8, 16, 24, 48, 96 or 256 cavities may be formed. As described above, in FIG. 5(a), four cavities are connected to facilitate fluid flow.

[0069] When the outer wall 310 is attached, coupled, bonded to (or integrated with) the substrate 320, the boundary at which the inner surface of the outer wall 310 meets the top surface of the substrate 320 forms an inner corner path. For example, in FIGS. 1 and 2, the inner corner path may be a radius shape. In FIG. 5(a), the inner corner path has a shape in

which four ellipses are connected. In FIGS. 5(b) and 5(c), the inner corner paths are formed with four separated ellipses.

[0070] In some embodiments, the microfluidic channel module 220 is disposed in each cavity. The microfluidic channel module 220 may have an elongated bar or long rod shape with top and bottom surfaces and both ends. In FIG. 1, one end of the microfluidic channel module 220 is attached to one position on the inner surface of the outer wall 310 and the other end is attached to another position on the inner surface of the outer wall 310, and therefore the microfluidic channel module may be disposed so as to cross the inside of the corresponding cavity.

[0071] In some embodiments, a part or all of the top surface of the microfluidic channel module 220 forms a partition 200 extending upward (that is, a direction of an opening to the cavity). In FIGS. 2 to 3, 7 and 9, the configuration includes a partition 200 in which a part of the top surface extends up to the top of the cavity. The partition 200 divides the cavity into the two chambers 510 and 520. Each chamber 510 or 520 may accommodate a different culture medium.

[0072] Meanwhile, when the microfluidic channel module 220 is disposed in a corresponding cavity, at least a part, preferably all, of the bottom surface of the microfluidic channel module 220 is disposed apart from the top surface of the substrate 320 forming a microfluidic channel (microfluidic flow path) facilitating fluid flow between the bottom surface and the top surface of the substrate 320 by capillary force. In some embodiments, by forming a recess, parallel to the microfluidic channel, on the bottom surface of the microfluidic channel module 220, a plurality of microfluidic channels with different heights is formed. For example, in FIG. 2, as one recess is formed in the bottom surface along a longitudinal direction of the microfluidic channel module 220, three microfluidic channels 221a, 221b and 222 with different heights are formed. However, the number and height of the microfluidic channels are not limited thereto, but may vary if necessary. That is, in some embodiments, the microfluidic device 100 includes n recesses and m protrusions on the bottom surface of the microfluidic channel module (where n is an integer number and m is an integer number). In some cases, n and m satisfy the following relationship $m = n+1$. In some cases, n and m satisfy the following relationship $m=n$. In some cases n and m satisfy the following relationship: $m = n-1$.

[0073] In FIG. 2, the microfluidic channels 221a, 221b and 222 may be filled with a fluid moved by capillary force, and have an open structure (e.g., sides of the microfluidic channels are not at least completely enclosed) in which the microfluidic channels are connected to

facilitate fluid exchange with a fluid in an adjacent microfluidic channel or the chamber 510 or 520 and at both sides of the microfluidic channel (e.g., right and left sides of 221a of FIG. 2). Therefore, the microfluidic channel of the microfluidic device manufactured as described in this application may be open (in that the microfluidic channel has no or partial side walls), reversible, and transient.

[0074] In addition, the inner corner path 340 may have a circular, elliptical, polygonal or atypical shape (e.g., FIG. 10). When the inner corner path 340 has a polygonal shape, since the inner corner paths have to be tangentially connected to perform continuous patterning of a fluid, a fillet having a radius (R) value of 0.05 mm or more may be preferably included at an outer wall corner corresponding to the part at which polygonal sides meet with each other.

[0075] The microfluidic device may be manufactured of an engineering plastic so as to have a contact angle of 90 degrees or less (based on distilled water (DW)).

[0076] Meanwhile, to provide a fluid into the microfluidic channel, for example, the microfluidic channel 222, or discharge a fluid from the microfluidic channel, in the top surface of the microfluidic channel module 220, one or more openings 231 may be formed by passing from the top surface to the corresponding microfluidic channel. Preferably, for example, as in the exemplary embodiment shown in FIG. 1, to provide a fluid into the microfluidic channel 222 formed by a recess or discharge a fluid therefrom, an opening 231 may be formed at each end of the microfluidic channel module 220 in the top surface corresponding to the vertically upper side of the microfluidic channel 222. With this configuration, the microfluidic channel 222 is connected to facilitate fluid flow with the chamber 520 through the opening 231.

[0077] In some embodiments, the inner diameter of the outer wall 310 is at least 3 mm, at least 4 mm, at least 5 mm, at least 6 mm, at least 7 mm, at least 8 mm, at least 9 mm, at least 10 mm, at least 11 mm, at least 12 mm, at least 13 mm, at least 14 mm, or at least 15 mm. In some embodiments, the inner diameter of the outer wall 310 is at most 3 mm, at most 4 mm, at most 5 mm, at most 6 mm, at most 7 mm, at most 8 mm, at most 9 mm, at most 10 mm, at most 11 mm, at most 12 mm, at most 13 mm, at most 14 mm, or at most 15 mm.

[0078] In some embodiments, the microfluidic channels 221a and the microfluidic channels 221b have a height of at least 0.05 mm, at least 0.06 mm, at least 0.07 mm, at least 0.08 mm, at least 0.09 mm, at least 0.1 mm, at least 0.11 mm, at least 0.12 mm, at least 0.13 mm, at least 0.14 mm, at least 0.15 mm, at least 0.16 mm, at least 0.17 mm, at least 0.18 mm, at least 0.19 mm, or at least 0.20 mm. In some embodiments, the microfluidic channels 221a and the microfluidic channels 221b have a height of at most 0.05 mm, at most 0.06 mm, at most 0.07

mm, at most 0.08 mm, at most 0.09 mm, at most 0.1 mm, at most 0.11 mm, at most 0.12 mm, at most 0.13 mm, at most 0.14 mm, at most 0.15 mm, at most 0.16 mm, at most 0.17 mm, at most 0.18 mm, at most 0.19 mm, or at most 0.20 mm.

[0079] In some embodiments, the microfluidic channels 221a and the microfluidic channels 221b have a width of at least 0.5 mm, at least 0.6 mm, at least 0.7 mm, at least 0.8 mm, at least 0.9 mm, at least 1.0 mm, at least 1.1 mm, at least 1.2 mm, at least 1.3 mm, at least 1.4 mm, at least 1.5 mm, at least 1.6 mm, at least 1.7 mm, at least 1.8 mm, at least 1.9 mm, or at least 2.0 mm. In some embodiments, the microfluidic channels 221a and the microfluidic channels 221b have a width of at most 0.5 mm, at most 0.6 mm, at most 0.7 mm, at most 0.8 mm, at most 0.9 mm, at most 1.0 mm, at most 1.1 mm, at most 1.2 mm, at most 1.3 mm, at most 1.4 mm, at most 1.5 mm, at most 1.6 mm, at most 1.7 mm, at most 1.8 mm, at most 1.9 mm, or at most 2.0 mm.

[0080] In some embodiments, the microfluidic channel 222 has a height of at least 0.05 mm, at least 0.1 mm, at least 0.2 mm, at least 0.3 mm, at least 0.4 mm, at least 0.5 mm, at least 0.6 mm, at least 0.7 mm, at least 0.8 mm, at least 0.9 mm, at least 1.0 mm, at least 1.1 mm, at least 1.2 mm, at least 1.3 mm, at least 1.4 mm, at least 1.5 mm, at least 1.6 mm, at least 1.7 mm, at least 1.8 mm, at least 1.9 mm, at least 2.0 mm, at least 2.1 mm, at least 2.2 mm, at least 2.3 mm, at least 2.4 mm, or at least 2.5 mm. In some embodiments, the microfluidic channel 222 has a height of at most 0.05 mm, at most 0.1 mm, at most 0.2 mm, at most 0.3 mm, at most 0.4 mm, at most 0.5 mm, at most 0.6 mm, at most 0.7 mm, at most 0.8 mm, at most 0.9 mm, at most 1.0 mm, at most 1.1 mm, at most 1.2 mm, at most 1.3 mm, at most 1.4 mm, at most 1.5 mm, at most 1.6 mm, at most 1.7 mm, at most 1.8 mm, at most 1.9 mm, at most 2.0 mm, at most 2.1 mm, at most 2.2 mm, at most 2.3 mm, at most 2.4 mm, or at most 2.5 mm.

[0081] In some embodiments, the microfluidic channel 222 has a width of at least 0.5 mm, at least 0.6 mm, at least 0.7 mm, at least 0.8 mm, at least 0.9 mm, at least 1.0 mm, at least 1.1 mm, at least 1.2 mm, at least 1.3 mm, at least 1.4 mm, at least 1.5 mm, at least 1.6 mm, at least 1.7 mm, at least 1.8 mm, at least 1.9 mm, or at least 2.0 mm. In some embodiments, the microfluidic channel 222 has a width of at most 0.5 mm, at most 0.6 mm, at most 0.7 mm, at most 0.8 mm, at most 0.9 mm, at most 1.0 mm, at most 1.1 mm, at most 1.2 mm, at most 1.3 mm, at most 1.4 mm, at most 1.5 mm, at most 1.6 mm, at most 1.7 mm, at most 1.8 mm, at most 1.9 mm, or at most 2.0 mm.

[0082] FIG. 3 is a schematic diagram illustrating a fluid patterning method in accordance with some embodiments. First, for first patterning, a first fluid 344 is provided to an arbitrary

part on the inner corner path 340 (e.g., shown in FIG. 2) in the second chamber 520. As shown in FIG. 3(c), a droplet of first fluid 344 provided on hydrophilic surface adjacent to the inner corner path 340 spreads along the inner corner path 340. In some embodiments, the provided fluid 344 flows in both directions along the inner corner path by capillary force (e.g., the provided fluid 344 spreads along the inner corner path to form a spread fluid 332 (e.g., FIG. 3(b)), which continuously flows along the microfluidic channels 221b and 221a to as fluids 330, and then sequentially patterns along the inner corner path 340 of the first chamber 510). Afterward, when a second fluid 334 for second patterning is injected through the opening 231, the microfluidic channel 222 formed by the recess is patterned or filled with the second fluid 334 (e.g., FIG. 3(a)). In some embodiments, a culture medium is injected into each of the chambers 510 and 520 (e.g., FIG. 3(a)).

[0083] FIG. 4 illustrates microfluidic channel modules 250 and 260 in accordance with some embodiments. In FIG. 4(a), four microfluidic channels 223a, 223b, 223c and 223d are formed to have sequentially increased heights by sequentially including step-like protrusions with different heights on the bottom surface of the microfluidic channel module 250. In the exemplary embodiment shown in FIG. 4(b), recesses 262a and 262b with different depths are sequentially formed in a step shape with different heights on the bottom surface of the microfluidic channel module 260.

[0084] As shown in FIG. 4(a), when the microfluidic channels 223a, 223b, 223c and 223d with different heights are adjacent to each other, a fluid may be sequentially patterned from the microfluidic channel 223a with the lowest height to a microfluidic channel with a higher height. While the difference in height of the microfluidic channels may vary according to purpose, the microfluidic channels are preferably formed to have a difference in height of 0.05 mm or more, and the highest microfluidic channel preferably has a height of 1 mm or less.

[0085] As the microfluidic channels are formed as shown in FIG. 4(b), the microfluidic channels 261a and 261b with the lowest height are included at the left and right sides of the drawing, and two microfluidic channels 262a and 262b with higher heights are sequentially disposed between them. In this configuration, the microfluidic channels 261a and 261b with the lowest height are first patterned at the left and right sides, and a fluid is injected through an opening (not shown in FIG. 4) located in the top surface of the microfluidic channel module 220, resulting in the patterning of the microfluidic channels 262a and 262b. The microfluidic channels 261a and 261b may have the lowest height of 500 μm or less, but are

not limited thereto, and the microfluidic channels may have various heights according to purpose or necessity.

[0086] FIG. 5 illustrates two or more microfluidic devices 100 implemented on one common substrate 600 in accordance with some embodiments.

[0087] In the exemplary embodiment shown in FIG. 5(a), the outer walls of the microfluidic devices are configured such that cavities of the microfluidic devices are connected to facilitate fluid flow between the cavities. In this configuration, since inner corner paths are connected such that a fluid can flow between the cavities, a fluid may be provided to an arbitrary position on an inner corner path of an arbitrary microfluidic device, and yet the fluid can flow to multiple cavities by capillary force and pattern microfluidic channels in the multiple cavities.

[0088] In FIGS. 5(b) and 5(c), cavities of the microfluidic device are not connected. In such configurations, the microfluidic devices may be patterned separately by fluid inlets 232 and fluid injection paths 242, which are separately formed (e.g., as shown in FIG. 5(c)), or patterned at one time by one fluid inlet 232 and a branched fluid injection path 241 (e.g., as shown in FIG. 5(b)). Each microfluidic device may be manufactured as a well type.

[0089] Referring to FIG. 6, in the microfluidic device 100 which has the first chamber 510 and the second chamber 520 divided by the partition 200 extending upward in the microfluidic channel module 220, a stopper portion 602 is included to prevent fluid flow between the first chamber 510 and the second chamber 520. The stopper portion 602 includes a hollow structure for stopping fluid patterning through the inner corner path 340. Applying a sealant (e.g., a gel) to the stopper portion 602 prevents the fluid flow between the microfluidic channel in the first chamber 510 and the microfluidic channel in the second chamber 520. In some embodiments, for additional patterning by blocking both open sides of a microfluidic channel, a curable (e.g., post-curable) material such as a gel is used for patterning.

[0090] Various types of fluid patterning may be performed using the microfluidic device 100 described herein. A fluid on the hydrophilic surface changes its shape and flows along the contact surface due to capillary force, and moves without a separate external force until the capillary force is in equilibrium. A patterning (edge-guided patterning; EGP) method through the inner corner path 340 of the microfluidic device 100 allows fluid flow along an inner corner having a right angle, acute angle or obtuse angle using such capillary force (FIG. 3(c)). In some embodiments, the EGP method provides a means capable of patterning a fluid at one time (refer to FIG. 3(b)). Specifically, a fluid provided at an arbitrary position on the inner

corner path 340 of the microfluidic device 100 moves along the hydrophilic surface due to capillary force, and further moves to the microfluidic channel 221b by a stronger capillary force caused by the microfluidic channel 221b at both ends thereof, thereby patterning a microfluidic channel with open sides. In addition, the fluid flowing to the microfluidic channel 221b further moves by a different inner corner path connected to the microfluidic channel 221b. According to such a method, desired fluid patterning may be realized through a single injection of a fluid.

[0091] In some embodiments, patterning may be performed step-by-step or the position and form of patterning may be controlled by adjusting an amount of the fluid provided for patterning (e.g., using a pipette 702). This is useful when precise adjustment of patterning thickness or width of a polymeric material serving as a cellular or extracellular matrix is needed. When an excessive amount of a fluid is injected, the fluid remaining after filling all flow paths further moves to a direction of a stronger capillary force, such that the thickness or width of a specific area increases. In some embodiments, as shown in FIG. 7(a), at least one edge in the bottom surface of a microfluidic channel module constituting a microfluidic channel is obliquely cut by a process such as chamfering. In such embodiments, when an excessive amount of a fluid is provided as shown in FIG. 7(b), the fluid flows along the oblique side. When a smaller amount of a fluid is injected as shown in FIG. 7(c), only some of the flow paths may be patterned with the fluid moving to a direction of a strong capillary force. This allows a subsequent provision of a different liquid to pattern one or more remaining flow paths.

[0092] FIG. 8 is a set of diagrams comparing (a) a microfluidic device according to the conventional art and (b) a microfluidic device in accordance with some embodiments. While the conventional microfluidic device has separate reservoirs 802 providing fluid 804 (e.g., culture medium) through microfluidic channels to cell location 806 (thus multiple reservoirs are exposed to air), the microfluidic device in accordance with some embodiments has a single-well shape, which includes at least partially open microfluidic channels.

[0093] FIG. 9 shows the direction of fluid flow allowed by an open-type microfluidic channel after two chambers divided by a partition are filled with liquids. In some embodiments, the microfluidic channels are filled with liquid-permeable material. In some embodiments, the first chamber is filled with a first liquid 904 and the second chamber is filled with a second liquid 906 that is distinct from the first liquid. In some embodiments, the first chamber and the second chamber are filled with a same liquid. In some embodiments, the first chamber is filled with a liquid of a first height and the second chamber is filled with

a liquid of a second height. In some embodiments, the gravitational force (and a pressure associated with it) causes flow of liquid from one chamber to the other chamber (e.g., from a chamber having a liquid with a greater height to a chamber having a liquid with a lower height, such as from the first chamber filled with the first liquid 904 to the second chamber filled with the second liquid 906). In some embodiments, the liquid flows between the chambers through other mechanisms (e.g., osmosis, etc.).

[0094] FIG. 11 illustrates an angiogenesis experiment performed using a device described herein. (a) Cell-seeding configuration for the angiogenesis experiment. From left to right: human umbilical vein endothelial cells (HUVECs) attached on the left side of the acellular fibrin matrix in the left channel (LC, h=100 μm). Lung fibroblasts (LFs) mixed in fibrin are patterned in the middle channel (MC). Acellular fibrin in the right channel (RC). (b) Fluorescence image of the angiogenic sprout at Day 2 showing endothelial tip cells invading towards the LF channel. Boundaries of the LC acellular matrix are denoted by the dotted lines. Scale bar=200 μm. (c) Fluorescence micrograph at Day 5 show that EC sprouts invaded about 700 μm. Scale bar=200 μm. (d) Confocal cross section image of the sprouts after Day 5 clearing showing open lumen. Scale bar=100 μm. (e) vascular endothelial (VE)-cadherin immunostained image confirms the strong expression of tight junction protein VE-cadherin in the vessels. Scale bar=50 μm.

[0095] In obtaining the results shown in FIG. 13, the following steps were taken.

Cell preparation

[0096] HUVECs (Lonza) were cultured in endothelial growth medium 2 (EGM-2, Lonze). LF (Lonza) were cultured in fibroblast growth medium 2 (FGM-2, Lonza). The cells were incubated at 37℃ in 5% CO2 for three days prior to loading in the devices. Cultured LFs and HUVECs are removed from the culture dish using 0.25% Trypsin-EDTA (Hyclone). LFs are then re-suspended in a bovine fibrinogen solution at a cell concentration of 5 × 106 cells ml-1 and HUVECs are re-suspended at a concentration of 7 × 106 cells ml-1 in EGM-2.

Cell seeding in the device

[0097] Before starting the cell seeding, the hydrophilicity of the inner surface of the device was improved by 1 minute of plasma treatment (Cute, Femto Science, Korea) with a power of 50 W. To avoid degradation of hydrophilicity, liquid solution patterning was started without exceeding 30 minutes after plasma treatment. Immediately after mixing the cell-free fibrinogen solution with thrombin (0.5 U/ml, Sigma), one of the inner wedges formed by the bottom and sidewalls of the device was selected and dropped by 3 μl. A small amount of

liquid moves along the hydrophilic wedge centered on the dropping position, and the remaining two channels (depth: 100 μm) except for the middle channel (MC) are filled, and they are left at room temperature for 4 minutes and clotted. As a result, the MC becomes a closed channel with only two holes at both ends, creating a situation where a new liquid can be loaded. Immediately after mixing thrombin (0.5 U/ml) with the fibrinogen solution containing LFs (cell concentration of 5 × 106 cells ml-1), the MC was filled with a pipette. After a 3-minute waiting period, a total of 200 μl of EGM-2 medium was loaded onto two reservoirs in semicircular form after the polymerization was completed. For fixation of LFs in the fibrin matrix, the device was incubated for 18 hours at 37℃ and 5% CO2. After the medium in the medium reservoir was removed, 20 μl of EGM-2 solution (cell concentration 5 × 106 cells ml-1) containing HUVECs prepared in advance was loaded into left reservoir (LR). To attach the HUVECs to the fibrin matrix lateral surface using gravity, the device was incubated in the incubator for 30 minutes at an angle of 90 degrees. Then, the entire reservoir of the device was filled with EGM-2 and stored in an incubator. After 3 days from the start of co-culture, the medium was replaced with fresh EGM-2.

Immunostaining

[0098] Co-cultured tissues in the device were fixed with 5% (w/v) paraformaldehyde (Biosesang) in PBS (Gibco) for 15 minutes, followed by permeabilization with a 20 minutes immersion in 0.15% Triton X-100 (Sigma). Samples were then treated with a 1-hour immersion in 3% BSA (Sigma) to prevent nonspecific antibody binding. Fluorescence-conjugated monoclonal mouse anti-human VE-cadherin (eBioscience) and anti-human CD31 (BioLegend) primary antibody dyes were prepared in a 1:200 dilution and applied to the tissue samples overnight at 4℃. DNA staining was done with a 1:1000 dilution of Hoechst 3342 (Molecular Probes) for 1 hour at room temperature. Imaging was done via confocal microscopy (Olympus FV1000) at 10x and 20x to produce a three-dimensional renderable projection of the angiogenic sprouts.

[0099] FIG. 12 shows example time-lapse fluorescence images of angiogenesis obtained using a device described herein.

[00100] FIG. 13 shows example perfusability test images obtained using a device described herein. Perfusable vessel was verified by flowing FITC-dextran dye from one side of channel.

[00101] FIG. 14 example lymphangiovasculogenesis obtained using a device described herein. Lymphangiovasculogenesis is formation of network by lymphatic endothelial cells (LECs).

[00102] FIG. 15 illustrates a microfluidic device in accordance with some embodiments.

[00103] The microfluidic device shown in FIG. 15 has a hollow structure where a hollow center of the structure is facing toward the substrate.

[00104] In some embodiments, the microfluidic device shown in FIG. 15 is made by injection molding.

[00105] In some embodiments, the hollow structure has a round cross-section (e.g., having a shape of a donut or a circle).

[00106] FIG. 15(A) shows the structure of the microfluidic device, which has a round shape. The microfluidic device is divided into cell patterning parts and media reservoir areas.

[00107] FIG. 15(B) shows a photograph of a device manufactured by injection molding.

[00108] FIG. 15(C) shows that the device is treated to have hydrophilic surfaces. The hydrogel patterning is carried out by a droplet of hydrogel on a surface, where the hydrogel is spontaneously drawn under the structure that acts as a "liquid guide" (e.g., by capillary force).

[00109] FIG. 15(D) shows that the device accommodates more than one hydrogel and media in some embodiments.

[00110] FIG. 15(E) shows images of an actual microfluidic device, viewed from the bottom through the patterning operations.

[00111] FIG. 16 shows example angiogenesis images obtained using a device described herein (e.g., the microfluidic device shown in FIG. 15). FIG. 16 shows that the device can serve as a microfluidic platform for angiogenesis studies.

[00112] FIG. 16(A) shows that, after the acellular fibrin gel is patterned under the guide structure, Normal Human Lung Fibroblasts (NHLFs) are loaded in the center, and the HUVECs are seeded on the outer edge. Since NHLFs, positioned at the center, secrete vascular endothelial growth factor radially, vascular sprouting also converges toward the center area. FIG. 16(B) is a confocal image of sprouting vessels. FIG. 16(C) is a fluorescence image of a part of the device. FIG. 16(D) a high-magnification image showing morphology of blood vessels. FIG. 16(G) is a confocal cross section image of the blood vessels, which confirms the formation of the lumen.

[00113] FIG. 17 shows example vasculogenesis images obtained using a device described herein. FIG. 17 shows that the device can serve as a microfluidic platform for vasculogenesis studies.

[00114] FIG. 17(A) shows that the HUVECs and fibrin gel mixture is patterned under the guide structure and the NHLFs are loaded at the center. Additional NHLFs are seeded on the outer edge. Since the central NHLFs secretes vascular endothelial growth factor in a radial manner, the vascular network is formed uniformly in a circular pattern.

[00115] FIG. 17(B) is a confocal image of vascular network. FIG. 17(C) is a fluorescence image of a part of the device. FIG. 17(D) is a high-magnification image showing morphology of blood vessels. FIG. 17(G) is a confocal cross section image of the blood vessels confirms the formation of the lumen.

[00116] FIG. 18 shows images confirming formation of vascular system using a device described herein. Immunostaining for ZO-1 (A) and VE-Cadherin (B) tight junction protein expression by CD 31 positive endothelial cells confirms formation of the engineered vascular system. Confocal micrographs of the engineered vessels stained for the major components of substrate membrane, Laminin (C) and Collagen IV (D), also confirm formation of the engineered vascular system.

[00117] FIG. 19 reproducibility of vascular system formed using a device described herein (e.g., the device shown in FIG. 15). The reproducibility of the outcome obtained from each microchip is important for this platform which is mass-produced through injection molding. To demonstrate reproducibility, vasculogenesis and angiogenesis experiments were carried out under the same conditions. Experimental results were collected and quantified based on vessel characteristics. In the case of vasculugenesis, the representative model was compared and analyzed for vessel area and total number of junctions. In terms of angiogenesis, the total vessel length and vessel area were analyzed. These results show that the vascular system formed using the device is highly reproducible.

[00118] FIG. 20 shows uniformity of vascular system formed using a device described herein (e.g., the device shown in FIG. 15). The device shown in FIG. 15 is characterized by a circle shape, which has the advantage of being capable of factor supply from the center radially. Eight pieces of each sample were divided identically, and the quantification was performed on each section based on the characteristics of the blood vessels. In the case of vasculogenesis, blood vessel density and total number of junctions were analyzed. And, angiogenesis samples were analyzed by vessel length and total number of junctions. The results show that the vascular system formed using the device has a high uniformity among the sections.

[00119] FIG. 21 shows images showing cell assays using a device described herein (e.g., the device shown in FIG. 1). FIG. 21(a) shows the configuration of patterned cells. HeLa cells

encapsulated in collagen were patterned between the lower rails and bottom surface and NK cell suspension was loaded in the channel formed by the patterned collagens. FIG. 21(b) shows NK-92 cells migrated into the gel by killing HeLa cells in the path of migration. Other cells left in the medium aggregated into spheroids.

[00120] FIG. 22 shows images obtained by real-time monitoring of cytotoxic activity of NK-92 cells using a device described herein. NK-92 cells move around the HeLa cells by making the edges of HeLa cells lose the connection with collagen to have a sphere shape for longer than one hour and dead signals appeared about two hours later of shrinkage.

[00121] FIG. 23 shows migration and cytotoxic activity of NK-92 cells in various collagen concentrations to reconstitute tumor-associated fibrosis, as monitored using a device described herein. FIG. 23(a) shows that NK-92 cells migrated inactively in the denser collagens and dead signals were frequently observed at the front of migration of NK-92 cells. FIG. 23(b) shows the populations of dead signals according to the distance from the side where NK-92 cells were adhered. The collagen block with width of 800 μm, patterned between a rail and the bottom surface, is divided into five regions according to the distance and the populations of dead signals were plotted. In 2 and 3.5 mg/ml of collagens, dead signals were mostly observed at the front of NK-92 cells migration. In 5 mg/ml of collagen, however, the environment was so stiff that HeLa cells had lower viability than other concentrations across all regions.

[00122] FIG. 24 shows migration and cytotoxic activity of NK-92 cells against various concentrations of HeLa cells, as monitored using a device described herein. After 18 hours of co-culture, NK-92 cells killed almost all of the HeLa cells patterned with 1 x 10^6 cells/ml. In case of the largest number of HeLa cells, migration of NK-92 cells seems to be limited due to the cytotoxic activity of NK-92 cells instead of migrating into the gel.

[00123] FIG. 25 shows a vascularized tumor spheroid formed by using a device described herein (e.g., the device shown in FIG. 15).

[00124] FIG. 26 illustrates a method of forming a vascularized tumor spheroid using a described herein (e.g., the device shown in FIG. 15) in accordance with some embodiments. A tumor spheroid was cultured in a u-shaped culture plate, and was subsequently co-cultured in the microfluidic device with fibroblasts to obtain a vascularized tumor spheroid.

[00125] FIG. 27 shows an image of a tumor spheroid formed by using a device described herein (e.g., the device shown in FIG. 15). Using a tissue clearing method, the tumor spheroid having approximate diameter of 1 mm was imaged.

[00126] FIG. 28 shows tumor spheroids that have been formed by using a device described herein. FIG. 28 shows that a tumor spheroid co-cultured with vascular endothelial cells is more effective in vasculogenesis than a tumor spheroid that has not been co-cultured with vascular endothelial cells.

[00127] FIG. 29 shows an effect of a tyrosine kinase inhibitor (e.g., axitinib) on a tumor spheroid, as monitored using a device described herein. Axitinib is a tyrosine kinase inhibitor that selectively inhibits vascular endothelial growth factor VEGFR-1, VEGFR-2, and VEGFR-3. Axitinib also inhibits platelet-derived growth factor (PDGF). FIG. 29 shows that axitinib has reduced the vessel area, the diameter of vessels, and the number of vessels interacting with the tumor spheroid.

[00128] FIG. 30 illustrates a structure of a device (called herein PMMB) for high-throughput experiment.

[00129] FIG. 30(a) is an example photograph of a PMMB device. FIG. 30(b) is a schematic diagram of a 96 well microplate with 96 independent PMMB devices for high-throughput experiment and drug screening. FIG. 30(c) is a schematic diagram illustrating a PMMB device including two hydrogel injection ports, two media channels, a hydrogel channel, and micro-posts in the top view (left) and side view (right). FIG. 30(d) shows the speed of hydrogel patterning on hydrophilic state on a PMMB device. The scale bar represents 2 mm.

[00130] FIG. 31 shows reconstruction of a three-dimensional neural network in the PMMB device. FIG. 31(a) is a schematic diagram illustrating a three-dimensional neural network. CNS neurons were seeded on both side of media channel. Axons of CNS neurons extended into gel channel, forming synapse. FIG. 31(b) Representative fluorescence confocal images of CNS neuron at DIV20 immunostained for Tuj1, synaptophyisin, and DAPI. Scale bar, 100 μm. Functional calcium activity of the three-dimensional neural network was analyzed by using the Oregon Green 488-BAPTA-1 AM at DIV 20. Randomly selected eight soma regions and four axon regions are measured. Representative image of neural network stained with BAPTA-1 AM (FIG. 31 (c)). Changes of the fluorescence intensity at twelve selected regions (FIG. 31(d), indicated by numbers shown in FIG. 31(c)) are shown. Scale bar, 100 μm.

[00131] FIG. 32 shows reconstruction of a three-dimensional blood brain barrier (BBB) with coculture of CNS neuron-astrocyte-HUVEC in the PMMB device. FIG. 32(a) is a schematic diagram illustrating three-dimensional reconstructed BBB model. HUVECs and LFs were firstly injected with the mixture of hydrogel into the gel channel. After 4 days, CNS cells including neurons and astrocytes were seeded into the media channel opposite to LFs. Astrocytic endfeet were anchored on vascular network. Representative fluorescence confocal

images of CNS neuron-astrocyte-HUVEC coculture immunostained with platelet-endothelia cell adhesion molecule-1[PECAM-1] (CD31) and glia fibrillary acidic protein (GFAP) at DIV 14 (of neuron). The confocal image in FIG. 32(c) showed enlarged boxed area of FIG. 32(b) and arrow heads (right) confirmed direct contact between astrocyte and vascular network. Scale bar, 100 μm. FIG. 32(d) shows example time-lapse microscopic photographs of HUVEC with or without astrocyte with 70 kDa FITC-dextran at time 0 and 7 min, respectively. FIG. 32(e) shows quantification results of BBB permeability. The permeability of only HUVECs is 2.6 ± 0.7, while coculture with astrocyte is 1.0 ± 0.2. Graph shows mean ± SEM values from ten independent experiments. Scale bar, 200 μm (unpaired, two-tailed-t test with Welch's correction).

[00132] FIG. 33 shows reconstruction of three-dimensional formation of myelin sheaths in the PMMB device. FIG. 33(a) is a schematic diagram illustrating three-dimensional coculture of PNS neruons-Schwann cells (SCs). SCs were firstly seeded into one hydrogel channel and after 3 hours, PNS neurons were then seeded into other side of media channel. SCs migrated and proliferated, while PNS neurons extend axons into gel channel. Over time, SCs contacted and wrapped around the axons of neurons, forming myelin sheath. FIG. 33(b) shows example fluorescence confocal images of PNS neuron-SC coculture immunostained with tubulin beta III (Tuj1), myelin basic protein (MBP), and DAPI at DIV 20. The expression of MBP became highly localized along the axons of PNS neurons (arrow heads). Scale bar, 200 μm. The level of MBP was determined by western blot analysis at DIV 20. The level of MBP expression on three-dimensional coculture of the PMMB was compared to three-dimensional coculture on a coverslip coated with Matrigel (2D). Representative immunoblots (FIG. 33(c)) and quantification (FIG. 33(d)) of MBP levels are shown. Protein levels were normalized against the level of ß-actin which was used as a loading control. Graph shows mean ± SEM values from three independent experiments. (unpaired, two-tailed-t test with Welch's correction).

[00133] In light of these principles and examples, we turn to certain embodiments.

[00134] In accordance with some embodiments, a device includes a substrate (e.g., substrate 320) having a top surface and a bottom surface opposite to the top surface; and one or more beams. A respective beam of the one or more beams (e.g., microfluidic channel module 220) has a bottom surface facing the top surface of the substrate and a top surface opposite to the bottom surface of the respective beam facing away from the top surface of the substrate. The respective beam is positioned adjacent to the substrate. At least a portion of the respective beam is spaced apart from the top surface of the substrate to define one or more microfluidic channels to enable movement of a fluid by capillary force between the bottom surface of the

respective beam and the top surface of the substrate along the one or more microfluidic channels. In some embodiments, the bottom surface of the respective beam is hydrophilic. In some embodiments, the top surface of the substrate is hydrophilic.

[00135] In some embodiments, the one or more beams includes a beam having an indented bottom surface (e.g., the microfluidic channel module 220 in FIG. 2). The indented bottom surface includes a first linear portion, a second linear portion, and a third linear portion. The second linear portion is located between the first linear portion and the third linear portion. The first linear portion is separated from the substrate by a first distance. The second linear portion is separated from the substrate by a second distance that is distinct from the first distance. The third linear portion is separated from the substrate by a third distance that is distinct from the second distance.

[00136] In some embodiments, the second distance is greater than the first distance and the third distance (e.g., the microfluidic channel module 220 in FIG. 2).

[00137] In some embodiments, the second linear portion of the beam includes one or more through-holes that extend from the second linear portion of the indented bottom surface to the top surface of the beam (e.g., through-holes 231 in FIG. 1).

[00138] In some embodiments, the second distance is greater than the first distance and the third distance is greater than the second distance (e.g., FIG. 4).

[00139] In some embodiments, the one or more beams include two or more beams that are separated from each other. In some embodiments, the device includes two beams that correspond to the first linear portion of the beam and the third linear portion of the beam without the second linear portion of the beam connecting the first linear portion of the beam to the third linear portion of the beam.

[00140] In some embodiments, a respective beam of the two or more beams has an indented bottom surface, the indented bottom surface including a first linear portion, a second linear portion, and a third linear portion. The second linear portion is located between the first linear portion and the third linear portion. The first linear portion is separated from the substrate by a first distance. The second linear portion is separated from the substrate by a second distance that is distinct from the first distance. The third linear portion is separated from the substrate by a third distance that is distinct from the second distance. For example, as shown in FIG. 5, the device includes multiple beams.

[00141] In some embodiments, the second distance is greater than the first distance and the third distance.

[00142] In some embodiments, the second distance is greater than the first distance and the third distance is greater than the second distance.

[00143] In some embodiments, the two or more beams include a first beam and a second beam that is substantially parallel to the first beam.

[00144] In some embodiments, the device includes one or more side walls located adjacent to the substrate.

[00145] In some embodiments, the one or more beams extend from the one or more side walls (e.g., side wall 310).

[00146] In some embodiments, the one or more side walls are in contact with the substrate. A respective side wall of the one or more side walls defines a path corresponding to a contact between the substrate and the respective side wall (e.g., inner corner path 340).

[00147] In some embodiments, the one or more side walls include a first side wall and a second side wall that is separated from the first side wall. The one or more beams include a first beam that extends from the first side wall and a second beam that extends from the second side wall.

[00148] In some embodiments, the one or more beams include a first beam and a second beam that is separate from the first beam. The first beam extends from a first wall location on a first side wall of the one or more side walls. The second beam extend from a second wall location on the first side wall that is distinct from the first wall location. The first side wall defines a first through-hole between (i) a first corner location that is located on the first side wall below the first wall location adjacent to a contact between the first side wall and the substrate and (ii) a second corner location that is located on the first side wall below the second wall location adjacent to a contact between the first side wall and the substrate.

[00149] In some embodiments, the first beam extends to a third wall location that is distinct from the first wall location and the second wall location. The second beam extends to a fourth wall location that is distinct from the first wall location, the second wall location, and the third wall location. The one or more side walls define a second through-hole between (i) a third corner location that is located on the one or more side walls below the third wall location adjacent to a contact between the one or more side walls and the substrate and (ii) a fourth corner location that is located on the one or more side walls below the fourth wall location adjacent to a contact between the one or more side walls and the substrate.

[00150] In some embodiments, a respective beam of the two or more beams has an indented bottom surface, the indented bottom surface including a first linear portion, a second linear portion, and a third linear portion. The second linear portion is located between the first linear

portion and the third linear portion. The first linear portion is separated from the substrate by a first distance. The second linear portion is separated from the substrate by a second distance that is greater than the first distance. The third linear portion is separated from the substrate by a third distance that is less than the second distance. The first linear portion extends from a first wall location on a first side wall of the one or more side walls. The third linear portion extends from a second wall location on the first side wall that is distinct from the first wall location. The first side wall defines a first through-hole (e.g., stopper portion 602) between (i) a first corner location that is located on the first side wall below the first wall location adjacent to a contact between the first side wall and the substrate and (ii) a second corner location that is located on the first side wall below the second wall location adjacent to a contact between the first side wall and the substrate.

[00151] In some embodiments, the first linear potion extends to a third wall location that is distinct from the first wall location and the second wall location. The third linear portion extends to a fourth wall location that is distinct from the first wall location, the second wall location, and the third wall location. The one or more side walls define a second through-hole (e.g., another stopper portion on the opposite side from stopper portion 602) between (i) a third corner location that is located on the one or more side walls below the third wall location adjacent to a contact between the one or more side walls and the substrate and (ii) a fourth corner location that is located on the one or more side walls below the fourth wall location adjacent to a contact between the one or more side walls and the substrate.

[00152] In some embodiments, the one or more side walls define one or more wells. The respective beam is coupled with a vertical divider (e.g., partition 200) to separate a respective well of the one or more wells into a first chamber on a first side of the vertical divider and a second chamber that is distinct from the first chamber, the second chamber being on a second side of the vertical divider that is opposite to the first side of the vertical divider.

[00153] In some embodiments, the bottom surface of the respective beam extends curvilinearly. For example, the respective beam is not linear (e.g., the respective beam is curved, such as forming a circle or an ellipse).

[00154] In some embodiments, the respective beam includes a hollow portion (e.g., FIG. 15), an open end of the hollow portion being positioned adjacent to the substrate. For example the respective beam has a shape of a vertical pipe, and one end of the pipe is positioned toward the substrate.

[00155] In some embodiments, the device includes one or more side walls, a respective side wall of the one or more side walls being tilted so that a first portion of the respective side

wall has a first distance to the respective beam and a second portion of the respective side wall that is located above the first portion of the respective side wall has a second distance to the respective beam that is greater than the first distance to the respective beam (e.g., the side wall in FIG. 15).

[00156] In some embodiments, the device includes a plurality of pillars extending from the top surface of the substrate and positioned at respective locations below the bottom surface of the respective beam (e.g., FIG. 30). A first subset of the plurality of pillars positioned along a first linear or curvilinear path on the top surface of the substrate. In some embodiments, a second subset of the plurality of the plurality of pillars positioned along a second linear or curvilinear path on the top surface of the substrate that is distinct from the first linear or curvilinear path.

[00157] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes flowing a liquid between the bottom surface of the respective beam of any device described herein and the top surface of the substrate of the device (e.g., FIG. 3).

[00158] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes flowing a first liquid between the first linear portion of any device described herein and the top surface of the substrate of the device (e.g., fluid 330 in FIG. 3). The method also includes flowing a third liquid between the third linear portion of the device and the top surface of the substrate of the device (e.g., FIG. 3 or FIG. 7). In some embodiments, the third liquid is identical to the first liquid (e.g., FIG. 3). In some embodiments, the third liquid is distinct from the first liquid (e.g., FIG. 7(c)).

[00159] In some embodiments, the method includes flowing a second liquid between the second linear portion of the device and the top surface of the substrate of the device (e.g., liquid 334 in FIG. 3).

[00160] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes flowing a first liquid between a first beam of the two or more beams of any device described herein and the top surface of the substrate of the device. The method also includes flowing a second liquid between a second beam of the two or more beams of the device and the top surface of the substrate of the device.

[00161] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids. The method includes providing a first liquid to a first location on the substrate of any device described herein adjacent to the one or more side walls.

[00162] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes providing a first liquid to a first side of the first through-hole so

that the first liquid flows between the first linear portion of any device described herein and the top surface of the substrate of the device. The method includes providing a second liquid to a second side of the first through-hole that is distinct from the first side of the first through-hole so that the second liquid flows between the third linear portion of the device and the top surface of the substrate of the device (e.g., FIG. 6).

[00163] In accordance with some embodiments, a method of covering a substrate with a pattern of liquids includes providing a first liquid to a first side of the first through-hole so that the first liquid flows between the first beam of any device described herein and the top surface of the substrate of the device. The method also includes providing a second liquid to a second side of the first through-hole that is distinct from the first side of the first through-hole so that the second liquid flows between the second beam of the device and the top surface of the substrate of the device.

[00164] In some embodiments, the method includes, subsequent to providing the first liquid and the second liquid, sealing the first through-hole (e.g., FIG. 6). The method also includes, subsequent to sealing the first through-hole, providing a third liquid so that the third liquid flows between the second linear portion of the device and the top surface of the substrate of the device.

[00165] In some embodiments, the method includes providing a second liquid to a second location on the substrate of the device adjacent to the one or more side walls. The second location is distinct and separate from the first location (e.g., FIG. 7(c)).

[00166] In accordance with some embodiments, a method includes, while a first liquid remains between the first linear portion of any device described herein and the substrate, a second liquid remains between the second linear portion of the device and the substrate, and a third liquid remains between the third linear portion of the device and the substrate, providing a fourth liquid to the first chamber (e.g., fluid 904 in Fig. 9).

[00167] In some embodiments, the method includes providing a fifth liquid to the second chamber (e.g., fluid 906 in FIG. 9).

[00168] In some embodiments, the fourth liquid has a first height in the first chamber and the fifth liquid has a second height in the second chamber that is less than the first height.

[00169] In some embodiments, at least one of the first liquid, the second liquid and the third liquid includes cells.

[00170] In accordance with some embodiments, a method includes causing angiogenesis or vasculogenesis using any device described herein.

[00171] In accordance with some embodiments, a method includes causing cellular reaction using any device described herein by: providing a first liquid between the first linear portion and the substrate; providing the first liquid between the third linear portion and the substrate; providing a second liquid between the second linear portion and the substrate, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and providing a third liquid distinct form the first liquid, the third liquid containing cells of a second type adjacent to the first linear portion so that the third liquid comes in contact with the first liquid.

[00172] In accordance with some embodiments, a method includes causing cellular reaction using any device described herein by providing a first liquid between the respective beam and the substrate; providing a second liquid in a region surrounded by the first liquid so that the second liquid comes in contact with the first liquid, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and providing a third liquid distinct from the first liquid adjacent to the respective beam so that the third liquid comes in contact with the first liquid, the third liquid containing cells of a second type.

[00173] In some embodiments, the method includes causing angiogenesis. The first liquid contains fibrin. The cells of the first type include fibroblast cells. The cells of the second type include vascular endothelial cells (e.g., FIG. 11 and FIG. 16).

[00174] In some embodiments, the method includes causing vascular genesis. The first liquid contains vascular endothelial cells. The cells of the first type include fibroblast cells. The cells of the second type include fibroblast cells (e.g., FIG. 17).

[00175] In some embodiments, the cells of the first type include cancer cells (e.g., HeLa cells); and the cells of the second type include lymphocytes (e.g., NK cells).

[00176] In accordance with some embodiments, a method for obtaining a vascularized tumor spheroid includes placing a tumor spheroid in any device described herein. The method also includes co-culturing the tumor spheroid with fibroblast cells (e.g., FIG. 26).

[00177] In accordance with some embodiments, a method includes placing a vascularized tumor spheroid in any device described herein, and providing a liquid containing kinase inhibitor (e.g., FIG. 29).

[00178] In accordance with some embodiments, a method includes forming a three-dimensional matrix of cells with any device described herein by: providing a first liquid between the bottom surface of the respective beam and the substrate; providing a second liquid on a first side of the first liquid, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and providing a third liquid on a second side

of the first liquid that is opposite to the first side, the third liquid being distinct from the first liquid (e.g., FIG. 30).

[00179] In some embodiments, the cells of the first type include neuron cells; and the third liquid contains cells of a second type, the cells of the second type including neuron cells (e.g., FIG. 31).

[00180] In some embodiments, the cells of the first type include neuron cells; and the third liquid contains cells of a second type, the cells of the second type including vascular endothelial cells and fibroblast cells (e.g., FIG. 32).

[00181] In some embodiments, the cells of the first type include neurolemmocytes; and the third liquid contains cells of a second type, the cells of the second type including neuron cells (e.g., FIG. 33).

[00182] The microfluidic device with the above-described characteristics has the following advantages. First, the microfluidic device can solve problems caused by low gas saturation in three-dimensional cell culture. That is, in the conventional art, since a culture medium in a reservoir is provided to cells in a microfluidic channel through a long and narrow culture medium channel, gas saturation in the culture medium is reduced while the gas provided from the top surface of the culture medium passes through the culture medium channel, and thus an environment disadvantageous for cells is provided. On the other hand, the microfluidic device is connected to facilitate fluid flow with a culture medium through both open sides of a microfluidic channel and an opening, and therefore a cell culture environment maintaining high gas saturation may be provided.

[00183] In addition, the microfluidic device provides rapid and simple fluid patterning. That is, in the microfluidic device, an inner corner path which facilitates fluid flow by capillary force is formed and connected with the microfluidic channel of the microfluidic channel module to facilitate fluid flow, and therefore a suitable amount of the fluid is provided to an arbitrary position on the inner corner path, resulting in easy patterning of the entire microfluidic channels and inner corner paths. This is compared with a need for three or more times of independent fluid injection, for example, when the same fluid is injected into three fluid channels, as in the conventional art. Accordingly, the microfluidic device provides a considerably excellent effect on experiment precision, time and utilization. As described above, since fluid patterning using the microfluidic device can obtain the same patterning result even when a fluid is applied to an arbitrary position on the inner corner path, it can be useful even when a uniform and reproducible repeated experiment is required. This is because the fluid patterning moves until the capillary force applied to the fluid along the

inner corner path of the microfluidic device is in equilibrium. When the inner corner path of the microfluidic device has the same contact angle, the fluid patterning can be uniformly performed regardless of external factors such as the experience and skill of an experimenter or operator.

[00184] In addition, the microfluidic device may allow patterning of a fluid to a desired area within several seconds, preferably, 1 second after the fluid is applied, and therefore is suitable for an environment requiring rapid and uniform patterning. For example, in three-dimensional co-culture of two or more types of cells, patterning is very important to prevent the mixing of fluids containing different cells. In this case, to fix cells to a specific position under an environment in which free mass transfer is possible, a polymer material, for example, fibrin gel is used together with cells. Here, to cure the fibrin gel mixed with the cells, a generally-used crosslinking agent is added, and for a stable and highly-reliable experiment, rapid and uniform fluid patterning is required.

[00185] In addition, since the microfluidic device may be manufactured of an engineering plastic, which is a hydrophilic material, it can be manufactured by curing a melted resin by injection molding, hot embossing or 3D printing, and therefore has an advantage of being applicable to economical mass-production.

[00186] As described above, the microfluidic device having the above-described structure and advantages does not need other external forces, for example, a pressure, except capillary force, in patterning of a fluid, does not require a separate sensor for precise control of a fluid injection position, and considerably reduces the probability of injection failure, and therefore the microfluidic device can be applied to cell culture using automation equipment.

[00187] A microfluidic device, which includes a microfluidic channel embedded in a chamber and open at both sides is manufactured using a material having a hydrophilic surface characteristic, and a fluid can be patterned in a microfluidic channel using capillary force. In accordance with some embodiments, an inner corner path and the microfluidic channel can be used in rapid and precise fluid patterning at one time by applying a suitable amount of the fluid to be patterned on the inner corner path of the microfluidic device. In addition, the microfluidic channel is incorporated or embedded in the lower portion of the chamber, and thus connected to facilitate fluid flow with a culture medium without passing through a long and narrow culture medium channel as shown in the conventional art. Therefore, since cells can easily use a gas entering from an air contact surface which is on the top surface of the culture medium in the chamber, an advantageous culture environment can be imparted to the

cells in the microfluidic channel. Therefore, the microfluidic device can be effectively used in three-dimensional culture of cells or tissue.

[00188] This application describes a microfluidic device which includes a microfluidic channel (which is often embedded in a culture medium chamber), and a structure which is formed by capillary force and facilitates fluid flow between an adjacent microfluidic channel and a culture medium. In addition, this application also describes a structure having several microfluidic devices on one common substrate. In addition, the microfluidic device may be manufactured of a hydrophobic engineering plastic by injection or molding. Accordingly, the microfluidic device may be effectively used in culture of cells, tissue or cells and tissue, required for three-dimensional culture, and therefore, it may be used in general industries such as biotechnology laboratories, cosmetics development and new drug development.

WHAT IS CLAIMED IS:

1. A microfluidic device having a microfluidic channel in which at least a surface in contact with a fluid is formed of a hydrophilic material, comprising:

a substrate having a top surface and a bottom surface;

outer walls which are attached to the top surface of the substrate such that one or more cavities with an open top are formed and which include an inner surface facing the cavity and an outer surface opposite to the inner surface;

inner corner paths formed by the boundary at which the inner surface of the outer wall meets the top surface of the substrate; and

a microfluidic channel module including a top surface, a bottom surface and both ends, disposed in the one or more cavities to cross the inside of the cavity by bonding the both ends to different positions on the inner surface of the outer wall,

wherein a part or all of the bottom surface of the channel module is spaced apart from the top surface of the substrate to form one or more microfluidic channels connected with the inner corner path so as to be able to move a fluid by capillary force between the bottom surface of the microfluidic channel module and the top surface of the substrate.

2. The microfluidic device according to claim 1, wherein a part or all of the top surface of the microfluidic channel module extends upward such that a partition which divides the cavity is formed.

3. The microfluidic device according to claim 1 or 2, wherein the inner corner path forms one or more closed curves.

4. The microfluidic device according to claim 3, further comprising:

when the closed curve has a polygonal shape, a fillet formed on the inner surface of the outer wall so as to have a radius (R) value of a corresponding inner corner path of 0.05 mm or more.

5. The microfluidic device according to claim 1 or 2, wherein the microfluidic channel module includes two or more microfluidic channels sequentially increased in height.

6. The microfluidic device according to claim 1 or 2, comprising:

one or more recesses, formed parallel to the microfluidic channel, on the bottom surface of the microfluidic channel module.

7. The microfluidic device according to claim 1 or 2, comprising:

two or more recesses, formed parallel to a microfluidic channel, on the bottom surface of a microfluidic channel module, and sequentially increased in depth.

8. The microfluidic device according to claim 6, wherein the microfluidic channel module includes one or more openings passing from the top surface thereof to the recess such that a cavity formed on the top surface and the recess are connected to facilitate fluid flow.

9. The microfluidic device according to claim 7, further comprising:

a culture medium inlet for injecting a culture medium into an upper portion of a recess and a culture medium outlet for discharging the culture medium.

10. The microfluidic device according to claim 7, wherein the microfluidic device has a well shape.

11. The microfluidic device according to claim 1, wherein two or more cavities are formed by the outer walls and the substrate, and
connected to facilitate fluid flow.

12. A method for patterning a fluid using the microfluidic device of claim 1 or 2, comprising:

applying a patterning fluid at one position on the inner corner path; and

performing patterning of the patterning fluid along the inner corner path and a microfluidic channel by capillary force.

13. A method for three-dimensional co-culture of one or more among cells and tissue using the microfluidic device of claim 1 or 2.

14. A microfluidic chip in which the two or more microfluidic devices of claim 1 or 2 are formed on a common substrate.

15. The microfluidic chip according to claim 14, which includes 4, 8, 16, 24, 48, 96 or 256 microfluidic devices.

16. The microfluidic chip according to claim 14 or 15, wherein the microfluidic devices are selectively connected to facilitate fluid flow.

17. A device, comprising:

a substrate having a top surface and a bottom surface opposite to the top surface; and

one or more beams, a respective beam of the one or more beams having a bottom surface facing the top surface of the substrate and a top surface opposite to the bottom surface of the respective beam facing away from the top surface of the substrate, the respective beam being positioned adjacent to the substrate,

wherein at least a portion of the respective beam is spaced apart from the top surface of the substrate to define one or more microfluidic channels to enable movement of a fluid by capillary force between the bottom surface of the respective beam and the top surface of the substrate along the one or more microfluidic channels.

18. The device of claim 17, wherein:

the one or more beams includes a beam having an indented bottom surface, the indented bottom surface including a first linear portion, a second linear portion, and a third linear portion;

the second linear portion is located between the first linear portion and the third linear portion;

the first linear portion is separated from the substrate by a first distance;

the second linear portion is separated from the substrate by a second distance that is distinct from the first distance; and

the third linear portion is separated from the substrate by a third distance that is distinct from the second distance.

19. The device of claim 18, wherein the second distance is greater than the first distance and the third distance.

20. The device of claim 19, wherein the second linear portion of the beam includes one or more through-holes that extend from the second linear portion of the indented bottom surface to the top surface of the beam.

21. The device of claim 18, wherein the second distance is greater than the first distance and the third distance is greater than the second distance.

22. The device of claim 17, wherein:

the one or more beams include two or more beams that are separated from each other.

23. The device of claim 22, wherein:

a respective beam of the two or more beams has an indented bottom surface, the indented bottom surface including a first linear portion, a second linear portion, and a third linear portion;

the second linear portion is located between the first linear portion and the third linear portion;

the first linear portion is separated from the substrate by a first distance;

the second linear portion is separated from the substrate by a second distance that is distinct from the first distance; and

the third linear portion is separated from the substrate by a third distance that is distinct from the second distance.

24. The device of claim 23, wherein the second distance is greater than the first distance and the third distance.

25. The device of claim 23, wherein the second distance is greater than the first distance and the third distance is greater than the second distance.

26. The device of any of claims 23-25, wherein the two or more beams include a first beam and a second beam that is substantially parallel to the first beam.

27. The device of any of claims 17-26, including:

one or more side walls located adjacent to the substrate.

28. The device of claim 27, wherein:

the one or more beams extend from the one or more side walls.

29. The device of claim 27 or 28, wherein:

the one or more side walls are in contact with the substrate, a respective side wall of the one or more side walls defining a path corresponding to a contact between the substrate and the respective side wall.

30. The device of any of claims 27-29, wherein:

the one or more side walls include a first side wall and a second side wall that is separated from the first side wall; and

the one or more beams include a first beam that extends from the first side wall and a second beam that extends from the second side wall.

31. The device of any of claims 27-29, wherein:

the one or more beams include a first beam and a second beam that is separate from the first beam;

the first beam extends from a first wall location on a first side wall of the one or more side walls;

the second beam extend from a second wall location on the first side wall that is distinct from the first wall location; and

the first side wall defines a first through-hole between (i) a first corner location that is located on the first side wall below the first wall location adjacent to a contact between the first side wall and the substrate and (ii) a second corner location that is located on the first side wall below the second wall location adjacent to a contact between the first side wall and the substrate.

32. The device of claim 31, wherein:

the first beam extends to a third wall location that is distinct from the first wall location and the second wall location;

the second beam extends to a fourth wall location that is distinct from the first wall location, the second wall location, and the third wall location;

the one or more side walls define a second through-hole between (i) a third corner location that is located on the one or more side walls below the third wall location adjacent to a contact between the one or more side walls and the substrate and (ii) a fourth corner location that is located on the one or more side walls below the fourth wall location adjacent to a contact between the one or more side walls and the substrate.

33. The device of any of claims 27-29, wherein:

a respective beam of the two or more beams has an indented bottom surface, the indented bottom surface including a first linear portion, a second linear portion, and a third linear portion;

the second linear portion is located between the first linear portion and the third linear portion;

the first linear portion is separated from the substrate by a first distance;

the second linear portion is separated from the substrate by a second distance that is greater than the first distance;

the third linear portion is separated from the substrate by a third distance that is less

than the second distance;

the first linear portion extends from a first wall location on a first side wall of the one or more side walls;

the third linear portion extends from a second wall location on the first side wall that is distinct from the first wall location; and

the first side wall defines a first through-hole between (i) a first corner location that is located on the first side wall below the first wall location adjacent to a contact between the first side wall and the substrate and (ii) a second corner location that is located on the first side wall below the second wall location adjacent to a contact between the first side wall and the substrate.

34. The device of claim 33, wherein:

the first linear potion extends to a third wall location that is distinct from the first wall location and the second wall location;

the third linear portion extends to a fourth wall location that is distinct from the first wall location, the second wall location, and the third wall location;

the one or more side walls define a second through-hole between (i) a third corner location that is located on the one or more side walls below the third wall location adjacent to a contact between the one or more side walls and the substrate and (ii) a fourth corner location that is located on the one or more side walls below the fourth wall location adjacent to a contact between the one or more side walls and the substrate.

35. The device of any of claims 27-34, wherein:

the one or more side walls define one or more wells; and

the respective beam is coupled with a vertical divider to separate a respective well of the one or more wells into a first chamber on a first side of the vertical divider and a second chamber that is distinct from the first chamber, the second chamber being on a second side of the vertical divider that is opposite to the first side of the vertical divider.

36. The device of any of claims 17-35, wherein:

the bottom surface of the respective beam extends curvilinearly.

37. The device of any of claims 17, 22, 27-29, and 35-36, wherein:

the respective beam includes a hollow portion, an open end of the hollow portion being positioned adjacent to the substrate.

38. The device of claim 37, including:

one or more side walls, a respective side wall of the one or more side walls being tilted so that a first portion of the respective side wall has a first distance to the respective beam and a second portion of the respective side wall that is located above the first portion of the respective side wall has a second distance to the respective beam that is greater than the first distance to the respective beam.

39. The device of any of claims 17-38, further comprising:

a plurality of pillars extending from the top surface of the substrate and positioned at respective locations below the bottom surface of the respective beam,

wherein a first subset of the plurality of pillars positioned along a first curvilinear path on the top surface of the substrate and a second subset of the plurality of the plurality of pillars positioned along a second curvilinear path on the top surface of the substrate that is distinct from the first curvilinear path.

40. A method of covering a substrate with a pattern of liquids, the method comprising:

flowing a liquid between the bottom surface of the respective beam of the device of any of claims 17-39 and the top surface of the substrate of the device.

41. A method of covering a substrate with a pattern of liquids, the method comprising:

flowing a first liquid between the first linear portion of the device of any of claims 18-21 and 23-26 and the top surface of the substrate of the device; and

flowing a third liquid between the third linear portion of the device and the top surface of the substrate of the device.

42. The method of claim 41, further comprising:

flowing a second liquid between the second linear portion of the device and the top surface of the substrate of the device.

43. A method of covering a substrate with a pattern of liquids, the method comprising:

flowing a first liquid between a first beam of the two or more beams of the device of claim 22 and the top surface of the substrate of the device; and

flowing a second liquid between a second beam of the two or more beams of the device and the top surface of the substrate of the device.

44. A method of covering a substrate with a pattern of liquids, the method comprising:

providing a first liquid to a first location on the substrate of the device of any of claims 27-39 adjacent to the one or more side walls.

45. A method of covering a substrate with a pattern of liquids, the method comprising:

providing a first liquid to a first side of the first through-hole so that the first liquid flows between the first beam of the device of claim 31 or 32 and the top surface of the substrate of the device; and

providing a second liquid to a second side of the first through-hole that is distinct from the first side of the first through-hole so that the second liquid flows between the second beam of the device and the top surface of the substrate of the device.

46. A method of covering a substrate with a pattern of liquids, the method comprising:

providing a first liquid to a first side of the first through-hole so that the first liquid flows between the first linear portion of the device of claim 33 or 34 and the top surface of the substrate of the device; and

providing a second liquid to a second side of the first through-hole that is distinct from the first side of the first through-hole so that the second liquid flows between the third linear portion of the device and the top surface of the substrate of the device.

47. The method of claim 46, further comprising:

subsequent to providing the first liquid and the second liquid, sealing the first through-hole; and

subsequent to sealing the first through-hole, providing a third liquid so that the third liquid flows between the second linear portion of the device and the top surface of the substrate of the device.

48. The method of claim 44, further comprising:

providing a second liquid to a second location on the substrate of the device adjacent to the one or more side walls, wherein the second location is distinct and separate from the first location.

49. A method, comprising:

while a first liquid remains between the first linear portion of the device of claim 35 and the substrate, a second liquid remains between the second linear portion of the device and the substrate, and a third liquid remains between the third linear portion of the device and the substrate, providing a fourth liquid to the first chamber.

50. The method of claim 49, further comprising:

providing a fifth liquid to the second chamber.

51. The method of claim 50, wherein:

the fourth liquid has a first height in the first chamber and the fifth liquid has a second height in the second chamber that is less than the first height.

52. The method of claim 49, wherein:

at least one of the first liquid, the second liquid and the third liquid includes cells.

53. A method, comprising:

causing angiogenesis or vasculogenesis using the device of any of claims 17-39.

54. A method, comprising:

causing cellular reaction using the device of any of claims 18-26 and 31-34 by:

providing a first liquid between the first linear portion and the substrate;

providing the first liquid between the third linear portion and the substrate;

providing a second liquid between the second linear portion and the substrate, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and

providing a third liquid distinct form the first liquid, the third liquid containing cells of a second type adjacent to the first linear portion so that the third liquid comes in contact with the first liquid.

55. A method, comprising:

causing cellular reaction using the device of claim 37 or 38 by:

providing a first liquid between the respective beam and the substrate;

providing a second liquid in a region surrounded by the first liquid so that the second liquid comes in contact with the first liquid, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and

providing a third liquid distinct from the first liquid adjacent to the respective beam so that the third liquid comes in contact with the first liquid, the third liquid containing cells of a second type.

56. The method of claim 54 or 55, including:

causing angiogenesis, wherein:

the first liquid contains fibrin;

the cells of the first type include fibroblast cells; and

the cells of the second type include vascular endothelial cells.

57. The method of claim 54 or 55, including:

causing vascular genesis, wherein:

the first liquid contains vascular endothelial cells;

the cells of the first type include fibroblast cells; and

the cells of the second type include fibroblast cells.

58. The method of claim 54 or 55, wherein:

the cells of the first type include cancer cells; and

the cells of the second type include lymphocytes.

59. A method for obtaining a vascularized tumor spheroid, the method comprising:

placing a tumor spheroid in the device of claim 37 or 38; and

co-culturing the tumor spheroid with fibroblast cells.

60. A method, comprising:

placing a vascularized tumor spheroid in the device of claim 37 or 38; and

providing a liquid containing kinase inhibitor.

61. A method, comprising:

forming a three-dimensional matrix of cells with the device of any of claims 17-39 by:

providing a first liquid between the bottom surface of the respective beam and the substrate;

providing a second liquid on a first side of the first liquid, the second liquid being distinct from the first liquid, the second liquid containing cells of a first type; and

providing a third liquid on a second side of the first liquid that is opposite to the first side, the third liquid being distinct from the first liquid.

62. The method of claim 61, wherein:

the cells of the first type include neuron cells; and

the third liquid contains cells of a second type, the cells of the second type including neuron cells.

63. The method of claim 61, wherein:

the cells of the first type include neuron cells; and

the third liquid contains cells of a second type, the cells of the second type including vascular endothelial cells and fibroblast cells.

64. The method of claim 61, wherein:

the cells of the first type include neurolemmocytes; and

the third liquid contains cells of a second type, the cells of the second type including neuron cells.

FIG. 1

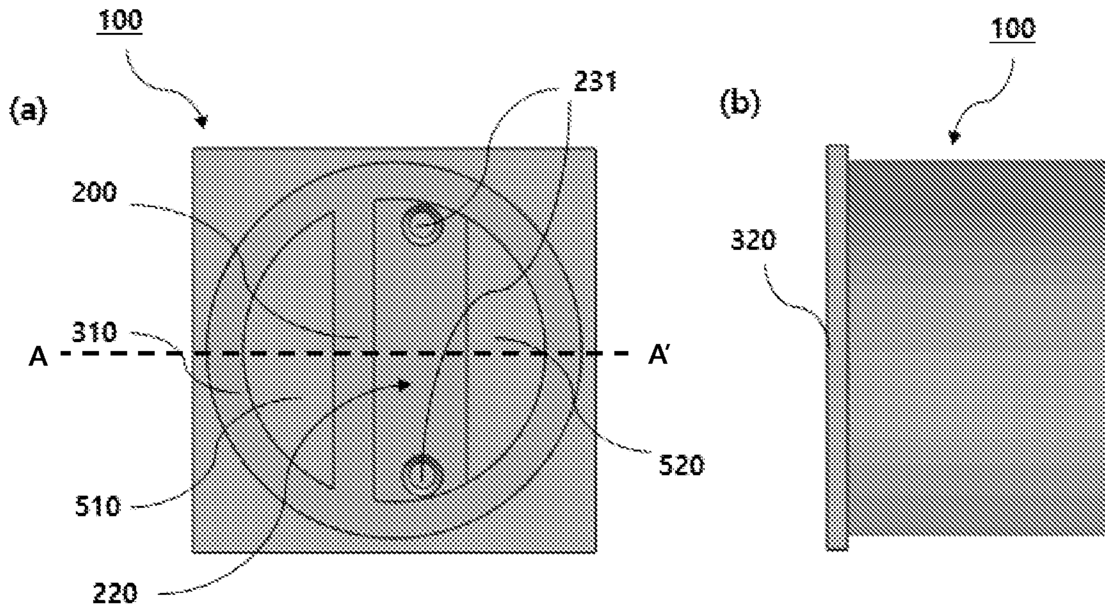


FIG. 2

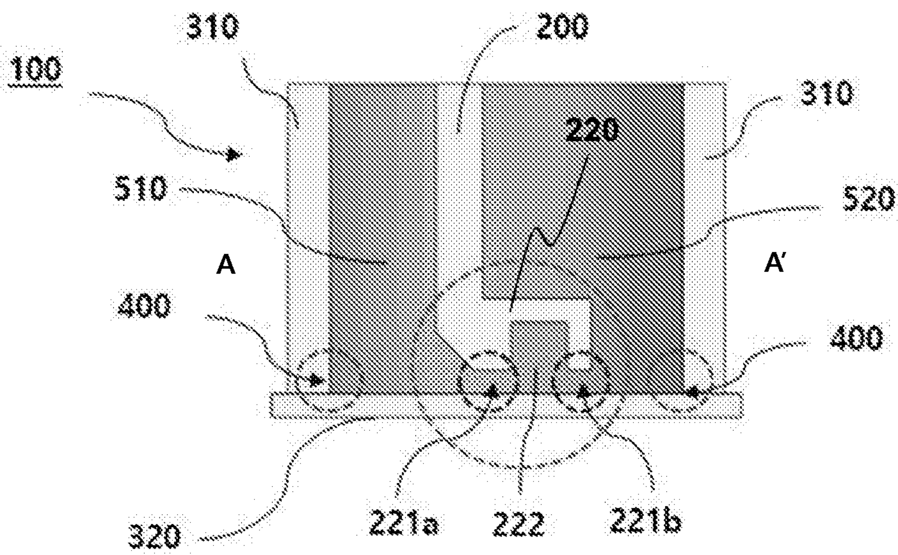
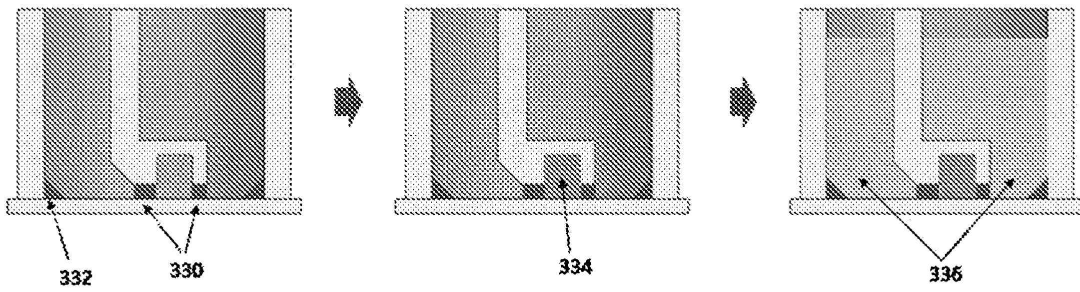


FIG. 3

(a)



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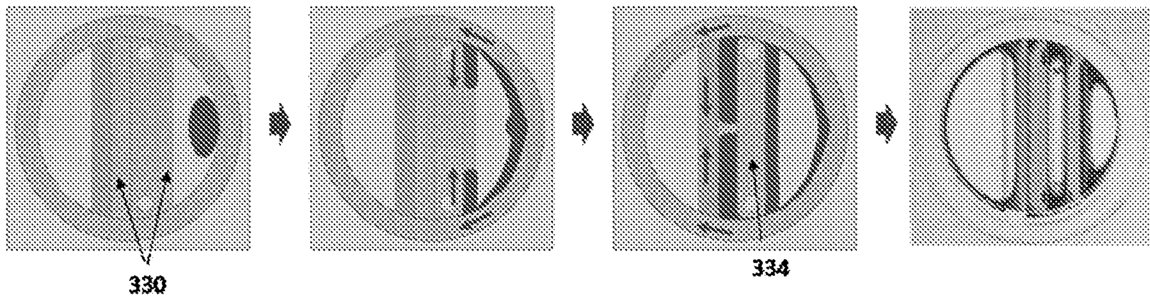


(c)

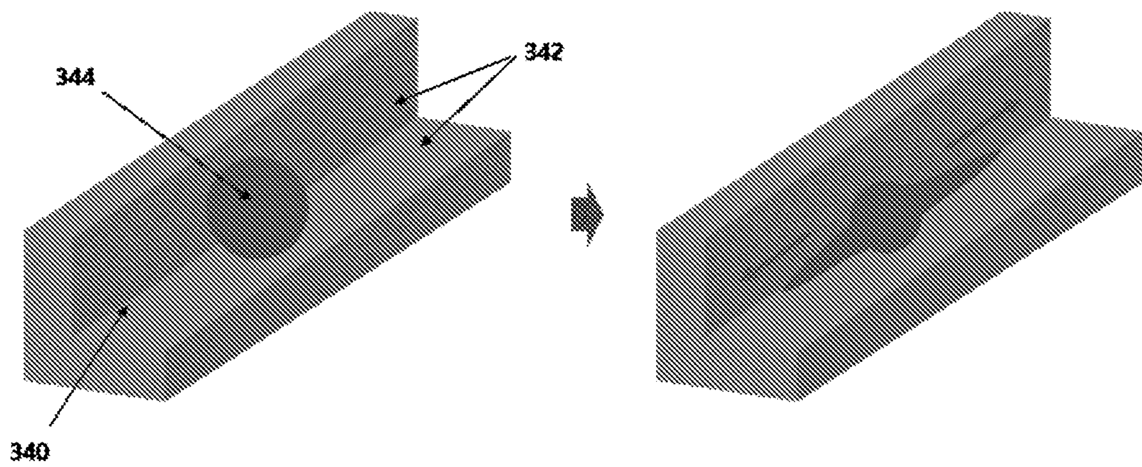


FIG. 4

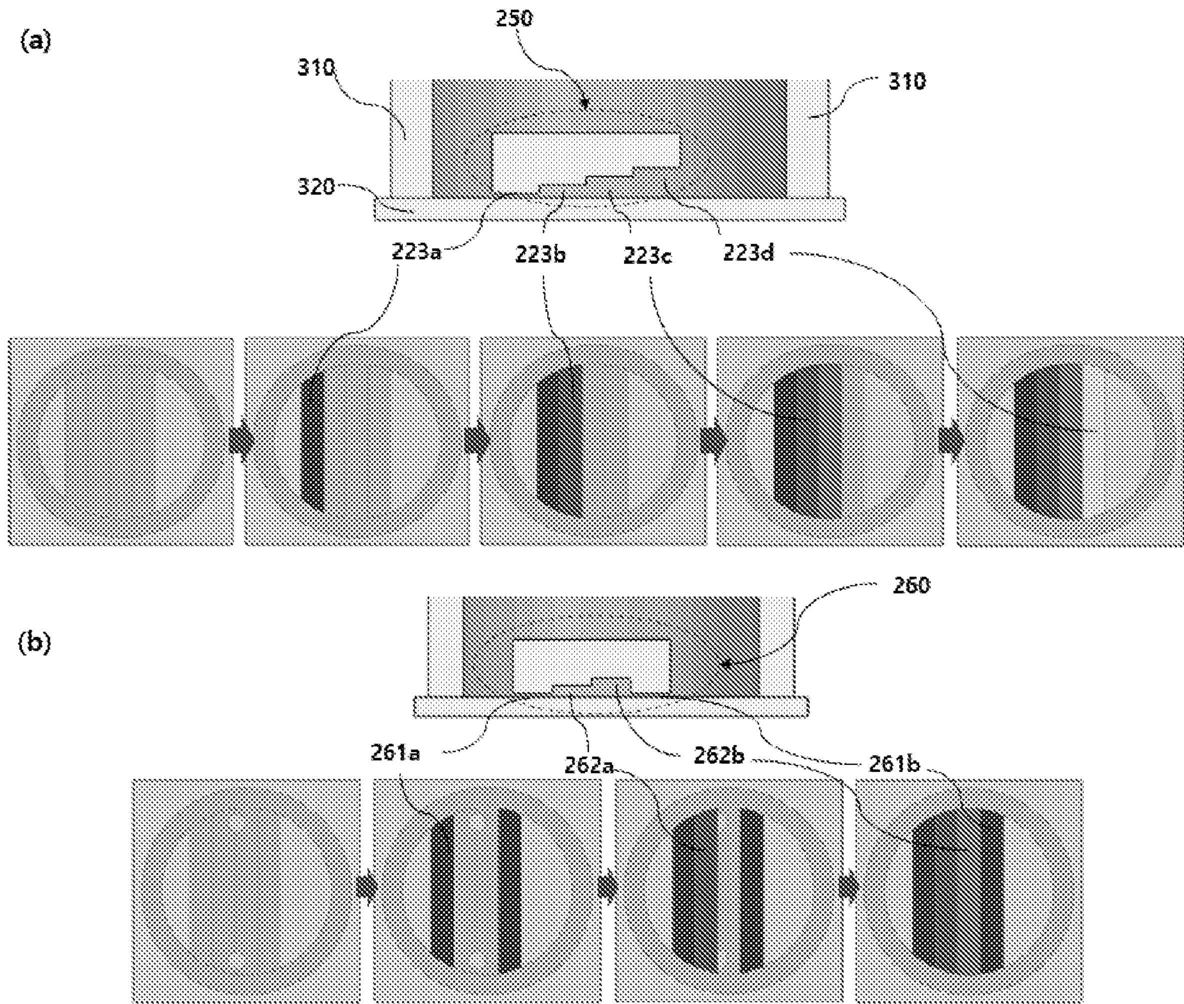


FIG. 5

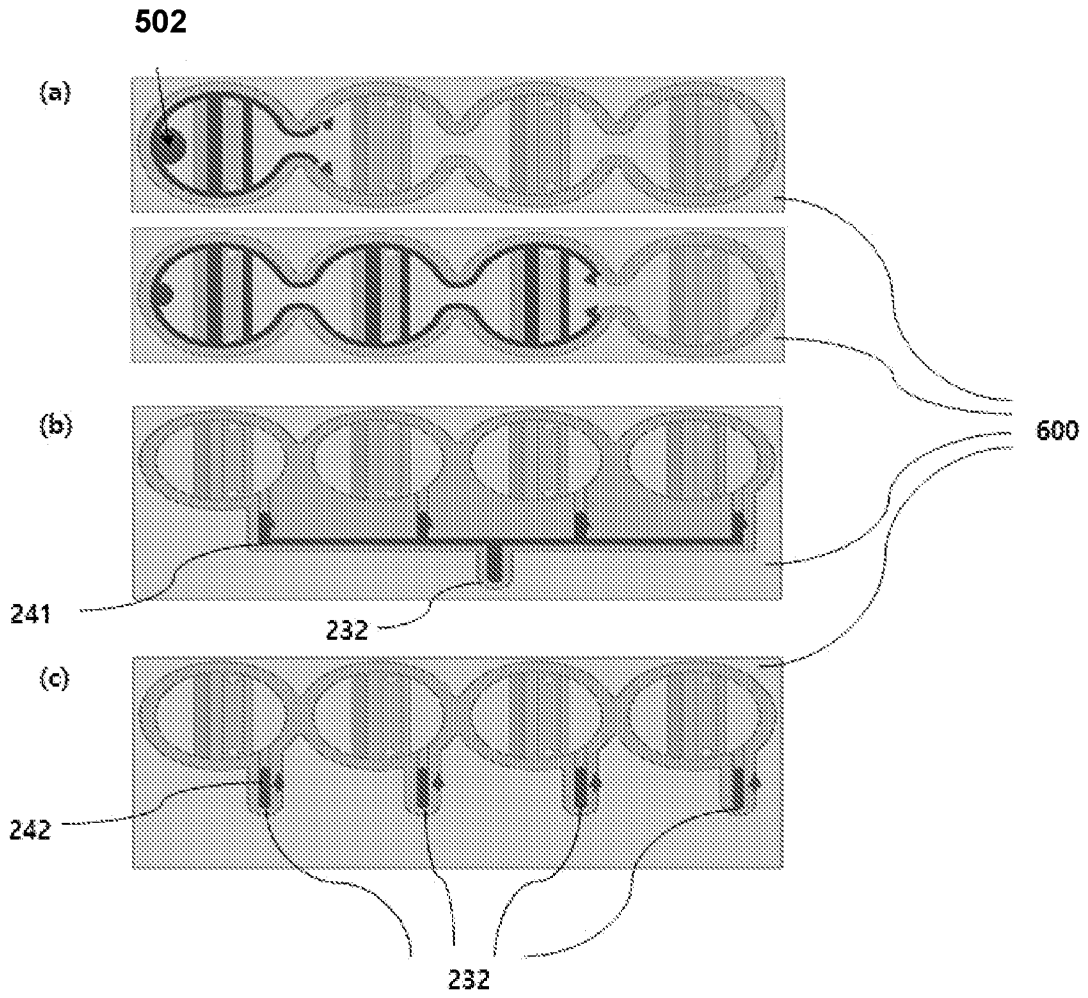


FIG. 6

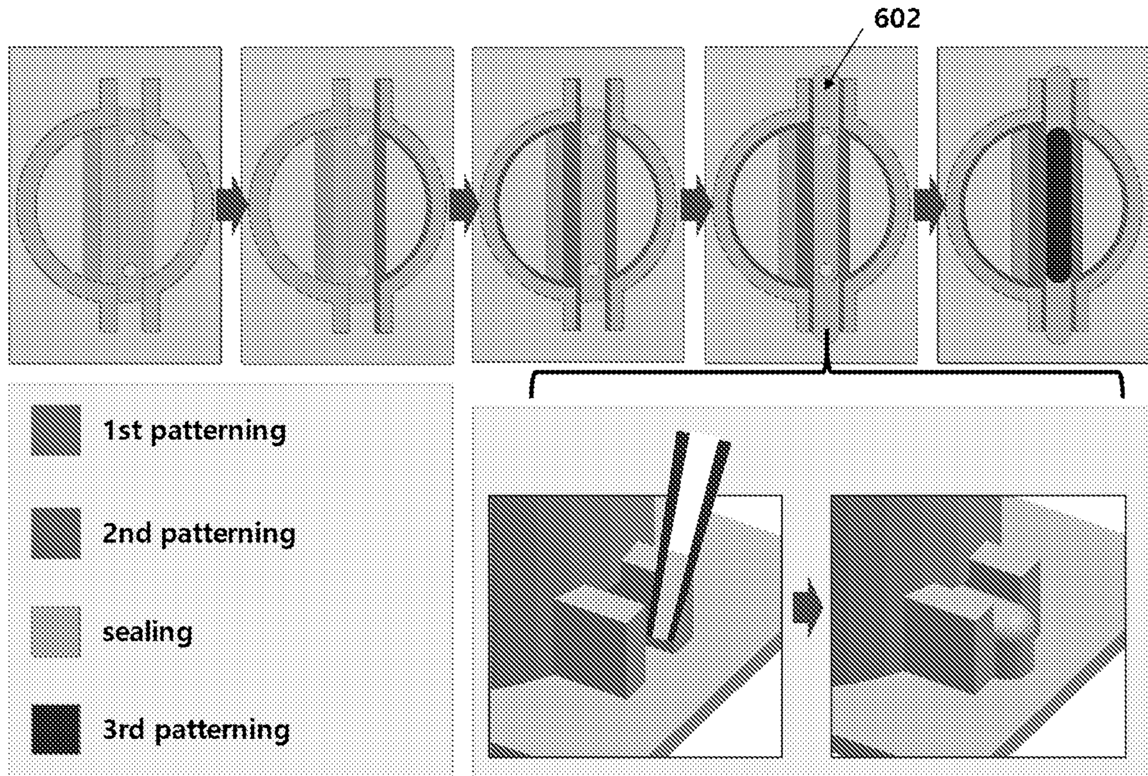
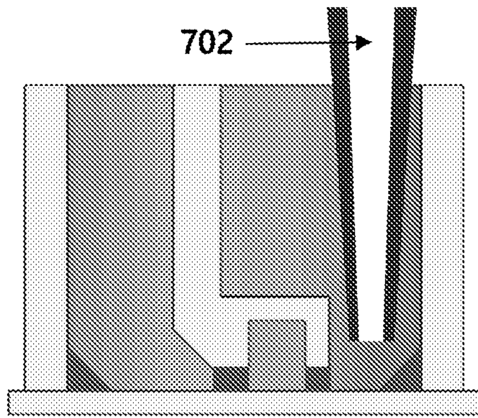
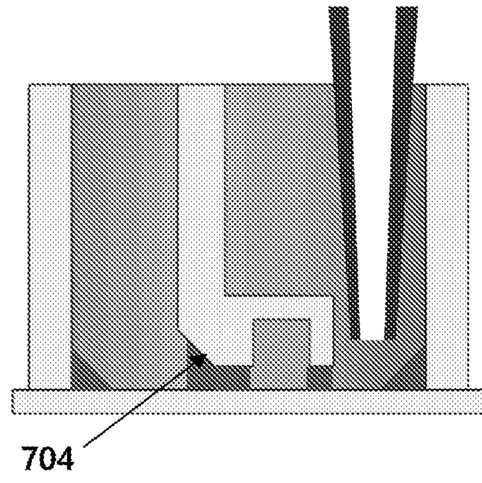


FIG. 7

(a)



(b)



(c)

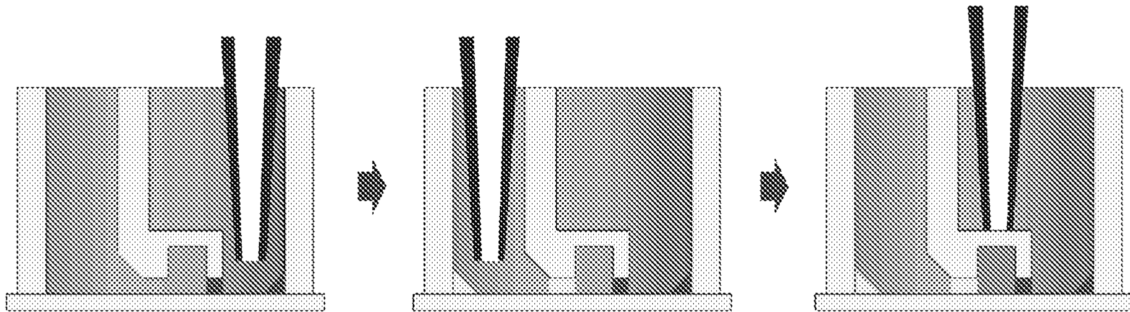
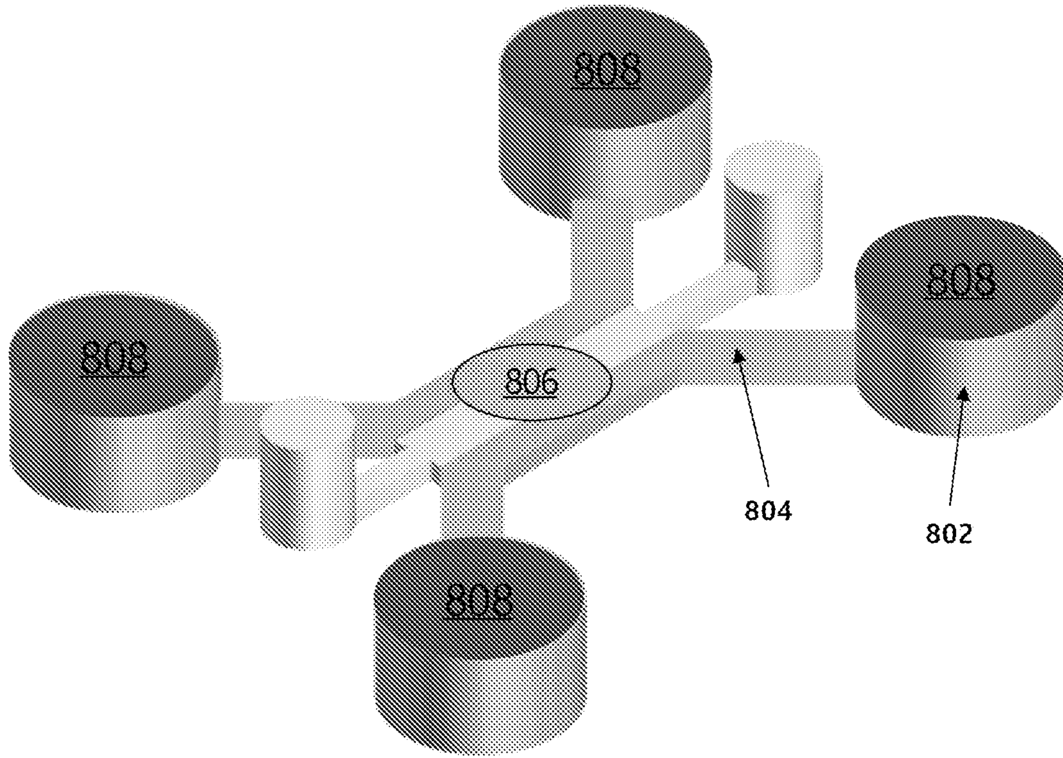


FIG. 8

(a)



(b)

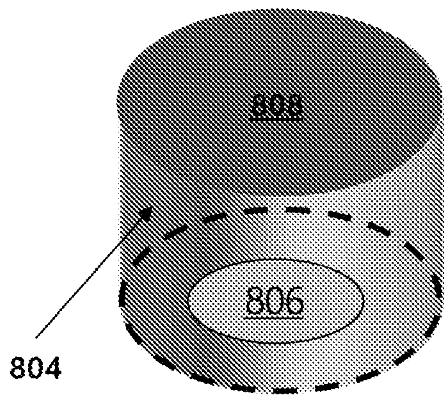


FIG. 9

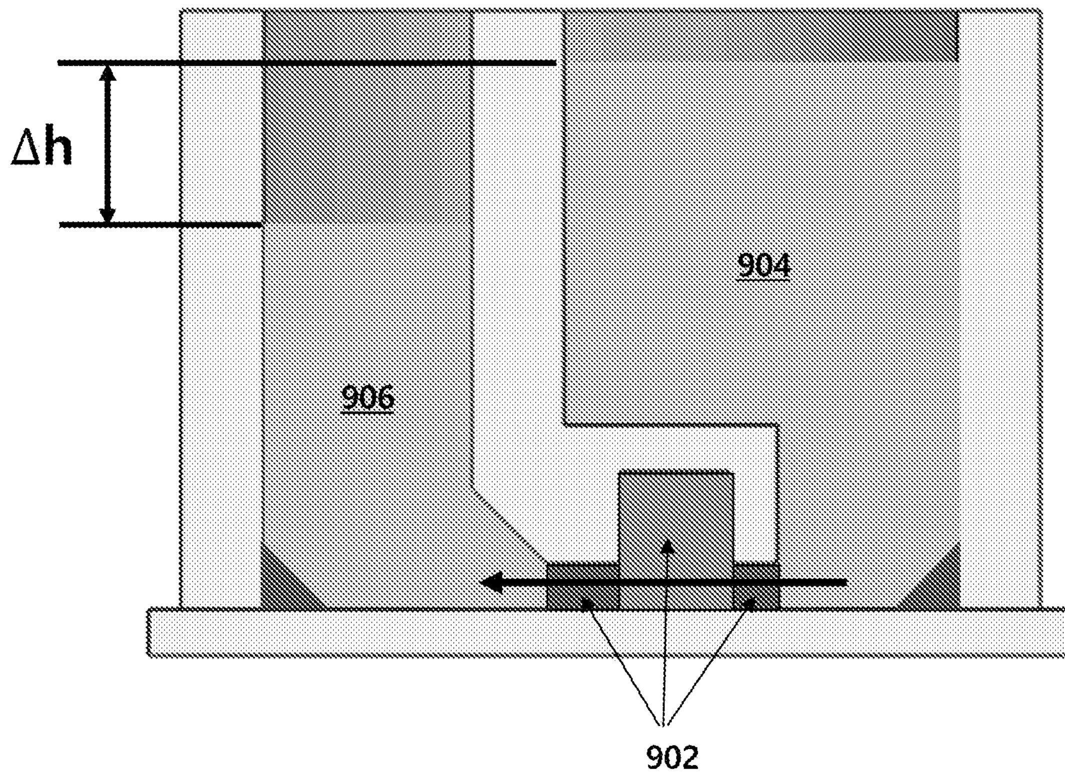
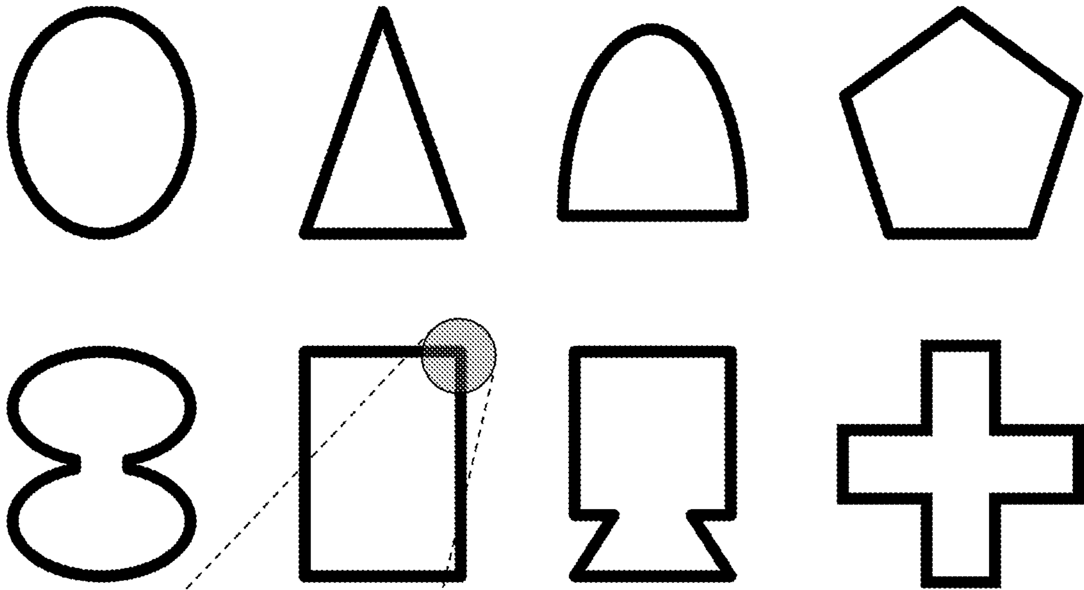


FIG. 10



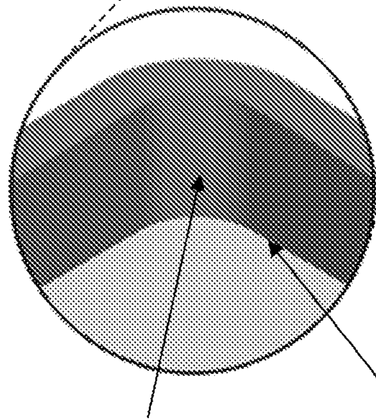


FIG. 11

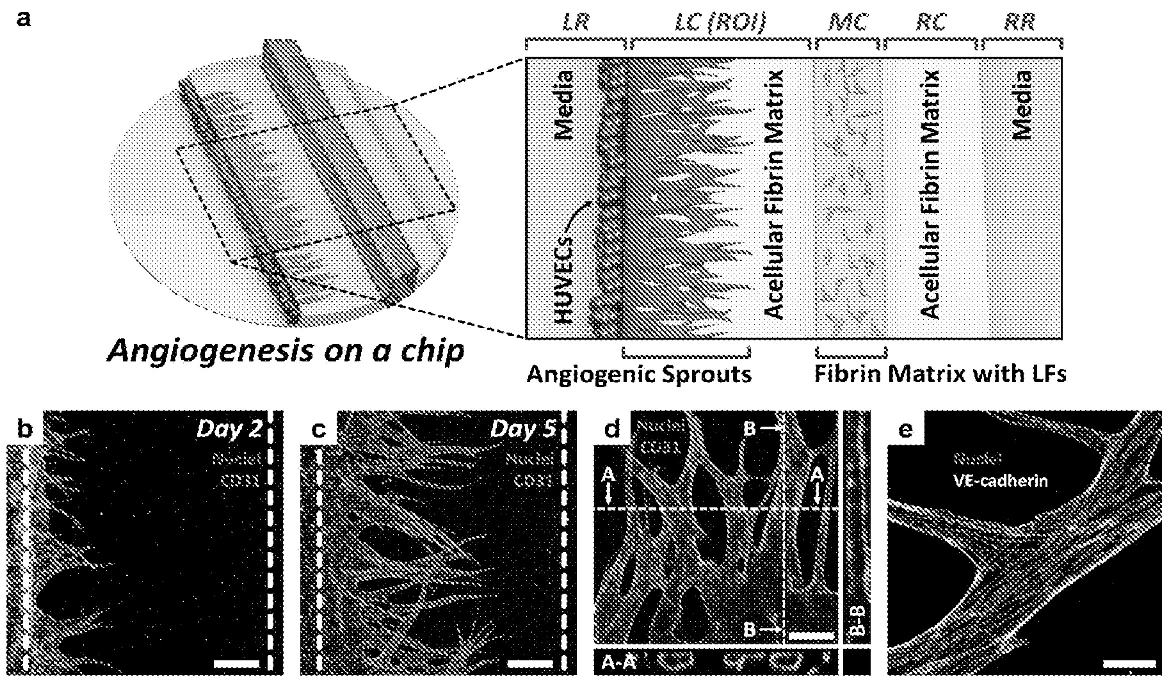


FIG. 12

FIG. 13

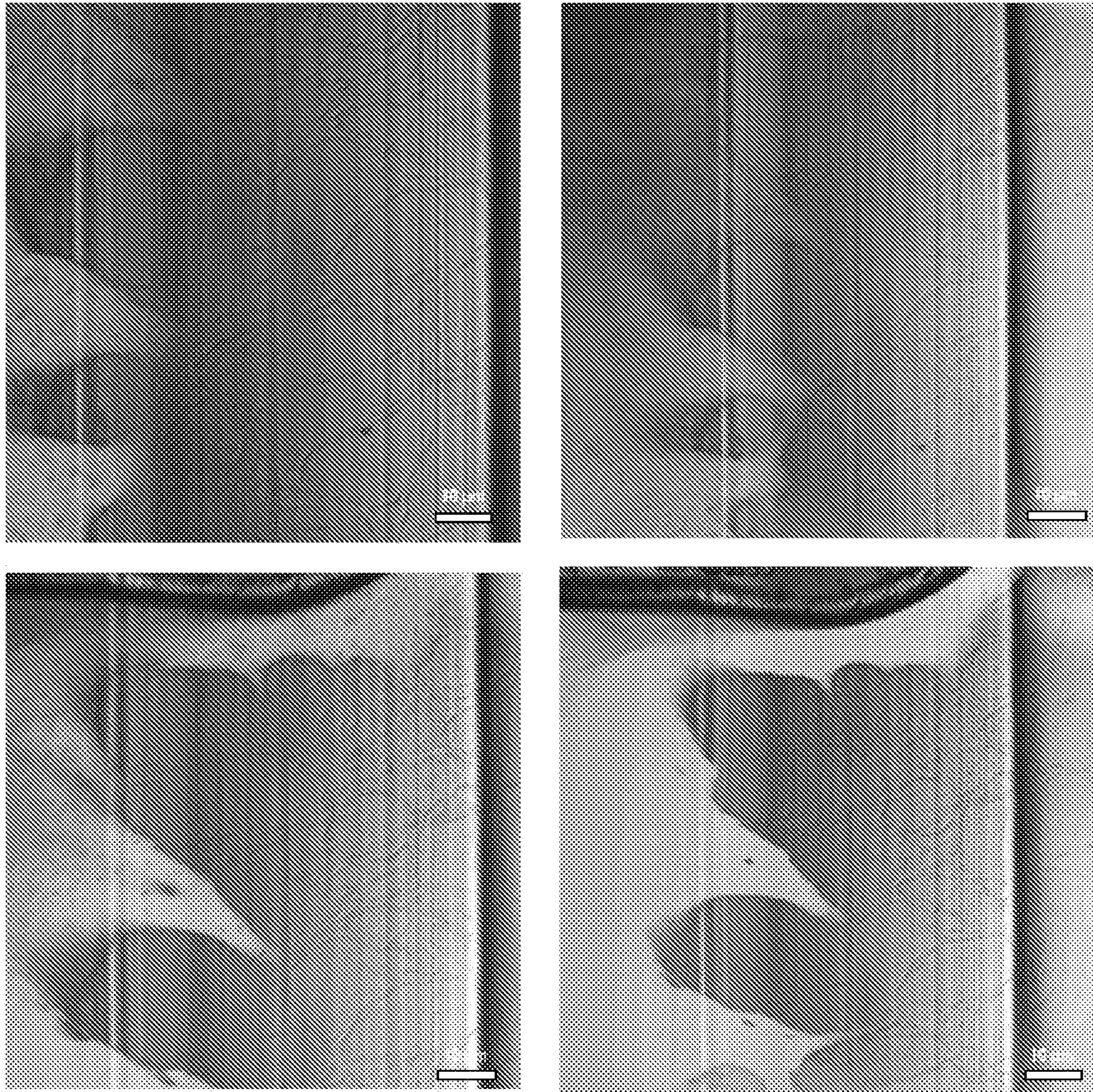
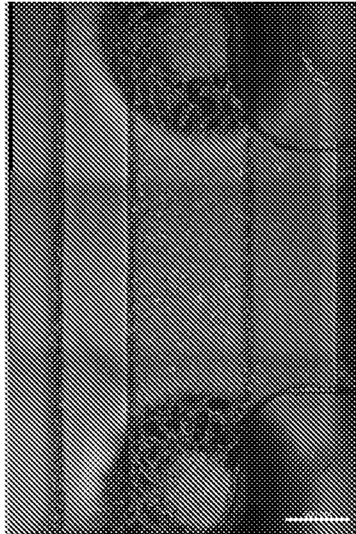
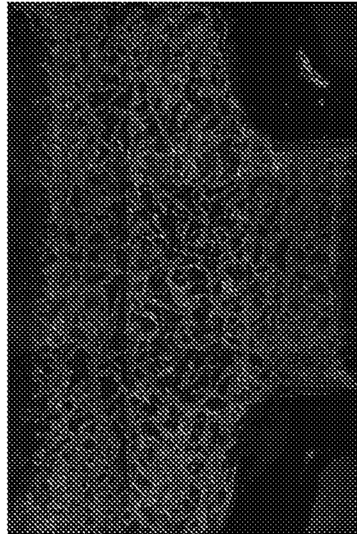
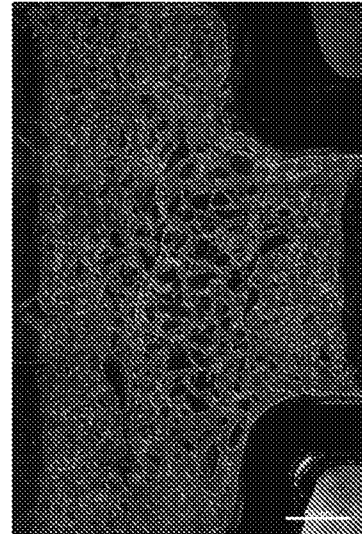
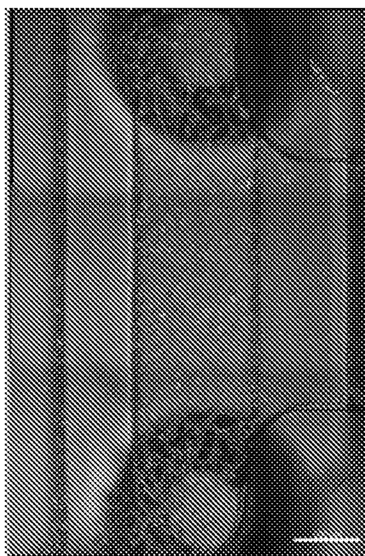
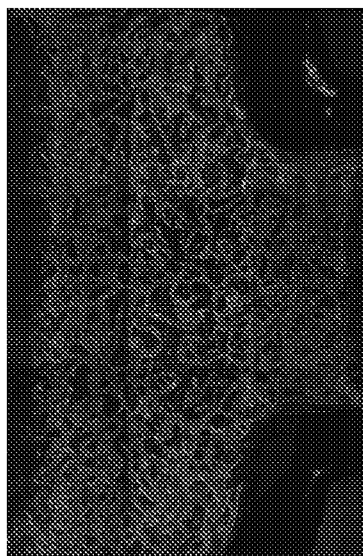
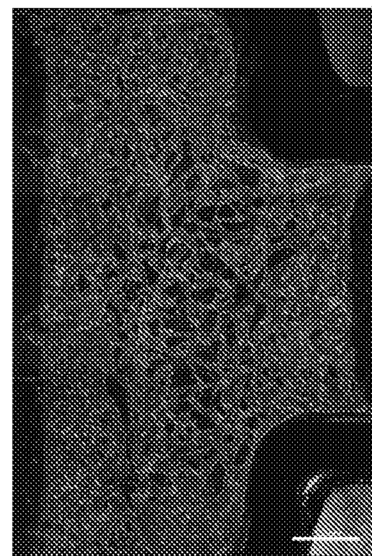


FIG. 14

(a) (b) (c)

Bright Filed image of **Day3** Lymphangiovasculogenesis

Confocal fluorescence image of **Day3** Lymphangiovasculogenesis

Confocal fluorescence image of **Day5** Lymphangiovasculogenesis

FIG. 15

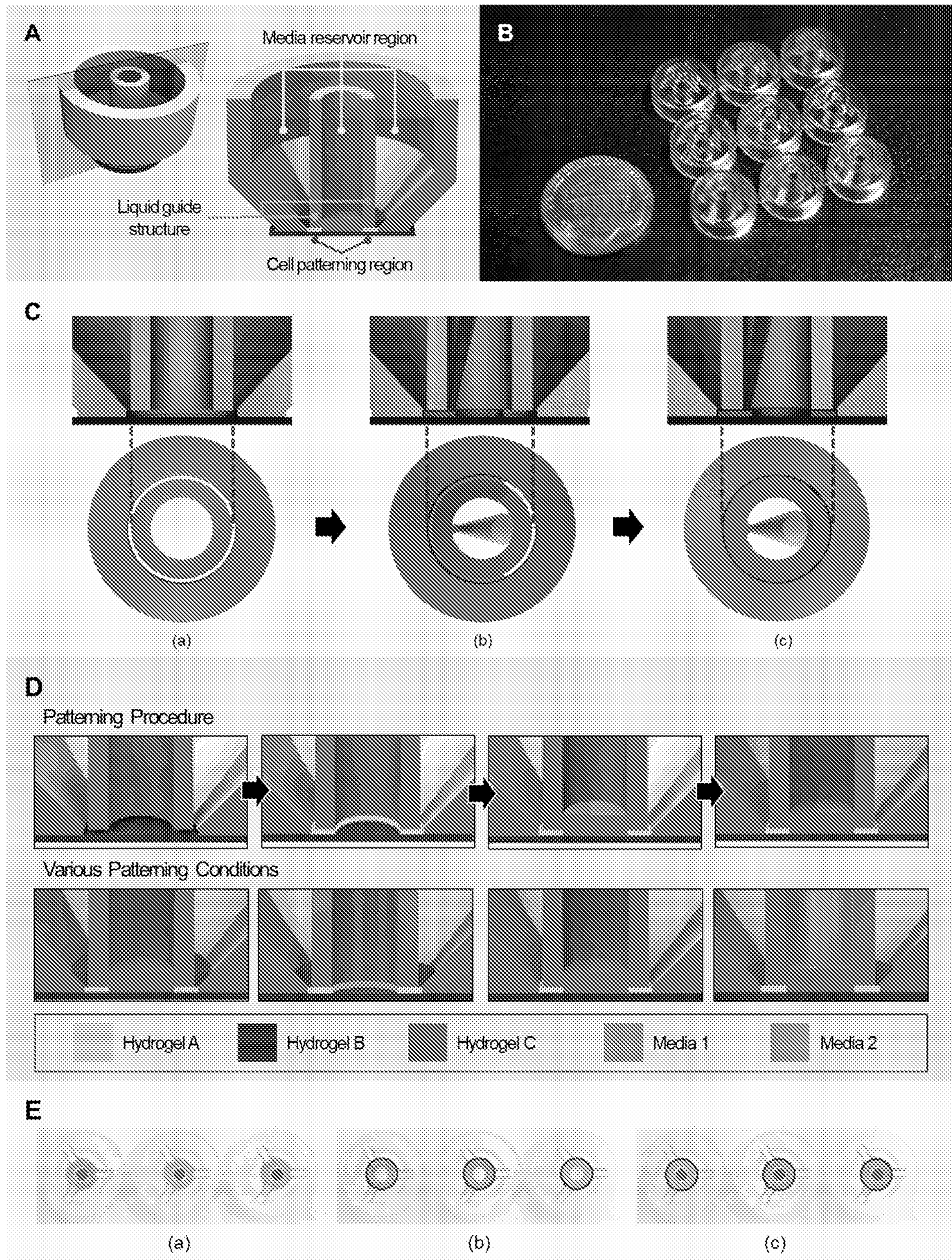


FIG. 16

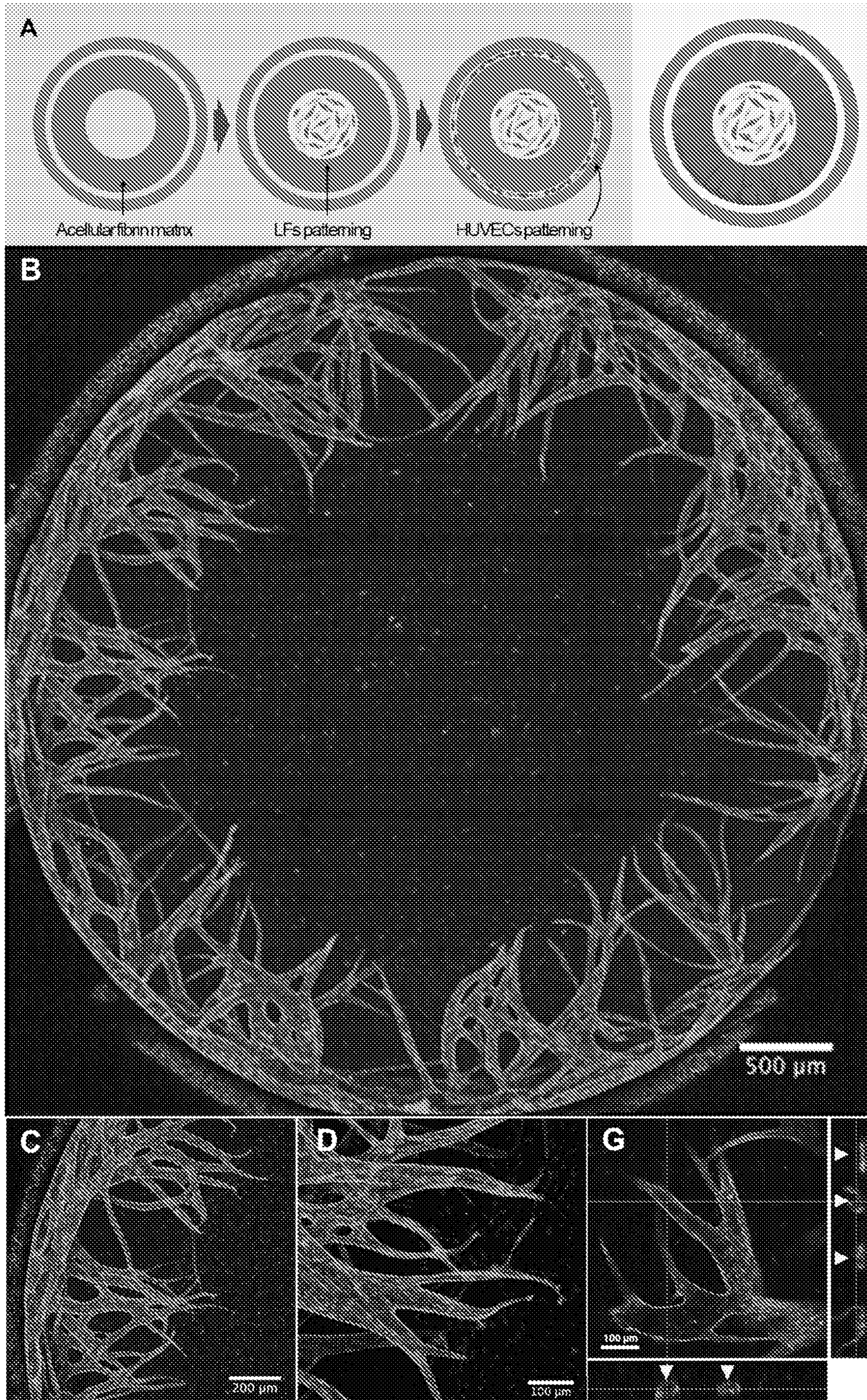


FIG. 17

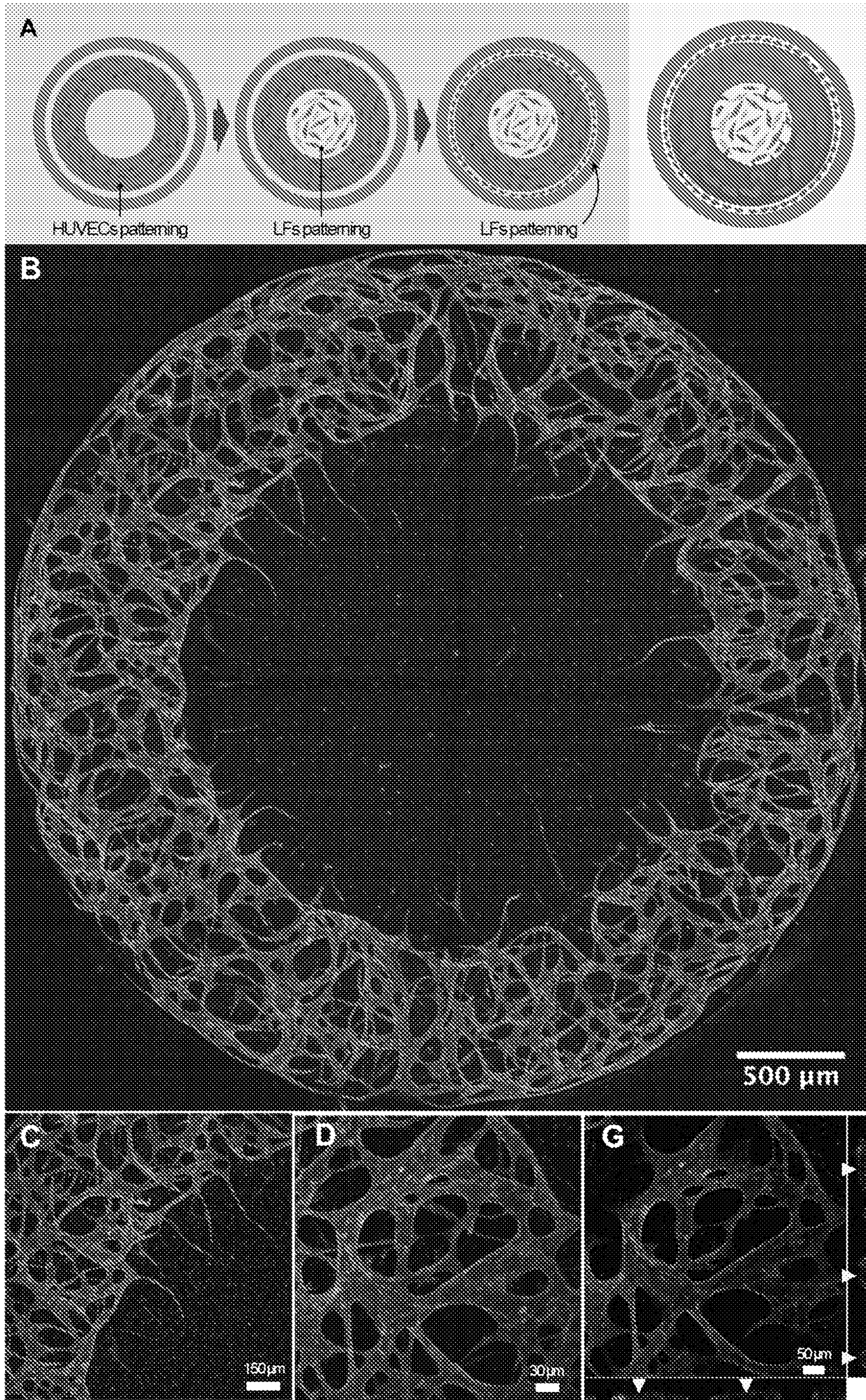


FIG. 18

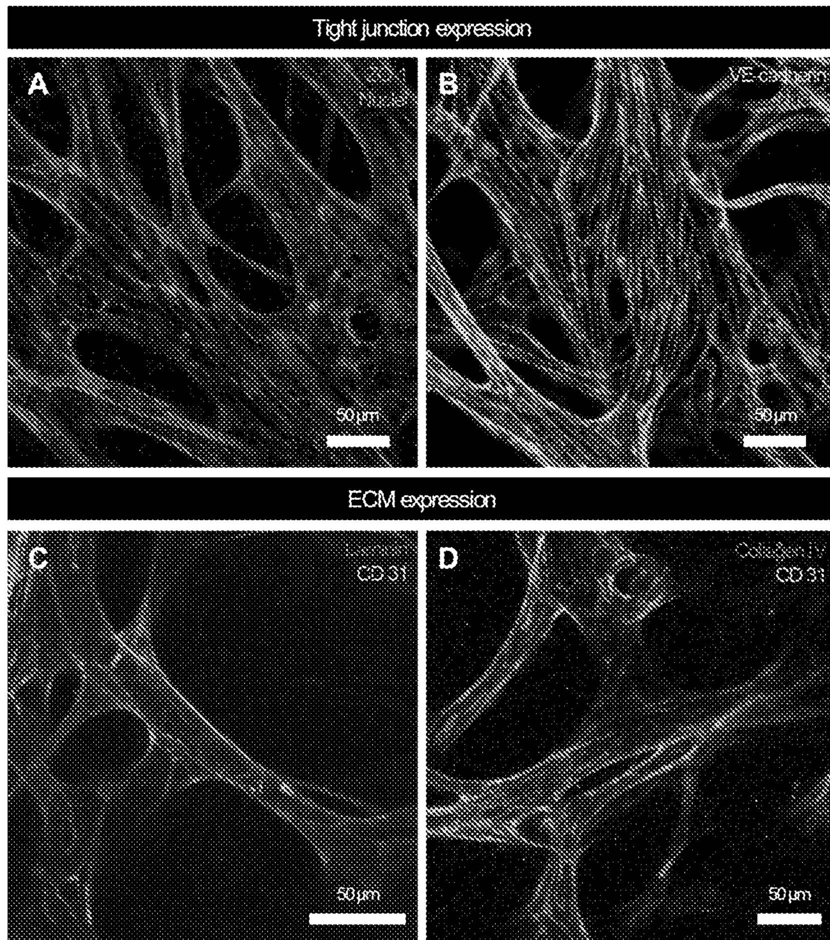
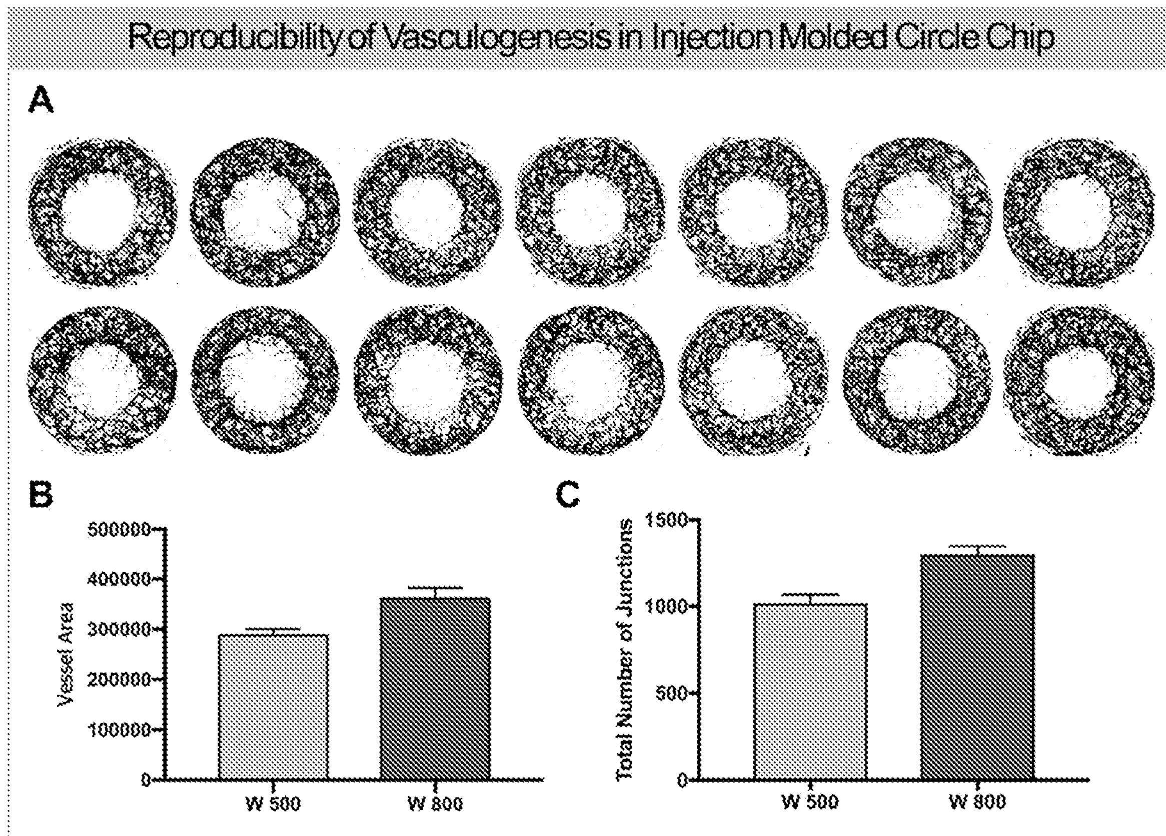


FIG. 19



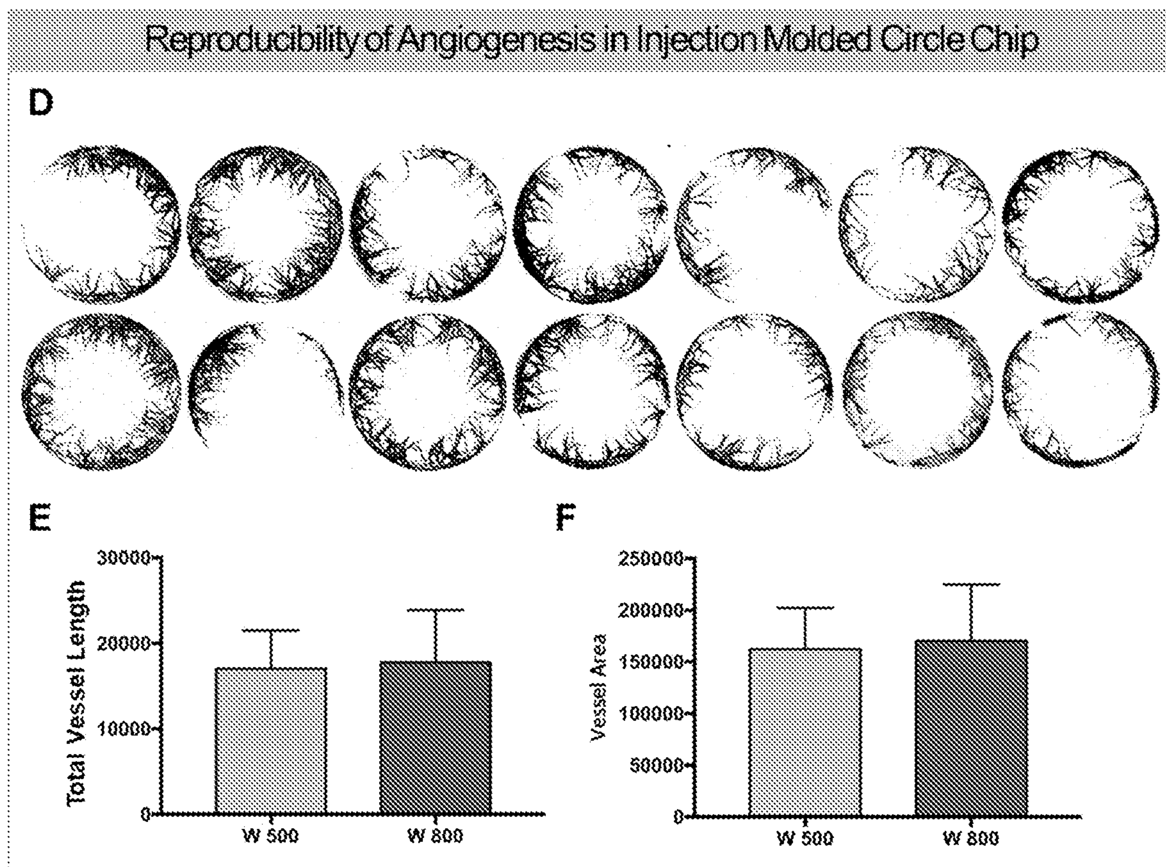


FIG. 20

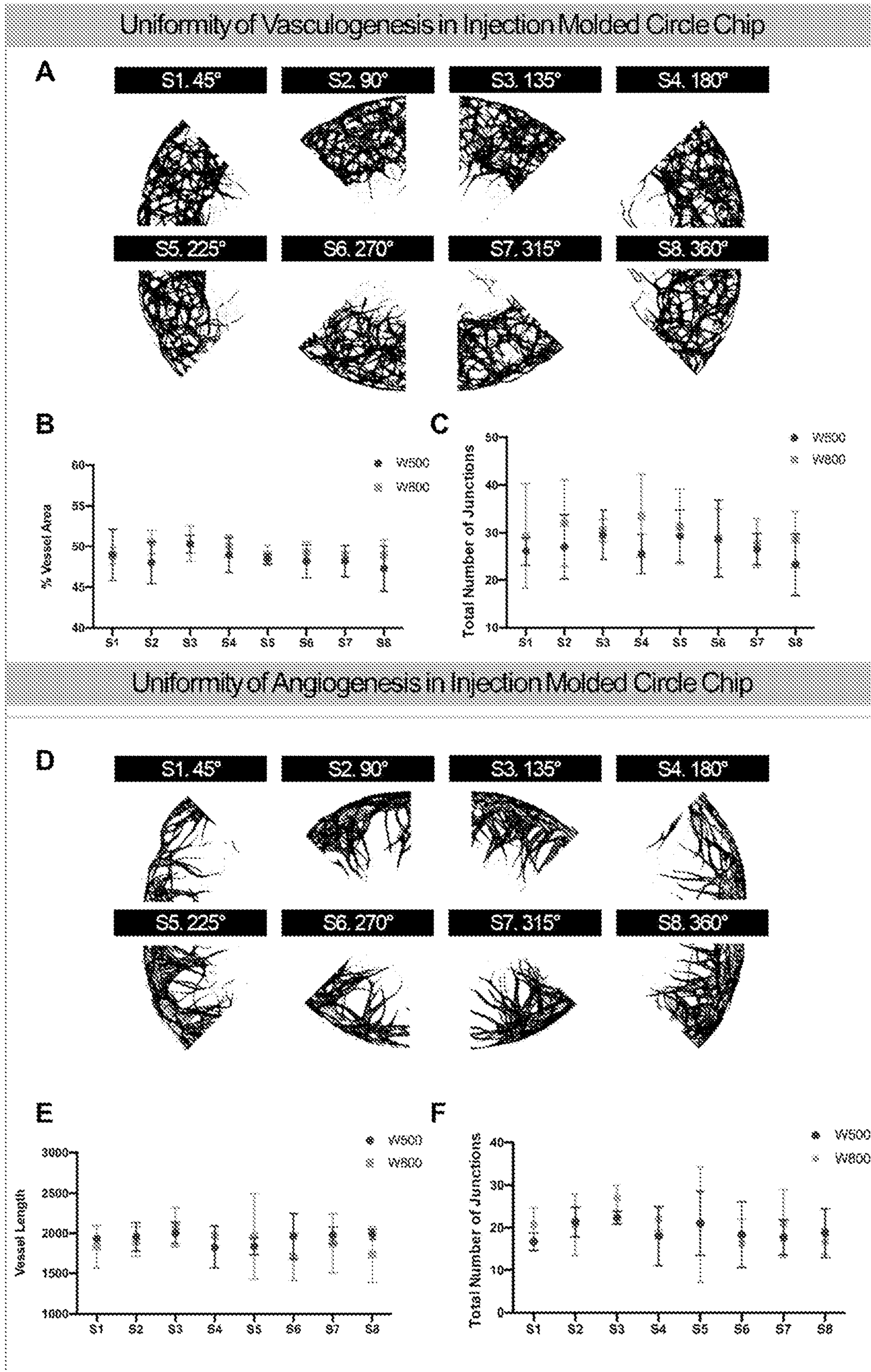


FIG. 21

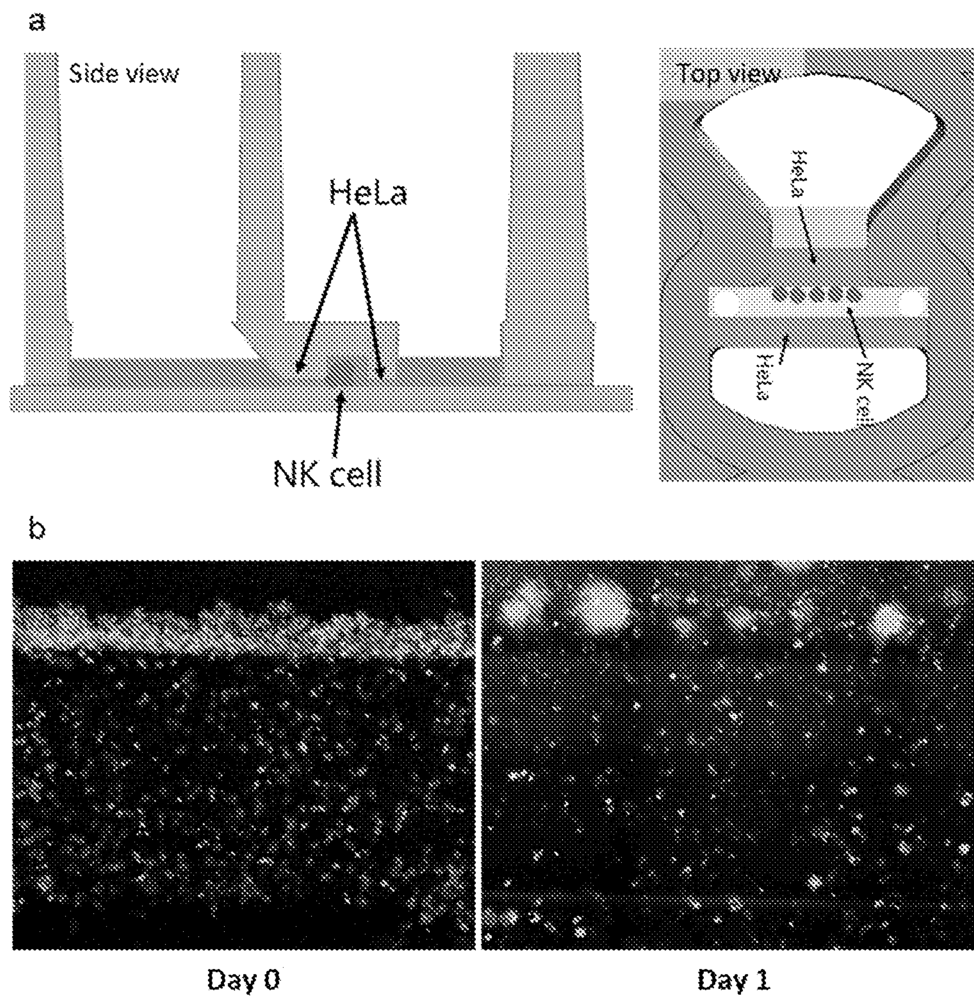


FIG. 22

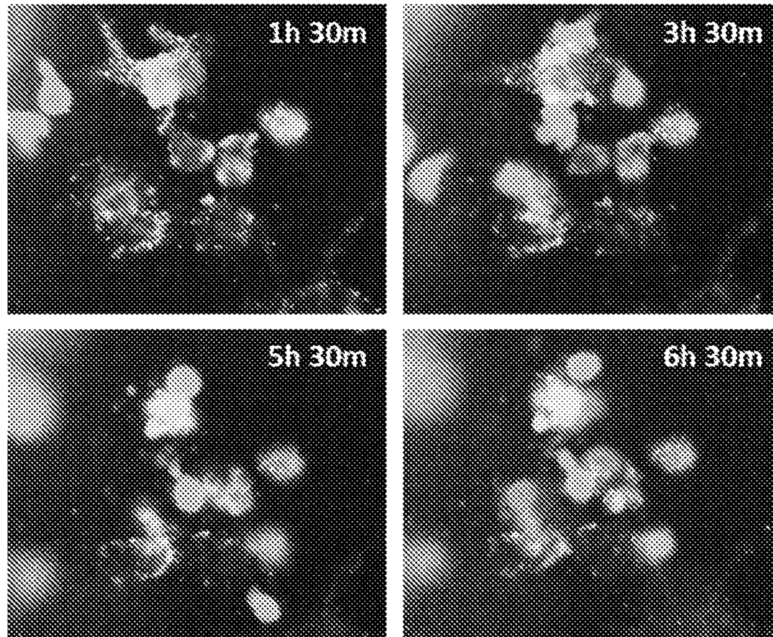


FIG. 23

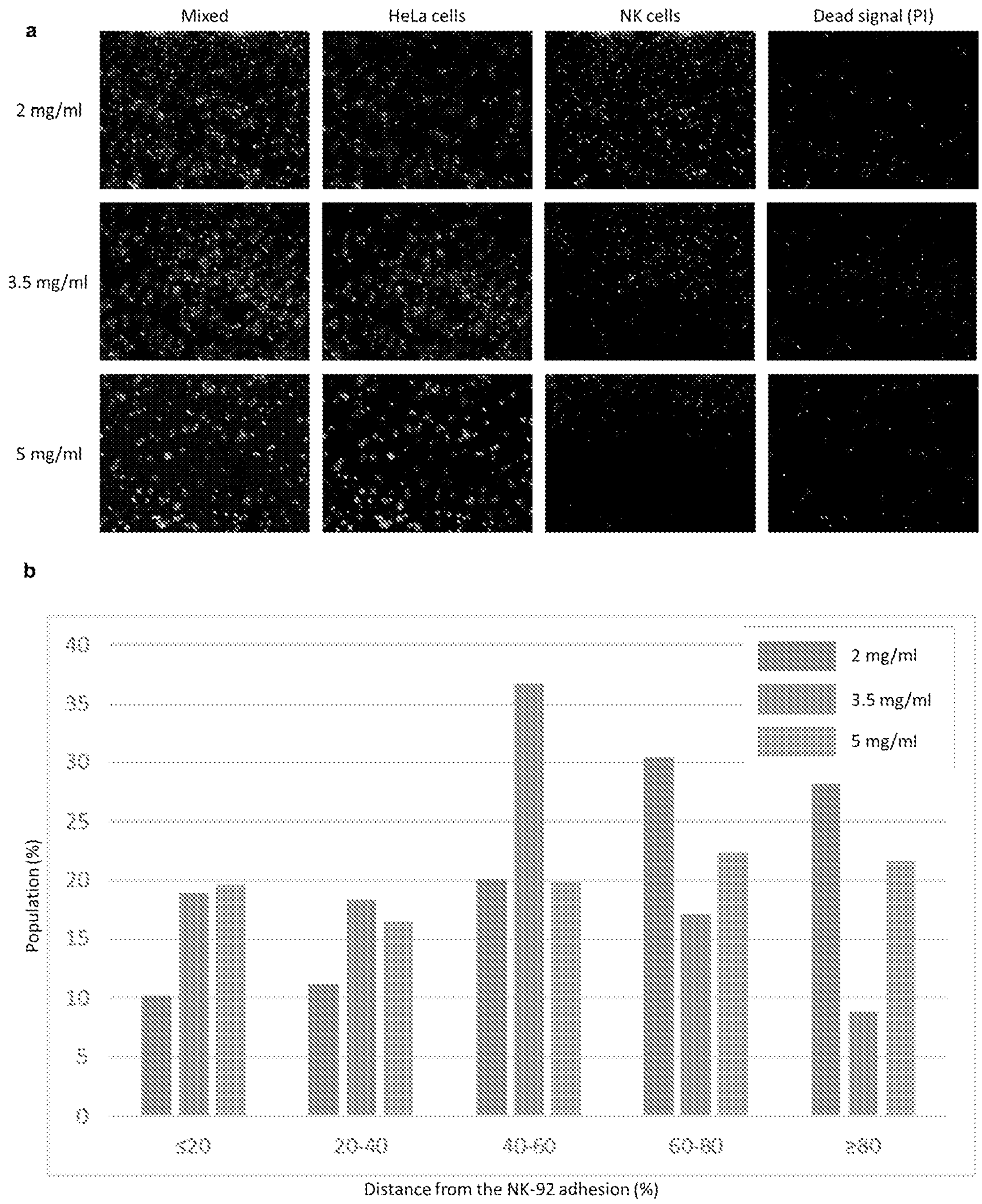


FIG. 24

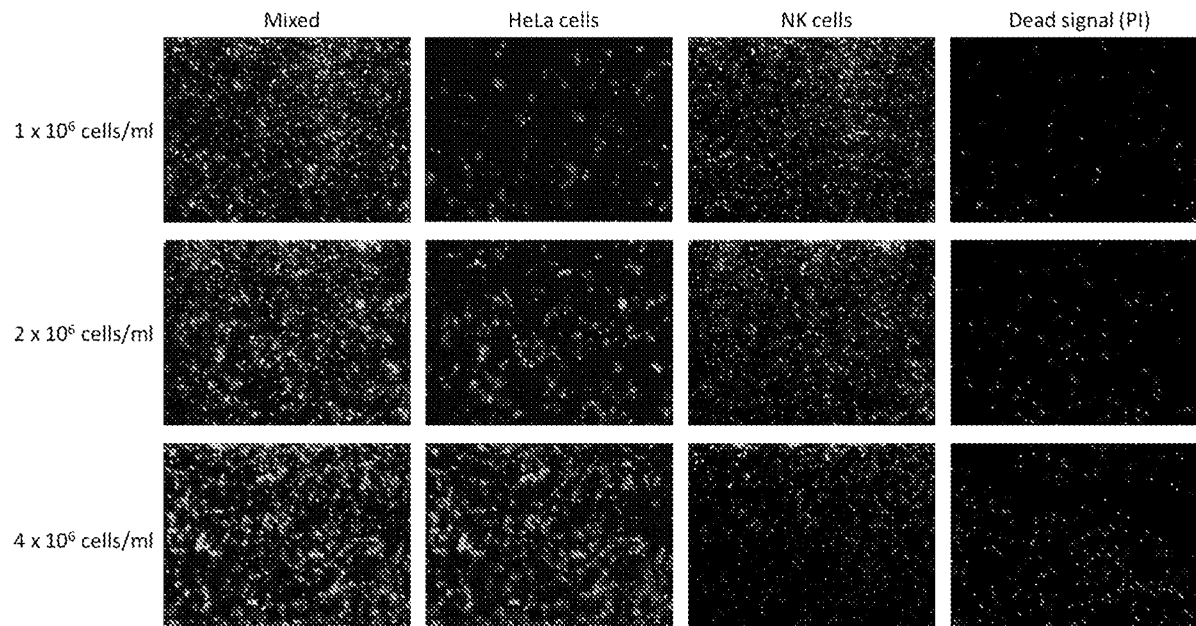


FIG. 25

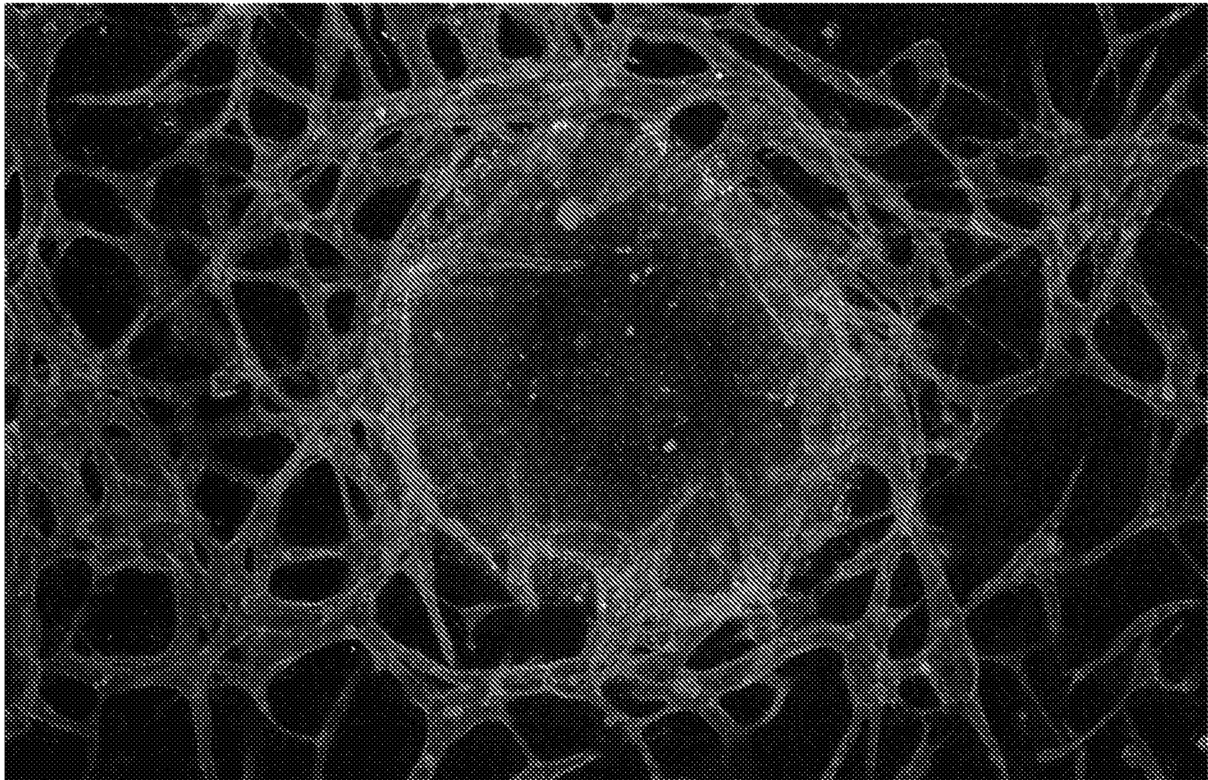


FIG. 26

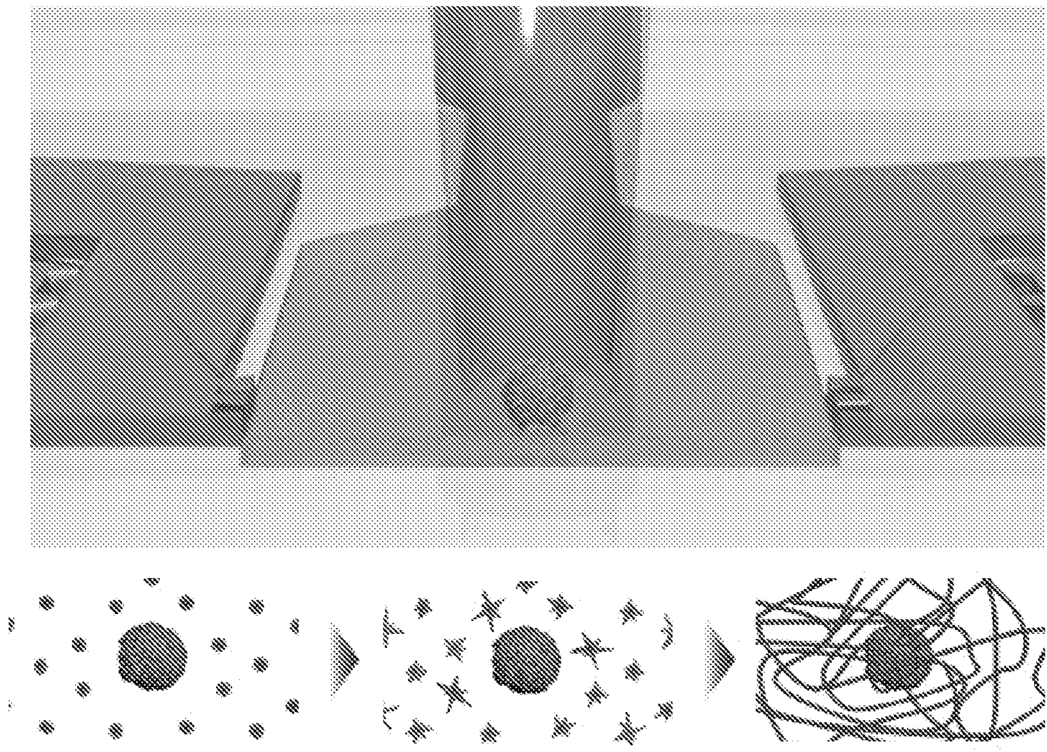


FIG. 27

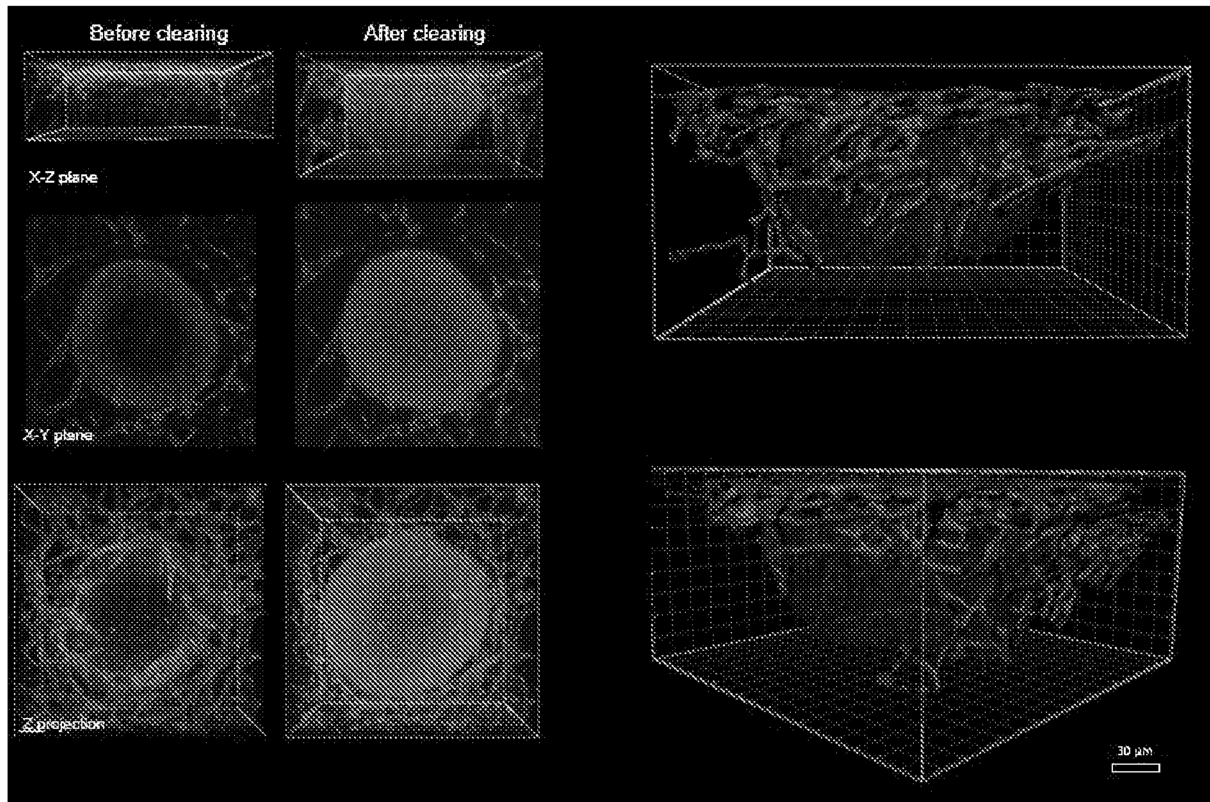


FIG. 28

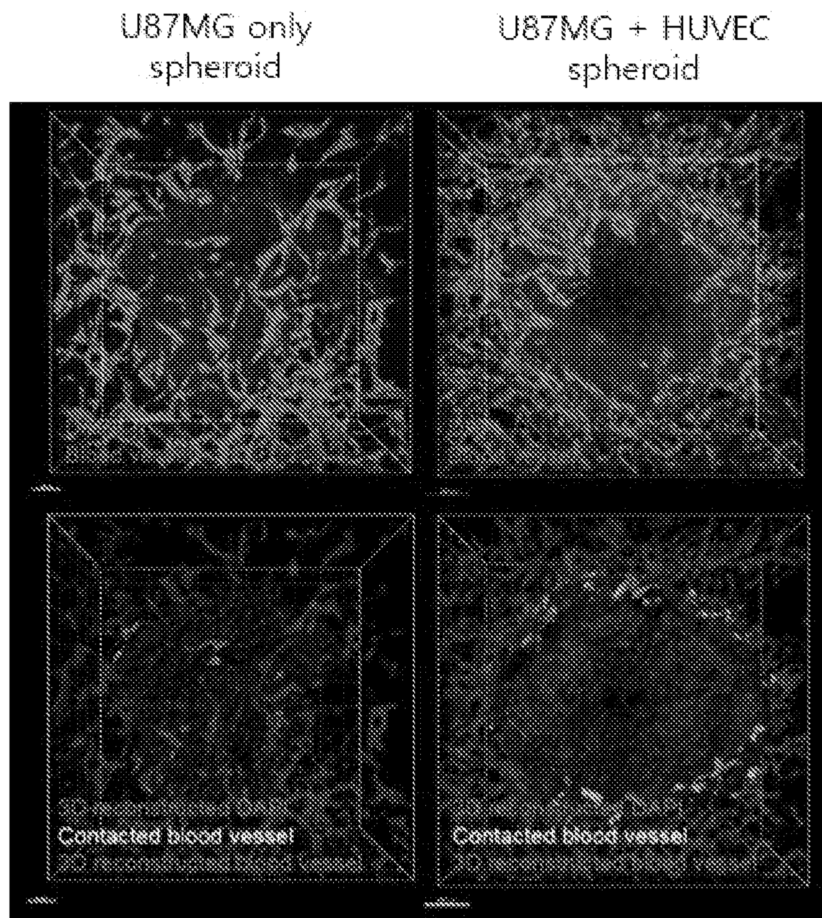


FIG. 29

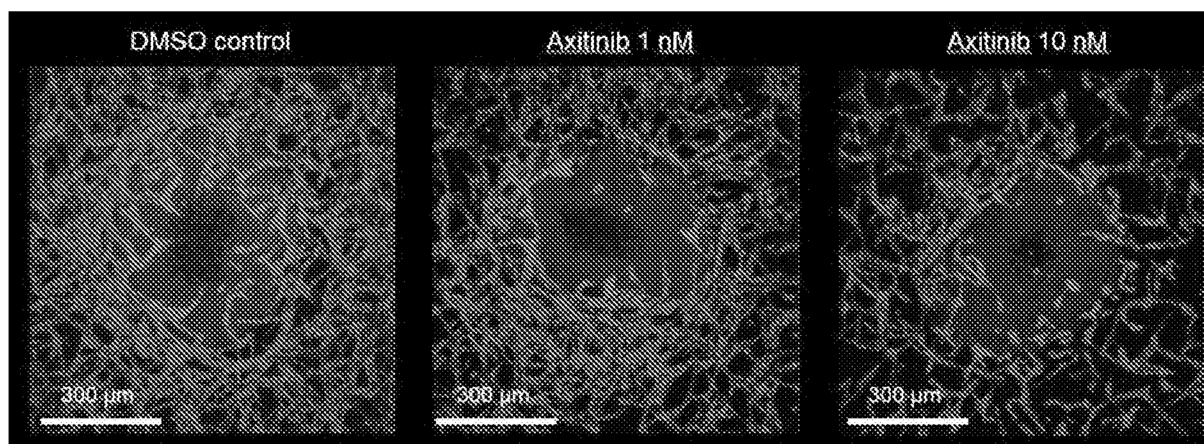


FIG. 30

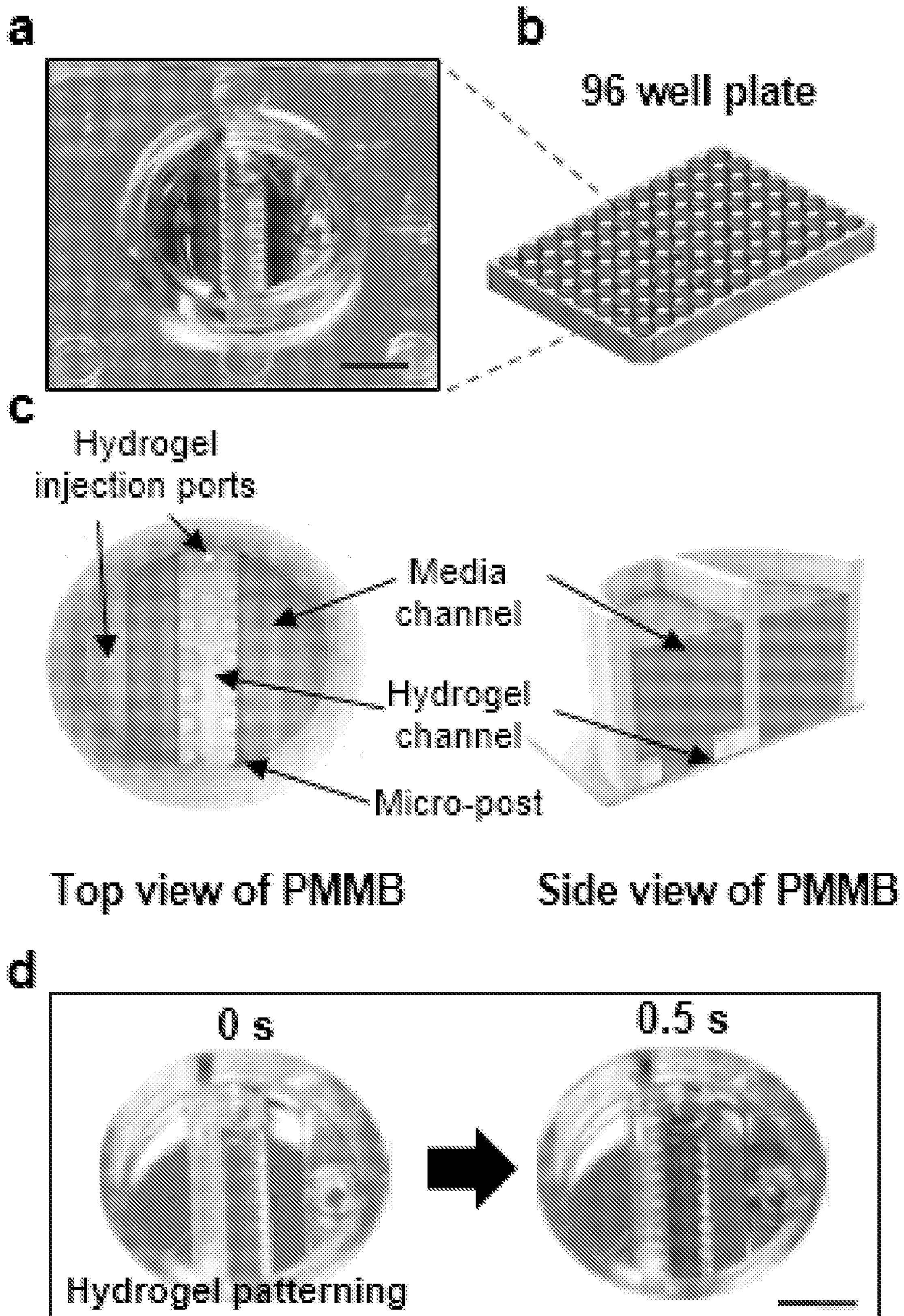


FIG. 31

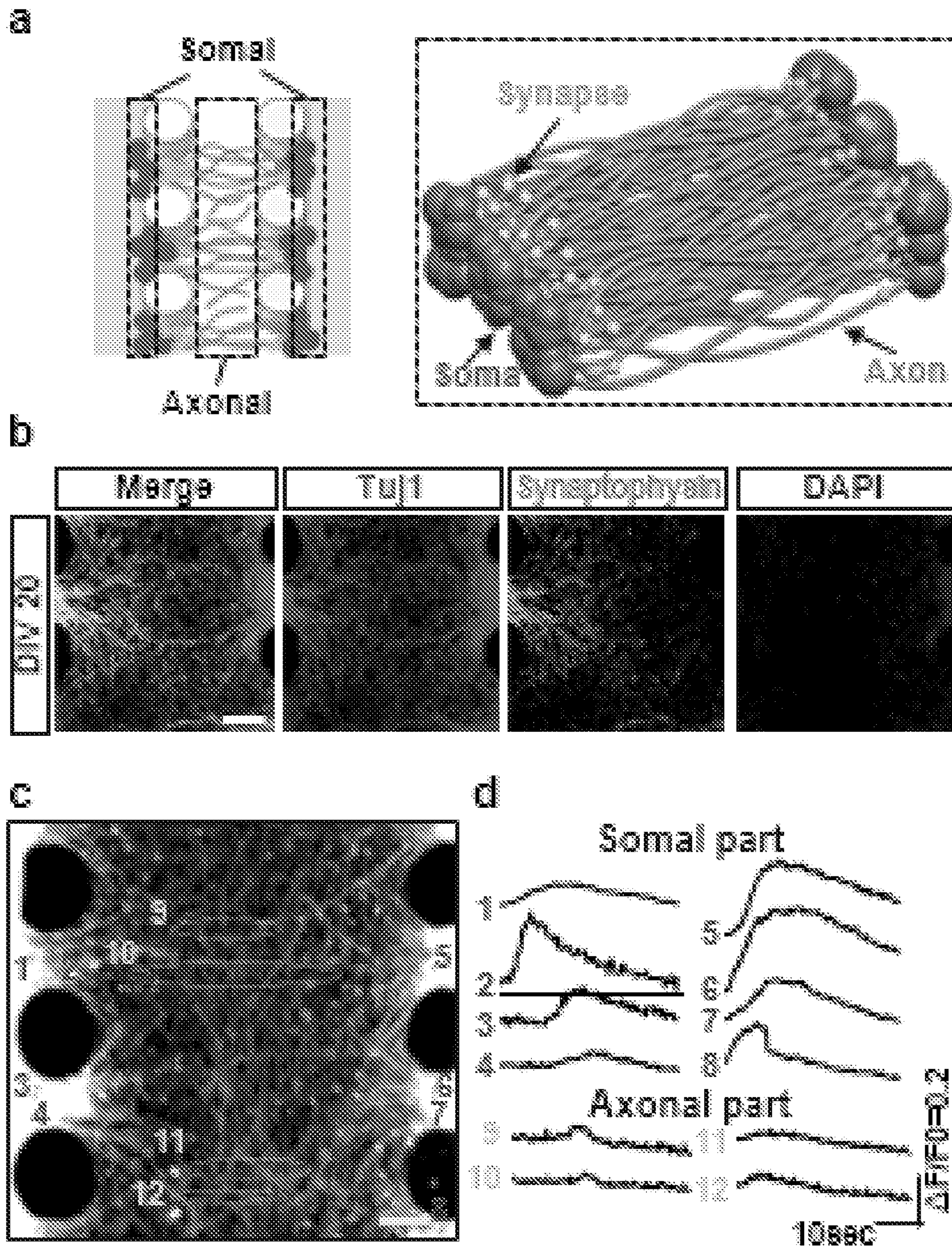


FIG. 32

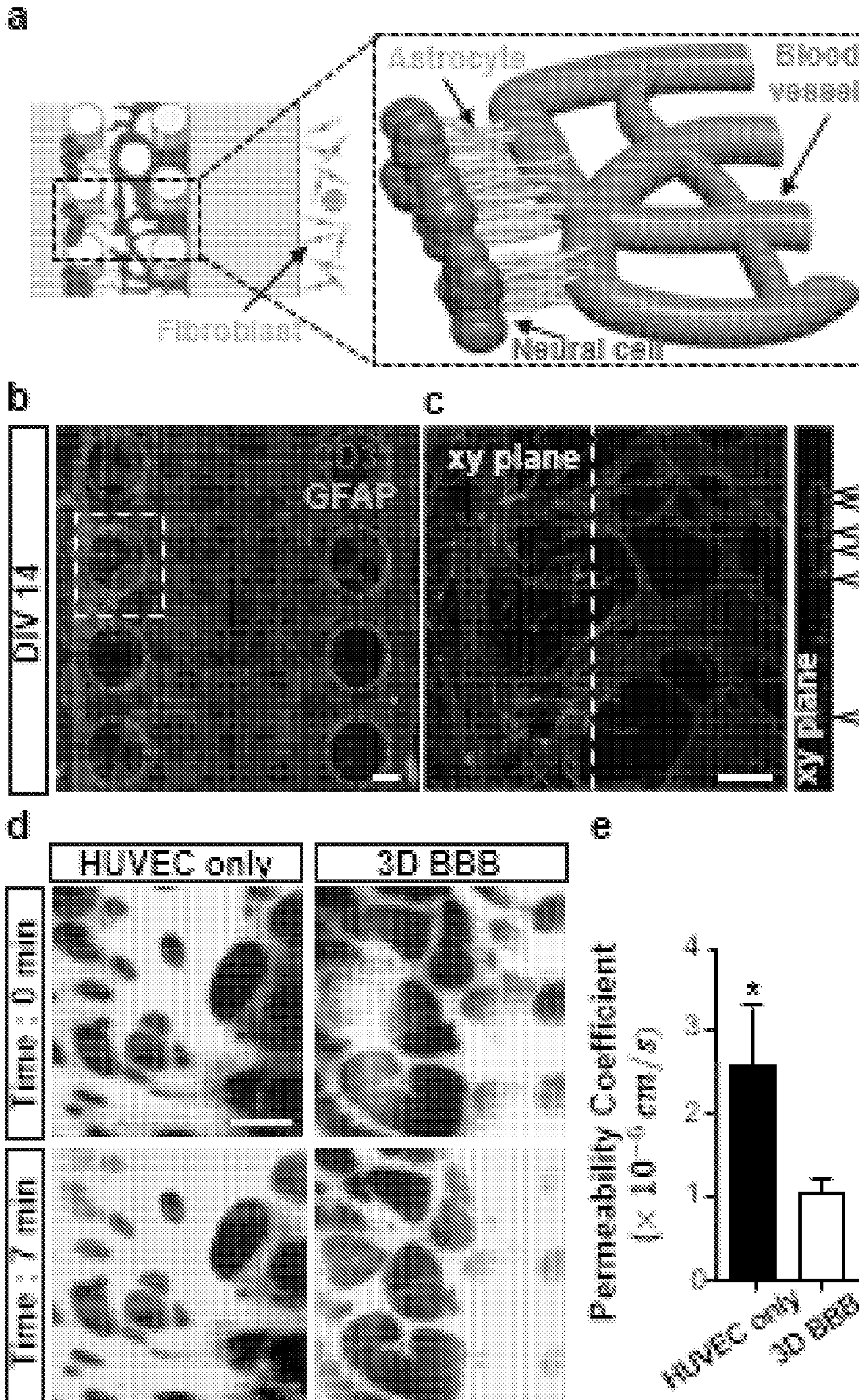
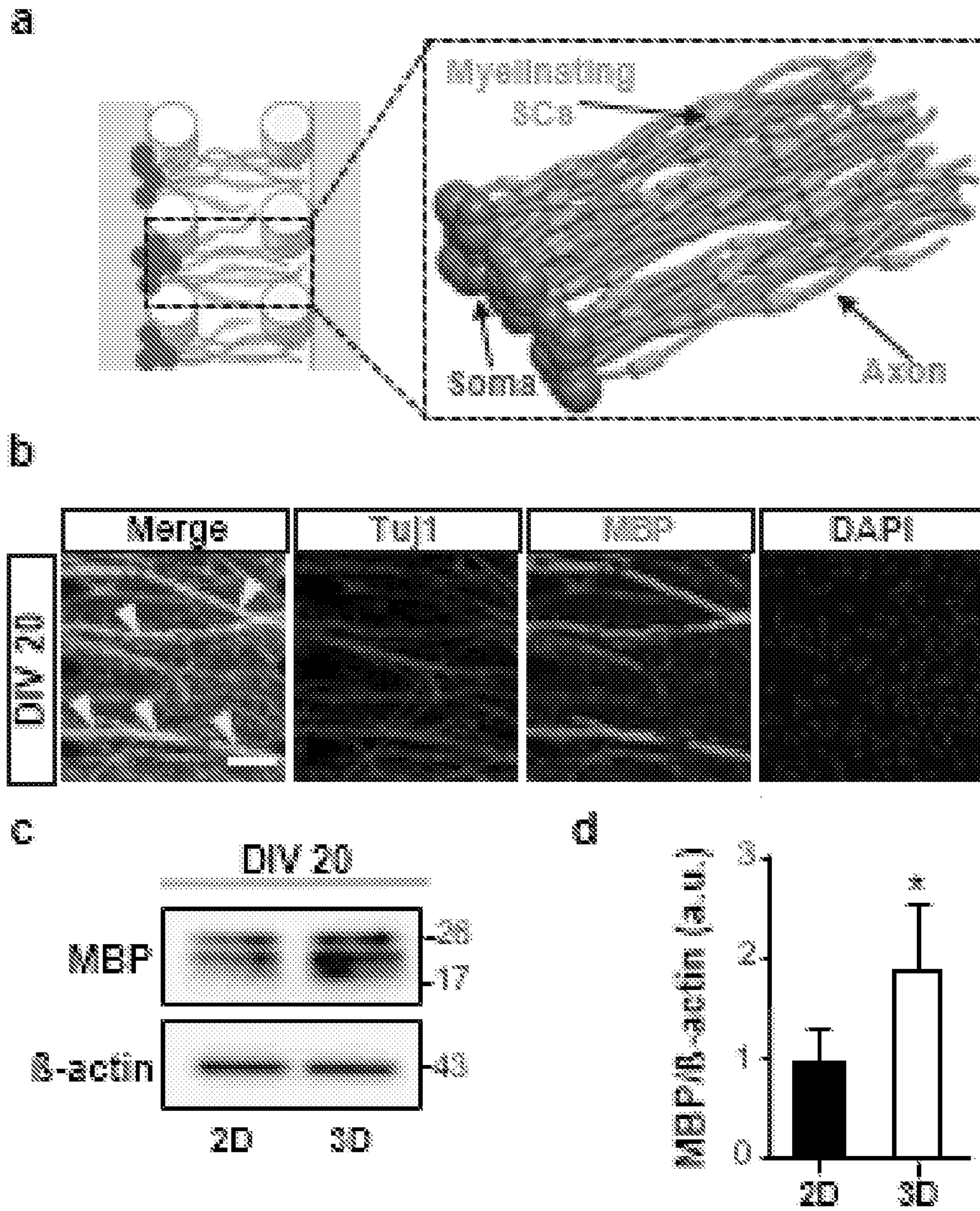


FIG. 33



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/38325

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 16, 27-42, 44-64
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Group I: Claims 1-15, directed to a microfluidic device having a microfluidic channel in which at least a surface in contac with a fluid is formed of a hydrophilic material, comprising: a substrate having a top surface and a bottom surface; outer walls which are attached to the top surface of the substrate such that one or more cavities with an open top are formed and which include an inner surface facing the cavity and an outer surface opposite to the inner surface; inner corner paths formed by the boundary at which the inner surface of the outer wall meets the top surface of the subtrate.

Continued in supplemental box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-15

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/38325

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2018.01)
CPC - B01L 2200/0652, B01L 2200/0668, B01L 2200/0673

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0089529 A1 (BARHOLM-HANSEN et al.) 15 April 2010 (15.04.2010) Abstract, para[0015]-[0016], [0022], [0166], [0196]-[0198] Figures 1-2	1-15
A	US 6210986 B1 (ARNOLD et al.) 03 April 2001 (03.04.2001) Title, Abstract, Col 6 lines 16-18, 62; Figures 2B, 6A	1-15
A	US 2004/0206399 A1 (HELLER et al.) 21 October 2004 (21.10.2004) Title, Abstract, para[0011], [0012], [0043], [0045]; Figures 1, 2A	1-15
A	US 2003/0026740 A1 (STAATS) 06 February 2003 (06.02.2003) Entire document	1-15
A	US 2007/0286774 A1 (BARHOLM-HANSEN et al.) 13 December 2007 (13.12.2007) Entire document	1-15
A	US 2002/0117517 A1 (UNGER et al.) 29 August 2002 (29.08.2002) Entire document	1-15

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 August 2018	24 OCT 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Continuation of:

Box NO. III. Observations where unity of invention is lacking

Group II: Claims 17-26 and 43, directed to a device, comprising: a substrate having a top surface and abottom surface opposite to the top surface; and one or more beams, a respective beam of the one or more beams having a bottom surface facing the top surface of the substrate and a top surface opposite to the bottom surface of the respective beam facing away from the top surface of the substrate, the respective beam being positioned adjacent to the substrate.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I requires a microfluidic channel in which at least a surface in contact with a fluid is formed of a hydrophilic material, comprising: outer walls which are attached to the top surface of a substrate such that one or more cavities with an open top are formed and which include an inner surface facing the cavity and an outer surface opposite to the inner surface; inner corner paths formed by the boundary at which the inner surface of the outer wall meets the top surface of the substrate; and a microfluidic channel module including a top surface, a bottom surface and both ends is disposed in the one or more cavities to cross the inside of the cavity by bonding the both ends to different positions on the inner surface of the outer wall, not required by group II.

Group II requires a device comprising one or more beams, a respective beam of the one or more beams having a bottom surface facing teh top surface of a substrate and a top surface opposite to the bottom surface of the respective beam facing away from the top surface of the substrate, the respective beam being positioned adjacent to the substrate., wherein at least a portion of the respective beam is spaced apart fromt he top surface of the substrate, not required by group I.

Common Technical Features:

Groups I-II share the technical feature of a device having a substrate having a top surface and a bottom surface, and a microfluidic channel that can move a fluid by capillary force along the top surface of the substrate.

However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by US 2010/0089529 A1 to BARHOLM-HANSEN et al. (hereinafter "Barholm-Hansen"). Barholm-Hansen discloses a device (Title, Microfluidic devices...) having a substrate having a top surface and a bottom surface (para[0196], ...base substrate 11...Figure 2, ...substrate 11 has a top surface and a bottom surface), and a microfluidic channel (Abstract, ...a microfluidic device having at least one flow path...para[0016], ...in most microfluidic devices the flow path may preferably be in the form of a closed flow path in the form of a channel...) that can move a fluid by capillary force along the top surface of the substrate (para[0197], ...the liquid sample flow direction is indicated with the arrow A...Figure 1, the flow path is on top of the substrate 4...para[0015], ...the term flow path is a pathway arranged in the microfluidic device along which path a liquid sample can flow either by means of capillary forces...).

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-II lack unity under PCT Rule 13.

Note:

Claims 16, 27-42, 44-64 have been excluded because they are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).