

5 APPLICATIONS OF BIOLOGICAL BLOCK PLATFORM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/296,276, filed January 4, 2022, which is expressly incorporated by reference herein in its entirety.

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TECHNICAL FIELD

Embodiments of the presently-disclosed invention relate generally to methods of growing of tissue cells *ex vivo*, in which one or more interlocking porous hydrogel blocks (IPHB) and/or one or more acellular substrates and joined together for the growth of one or more cell types of interest, which may include the formation of unique interfaces between different cell types of interest and/or the collection of one or more biologics produced or secreted from one or more of the cells of interest.

BACKGROUND

Cells (e.g., human, animal, plant, bacteria, fungi) are cultivated traditionally on plastic substrates, such as petri dishes, mono-plates, well-plates, flasks, and scaffolds. However, each of these cell culture vessels are discrete and finite or limited in surface area for cell propagation. For example, these *in vitro* expansion systems include the use of rigid, 2D plastic culture vessels to expand the cells as a monolayer and require multiple protease-dependent sub-culturing (passaging) events to achieve large enough cell quantities. Evidence suggests that traditional 2D culture systems are not ideal for stem cell expansion and can result in a loss of multipotency, reduce viability, induce senescence and decrease secretion of regenerative factors. These types of vessels, moreover, do not mimic the internal environments from which the cells of interest naturally grow (e.g., human body from which different cell types arise). While these cell culture vessels enable easy cultivation of cells, the cells must be dissociated using a toxin to the cells and transferred to additional cell culture vessels upon outgrowing the original cell culture vessel. This process, as referenced above, is known as sub-culturing and is also known as passaging. The process of propagating cells is time-consuming, prone to error, laborious, expensive. As primary cells derived directly from tissue sub-cultured over and over, these primary cells eventually lose native characteristics (phenotype) and function. At a certain point, primary cells will stop dividing and will no longer function as they did when first extracted from primary tissue. This ceasing of function

5 is known as senescence, and may limit the lifetime of propagation of cells from an initial sample of a primary cell of interest.

Moreover, connective tissues such as bone, cartilage, skin, nerve, lung, muscle, and many others contain cells that secrete and organize extracellular matrix (ECM) proteins to create scaffolds to allow cells to migrate, proliferate, and organize to form tissues. The
10 interplay between the organization of composition and structure of ECM is well documented. However, approaches for developing interfaces between such different cell types for therapeutic purposes or for analysis is currently lacking.

Therefore, there remains a need in the art for flexible platforms for methods of growing one more cells of interest, such as the growth of interfaces between different cell
15 types of interest.

SUMMARY OF INVENTION

One or more embodiments of the invention may address one or more of the aforementioned problems. Certain embodiments according to the invention provide a method
20 of growing tissue cells *ex vivo*, in which the method may comprise the following: (i) interlocking together a plurality of interlocking porous hydrogel blocks (IPHB) including a first IPHB and a second IPHB, wherein each of the plurality of IPHBs include a respective three-dimensional (3D) macrostructure defined by a respective continuous polymeric matrix material and a respective network of microporous channels and/or chambers extending
25 throughout the respective continuous polymeric matrix material, and wherein the respective 3D macrostructures each comprise a respective top surface, a respective bottom surface, and a respective thickness defined by at least one respective side edge extending from the respective top surface to the respective bottom surface, and wherein the respective 3D macrostructure structures each include at least one respective interlocking-male component and at least one respective interlocking-female component; (ii) seeding the first IPHB with a
30 first cell type of interest; (iii) seeding the second IPHB with a second cell type of interest, wherein the first cell type of interest is different than the second cell type of interest; (iv) feeding the first cell type of interest with a first culture media, and allowing the first cell type of interest to propagate throughout a first network of microporous channels and/or chambers
35 towards the second IPHB; (v) feeding the second cell type of interest with a second culture media, and allowing the second cell type of interest to propagate throughout a second network of microporous channels and/or chambers towards the first IPHB; and (vi) forming a first interface between the first cell type and the second cell type. In accordance with certain

- 5 embodiments of the invention, the plurality of IPHBs may be joined with one or more
acellular substrates for tissue cell growth.

BRIEF DESCRIPTION OF THE DRAWING(S)

10 The invention now will be described more fully hereinafter with reference to the
accompanying drawings, in which some, but not all embodiments of the invention are shown.
Indeed, this invention may be embodied in many different forms and should not be construed
as limited to the embodiments set forth herein; rather, these embodiments are provided so that
this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements
throughout, and wherein:

15 Figure 1 is a visual illustration of a method in accordance with certain embodiments
of the invention;

Figure 2 illustrates two (2) separate IPHBs in accordance with certain embodiments of
the invention;

20 Figure 3 illustrates three (3) interlocked IPHBs in accordance with certain
embodiments of the invention;

Figure 4 depicts top and side views of an IPHB and illustrates the continuous
polymeric matrix material and the network of microporous channels and/or chambers
extending throughout the continuous polymeric matrix material, which are exposed at the
surfaces of the IPHB, in accordance with certain embodiments of the invention;

25 Figure 5 illustrates a IPHB including an interlocking-male component located on a top
surface and an interlocking-female component protruding into the bottom surface in
accordance with certain embodiments of the invention;

30 Figure 6 illustrates another IPHB including an interlocking-male component located
on a top surface and an interlocking-female component protruding into the bottom surface in
accordance with certain embodiments of the invention;

Figure 7 illustrates cell migration from a first IPHB to a second IPHB when
interlocked together in accordance with certain embodiments of the invention;

Figures 8A-8F illustrate cell growth patterns within a microchannel in accordance
with certain embodiments of the invention;

35 Figure 9 is a schematic illustrating the nomenclature and conceptual cell migration of
a plurality of IPHBs in accordance with certain embodiments of the invention;

Figure 10 is a schematic illustrating additional options for adding a varying number
and positioning of additional IPHBs to those of Figure 9;

5 Figure 11 is a schematic for an infiltration assay configuration of a series of interlocked empty IPHBs with four (4) seeded IPHBs interlocked as side-chains to the series of interlocked empty IPHBs;

 Figure 12 is a schematic for a dual-infiltration assay configuration of a series of interlocked empty IPHBs with a first set of four (4) IPHBs seeded with a first cell type
10 interlocked as side-chains to the series of interlocked empty IPHBs and a second set of four (4) IPHBs seeded with a second cell type, which may produce or secrete a therapeutic agent interlocked as side-chains on an opposite side of the series of interlocked empty IPHBs;

 Figure 13 illustrates a multi-infiltration assay configuration in which eight IPHBs are attached as side-chains to a series of interlocked empty IPHBs, in which each IPHB attached
15 as a side-chain is seeded with a different cell type, each of which may produce or secrete a different therapeutic agent;

 Figure 14 illustrates a migration assay configuration in which a series of seeded IPHBs have eight (8) side-chains of unseeded or empty IPHBs attached thereto;

 Figure 15 illustrates a dual-migration assay configuration in which a series of seeded
20 IPHBs include a first group of IPHBs are seeded with a first cell type and a second group of IPHBs are seeded with a second cell type, in which eight (8) side-chains of unseeded or empty IPHBs are attached thereto;

 Figure 16 illustrates a multi-migration assay configuration in which a series of seeded IPHBs, in which each IPHB is seeded with a different cell type, and eight (8) side-chains of
25 unseeded or empty IPHBs are attached thereto;

 Figure 17 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which each chamber is filled with a group of four (4) interlocked IPHBs seeded with a different cell type (e.g., each chamber has its own cell type therein);

 Figure 18 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and
30 spacers, in which the IPHBs are configured as a one-way paracrine signaling assay;

 Figure 19 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a two-way paracrine signaling assay;

 Figure 20 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a paracrine signaling assay;

35 Figure 21 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a four-way paracrine signaling assay;

 Figure 22 illustrates an acellular matrix in accordance with certain embodiments of the invention;

5 Figure 23 illustrates a cuboidal mold having a network of microchannels and/or micropods 3D printed therein or disposed therein;

 Figure 24 illustrates the seeding of a cell type of interest onto the network of microchannels and/or micropods, and growth of the extracellular matrix of the cell type of interest to fill the cuboidal mold;

10 Figure 25 illustrates the decellularized tissue and subsequent degradation or dissolution of the hydrogel material to form an intermediate acellular substrate;

 Figure 26 illustrates the intermediate acellular substrate being lyophilized and subsequently removed from the cuboidal mold followed by sterilization;

 Figure 27 illustrates the acellular matrix formed in accordance with certain
15 embodiments of the invention;

 Figure 28-31 illustrate different configurations of a plurality of joined acellular substrates in accordance with certain embodiments of the invention;

 Figure 32A illustrates the macrostructure of the hydrogel system, in which (i) the left image is a single photographic image of the $\sim 1\text{-cm}^3$ 3D hydrogel system; (ii) the middle
20 image is a photographic image of four hydrogels connected to each other within a six-well plate; and (iii) the right image is a photographic image of four hydrogels, connected with annotations depicting the migration of cells out/from an initially seeded hydrogel into/toward supplementally attached hydrogels without cells;

 Figure 32B illustrates the microstructure of the hydrogel system, in which (i) the left
25 image is a confocal microscopy image of a cross-section of the internal structure of the hydrogel depicting adipose-derived mesenchymal stem/stromal cell (ASCs) migrating and proliferating within the porous architecture where (a) the small white arrow indicates polymeric struts of the hydrogel, (b) Blue = Hoechst, (c) green = phalloidin, and (d) red = Mitotracker; (ii) the middle image is a fluorescent image of ASCs lining an individual pore
30 within the microstructure of the hydrogel system where (a) Blue = Hoechst, (b) green = wheat germ agglutinin, and (c) Red = Mitotracker; and (iii) the right image is a confocal microscopy z-stack image of ASC migrating from an initially seeded hydrogel (Hydrogel #1) into a newly attached hydrogel (Hydrogel #2), in which the large white arrows indicate directionality of ASC migration, while the white dashed line indicates the junction of the two
35 attached hydrogels;

 Figure 32C illustrates three images depicting a 3D z-stack of images within a single pore channel to highlight (middle image) the 3D networks formed by ASCs and (right image) cellular extension protruding from the cells, while the left image is a low-magnification

5 image of the entire stained hydrogel where all images were acquired by Nikon on their AXR Confocal Imaging System in which Blue = Hoechst, Green = phalloidin, and Pink = Mitotracker;

Figures 33A-33B illustrate results of ASCs that were seeded at passage 1 (P1) within the 3D hydrogel system or traditional 2D culture, in which ASCs were continuously subcultured and assessed at P2, P6 and P10 for 2D culture while the ASCs in the 3D hydrogel system were compared with their respective 2D counterparts via passage-equivalent time points. Figure 23A illustrates that at each respective time point (P2, P6 and P10), representative images of 2D (leftmost columns) and 3D (right-most columns) cultured ASCs stained for either CD73 (left), CD90 (middle) or CD105 (right) are depicted. CD marker staining is denoted by green in the images. Samples were counterstained with Hoechst (blue) and phalloidin (not shown). Figure 23B illustrates quantification of imaging data performed and total percentage of positive cells denoted by box-and-whiskers plots for each marker, in which each individual point indicates quantification of a single image of ASCs in 2D (black circles) or 3D (teal diamonds) cultures using a 20 X objective. Samples were analyzed in quadruplicate (n = 4). Scale bar = 100 μ m. Error bars are standard error of the mean, while *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001;

Figures 34A-34N illustrate the reduced induction of senescence in 3D hydrogel over time as compared to a traditional 2D culture system, in which ASCs were seeded at passage 1 (P1) within the 3D hydrogel system or traditional 2D culture and the ASCs were continuously subcultured and assessed at P2, P6 and P10 for 2D culture. The ASCs in the 3D hydrogel system were compared with their respective 2D counterparts via passage-equivalent time points. Three additional hydrogels were added to each individual hydrogel at the 2-week mark and left for the remainder of the culture period to provide adequate surface area for continuous cell growth. Figure 34A illustrates that at each respective time point (P2, P6 and P10), representative images of 2D (top row) and 3D (bottom row) cultured ASCs stained for β -galactosidase (green) are depicted. Samples were counterstained with Hoechst (blue) and phalloidin (not shown). Figure 34B illustrates the quantification of imaging data performed and total percentage of positive cells denoted by box-and-whiskers plots for each marker. Each individual point indicates quantification of a single image of ASCs in 2D (black circles) or 3D (teal diamonds) using a 20 X objective. Samples were analyzed in quadruplicate (n = 4). Scale bar = 100 μ m. Error bars are standard error of the mean and ***p < 0.001; ****p < 0.0001; and

5 Figures 35A-35D illustrate retention of wound healing capacity in adipose-derived mesenchymal stem/stromal cell-conditioned media (ASC-CM) from 2D and 3D from each respective time point that was used to treat keratinocytes, which were then assessed for changes in their migratory, metabolic and proliferative activity. Figures 35A and 35B illustrate migratory activity that was assessed via a scratch assay, in which Figure 35A illustrates whole-well image scans that were acquired, and representative images of the wound images are provided, wherein white solid lines denote original wound boundaries and black solid lines outline the remaining wound area. Figure 35B illustrates the average wound area after 22 h was determined and performed in triplicate. Figure 35C illustrates metabolic activity that was quantified via PrestoBlue and is displayed as an average value of relative fluorescent units. Figure 35D illustrates proliferative activity that was quantified via PicoGreen and the average cell number was determined. Keratinocytes treated with 2D ASC-CM are denoted with black bars; keratinocytes treated with 3D ASC-CM are denoted with teal bars. Scale bar = 500 μ m. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Error bars are standard error of the mean.

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DETAILED DESCRIPTION

The invention now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the invention are shown. Indeed, this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. As used in the specification, and in the appended claims, the singular forms “a”, “an”, “the”, include plural referents unless the context clearly dictates otherwise.

The presently-disclosed invention relates generally to methods of growing tissue cells *ex vivo*, in which a plurality of biological blocks may be interlocked or otherwise joined together to provide a tailored environment for the continuous growth of one or more different cell types of interest. For instance, the biological blocks comprise substrates that contain the structure components and cues to instruct cells on how to behave to form tissues. Each tissue, for example, has a different composition and organizational structure to instruct cell types how to arrange, organize, and function. The biological blocks (e.g., IPHBs and/or acellular substrates) may be manufactured using tissue components as source material to create a unique scaffold that possess the same (or similar) physical, chemical, and structural components of native tissue. By way of example, the tissue components can comprise ECM

5 components, such as Glycosaminoglycans (GAGs); Proteoglycans; Hyaluronic acid (HA);
 Heparin sulfate (HS); Chondroitin sulfate (CS); Keratan sulfate (KS); Collagen Types (e.g.,
 Fibrillar (I, II, III, V, XI), Facit (IX, XII, XIV), Short chain (VIII, X), Basement membrane
 (IV), and Other (VI, VII, XIII); Elastin; DNA; RNA; Fibronectins and glycoproteins;
 Laminin-111 (Laminin-1); Laminin-211 (Laminin-2); Laminin-121 (Laminin-3); Laminin-
 10 221 (Laminin-4); Laminin-332/ Laminin-3A32 (Laminin-5/ Laminin-5A); Laminin-3B32
 (Laminin-5B); Laminin-311 / Laminin-3A11 (Laminin-6/ Laminin-6A); Laminin-321 /
 Laminin-3A21 (Laminin-7/ Laminin-7A); Laminin-411 (Laminin-8); Laminin-421 (Laminin-
 9); Laminin-511 (Laminin-10); Laminin-521 (Laminin-11); Laminin-213 (Laminin-12);
 Laminin-423 (Laminin-14); Laminin-522; Laminin-523 (Laminin-15); Peptides; Lipids and
 15 Phospholipids; Carbohydrates; Phosphates; Saturated Fats; Unsaturated Fats; Trans Fats;
 Cholesterols; Organelles (e.g., Mitochondria, Smooth Endoplasmic Reticulum, Rough
 Endoplasmic Reticulum, Golgi body/apparatus, Lysosome, Endosome, Vesicles,
 Peroxisomes, Nucleolus, Nucleus, and Ribosome); and Cytoskeleton Components (e.g.,
 Actin). The biological blocks may be made such that they may be connected together for the
 20 purpose of fusing and forming tissues. The ability to interconnect a variety of customized
 biological blocks to provide further customized configurations to enable interplay or
 interaction between multiple different cell types of interest for the purpose of producing
 tissue (e.g., micro tissues) from custom substrates based on the end user's application, such
 as the formation of unique configurations utilizing specific tissue ECM. Moreover, the
 25 biological blocks may be connected or joined together in a myriad of different configurations
 in the X-Y plane, X-Z plane, or Y-Z plane. Furthermore, the interactions of cells between the
 biological blocks changes depending on which configuration the Bio-biological blocks are
 linked together to provide a 3D scaffold for cell growth and propagation. For example, a
 single tissue may be formed, or to create a tissue construct in which two different tissues may
 30 interface based on how the biological blocks are joined together.

Certain embodiments according to the invention provide a method of growing tissue
 cells ex vivo, in which the method may comprise the following: (i) interlocking together a
 plurality of interlocking porous hydrogel blocks (IPHB) including a first IPHB and a second
 IPHB, wherein each of the plurality of IPHBs include a respective three-dimensional (3D)
 35 macrostructure defined by a respective continuous polymeric matrix material and a respective
 network of microporous channels and/or chambers extending throughout the respective
 continuous polymeric matrix material, and wherein the respective 3D macrostructures each
 comprise a respective top surface, a respective bottom surface, and a respective thickness

5 defined by at least one respective side edge extending from the respective top surface to the
respective bottom surface, and wherein the respective 3D macrostructure structures each
include at least one respective interlocking-male component and at least one respective
interlocking-female component; (ii) seeding the first IPHB with a first cell type of interest;
(iii) seeding the second IPHB with a second cell type of interest, wherein the first cell type of
10 interest is different than the second cell type of interest; (iv) feeding the first cell type of
interest with a first culture media, and allowing the first cell type of interest to propagate
throughout a first network of microporous channels and/or chambers towards the second
IPHB; (v) feeding the second cell type of interest with a second culture media, and allowing
the second cell type of interest to propagate throughout a second network of microporous
15 channels and/or chambers towards the first IPHB; and (vi) forming a first interface between
the first cell type and the second cell type. In accordance with certain embodiments of the
invention, the plurality of IPHBs may be joined with one or more acellular substrates for
tissue cell growth.

In this regard, the methods in accordance with certain embodiments of the invention
20 enable customizable cell cultivation methods utilizing a platform where cells of interest may
continuously propagate on a substrate (e.g., a 3D scaffold) without the need for sub-culture.
In accordance with certain embodiments of the invention, the methods of cultivating cells of
interest utilize one or more biological blocks, such as one or more IPHBs and/or one or more
acellular substrates, that can be interlocked or otherwise joined with each other to provide
25 continuous 3D growth of a variety of cells and/or tissues. In this regard, the biological
blocks, such as one or more IPHBs and/or one or more acellular substrates, provide a
substrate that is modular, and connect or link together, for example, in a manner similar to the
attachment of puzzle pieces. Cells grown on one biological block, such as an IPHB and/or an
acellular substrate, may migrate, and propagate on a second biological block, such as an IPHB
30 and/or an acellular substrate, when the second biological block, such as an IPHB and/or an
acellular substrate, is linked or connected to the original biological block, such as an IPHB
and/or an acellular substrate. Additional biological blocks, such as one or more IPHBs and/or
one or more acellular substrates, (e.g., scaffolding substrates) may be connected or linked to
the original IPHB or IPHBs linked or connected to the original IPHB. Beneficially, therefore,
35 cells may continue propagating as long as additional biological blocks, such as an IPHB and/or
an acellular substrate, are linked or connected together. As such, the system of
interconnected biological block, such as an IPHB and/or an acellular substrate, may define an
aggregate 3D network of microporous channels and/or chambers extending in a continuous

5 manner throughout the entirety of the interconnected biological block, such an IPHB and/or an acellular substrate, (e.g., each biological block has its own 3D network of microporous channels and/or chambers, which may be the same or different from any of the others).

In accordance with certain embodiments, the methods of cell cultivation are devoid of any sub-culturing step, such methods of avoiding sub-culture preserves cell characteristics for
10 twice as long or greater than when cells are grown in a vessel that requires sub-culturing. The modular IPHBs (e.g., substrates) may be two-dimensional (2D) or 3D, while a 3D configuration may be more desirable. As noted above and discussed in more detail below, the IPHBs may contain void spaces, channels, or be permeable to gas or liquid substrates. In accordance with certain embodiments of the invention, the continuous polymeric material of
15 the IPHBs may be made mostly or exclusively hydrogel materials comprising or consisting of synthetic polymers, biologically derived polymers, and/or tissue derived components such extracellular matrix. By use of hydrogel materials, the IPHBs may be individually tailored to have characteristics that mimic the environment of virtually any environment within, for example, the human body to enable cells to grow more natively, and maintain cell
20 characteristics for a longer period than when grown in cell culture vessel that requires sub-culturing. Moreover, different IPHBs may be combined together (e.g., interlocked) to allow different types of cells to mix to form tissues or produce multi-cell organoids. IPHBs may be fixed in place to preserve cells for analysis or substrates may be degraded to release cells for analysis in accordance with certain embodiments of the invention.

25 In accordance with certain embodiments of the invention, the methods of cultivating cells may enable end-users to continuously culture virtually any cell type without stopping and without sub-culturing. This, beneficially, enables continuity of experiments and continuity of cell production not realized by traditional platforms. Moreover, the methods of cultivating cells

30 minimizes human interaction, which reduces human errors, contamination risks, and other inconsistencies since the need for sub-culturing is eliminated. Furthermore, the methods utilizing the biological blocks, in accordance with certain embodiments of the invention, may be compatible with current cell culture trays and plates, which means these methods may be used in automated systems that can transfer standard cell culture trays and
35 plates between incubators and liquid handlers for dispensing and aspirating cell media. With less manual operation and sub-culturing, the methods disclosed herein may also dramatically reduce the consumption of consumables including media and plasticware. The methods utilizing the biological blocks, in accordance with certain embodiments of the invention, also

5 permits uniform nutrient and gas exchange through the IPHB (e.g., hydrogel substrate) based on the microchannel architecture built into the IPHB. These methods utilizing IPHBs, for example, enable customization to facilitate growth of specific cell types, such as the hydrogel material forming the continuous polymeric network and/or the structure of the network of microporous channels and/or chambers extending throughout the continuous polymeric
10 matrix material of the IPHB. Moreover, methods in accordance with certain embodiments of the invention permit the culture of multiple different cell types in combination, permits cells to secrete and form extracellular matrices that can be harvested as raw materials for therapeutic or diagnostic purposes, promotes the secretion of biological products from cells into the luminal space of the microchannels for collection.

15 In accordance with certain embodiments of the invention, the IPHBs permit perfusion of liquid media and/or gases through the microchannels thereof, and permits cells to organize according to the design of the microarchitecture for the purpose of forming specific tissue structures. Beneficially, methods of cell cultivation in accordance with certain embodiments of the invention overcomes the problem of spheroid necrosis by enabling spheroids to unwind
20 within the microchannels and form cylinders and other higher-order structures. In this regard, this present methodology facilitates the formation of native microenvironments that promote cell proliferation, migration, viability, and function. Moreover, the flexible and robust nature of the methods utilizing the IPHB-based system may permit custom growth of cell cultures.

25 In accordance with certain embodiments of the invention, the methods of cell cultivation may be applied to the production of biologics, such as exosomes, extracellular vesicles, growth factors, monoclonal antibodies, peptides, proteins, viral particles, oligonucleotides, and organelles. In this regard, cells that produce or secrete a biologic of interest may be cultivated as disclosed herein, and the produced or secreted biologic may be
30 isolated for further processing, use, and/or analysis. Additional applications for which methods of cell cultivation may be applied include, for example, tissue formation, organoid formation, filtration, regenerative medicine, auto-graft generation, meat production, cell culture, plant culture, protein production, physiological modeling, infectious disease, wound healing, cellular reprogramming, disease modeling, cancer research, bioreactor operations,
35 mass cell production, and microenvironment formation.

As noted above, the presently-disclosed invention relates generally to methods of cultivating cells. The method, in accordance with certain embodiments of the invention may comprise the following steps: (i) providing an initial scaffolding comprising a first

5 interlocking porous hydrogel block (IPHB), wherein the first IPHB comprises a three-dimensional (3D) macrostructure defined by a continuous polymeric matrix material and a network of microporous channels and/or chambers extending throughout the continuous polymeric matrix material, and wherein the 3D macrostructure comprises a top surface, a bottom surface, and a thickness defined by at least one side edge extending from the top
10 surface to the bottom surface, and wherein the 3D macrostructure structure includes at least one interlocking-male component and at least one interlocking-female component; (ii) seeding the first IPHB with one or more cells of interest; (iii) feeding the one or more cells of interest with a first culture media, and allowing the one or more cells of interest to propagate throughout the network of microporous channels and/or chambers; (iv) expanding the initial
15 scaffolding by interlocking a second IPHB to the first IPHB, wherein the at least one interlocking-male component or at least one interlocking-female component of the first IPHB is joined to a corresponding interlocking-male component or corresponding interlocking-female component of the second IPHB; and (v) allowing the one or more cells of interest to propagate from the first IPHB into the second IPHB, and feeding the one or more cells of
20 interest located inside the second IPHB with the first culture media or a second culture media.

Figure 1, for instance, illustrates one example method of cultivating cells 200 including an optional step 210 of coating a first IPHB 1 (e.g., a primary or seeding block) with a compatibilizer that may promote or facilitate adhesion of the cells of interest to the polymeric matrix material within the inside of the first IPHB (e.g., the walls defining the
25 network of microporous channels and/or chambers extending throughout the continuous polymeric matrix material). As shown in Figure 1, the coating step with the compatibilizer may be performed simply by applying a flowable or liquid composition including the compatibilizer to the top surface of the first IPHB and allowing the composition to diffuse and/or flow into and through the network of microporous channels and/or chambers
30 extending throughout the continuous polymeric matrix material. In accordance with certain embodiments of the invention, a gas or combination of the liquid composition and gas may be conveyed through the first IPHB for the purposes of changing the texture of the network of microporous channels and/or chambers for facilitating cell adhesion, cell migration, cell proliferation, or other cell behavior and function. In this regard, the first IPHB may be
35 bathed or soaked in the composition having the compatibilizer to ensure desirable coating of the network of microporous channels and/or chambers with the compatibilizer. Figure 1 also illustrates that the operation 220 of seeding the first IPHB with one or more cells of interest (e.g., a first type of cells for culturing). The nature of the cells is generally not critical as the

5 IPHB is agnostic and will accept any cell line. Step 230 may comprise feeding the now seeded cells of interest that are present within the first IPHB. In this regard, one or more culture mediums may be used according with the desired protocol for growth of the seeded cells. Again, the feeding operation may be conducted by applying the culture media to the top surface of the IPHB and allowing the culture media to diffuse and/or flow through the
10 IPHB. Additionally or alternatively, the IPHB may be soaked or submerged in the culture media. As shown in Figure 1, optional step 240 may comprise expanding the initial scaffolding associated with the first IPHB by interlocking a second IPHB and a third IPHB to the first IPHB. Step 250 comprises harvesting the cultured cells from within the IPHB(s). Depending on the nature of the hydrogel material forming the continuous polymeric matrix
15 material, harvesting may comprise degrading the hydrogel material to expose the cells, or the cells may be flushed out with a retrieval reagent in liquid form that is passed through the IPHB(s).

In accordance with certain embodiments of the invention, the method may further comprising a step of harvesting at least a portion of the one or more cells located throughout
20 the network of microporous channels and/or chambers of the first IPHB. The step of harvesting at least a portion of the one or more cells located throughout the network of microporous channels and/or chambers of the first IPHB may comprise flushing them out of the first IPHB with a fluid medium. Depending on the IPHB being used, end-users may utilize, for example, Trypsin and Accutase based products to remove cells from the network
25 of microporous channels and/or chambers. In accordance with certain embodiments of the invention, an enzymatic reagent physically cuts the bonds in the hydrogel material forming the continuous polymeric matrix material to degrade the IPHB and gently release the cells located within the network of microporous channels and/or chambers. Additionally or alternatively, the step of harvesting at least a portion of the one or more cells located
30 throughout the network of microporous channels and/or chambers of the first IPHB may comprise degrading the 3D macrostructure of the first IPHB. As noted herein, degradable materials may include naturally occurring biopolymers, such as collagen, hyaluronan, gelatin, and nucleic acids. These type of materials, or combinations of these materials can be degraded with corresponding enzymes such as collagenase (e.g., works for collagen and
35 gelatins), hyaluronase, and Dnase/Rnase.

In accordance with certain embodiments of the invention, the methods may optionally comprise a step of coating an interface between the continuous polymeric matrix material and the network of microporous channels and/or chambers extending throughout the continuous

5 polymeric matrix material with a compatibilizer, such as described and disclosed herein. The compatibilizer, for example, may be selected to promote adhesion of a primary cell of interest to the IPHB. In this regard, the coating step would occur prior to seeding of the IPHB.

In accordance with certain embodiments of the invention, the IPHBs can be interlocked with each other to provide continuous 3D growth of a variety of cells and/or
10 tissues. Hydrogels are insoluble polymer matrices that can be engineered to hold up to 96% water content by mass, such as up to 40, 50, 60, 70, 80, 90, and 95% water content by mass). A variety of different polymers can be used individually or in combination to create unique hydrogels. Through cross-linking of polymers via light, temperature shift, or chemical
15 reaction, hydrogels can be tailored to exhibit different mechanical properties, diffusion gradients, osmotic pressures, chemical formulations, and structures such as pores and fibers of varying shapes and sizes. Hydrogels may also be degradable or non-degradable. Hydrogels are versatile in their ability to be used in different applications, such as soft contact lenses to provide optics to correct a patient's vision. Hydrogels have been used in wound
20 healing applications as a dressing and have also been used as bioinks in Life Science applications to create unique structural scaffolds for micro-fluidic experiments or provide a substrate for cells to be cultured on or in.

The IPHBs, in accordance with certain embodiment of the invention, may be joined together or interlocked together via at least one interlocking-male component and at least one interlocking-female component. For example, the at least one interlocking-male component
25 of a first IPHB is configured to be received within a corresponding at least one interlocking-female component of a second IPHB. In addition to the joining of the IPHBs via their 3D macrostructure, the microstructure of network of microporous channels and/or chambers (e.g., void spaces). Most hydrogels are solid materials. However, by introducing void spaces and microchannels into the hydrogel, liquid, gas, and cell migration can be directed for the
30 purpose of expanding the hydrogels together to form a continuous substrate. This feature, in accordance with certain embodiments of the invention, may be particularly beneficial since microstructure of network of microporous channels and/or chambers (e.g., void spaces) allow for a second medium to be used to interlock the IPHBs (e.g., hydrogels) together, and create a larger or expanded material for cell and/or tissue growth.

35 Hydrogels may be formed by crosslinking any synthetic polymer, biological polymer, tissue component (derived from human, animal, plant, or combination thereof), or combination thereof in the presence of water using a free-radical mediated reaction (e.g., photo reaction, chemical reaction) or reaction as a result of change in temperature.

5 The IPHBs (e.g., hydrogels), in accordance with certain embodiments of the invention, may be suitable for a variety of applications, such as producing or growing cell cultures, biologics, exosomes, extracellular vesicles, growth factors, monoclonal antibodies, peptides, proteins, viral particles, oligonucleotides, organelles, organoid formation, plant growth, drug delivery, tissue formation, ex vivo modeling, electrical conduction, wound
10 healing, cellular reprogramming, filtration, optics, microfluidics, custom network of microchannels, custom scaffold architecture construction, custom extracellular matrix derived scaffolds, dissolvable hydrogels, custom tissue formation, accepts patient cells, custom configurations, modular, and microenvironment manipulation.

 In accordance with certain embodiments of the invention, the IPHBs allow for cells to
15 grow in a more native physiological-like environment compared to culture in a 2D plastic cell culture vessel. For instance, the IPHBs may be tuned or configured to mimic an original tissue environment from which specific cells arise and grow, unlike plastic cell culture vessels and other technologies that are not customizable and modular. Additionally, certain embodiments of the invention provide for the combination of multiple IPHB (e.g., hydrogel)
20 substrates to be joined that are like or unlike to form an expanded continuous hydrogel substrate for cell production and/or biologics production. For example, like or unlike hydrogels (e.g., IPHB) may be joined together to create unique and custom microenvironments for cells to grow in, unlike other cell culture vessels or technologies. Moreover, the IPHBs can allow for the formation of

25 spheroids and organoids without developing a necrotic core inside the IPHB's network of microchannels. For example, the IPHBs, in accordance with certain embodiments of the invention, allows spheroids and organoids to unwind and form tubes, cylinders, and other sophisticated structures where nutrients and gases may evenly diffuse to cells within the IPHB (e.g., hydrogel). Beneficially, for instance, the IPHBs may permit even nutrient and
30 gas exchange for healthy cell growth unlike other technologies that claim to mass produce cells. In accordance with certain embodiments of the invention, the IPHBs may be modified to suite a wide variety of different cell types. In accordance with certain embodiments of the invention, the IPHBs enables cells to secrete extracellular matrix and create natural microenvironments that promote cell growth, migration, viability, and function. Still further,
35 the IPHBs beneficially eliminate the need to sub-culture cells. Moreover, the use of the IPHBs is easy to use as they can be provided in a pre-formed format, and does not require sophisticated changes in temperature, pH, or chemical exposure to use. The IPHBs, for example, may enable users to achieve one or more of the following: grow custom cell

5 cultures, grow multiple cell types in parallel or sequence, combine cell cultures to create complex tissues, mass produce cells without ever stopping production of cells, use the same substrates to produce cells from the benchtop all the way through clinical trials and for industrial production, and use less media and fewer consumables than present technologies, reduce human error and risks of contamination by reducing or eliminating human touch
10 points in the production of cells. In accordance with certain embodiments of the invention, the IPHBs provide a modular platform for any of the above-referenced applications.

Certain embodiments according to the invention provide an interlocking porous hydrogel block (IPHB) comprising a three-dimensional (3D) macrostructure defined by a continuous polymeric matrix material and a network of microporous channels and/or
15 chambers extending throughout the continuous polymeric matrix material. The 3D macrostructure may comprise a top surface, a bottom surface, and a thickness defined by at least one side edge extending from the top surface to the bottom surface, in which the 3D macrostructure includes at least one interlocking-male component and at least one interlocking-female component. In accordance with certain embodiments of the invention,
20 the at least one interlocking-male component of a first IPHB is configured to be received within a corresponding at least one interlocking-female component of a second IPHB.

Figure 2, for instance, illustrates two (2) separate IPHBs 1 in accordance with certain embodiments of the invention. Each of these IPHBs include a top surface 12, a bottom surface 14 and at least one side edge 16. The particular IPHBs 1 shown in Figure 2 include at
25 least one interlocking-male component 50 and at least one interlocking-female component 60. Figure 3 illustrates three (3) interlocked IPHBs 1 in accordance with certain embodiments of the invention. The IPHBs 1 shown in Figure 3 each include a first interlocking-male component 51, a second interlocking-male component 52, a first interlocking-female component 61, a second interlocking-female component 62.

30 Figure 4 depicts top and side views of an IPHB 1 and illustrates the continuous polymeric matrix material 10 and the network of microporous channels and/or chambers 30 extending throughout the continuous polymeric matrix material, which are exposed at the surfaces of the IPHB, in accordance with certain embodiments of the invention. As illustrated by Figure 4, a seeded IPHB may enable cells 33 to grow and migrate throughout
35 the network of microporous channels and/or chambers 30 in a three-dimensional manner.

In accordance with certain embodiments of the invention, the at least one interlocking-male component includes a first interlocking-male component extending outwardly from the at least one side edge. For example, the at least one side edge may

5 include a first side edge and a second side edge, in which the at least one interlocking-male component includes a first interlocking-male component extending outwardly from the first side edge and a second interlocking-male component extending outwardly from the second side edge. The interlocking-male components extending outwardly from the side edges, for instance, are configured to interlock or join to corresponding interlocking-female components
10 of other IPHBs to form an expanding continuous 3D scaffolding system with the interlocked or joined IPHBs expanding outwardly in an x-y plane. In accordance with certain embodiments of the invention, the at least one interlocking-male component may also include a third interlocking-male component extending outwardly from the top surface. In this regard, the interlocking-male components extending outwardly from the top surface, for
15 instance, are configured to interlock or join to corresponding interlocking-female components located on a bottom surface of other IPHBs to form an expanding continuous 3D scaffolding system with the interlocked or joined IPHBs expanding in a z-direction that is perpendicular to the x-y plane. In accordance with certain embodiments of the invention, for example, a plurality of IPHBs may be interlocked or joined together in both the x-y plane and stacked
20 upon themselves in the z-direction. As noted above, the at least one interlocking-female component may include a first interlocking-female component extending inwardly from the at least one side edge towards an interior portion of the 3D macrostructure. For example, the at least one side edge may include a third side edge and a fourth side edge, in which the at least one interlocking-female component includes a first interlocking-female component
25 extending inwardly from the third side edge towards an interior portion of the 3D macrostructure and a second interlocking-female component extending inwardly from the fourth side edge towards an interior portion of the 3D macrostructure. In such example embodiments, the first side edge and the third side edge may define a first pair of opposing side edges, while the second side edge and the fourth side edge may define a second pair of
30 opposing side edges. In accordance with certain embodiments of the invention and as noted above, at least one interlocking-female component may include a third interlocking-female component extending inwardly from the bottom surface towards an interior portion of the 3D macrostructure. In this regard, a plurality of IPHBs may be interlocked or joined together in both the x-y plane and stacked upon themselves in the z-direction.

35 In accordance with certain embodiments of the invention, the interlocking feature of the IPHBs enable the custom formation of continuous 3D scaffolds for the growth or a variety of cells and/or tissues, in which the number of particular cells being seeded and/or grown in the continuous 3D scaffold is not limited. Such flexibility in the relative positioning

5 and interlocking of the different IPHBs enable the custom growth of multiple types of cells that may form complex interfaces between different types of cells. For example, a plurality of interlocked IPHBs defining a continuous 3D scaffold (e.g., continuous network microporous channels and/or chambers extending throughout the plurality of interlocked IPHBs) may include from 1 to 20 different cell and/or tissue types being grown
10 simultaneously, such as at least about any of the following: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 different cell and/or tissue types being grown simultaneously, and/or at most about any of the following: 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, and 10 different cell and/or tissue types being grown simultaneously.

Figure 5, for example, illustrates a IPHB 1 including an interlocking-male component
15 53 located on a top surface 12 and an interlocking-female component 63 protruding into the bottom surface 14 of the IPHB in accordance with certain embodiments of the invention. In this regard, the interlocking-male component 53 located on a top surface 12 and an interlocking-female component 63 protruding into the bottom surface 14 of the IPHB may be configured to engage and interlock with a separate IPHB as described herein.

20 In accordance with certain embodiments of the invention, the 3D macrostructure with the exception of the at least one interlocking-male component and at least one interlocking-female component may define a cube, a square prism, or a triangular prism. The 3D macrostructure with the exception of the at least one interlocking-male component and at least one interlocking-female component, in accordance with certain embodiments of the
25 invention, define a polygonal prism having from 3 to 12 side edges, such as at least about 3, 4, 5, 6, 7, and 8 side edges, and/or at most about any of the following: 12, 11, 10, 9, and 8 side edges. In accordance with certain embodiments of the invention each side may include either an interlocking-male component and/or an interlocking-female component. Alternatively, some of the side edges may be devoid of an interlocking-male component and
30 an interlocking-female component.

In accordance with certain embodiments of the invention, the at least one side edge includes a first side edge, a second side edge, and an arcuate side edge located between and adjacent the first side edge and the second side edge. For example, the first side edge may include the at least one interlocking-male component extending outwardly from the first side
35 edge and the second side edge may include the at least one interlocking-female component extending inwardly from the second side edge towards an interior portion of the 3D macrostructure. In this regard, each IPHB may define a pie-like shape that when assembled or interlocked together forms a circle (e.g., cylinder since each IPHB has a thickness). Such

5 configurations of the IPHBs may be desirable for use with circular culture wells. Additionally or alternatively, the at least one interlocking-male component includes a second interlocking-male component extending outwardly from the top surface, and/or the at least one interlocking-female component includes a second interlocking-female component extending inwardly from the bottom surface towards an interior portion of the 3D
10 macrostructure. In this regard, a plurality of IPHBs may be stacked upon each other in a z-direction to form a thicker cylinder or semi-cylinder. In accordance with certain embodiments of the invention, the 3D macrostructure with the exception of the at least one interlocking-male component and at least one interlocking-female component may define a semi-cylinder, such as 1/8th of a cylinder to 1/2 of a cylinder, such as 1/8th, 1/4th, 1/3rd, or
15 1/2 of a cylinder.

Figure 6, for example, illustrates a semi-cylinder shaped IPHB 100 including an interlocking-male component 153 located on a top surface 112 and an interlocking-female component 163 protruding into the bottom surface 114 of the IPHB in accordance with certain embodiments of the invention. The semi-cylinder shaped IPHB 100 illustrated by
20 Figure 6 includes a first interlocking-male component 150 extending from a second side edge 118 and a first interlocking-female component 160 protruding into a second side edge 116. The first side edge 116 and the second side edge 118 of the IPHB 100 shown in Figure 6 are connected via an arcuate side edge 119 at one end of the IPHB. In this regard, the interlocking-male component 153 located on a top surface 112 and an interlocking-female
25 component 163 protruding into the bottom surface 114 of the IPHB may be configured to engage and interlock with a separate IPHB as described herein.

In accordance with certain embodiments of the invention, the at least one interlocking-male component may occupy or overlap from about 5% to about 50% of the macroscopic surface area of the surface (e.g., side edge, top surface, or bottom surface) upon
30 which it extends from, such as at about any of the following: 10, 15, 20, and 25%, and/or at most about any of the following: 50, 45, 40, 35, 30, and 25%. Additionally or alternatively, the at least one interlocking-female component may occupy or overlap from about 5% to about 50% of the macroscopic surface area of the surface (e.g., side edge, top surface, or bottom surface) upon which it penetrated into, such as at about any of the following: 10, 15,
35 20, and 25%, and/or at most about any of the following: 50, 45, 40, 35, 30, and 25%.

In accordance with certain embodiments of the invention, the bottom surface may have a rougher texture relative to the top surface. For example, the top surface may be relatively smooth relative to the bottom surface which may have a textured structure. The

5 textured structure at the bottom surface, for example, may facilitate the flow of a culture medium or washing medium through the entirety of the IPHB by providing structural spacers to facilitate the drainage of the culture medium or washing medium from the IPHB. The textured surface of the bottom surface, for example, may include a plurality of minor protrusions, such as individual nubs or ridges that function as short spacers. The plurality of
10 minor protrusions, however, may be significantly smaller in size compared to the at least one interlocking-male component, such as being at most about 1/10th the size of the at least one interlocking-male component. In this regard, the minor protrusions may generally not provide any interlocking functionality in accordance with certain embodiments of the invention.

15 In accordance with certain embodiments of the invention, the top surface of the IPHB may comprise a macroscopic surface area from about 0.25 cm² to about 25 cm², such as at least about any of the following: 0.25, 0.5, .75, 1, 1.5, 2, 5, 8, 10, and 12 cm², and/or about any of the following: 25, 22, 20, 18, 15, and 12 cm². Additionally or alternatively, the bottom surface may comprise a macroscopic surface area from about 0.25 cm² to about 25
20 cm², such as at least about any of the following: 0.25, 0.5, .75, 1, 1.5, 2, 5, 8, 10, and 12 cm², and/or about any of the following: 25, 22, 20, 18, 15, and 12 cm². Additionally or alternatively, the thickness of the 3D macrostructure may be from about 0.5 cm to about 3 cm, such as at least about any of the following: 0.5, 0.75, 1, 1.25, and 1.5 cm, and/or at most about any of the following: 3, 2.5, 2, and 1.5 cm.

25 As noted above, the each of the at least one interlocking-female components may be configured to receive a corresponding at least one interlocking-male component of a second IPHB. In this regard, a plurality of IPHBs may be joined or interlocked together in an individually sequential addition to expand the 3D scaffold as desired along the x-y plane and alone the z-direction. For example, cell growth in a plurality of interconnected IPHBs may
30 be continued in the z-direction by stacking layers of additional IPHBs on top of a first layer of IPHBs.

In accordance with certain embodiments of the invention, the continuous polymeric matrix material may be non-degradable. In this regard, the cells and/or tissue produced in the IPHB may need to be flushed out of the interior network of the network of microporous
35 channels and/or chambers for further analysis, purification, or development. Additionally or alternatively, the continuous polymeric matrix material may be selectably degradable. For example, hydrogel formulations may be rendered biodegradable, such as by insertion of enzyme-sensitive sequences or utilization of native matrix-derived compounds. For example,

5 the continuous polymeric matrix material may comprises a selectably degradable hydrogel material comprising one or more degradable polymers, such as one or more biopolymers derived from a living organism. The one or more biopolymers derived from a living organism, for example, may comprise a polynucleotide, polysaccharide, polypeptide, or any combination thereof. In accordance with certain embodiments of the invention, the one or
10 more biopolymers may comprise collagen, gelatin, laminin, alginate, glycosaminoglycans, oligonucleotides (e.g., DNA, RNA), carbohydrates, lipids, cellulose, alginate, and proteins that can be gently and degraded, such as with the use of protein specific enzymes, ionic solvents, neutral detergents, weak acids, and peroxides to disrupt the biopolymer chains. In accordance with certain embodiments of the invention, the one or more biopolymers may
15 comprise degradable monomers comprising esters, such as hydroxybutyrate, lactic acid, glycolic acid, and caprolactone; anhydrides, such as adipic acid, and sebacic acid; saccharides, such as cellulose, alginate, pectin, dextrin, chitosan, hyaluronan, Chondroitin sulfate, and heparin; proteins; nucleotides (DNA, RNA); peptides, such as collagen, gelatin, silk, and fibrin; urethanes; phosphates; carbonates; and vinyl chlorides. In accordance with
20 certain embodiments of the invention, the selectably degradable hydrogel material may further comprise a synthetic polymer, such as a polyester, a polyanhydride, a polycarbonate, a polyurethane, a polyphosphate or combinations thereof. The continuous polymeric matrix material, in accordance with certain embodiments of the invention, may comprise a 3D cross-linked polymer network, a non-crosslinked polymer network, or a combination thereof.

25 The continuous polymeric matrix material, in accordance with certain embodiments of the invention, may comprise a swellable hydrogel material. The swellable hydrogel material may comprise a radically mediated reaction product of at least a first monomer including an acrylate or methacrylate functional groups and a second monomer or oligomer including at least two (2) free-radically polymerizable functional groups. For example, the at
30 least two (2) free-radically polymerizable functional groups may independently from each other comprise an acrylate or methacrylate group, an allylic group, an alkynyl, a vinyl nitrile, a vinyl ether, a vinyl ester, a vinyl amide, a styrenic group, a maleate group, a fumarate group, or a norbornene group. In accordance with certain embodiments of the invention, at least one of the first monomer or the second monomer may comprise polyethylene glycol
35 functionality (e.g., $\text{—O}(\text{C}_2\text{H}_4\text{O})_n\text{H}$; where n has a value from 1 to 100, polypropylene glycol functionality (e.g., $\text{—O}(\text{C}_3\text{H}_6\text{O})_n\text{H}$; where n has a value from 1 to 100, and/or glycerol functionality incorporated into a backbone of the monomer and/or grafted onto the monomer as a side-chain or a component of a side chain. By way of example only, the at least one of

5 the first monomer or second monomer comprises 2-Hydroxyethyl acrylate (HEA), Poly(ethylene glycol) methyl ether acrylate (MPEGA), N-Methylacetamide (NMA), or Poly(ethylene glycol) diacrylate (PEGDA). In accordance with certain embodiments of the invention, non-limiting examples of non-degradable monomers that may be utilized in the hydrogel materials may include polyolefins (e.g., ethylene, propylene), styrene, nylon (e.g.,
10 amides), and/or acrylics. In accordance with certain embodiments of the invention, non-limiting examples of degradable monomers that may be utilized in the hydrogel materials may include esters (e.g., hydroxybutyrate, lactic acid, glycolic acid, caprolactone), anhydrides (e.g., adipic acid, sebacic acid) saccharides (e.g., cellulose, alginate, pectin, dextrin, chitosan, hyaluronan, Chondroitin sulfate, heparin), proteins, nucleotides (e.g., DNA,
15 RNA), peptides (e.g., collagen, gelatin, silk, fibrin), urethanes, phosphates, carbonates, and vinyl chlorides. Additionally or alternatively, a third monomer comprising a cross-linking agent may be incorporated into the continuous polymeric matrix material. Additionally or alternatively, the swellable hydrogel material may comprise one or more natural polymers, such as plant-derived polymers (e.g., cellulosic-polymers) and animal-derived polymers.

20 In accordance with certain embodiments of the invention, the continuous polymeric matrix material may mimic a natural tissue of interest by including one or more physical properties within about 20%, such as within about 15%, 10%, 8%, 5%, 3%, or 1%, of the natural tissue of interest, wherein the one or more physical property of interest includes softness and tension. For example, the one or more physical properties may comprise an
25 elastic and/or compressive modulus, a storage modulus at 1Hz, loss of modulus at 1 Hz, and/or protein/chemical coating (e.g., Collagen Type I, II, III, IV, Laminin I, II, Hyaluronan, Gelatin, Fibrin, Fibronectin, etc.). By way of example only, native adipose tissue has a storage modulus at 1Hz from 50-100 kPa, a loss of modulus at 1 Hz of 10-20 kPa, and an elastic and/or compressive modulus of 3 kPa. In this regard, for example, a IPHB may have a
30 storage modulus at 1Hz of about 110 kPa, a loss of modulus at 1 Hz of about 22 kPa, and an elastic and/or compressive modulus of about 3 kPa. The IPHB, for example, may be analyzed with a Dynamic Mechanical Analyzer (DMA; TA Instruments, RSA3) setup at to assess mechanical and physical properties. A 5-mm biopsy punch may be used to isolate a circular hydrogel sample to prevent force-concentrating points. DMA may be performed via
35 a dynamic cylindrical compression analysis with a rate of compression of 0.005-mm/sec and a frequency sweep at one 1-Hz. For example, the particular chemical constituents and/or degree of crosslinking may be altered to tailor one or more physical and/or mechanical properties of the resulting continuous polymeric matrix material to mimic or mirror those

5 associated with a natural tissue of interest. Additionally or alternatively, the surface
topography/texture of the network of microporous channels and/or chambers and/or the
outside of the IPHBs can be manipulated. Most of these surfaces may be smooth, grooves,
bumps, mounds, divots, and other surface irregularities may be introduced to alter the flow of
liquid or gas through the network of microporous channels and/or chambers. Such surface
10 irregularities, for example, may introduce turbulence to help slow the flow of liquids or gases
throughout the network of microporous channels and/or chambers. By way of example only,
the surface irregularities may be significantly smaller in size compared to the average
diameter of the network of microporous channels and/or chambers, such as being at most
about 1/4th to about 1/10th the size of the average diameter of the network of microporous
15 channels and/or chambers.

In accordance with certain embodiments of the invention, the continuous polymeric
matrix material is formed via an additive manufacturing technique, such as 3D printing or
digital light synthesis printing. In this regard, the network of microporous channels and/or
chambers is structured to mimic the morphology of a natural tissue of interest, such as by
20 varying the geometry and dimensions of the network of microporous channels and/or
chambers to mirror the morphology of the natural tissue of interest. For instance, the
morphology of a natural tissue of interest may be readily ascertained by one of skill in the art,
and this morphology may be duplicated via a 3D printing or digital light synthesis printing
operation to form an IPHB having a network of microporous channels and/or chambers that
25 mimics the morphology of the natural tissue of interest.

In accordance with certain embodiments of the invention, the average diameter of the
network of microporous channels and/or chambers may comprise from about 100 to about
800 microns, such as at least about any of the following: 100, 120, 150, 180, 200, 220, and
250 microns, and/or at most about any of the following: 800, 780, 750, 720, 700, 680, 650,
30 620, 600, 580, 550, 520, 500, 480, 450, 420, 400, 380, 350, 320, 300, 280, and 250 microns.
Additionally or alternatively, the network of microporous channels and/or chambers may
comprise at least about 40% by volume of the 3D macrostructure, such as from at least about
any of the following: 40, 50, 60, and 70% by volume of the 3D macrostructure, and/or at
most about any of the following: 90, 85, 80, 75, and 70% by volume of the 3D
35 macrostructure.

In accordance with certain embodiments of the invention, an interface between the
network of microporous channels and/or chambers and continuous polymeric matrix material
may comprise a coating of a compatibilizer selected to promote adhesion of a primary cell of

5 interest. This coating may be applied subsequent to formation of the IPHB. By way of example, the coating comprising the compatibilizer may comprise a biological coating including, for example, Collagen I (e.g., Human Mesenchymal Stem Cells [from Adipose, Bone Marrow, Umbilical Cord], Human Neonatal Dermal Fibroblasts, Human Adult Dermal Fibroblasts, Human Keratinocytes, Human Myocytes, Human Osteoblasts, Human

10 Osteocytes, Human Chondrocytes, Bovine Myocytes, Porcine Hepatocytes, Porcine Chondrocytes, Porcine Osteocytes, Equine Muscle Derived Stem Cells); Laminin I (e.g., Human Induced Pluripotent Stem Cells, Mouse Dorsal Root Ganglia); Hyaluronan (e.g., Porcine Hepatocytes, Human Dermal Adult Fibroblasts); Gelatin (e.g., Human Mesenchymal Stem Cells [from Adipose, Bone Marrow, Umbilical Cord], Human Neonatal Dermal

15 Fibroblasts, Human Adult Dermal Fibroblasts, Human Keratinocytes, Human Myocytes, Human Osteoblasts, Human Osteocytes, Human Chondrocytes, Human T cells (CD8+), Human T cells (CD4+), Human Macrophages, Bovine Myocytes, Porcine Hepatocytes, Porcine Chondrocytes, Porcine Osteocytes, Equine Muscle Derived Stem Cells); Fibrin (e.g., Human Keratinocytes); Fibronectin (e.g., human Mesenchymal Stem Cells [from Adipose,

20 Bone Marrow, Umbilical Cord], Human Neonatal Dermal Fibroblasts, Human Adult Dermal Fibroblasts, Human Keratinocytes, Human Osteoblasts, Human Osteocytes, Human Chondrocytes); or any combinations thereof.

In another aspect, the present invention provides a scaffolding system comprising a plurality of IPHBs, such as those described and disclosed herein. In accordance with certain

25 embodiments of the invention, for instance, the plurality of IPHBs includes a first IPHB including a first interlocking-male component and a second IPHB including a second interlocking-female component, in which the second interlocking-female component is configured to receive the first interlocking-male component. In accordance with certain

embodiments of the invention, for example, the first interlocking-male component may be

30 inserted into the second interlocking-female component, in which the first IPHB and the second IPHB are each provided in a swollen state thereby improving interlocking of the first IPHB and the second IPHB. In this regard, the swollen state may be provided due to the absorbance of a liquid, such as water or a culture medium. As the first and second IPHBs swell during an interlocked state, the frictional forces between the mated interlocking-male

35 and interlocking-female component(s) increases to increase the force required to separate the IPHBs.

In accordance with certain embodiments of the invention, the first IPHB has a first network of microporous channels and/or chambers and the second IPHB has a second

5 network of microporous channels and/or chambers, in which a first portion of the first
network of microporous channels and/or chambers at least partially overlaps with a first
portion of the second network of microporous channels and/or chambers when the first IPHB
and the second IPHB are interlocked and define an aggregate continuous network of
microporous channels and/or chambers. In this regard, the density of microporous channels
10 at the surfaces of the IPHBs is sufficiently large, as noted above, such that partial overlap of a
least portions of the microporous channels at the surfaces of the respective IPHBs enables
formation of a continuous aggregate continuous network of microporous channels and/or
chambers extending throughout the each IPHB that may be interlocked. As noted above, a
plurality of IPHBs may be interlocked together along that x-y plane and/or along the z-
15 direction.

In accordance with certain embodiments of the invention, the first IPHB may be
seeded with a first primary cell and the second IPHB is seeded with a second primary cell,
wherein the first primary cell is different than the second primary cell. As noted above, each
of the IPHBs may be seeded by a different and/or unique primary cell or a combination of a
20 plurality of primary cells. Alternatively, for mass production of a given cell of interest, each
IPHB may be seeded with the same primary cell.

By way of example only, one or more of the IPHBs may be seeded and enable cell
growth of any of the following: (1) Human Stem Cells, such as Human Wharton's Jelly Cells
(MSC), Human Bone Marrow Derived Mesenchymal Stem Cells (MSC), Human Adipose
25 Derived Mesenchymal Stem Cells (MSC), Human Skin Derived Induced Pluripotent Stem
Cells (iPSC), Human Blood Cell Derived Induced Pluripotent Stem Cells (iPSCs), Human
CD4+ T Cells, Human CD8+ T Cells; (2) Primary Mammalian Cells, such as HepG2 Cells
(Liver Carcinoma Cells), Human Adult Dermal Fibroblasts (Primary Cells), Human Neonatal
Dermal Fibroblasts (Primary Cells), Human Adult Keratinocytes (Primary Cells), Mouse
30 Dorsal Root Ganglia (Primary Neural Cells), Bovine Myocytes (Primary Cell Line), Primary
Porcine Hepatocytes, Porcine Chondrocytes, Porcine Osteocytes, Equine Muscle Derived
Stem Cells (Primary MSCs), Primary Snail Cells, Human Macrophages; (3) Immortalized
Mammalian Cell Lines, such as UB-OC2 Cells (Mouse Cochlear Epithelium), Human
Myoblastoma (Muscle Tumor), PC3 (Prostate Cancer), CHO (Chinese Hamster Ovary Cell),
35 HEK293 (Human Embryonic Kidney Cell), SHSY5Y (Neuronal Tumor), PANC-1 (Human
Pancreatic Cancer), HeLa (Cervical Cancer), A549 (Lung Cancer), A673 (Muscle Cancer);
and (4) Primary Plant Cells, such as Rosemary, Tobacco, and Tomato.

5 Figure 7 illustrates cell 33 migration from a first IPHB 1 to a second IPHB 2 when interlocked together in accordance with certain embodiments of the invention. As illustrated in Figure 7 the first IPHB 1 may be seeded with cells 33 and then joined to an empty (e.g., devoid of cells) second IPHB 2. Once joined together, the cells 33 migrate from the first IPHB 1 into the second IPHB 2 until an equilibrium is reached. After reaching equilibrium in
10 both IPHBs, the cells 33 will continue to proliferate.

 As noted above, the methods of cultivating cells in accordance with certain embodiments of the invention utilize one or more IPHB(s). By way of example, for instance, the method of cultivating cells may comprise providing an initial scaffolding comprising a first IPHB and seeding the first IPHB with one or more cells of interest (e.g., a first cell type).
15 Once seeded, the one or more cells may be provided appropriate nutrition via one or more culture medias, which may reach the seeded cells via the microporous network formed in the first IPHB. In this regard, old or exhausted culture media may be flushed out and/or sucked out of the IPHB and/or the well that the IPHB may be housed within. After removal of old or exhausted culture media, fresh culture media may be applied to the IPHB. For instance, a
20 first culture media may be desired at the outset of growth upon seeding, but a modified (different) culture media may be preferred once cell growth is well established and propagation of the cells through the IPHB is well established. In this regard, the method may comprise feeding the one or more cells of interest to facilitate or allow the one or more cells of interest to propagate throughout the network of microporous channels and/or chambers. As
25 the cells grow and propagate throughout the first IPHB, the initial scaffolding may be expanded by interlocking a second IPHB to the first IPHB. In this regard, the first IPHB has a first network of microporous channels and/or chambers and the second IPHB has a second network of microporous channels and/or chambers that at least partially overlap at an interface between the first IPHB and the second IPHB. In this regard, the one or more cells
30 of interest are allowed to propagate from the first IPHB into the second IPHB, where culture media may be provided to facilitate the growth and propagation of the cell line throughout the second IPHB.

 In accordance with certain embodiments of the invention, the one or more cells of interest that have been seeded into the network microporous channels and/or chambers may
35 propagate within the network in a variety of patterns. As illustrated in Figure 8A, for instance,

 the one or more cells of interest 33 (e.g., seeded in an IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a winding

5 pattern wherein the one or more cells of interest wind back-and-forth in a 3D configuration across at least a portion of individual microporous channels as the one or more cells of interest propagate from an interior portion of the first IPHB towards one or more exterior surfaces, such as the top surface, the bottom surface, and/or or the at least one side edge. As illustrated in Figure 8B, for instance, the one or more cells of interest 33 (e.g., seeded in an

10 IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a spiral pattern adhered to walls of the network of microporous channels and/or chambers as the one or more cells of interest propagate from an interior portion of the first IPHB towards one or more exterior surfaces, such as the top surface, the bottom surface, and/or or the at least one side edge. As illustrated in Figure 8C, for instance, the one or more

15 cells of interest 33 (e.g., seeded in an IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a network of nodes and branches defining open regions therebetween as the one or more cells of interest propagate from an interior portion of the first IPHB towards one or more exterior surfaces, such as the top surface, the bottom surface, and/or or the at least one side edge. As illustrated in Figure 8D,

20 for instance, the one or more cells of interest 33 (e.g., seeded in an IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a continuous cell sheet adhered to walls of the network of microporous channels and/or chambers as the one or more cells of interest propagate from an interior portion of the first IPHB towards one or more exterior surfaces, such as the top surface, the bottom surface, and/or or the at least one side edge. As illustrated in Figure 8E, for instance, the one or more

25 cells of interest 33 (e.g., seeded in an IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a spheroid. As illustrated in Figure 8F, for instance, the one or more cells of interest 33 (e.g., seeded in an IPHB) grow and propagate through the network of microporous channels and/or chambers 30 and define a

30 comet-like structure having a spheroid structure and a spiral structure emanating from the spheroid structure as the one or more cells of interest propagate from an interior portion of the first IPHB towards one or more exterior surfaces, such as the top surface, the bottom surface, and/or or the at least one side edge.

As noted above, the methods in accordance with certain embodiments of the invention

35 enable 3D cell growth and propagation formations that uniquely resemble in vivo growth and propagation. In this regard, Figure 9 is a schematic illustrating the nomenclature and conceptual cell migration of a plurality of IPHBs in accordance with certain embodiments of the invention. As shown in Figure 9, an initially seeded IPHB 300 may be referenced as the

5 prime or seed block (I). Once the seed block 300 reaches the desired confluency, which is cell phenotype dependent and may take from one to two weeks, it can be expanded by interlocking, for example, two (2) secondary (II) IPHBs 302a, 302b directly to the seed block 300. The number of secondary, tertiary, etc. may depend on the ultimate goal for cell and/or tissue growth. Figure 9 illustrates a single tertiary (III) IPHB directly 304a attached to both
10 of the secondary (II) 302a, 302b IPHBs. Figure 10 is a schematic illustrating additional options for adding a varying number and positioning of additional IPHBs to those of Figure 9. Figure 10 illustrates three (3) example additions including a variety of configurations including one example having a ; quaternary (IV) IPHB 308. As illustrated by Figures 9-10, the methods utilizing a plurality of IPHBs provide a myriad of different continuous growth
15 scaffolds for customizing a growth protocol.

In accordance with certain embodiments of the invention, the method comprises a chain-cultivation method comprising the sequential addition of a plurality of secondary blocks to the first IPHB. The plurality of secondary blocks may include the second IPHB and a third IPHB interconnected directly to the second IPHB such that the second IPHB is located
20 directly between the first IPHB and the third IPHB, and wherein the plurality of secondary IPHBs are initially devoid of cells. In this regard, the cells of interest located in the first IPHB are harvested after cell propagation from the first IPHB to the second IPHB, and the cells of interest located in the second IPHB are harvested after cell propagation from the second IPHB to the third IPHB. Such methods, therefore, enable the continual growth and
25 harvesting of the cells of interest, particularly without the need for re-seeding or sub-culturing. In accordance with certain embodiments of the invention, the number of secondary blocks (i.e., secondary IPHBs) may be from at least 2, such as at least about any of the following: 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 blocks.

In accordance with certain embodiments of the invention, the method comprises a
30 multi-cell cultivation method, in which the one or more cells of interest seeded in the first IPHB comprises a first cell type and the second IPHB is seeded with a second cell type. In this regard, the first cell type is different than the second cell type. In this regard, the cells of the first cell type (e.g., bone cells) and cells of the second cell type (e.g., cartilage cells) are allowed to propagate towards each other and form a first interface between the first cell type
35 and the second cell type. In accordance with certain embodiments of the invention, the method may further comprise a step of degrading each of the IPHBs to expose each cell type and the first interface. In this regard, the each cell type and the first interface may be isolated for further analysis and/or use. In accordance with certain embodiments of the invention, the

5 method may further comprise interlocking a third IPHB directly together with the second IPHB, wherein the second IPHB is located directly between the first IPHB and the third IPHB, and seeding the third IPHB with a third cell type that is different from the first cell type and the second cell type. The method may also comprise allowing and/or growing the cells of the second cell type and cells of the third cell type to propagate towards each other
10 and form a second interface between the second cell type and the third cell type. In accordance with certain embodiments of the invention, the method may further comprise a step of degrading each of the IPHBs to expose each cell type, the first interface, and the second interface.

As noted above, the one or more cells of interest may produce or secrete a therapeutic
15 agent of interest, such as a biologic. For example, the therapeutic agent may comprise exosomes, extracellular vesicles, growth factors, monoclonal antibodies, peptides, proteins, viral particles, oligonucleotides, organelles, or combinations thereof. In this regard, various configurations of multiple IPHBs may be interlocked to provide an aggregate scaffolding network, in which multiple cell lines, such as those that may produce or secrete a therapeutic
20 agent, are seeded in different IPHBs.

As noted above, the methods in accordance with certain embodiments of the invention may include interlocking a plurality of IPHBs in a myriad of different configurations to provide a wide variety of continuous growth scaffolds for customizing a growth protocol for a wide variety of different cell lines. Figures 11-21 illustrate just a few non-limiting example
25 configurations in accordance with certain embodiments of the invention. Figure 11, for instance, is a schematic for an infiltration assay configuration of a series of interlocked empty IPHBs 410 (e.g., IPHBs that are initially devoid of seeded cells) with four (4) seeded IPHBs 420 interlocked as side-chains to the series of interlocked empty IPHBs. All of the IPHBs are housed within a tray for housing the IPHBs and/or cell culture media. In this regard, the
30 seeded cells in the seeded IPHBs 420 are grown and propagate into the series of interconnected empty IPHBs. Figure 12 is a schematic for a dual-infiltration assay configuration of a series of interlocked empty IPHBs 410 with a first set of four (4) IPHBs seeded with a first cell type 420 interlocked as side-chains to the series of interlocked empty IPHBs and a second set of four (4) IPHBs seeded with a second cell type 430, which may
35 produce or secrete a therapeutic agent interlocked as side-chains on an opposite side of the series of interlocked empty IPHBs. In this regard, the series of initially interlocked empty IPHBs 410 (e.g., devoid of seeded cells) can be penetrated simultaneously from cell lines from IPHBs 420 and IPHBs 430. In this particular configuration, the first and second cell

5 types may form a plurality in interfaces and/or regions of interaction with each other in the initially empty IPHBs 410. Figure 13, for example, illustrates a multi-infiltration assay configuration in which eight IPHBs 420, 425, 430, 435, 440, 445, 450, 455 are attached as side-chains to a series of interlocked empty IPHBs 410, in which each IFPB attached as a side-chain is seeded with a different cell type, each of which may produce or secrete a
10 different therapeutic agent. In this regard, the growth and propagation of multiple cell lines may be simultaneously grown and propagated into the series of interlocked initially empty IPHBs 410. In this regard, a plurality of different interfaces between the different cell types or cell lines may also be formed throughout the series of interlocked initially empty IPHBs.

Figure 14 illustrates a migration assay configuration in which a series of seeded
15 IPHBs 420 have eight (8) side-chains of unseeded or initially empty IPHBs 410 attached thereto. Such a configuration, for example, may provide a multi-chain culturing method, such as noted above. That is, the cells of interest seeded in IPHBs 420 are grown and propagate outwardly through each of the initially empty IPHBs 410, in which earlier IPHBs penetrated by cell growth (those closest to the seeded IPHBs 420) can be harvested while the
20 cell line growth is simultaneously grown in the subsequent IPHBs (e.g., those farther away from the seeded IPHBs 420). Such an approach, for instance, may provide a mass production of cells of interest in which cells may be harvested without ceasing the continued 3D growth of the cells of interest.

Figure 15 illustrates a dual-migration assay configuration in which a series of seeded
25 IPHBs include a first group of IPHBs are seeded with a first cell type 420 and a second group of IPHBs are seeded with a second cell type 430, in which eight (8) side-chains of unseeded or initially empty IPHBs 410 are attached thereto. In this regard, such a configuration may provide a mass production of a plurality of different cell types of interest in which each type of cells may be harvested without ceasing the continued 3D growth of the plurality of
30 different cells of interest. Figure 16 illustrates a multi-migration assay configuration in which a series of seeded IPHBs 420, 425, 430, 435, 440, 445, 450, 455, in which each IPHB is seeded with a different cell type, and eight (8) side-chains of unseeded or initially empty IPHBs 410 are attached thereto. As shown in Figure 16, each of the different cell types may produce or secrete a therapeutic agent of interest.

35 Figure 17 illustrates an 8-chamber omni tray filled with a plurality of IPHBs 420, 425, 430, 435, 440, 445, 450, 455, and spacers, in which each chamber is filled with a group of four (4) interlocked IPHBs seeded with a different cell type (e.g., each chamber has its own cell type therein). Such a configuration, enables the production of multiple cell lines,

5 which may produce or secrete a therapeutic of interest for isolation, in the same tray over the same period of time. Figure 18 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a one-way paracrine signaling assay. Figure 19 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a two-way paracrine signaling assay. Figure 10 20 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as paracrine signaling assay. Figure 21 illustrates an 8-chamber omni tray filled with a plurality of IPHBs and spacers, in which the IPHBs are configured as a four-way paracrine signaling assay.

The foregoing example configurations are merely illustrative of a few possible
15 methods according to certain embodiments of the invention. That is, the foregoing example configurations and figures are merely illustrative and non-limiting. Moreover, despite the foregoing example configurations illustrating the use of multiple IPHBs, in accordance with certain embodiments of the invention one or more of the IPHBs may be substituted with an acellular substrate, such as those described in and disclosed herein.

20 Certain embodiments according to the invention provide a method of growing tissue cells *ex vivo*, in which the method may comprise the following: (i) interlocking together a plurality of interlocking porous hydrogel blocks (IPHB) including a first IPHB and a second IPHB, wherein each of the plurality of IPHBs include a respective three-dimensional (3D) macrostructure defined by a respective continuous polymeric matrix material and a respective
25 network of microporous channels and/or chambers extending throughout the respective continuous polymeric matrix material, and wherein the respective 3D macrostructures each comprise a respective top surface, a respective bottom surface, and a respective thickness defined by at least one respective side edge extending from the respective top surface to the respective bottom surface, and wherein the respective 3D macrostructure structures each
30 include at least one respective interlocking-male component and at least one respective interlocking-female component; (ii) seeding the first IPHB with a first cell type of interest; (iii) seeding the second IPHB with a second cell type of interest, wherein the first cell type of interest is different than the second cell type of interest; (iv) feeding the first cell type of interest with a first culture media, and allowing the first cell type of interest to propagate
35 throughout a first network of microporous channels and/or chambers towards the second IPHB; (v) feeding the second cell type of interest with a second culture media, and allowing the second cell type of interest to propagate throughout a second network of microporous channels and/or chambers towards the first IPHB; and (vi) forming a first interface between

5 the first cell type and the second cell type. In accordance with certain embodiments of the invention, the plurality of IPHBs may be joined with one or more acellular substrates for tissue cell growth. In accordance with certain embodiments, one or more (e.g., all) of the IPHBs may be replaced with one or more acellular substrates, such as those described and disclosed herein.

10 In accordance with certain embodiments of the invention, the first network of microporous channels and/or chambers has a first structure and the second network of microporous channels and/or chambers, wherein the first structure is different than the second structure. By way of example, the first network of microporous channels and/or chambers, the second network of microporous channels and/or chambers, or both may independently
15 from each other have an average diameter comprising from about 100 to about 800 microns, such as at least about any of the following: 100, 120, 150, 180, 200, 220, and 250 microns, and/or at most about any of the following: 800, 780, 750, 720, 700, 680, 650, 620, 600, 580, 550, 520, 500, 480, 450, 420, 400, 380, 350, 320, 300, 280, and 250 microns. Additionally or alternatively, the first network of microporous channels and/or chambers, the second network
20 of microporous channels and/or chambers, or both independently from each other may comprise at least about 40% by volume of the respective 3D macrostructure, such as from at least about any of the following: 40, 50, 60, and 70% by volume of the respective 3D macrostructure, and/or at most about any of the following: 90, 85, 80, 75, and 70% by volume of the respective 3D macrostructure.

25 In accordance with certain embodiments of the invention, a first continuous polymeric matrix material of the first IPHB comprises a non-degradable hydrogel material or a or a selectably degradable hydrogel material, such as described above. Additionally or alternatively, the second continuous polymeric matrix material of the second IPHB comprises a non-degradable hydrogel material or a selectably degradable hydrogel material, such as
30 described above. In accordance with certain embodiments of the invention, the first continuous polymeric matrix material of the first IPHB, the second continuous polymeric matrix material of the second IPHB, or both comprise a non-degradable hydrogel material comprising one or more synthetic polymers, such as synthetic polymers derived from petroleum.

35 In accordance with certain embodiments of the invention, the first continuous polymeric matrix material of the first IPHB, the second continuous polymeric matrix material of the second IPHB, or both comprise a selectably degradable hydrogel material comprising one or more degradable polymers, such as one or more biopolymers derived from a living

5 organism (as described above). For example, the one or more biopolymers derived from a living organism comprises a polynucleotide, polysaccharide, polypeptide, or any combination thereof. In accordance with certain embodiments of the invention, the one or more biopolymers comprises collagen, gelatin, laminin, alginate, glycosaminoglycans, oligonucleotides (e.g., DNA, RNA), carbohydrates, lipids, cellulose, alginate, and proteins
10 that can be gently and degraded, such as with the use of protein specific enzymes, ionic solvents, neutral detergents, weak acids, and peroxides to disrupt the biopolymer chains. In accordance with certain embodiments of the invention, the one or more biopolymers comprises degradable monomers comprising esters, such as hydroxybutyrate, lactic acid, glycolic acid, and caprolactone; anhydrides, such as adipic acid, and sebacic acid;
15 saccharides, such as cellulose, alginate, pectin, dextrin, chitosan, hyaluronan, Chondroitin sulfate, and heparin; proteins; nucleotides (DNA, RNA); peptides, such as collagen, gelatin, silk, and fibrin; urethanes; phosphates; carbonates; and vinyl chlorides. In accordance with certain embodiments of the invention, the selectably degradable hydrogel material further comprises a synthetic polymer, such as a polyester, a polyanhydride, a polycarbonate, a
20 polyurethane, a polyphosphate or combinations thereof.

In accordance with certain embodiments of the invention, the first continuous polymeric matrix material of the first IPHB is formed from a non-degradable hydrogel material and the second continuous polymeric matrix material of the second IPHB is formed from a selectably degradable hydrogel material. For example, a configuration of multiple
25 biological blocks may include a plurality of different degradable and/or non-degradable biological blocks. An end-user, for instance, may find it desirable to degrade just a portion of the configuration of biological blocks for retrieval of a section of the grown tissue while not interfacing with the tissue structure in the other biological blocks.

In accordance with certain embodiments of the invention, the methods may further
30 comprise interlocking a third biological block (e.g., a third IPHB) directly to the second IPHB, and seeding the third IPHB with a third cell type of interest, wherein the third cell type of interest is different than the first cell type of interest and the second cell type of interest. In this regard, the configuration of interlocked or otherwise joined biological blocks may simultaneously grow a plurality of different cell types of interest as well as form custom
35 interfaces from the plurality of different cell types of interest. The method, for example, may further comprise a step of feeding the third cell type of interest with a third culture media, and allowing the third cell type of interest to propagate throughout a third network of

5 microporous channels and/or chambers towards the second IPHB; and forming a second interface between the first cell type and the second cell type.

In accordance with certain embodiments of the invention, a plurality of different IPHBs (e.g., each may be formed from a different hydrogel material and/or have a different microarchitecture based on different cell morphologies) can be connected in a single culture
10 if desired. For example, if cell type 1 is seeded in dissolvable IPHB, and then a non-degradable/dissolvable IPHB is connected to the dissolvable IPHB, and then a partially dissolvable IPHB is connected to the original dissolvable IPHB, then cell type 1 can propagate throughout all three IPHB types. In this regard, a chain culture can be continued in three different directions with each type of IPHB. When the end-user is ready, the end-user
15 can remove the dissolvable IPHB originally seeded with cell type 1, and apply an enzymatic reagent to dissolve the IPHB originally seeded with cell type 1, and gently release the cells for analysis.

Similarly, the end-user can take one of the non-degradable/dissolvable that was originally connected to a dissolvable IPHB, and after cell type 1 has propagated into the non-
20 degradable/dissolvable (and subsequent connected non-degradable/dissolvable), the end-user can remove the non-degradable/dissolvable, fix it, embed in paraffin or flash freeze and section the non-degradable/dissolvable for further analysis, such as staining. Similarly, the end-user can take one the partially dissolvable IPHBs that was originally connected to a dissolvable IPHB, and after cell type 1 had propagated into the partially dissolvable IPHB
25 (and subsequent connected partially dissolvable IPHB), the end-user can remove the partially dissolvable IPHB, and use Trypsin or Accutase to dislodge cells or other biologics from inside the partially dissolvable IPHB. In this regard, even though the interconnected IPHBs (e.g., two or more different types of IPHBs) have different hydrogel formulations, they are structurally the same on a macroscopic level, and can be connected together, so the end-user
30 can essentially perform multiple experiments simultaneously, and with one cell type.

Additionally, the end-user can design experiments as complex as desired, where multiple cell types can be introduced into each IPHB type for different outcomes and applications. Each IPHB type can be used for each of the assays described and disclosed herein.

35 For example, the dual infiltration assay can be performed with IPHBs formed from three different hydrogel materials (e.g., dissolvable/degradable, non-dissolvable/degradable, and partially dissolvable). The assay operates the same regardless of IPHB type, but the user can obtain results differently by using different harvest procedures, based on which IPHB

5 type was used. For example, if the assay is performed with dissolvable/degradable IPHBs, at the conclusion of the assay, the end user will remove the dissolvable/degradable IPHBs that were originally left empty at the beginning of the assay, and gently dissolve the dissolvable/degradable IPHBs to see if the cell(s) of interested infiltrated into the originally empty dissolvable/degradable IPHBs. Similarly, if non-dissolvable/degradable IPHBs are
10 used, the end user could remove the non-dissolvable/degradable IPHBs that were originally empty and fix the non-dissolvable/degradable IPHBs, embed them in paraffin or freeze them, and section the non-dissolvable/degradable IPHBs, and then use a nuclear stain and background stain to see physically where in the 3D volume of the IPHBs the cells migrated. Likewise, the end-user could take the other non-dissolvable/degradable IPHBs through which
15 cells propagated to analyze which micro channel pathways the cells utilized most to migrate through the non-dissolvable/degradable IPHBs. In the case of the dual infiltration assay, using dissolvable/degradable IPHBs allows the end-user to obtain a “Yes/No” answer over time on if the cells migrated into the empty dissolvable/degradable IPHBs, and how many cells migrated in. Whereas if non-dissolvable/degradable IPHBs are used, the end-user gets
20 quantifiable and qualitative information, because the end-user has the option to examine where they moved spatially in the non-dissolvable/degradable IPHBs over time, so there is a behavior component that can be observed.

In accordance with certain embodiments of the invention, the method may comprise a step of harvesting at least a portion of first cells from the first cell type of interest located
25 throughout the first network of microporous channels and/or chambers of the first IPHB. The first continuous polymeric matrix material of the first IPHB, in accordance with certain embodiments of the invention, may comprise a non-degradable hydrogel material, and the step of harvesting comprises forming artificial tissue samples by cutting the first IPHB into a plurality of sections exposing at least a portion of the cells of the first cell type of interest at a
30 surface of the artificial tissue sample. In accordance with certain embodiments of the invention, the method may further comprise freezing the first IPHB before or after forming artificial tissue samples. The artificial tissue samples, for example, can be stored and subsequently used for downstream testing in a manner similar to native tissue samples.

In accordance with certain embodiments of the invention, the second continuous
35 polymeric matrix material of the second IPHB comprise a non-degradable hydrogel material, and the step of harvesting comprises harvesting (i) at least a portion of first cells from the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first IPHB, (ii) at least a portion of second cells from the second cell type of

5 interest located throughout the second network of microporous channels and/or chambers of the second IPHB, and (iii) at least a portion of first interfacing cells forming the first interface by forming multi-cell containing artificial tissue samples by cutting the interlocked first IPHB and second IPHB into a plurality of sections exposing the cells of (i)-(iii) at a surface of each multi-cell containing artificial tissue sample. The method, for example, may further comprise
10 freezing the interlocked first IPHB and second IPHB before or after forming each multi-cell containing artificial tissue sample. For instance, the multi-cell containing artificial tissue sample may be formed and subsequently frozen for storage and later utilization. In accordance with certain embodiments of the invention, the third continuous polymeric matrix material of the third IPHB comprise a non-degradable hydrogel material, and the step of
15 harvesting comprises additionally harvesting (iv) at least a portion of third cells from the third cell type of interest located throughout the third network of microporous channels and/or chambers of the third IPHB, and (v) at least a portion of second interfacing cells forming the second interface by forming multi-cell containing artificial tissue samples by cutting the interlocked first IPHB, second IPHB, and third IPHB into a plurality of sections exposing the
20 cells of (i)-(v) at the surface of each multi-cell containing artificial tissue sample. Similarly, the method may comprise freezing the interlocked first IPHB, second IPHB, and third IPHB before or after forming each multi-cell containing artificial tissue sample. For instance, the multi-cell containing artificial tissue sample may be formed and subsequently frozen for storage and later utilization.

25 In accordance with certain embodiments of the invention, the step of harvesting at least a portion of first cells from the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first IPHB comprises flushing them out of the first IPHB with a fluid medium, such as described above. Additionally or alternatively, the step of harvesting at least a portion of first cells from the first cell type of
30 interest located throughout the first network of microporous channels and/or chambers of the first IPHB degrading the 3D macrostructure of the first IPHB, such as described above.

In accordance with certain embodiments of the invention, the plurality of IPHBs interlocked together may vary from at least 2 IPBHs, such as at least about any of the following: 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 IPBHs.

35 In accordance with certain embodiments of the invention, the first cell type of interest produces or secretes a first therapeutic of interest, such as a first biologic. Additionally or alternatively, the second cell type of interest produces or secretes a second therapeutic of interest, such as a second biologic. Additionally or alternatively, the third cell type of interest

5 produces or secretes a third therapeutic of interest, such as a third biologic. In this regard, for example, one or more therapeutic agents, such as those described herein, may be produced in accordance with certain embodiments of the invention.

In accordance with certain embodiments of the invention, the method may further comprise positioning or interlocking a first acellular substrate directly adjacent the first IPHB
10 or directly between the first IPHB and the second IPHB, wherein the first acellular substrate comprises an acellular 3D macrostructure defined by a continuous matrix of extracellular matrix (ECM) material associated with a first cell type of interest and network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material associated with a first cell type of interest, and wherein the acellular 3D
15 macrostructure comprises a top surface, a bottom surface, and a thickness defined by at least one side edge extending from the top surface to the bottom surface. Figure 22, for instance, illustrates an acellular matrix 750 in accordance with certain embodiments of the invention. In Figure 22, the continuous matrix of ECM material is rendered transparent to illustrate the network of microporous channels and/or chambers.

20 Although not illustrated in Figure 22, the first acellular substrate may further comprise at least one interlocking-male component and at least one interlocking-female component in a manner similar to the IPHBs described and disclosed herein.

In accordance with certain embodiments of the invention, the method may further comprise a step of seeding the first acellular substrate with a fourth cell type of interest, in
25 which the fourth cell type of interest is different than the first cell type of interest. In accordance with certain embodiments, the fourth cell type of interest may be different than the second cell type of interest and the third cell type of interest. In accordance with certain embodiments of the invention, the method may comprise feeding the fourth cell type of interest with a fourth culture media, and allowing the fourth cell type of interest to propagate
30 throughout the network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material towards the first IPHB. In this regard, the method may comprise forming a third interface between the first cell type of interest and the fourth cell type of interest.

In accordance with certain embodiments of the invention, the method may comprise
35 a step of harvesting at least a portion of fourth cells from the fourth cell type of interest located throughout the network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material. For example, the step of harvesting may comprise forming artificial tissue samples by cutting the first acellular substrate into a

5 plurality of sections exposing at least a portion of the fourth cells of the fourth cell type of interest at a surface of the artificial tissue sample. The method may also comprise freezing the first acellular substrate before or after forming artificial tissue samples in a manner noted above.

10 In accordance with certain embodiments of the invention, the step of harvesting may comprise harvesting (i) at least a portion of first cells from the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first IPHB, (ii) at least a portion of fourth cells from the fourth cell type of interest located throughout network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material of the first acellular substrate, and (iii) at least a portion
15 of third interfacing cells forming the third interface by forming multi-cell containing artificial tissue samples by cutting the interlocked or adjacent first IPHB and first acellular substrate into a plurality of sections exposing the cells of (i)-(iii) at a surface of each multi-cell containing artificial tissue sample.

20 In accordance with certain embodiments of the invention, the fourth cell type of interest comprises a native cell from a living organism, such as a mammal or plant. Additionally or alternatively, a plurality of acellular substrates, including the first acellular substrate, that are interfaced with one or more IPHBs may vary from at least 1 acellular substrate, such as at least about any of the following: 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 acellular substrates.

25 Figures 23-26 illustrate a general method for making an acellular substrate. Figure 23 illustrates a cuboidal mold 700 having a network of microstrands and/or micropods 705 3D printed therein or disposed therein. The network of microstrands and/or micropods 705 may be formed from a degradable or dissolvable hydrogel material, such as those described and disclosed herein. Figure 24 illustrates the seeding of a cell type of interest 710 onto the
30 network of microchannels and/or micropods, and growth of the extracellular matrix 712 of the cell type of interest to fill the cuboidal mold, which may include at least one interlocking-male component and/or at least one interlocking-female component (not shown). After the cuboidal mold is filled with the extracellular matrix material, a decellularized tissue 715 is produced as illustrated in Figure 25 as well as a subsequent degradation or dissolution of the
35 hydrogel material (e.g., the network of microstrands and/or micropods) to form an intermediate acellular substrate 720. Figure 26 illustrates the intermediate acellular substrate being lyophilized and subsequently removed from the cuboidal mold followed by sterilization. Figure 27 illustrates the resulting acellular matrix formed in accordance with

5 certain embodiments of the invention. Figure 28-31 illustrate different configurations 760 of
a plurality of joined acellular substrates in accordance with certain embodiments of the
invention. As illustrated by Figures 28-31, the acellular substrates may be interlocked or
otherwise joined to together in a multitude of different 3D geometric shapes.

In accordance with certain embodiments of the invention, a method of producing an
10 acellular substrate can utilized a unique closed system where liquids are applied and aspirated
as needed. Inverse hydrogel molds (e.g., the network of microstrands and/or micropods) are
first deposited into cubic centimeter molds. The inverse hydrogels, for example, may be the
inverse structure of any of the IPHBs, which may be configured based on a particular tissue
morphology of interest. Stated differently, an IPHB may form a continuous matrix of a
15 hydrogel material with a network of microchannels extending therethrough, while the
acellular substrate will be opposite (i.e., the hydrogel material is used to form a network of
microstrands that correspond to the microchannels of the IPHB). After formation of the
inverse hydrogel molds (e.g., the network of microstrands and/or micropods), sterile saline
may be applied twice for a duration of about 5 minutes each. Afterward, cell media
20 containing 1 million cells per mL are applied to each mold (i.e., the network of microstrands
and/or micropods). Media is exchanged, for example, every 48 hours. After a minimum of 7
days, cell media is removed, and the molds are washed with sterile saline. Sodium dodecyl
sulfate (SDS) and sodium palmate (SP) are applied to each mold for up to 72 hours with
gentle agitation. The SDS and SP are aspirated, and the ECM is dialyzed with sterile
25 deionized water (DI) for up to 72 hours. The ECM is then cycled between -170°C and 37°C
over six days in 24 hour increments. SDS and SP are applied to each mold for up to 72 hours
with gentle agitation. The SDS and SP are aspirated, and the ECM is dialyzed with sterile DI
water for up to 72 hours. The inverse mold is then dissolved via photo lysis. ECM is washed
with sterile saline and 10% antibiotics 3 times for 60 minute durations under UV light. ECM
30 is washed with sterile saline. ECM is then packaged and lyophilized for storage.

Examples

The present disclosure is further illustrated by the following examples, which in no
way should be construed as being limiting. That is, the specific features described in the
following examples are merely illustrative and not limiting.

35 In this example, a 3D hydrogel system is demonstrated that improves expansion
outcomes of MSC populations, such as adipose-derived MSCs (ASCs). The customizable 3D
hydrogel system was bioprinted and was formulated to be a bioinert substrate that closely
mimicked native adipose tissue mechanics and ultimately acts like a tailored bioreactor for

5 MSC expansion and collection of biologics. The 3D hydrogel system was constructed with a unique ‘puzzle-piece’ macrostructure design (e.g., a plurality IPHBs) that enables easy addition of supplementary hydrogels (e.g., a IPHB). Additionally, the unique porous microarchitectural design permits mass transport and promotes cellular migration and proliferation, eliminating the need to subculture cells via cellular migration between
10 hydrogels within the microchannels. The initial utilization of a bioinert substrate allowed for the investigation and observation of the potential role of the mechanical and dimensional properties of the 3D system on ASC senescence and stem-like phenotypic properties without introducing a bioactive substrate. It was believed that the softer, 3D hydrogel substrate would provide a more natural mechanical environment for the ASCs and result in the
15 retention of nonsenescent stem-like ASC populations, relative to traditional 2D culture methodologies. Moreover, the unique architectural design would allow for continuous expansion of ASCs via cellular migration between the attached hydrogels (e.g., attached IPHBs), thus eliminating exposure of cells to negative 2D subculturing procedures and subsequent sequelae.

20 **Materials & methods**

Cell culture

Human ASCs (Lot #18TL212639, 23-year-old female, Black), human keratinocytes (KCs; Lot #18TL318559, 62-year-old male, Caucasian) obtained from Lonza (Basel, Switzerland) were utilized for this study. MSC-GM Mesenchymal Stem Cell Growth
25 Medium BulletKit™ was obtained from Lonza (#PT-3001) and used for ASCs. MesenCult™-ACF Plus Medium Kit (Stem Cell Technologies, BC, Canada; Cat. #05445) was used as the serum-free medium. DermaLife K Keratinocyte Medium Complete Kit was obtained from Lifeline Cell Technologies (MD, USA; #LL-0007) and used for KCs.

3D-printed hydrogel cell culture system

30 The bioinert 3D hydrogel system (e.g., IPHB) was approximately 1 x 1 x 1 cm and is a polyethylene glycol (PEG)-based system containinh a unique microarchitectural design and was fabricated to resemble the mechanics of native adipose soft tissue and demonstrated no significant changes in mechanical properties over 3 months. The 3D hydrogels were placed into a glass six-well culture plate for culturing. Fibronectin is a commonly selected coating
35 substrate for ASCs due to their natural secretion of fibronectin. Because the cells do not efficiently adhere/attach to the PEG-based polymer, both the 2D culture plastic/glass and 3D hydrogel were coated with fibronectin at a concentration of 5 µg/cm² to enhance the initial cell attachment. The concentration of fibronectin was standardized to surface area due to the

5 inherent surface-area-to-volume differences between 2D and 3D systems. The approximate surface area of the 3D hydrogel was calculated from the 3D model used for bioprinting.

Expansion of ASCs & KCs

ASCs and KCs were seeded within a T-150 flask and cultured until ~80% confluence before subculturing (passaging). Subculturing of cells was performed by removing culture
10 media, washing thrice with Hanks' balanced salt solution (HBSS, MA, USA; calcium-free, magnesium-free) and incubating with 0.05% Trypsin/EDTA (Lonza; Cat. #CC-3232) at 37°C for 5 min. Trypsin was neutralized with serum and the cells were centrifuged at 500 x g for 5 min, then pelleted and resuspended for reseeding on new 2D tissue culture plastic vessels. ASCs at passage 1 (P1) were reseeded onto 2D culture plastic or onto/within the bioprinted
15 3D hydrogel (e.g., IPHB) system via dropwise addition of a concentrated cell solution to the surface of the hydrogels. This process was repeated with the residual cell solution five times to ensure efficient cell seeding. This repetitive seeding process allowed for the cells to distribute throughout the microporous structure within the hydrogel system. Given that the increased surface area and attachment of additional hydrogels eliminated the need for
20 subculturing for this study, a passage-equivalence time point was utilized to allow for analogous comparison with 2D culture. Thus, a passaging event typically occurred every 4–5 days in 2D culture for ASCs but not in 3D culture. After 4–5 days of 2D culture for P2 ASCs, the cells were then subcultured and considered to be P3 in 2D, and the 3D ASCs were then considered P3 passage-equivalent. Culture expansion was determined based on the known 2D
25 and 3D surface areas, initial cell seeding density and average population doubling time of 2.25 days (experimentally determined in 2D; data not shown) for the ASCs in order to standardize cell numbers. ASCs were seeded at a standardized concentration of 5000 cells/cm² for assays. Media supplementation was standardized to 150 µl/cm² for ASC expansion to account for dilutional differences in surface-area-to-volume ratio between 2D
30 and 3D culture.

ASC phenotype characterization

The MSC stem-like phenotype was evaluated for the ASCs at P1/2/6/10 via immunolabel characterization of three key MSC surface markers (CD73/90/105). ASCs were either continuously subcultured in a T-150 flask or allowed to expand within the 3D hydrogel
35 system. At each respective passage time point, cells were seeded in 2D at a standardized density of ~5000 cells/cm² onto a 96-well glass culture plate (Cellvis, CA, USA; Cat. #P96-1.5H-N) for 2 days, fixed, then assessed for ASC phenotype via immunolabeling for surface CD markers. For ASCs within the 3D culture system, cells were fixed and stained in situ.

5 Positive staining for CD73/90/105 and negative staining for CD34/45 was used to denote a stem-like MSC phenotype for this study. After fixation with 4% paraformaldehyde, cells were washed thrice with HBSS and placed in blocking buffer (1% donkey serum in HBSS) for 1 h. After blocking, primary antibodies for CD73 (Abcam, MA, USA; Cat. #133582; 1:100), CD90 (Abcam; Cat. #181469; 1:100), CD105 (Abcam; Cat. #231774; 1:100), CD34
10 (Abcam; Cat. #81289; 1:200) or CD45 (Abcam; Cat. #40763; 1:200) were added and the cells incubated overnight at 4°C. The next day cells were washed thrice and secondary antibodies were applied for 1 h, followed by three additional washes. Cells were counterstained with Hoechst 33342 (Invitrogen, MA, USA; Cat. #H3570; 1:1000) and Alexa Fluor[®] 647 Phalloidin (Invitrogen; Cat. #A22287; 1:1000). Immunofluorescence was assessed with a
15 Revolve microscope using filters for 4',6-diamidino-2-phenylindole (DAPI; EX-380/30, EM-450/50), fluorescein isothiocyanate (EX-470/40, EM-525/50); Texas Red (EX-560/40, EM-630/75) and Cy5 (EX-630/40, EM-700/75) (Echo, CA, USA) and 20X objective (Olympus, Tokyo, Japan; UPlanSApo, 0.75NA). ASC stem-like phenotypic quantification was carried out in quadruplicate (n = 4), with images taken from a total of ten random fields of view per
20 biological replicate (2D = per well; 3D = per hydrogel), to achieve up to 40 total measured values for each sample. Total nuclei were counted, and total positive cells were evaluated. Phalloidin counterstain was used to aid in localization of positive staining. ASCs characterized at P1 were used as a baseline for comparison.

ASC senescence characterization

25 Similar to the ASC phenotyping methodology above, assessment of ASC senescence was performed. ASCs were continuously subcultured in a T-150 culture flask until each respective assay time point, when they were seeded onto a 96-well glass culture plate. ASCs in the 96-well plate were allowed to acclimate in serum-based media for 2 days, fixed, then assessed for senescent activity via immunofluorescent labeling of β -galactosidase activity
30 with the CellEvent[™] Senescence Green Detection Kit (Invitrogen; Cat. #C10850), per manufacturer's instructions. ASCs in the 3D system were assessed simultaneously at the P2/6/10 passage-equivalent time points. Similar to the method above, cells were counterstained with Hoechst and Phalloidin. Senescence characterization was carried out in quadruplicate (n = 4), with images taken from a total of ten random fields of view per
35 biological replicate, to achieve up to 40 total measured values per sample. Total nuclei were counted, and total senescent positive and negative cells were determined.

Isolation of ASC-conditioned media

5 Media supplementation was standardized for ASC expansion to account for dilutional differences in surface-area-to-volume ratio between 2D and 3D cultures. Media were changed every 2 days. For ASC-conditioned medium (ASC-CM) collection, MSC-GM was removed and cells were washed with HBSS thrice, then cultured with serum-free MSC media for 48 h before collection (for both 2D and 3D cultures). Collected ASC-CM was then centrifuged at
10 1500 x g for 10 min to eliminate cell debris, Steriflip-filtered with a 0.22- μ m filter and stored at -80° C until use.

KC activity after ASC-CM treatment

ASC-CM was collected at each respective time point per the protocol above. ASC-CM was then placed on KCs for up to 24 h to assess its capacity to modulate metabolic,
15 proliferative or migratory activity to evaluate wound healing capabilities of the ASC-CM. For these studies, ASC-CM was added at a 1:1 ratio with KC growth medium. Experimental assays were performed per the manufacturer's instructions. PrestoBlue fluorescence was obtained at 560/590 nm (n = 4) and used for evaluation of metabolic activity after 24 h of ASC-CM treatment. Hoechst was added to PrestoBlue samples and values were displayed as
20 average relative fluorescence unit values of PrestoBlue/Hoechst signal in order to control for potential differences in cell numbers and obtain approximate metabolic activity per cell. PicoGreen fluorescence was obtained at 485/535 nm (n = 4) and used for evaluation of cell number as a surrogate measurement of KC proliferation after 24 h of ASC-CM culture. Total cell numbers per 96-well plate were calculated based on an average DNA content of 7.7
25 pg/cell. KC scratch assays were performed to evaluate changes in wound size as a surrogate measurement of KC migration (n = 3). Migration images were taken using an ImageXpress Micro XLS Imaging System (Molecular Devices, CA, USA). The entire wound was imaged for each wound triplicate and three different wound regions per wound triplicate were used to calculate wound area. The three wound area values were averaged per triplicate and per time
30 point for each group and displayed as percentage wound area recovered (n = 3).

Statistical analysis

All data are reported as means with standard error of the mean. Characterization analyses of ASC populations with immunolabeling for senescence and CD markers were evaluated with a two-way analysis of variance. KC metabolic, proliferative and migratory
35 activities were evaluated with a two-way analysis of variance. Data were tested for normality via Shapiro–Wilk and Kolmogorov–Smirnov tests and plotted with a QQ plot. GraphPad Prism 9.0.2 software (GraphPad, CA, USA) was used for the analyses, and a p-value < 0.05 was considered significant.

5 **Results**

Unique 3D hydrogel (IPHB) design eliminates subculturing

The ~1-cm³ 3D-printed hydrogel system contains a unique ‘puzzle-piece’ macrostructure that allows the continuous addition of supplementary hydrogels (Figure 32A) and promotes the migration of cells from the primary seeded hydrogel into the newly attached hydrogels (Figures 32A & B). ASCs were cultured within/onto a single hydrogel system for 2 weeks and allowed to migrate throughout the hydrogel. Subsequently, an additional hydrogel was added for 5 days, and cells were allowed to migrate to the newly attached hydrogel. The cells were then stained and assessed for migration and proliferation between the two hydrogels (Figure 32B). ASCs were seen lining the porous channels beyond the superficial surface within the internal structure and can be seen forming networks within the hydrogel pores (Figure 32B & C). Cells were able to migrate both across the surface of attached hydrogels and within the microchannels, and can be seen with numerous cellular extensions and focal adhesions protruding from the cells to aid in migration (Figure 32C). The interaction of ASCs in 3D and the formation of 3D networks within the hydrogel microarchitecture were readily identified. Notably, ASCs could be seen lining the surface of the hydrogel and migrating down into one of the pore channels, ultimately interacting in three dimensions with other cell populations within the pore.

Retention of stem-like MSC surface markers in 3D hydrogel over time

Evaluation of stem-like ASC populations over time was performed via immunolabeling quantification of the CD markers CD73/90/105 (Figure 33A). The prevalence of stem-like CD markers declined over time in both 2D and 3D systems; however, 2D culture had a significantly enhanced rate of loss of these markers relative to the 3D system (Figure 33B). Similarly, there was an apparent decrease in intensity of positively stained cells over time in both 2D and 3D systems. Culture within the 3D system resulted in a significant retention at all passage time points for all three positive stem-like markers, except for the initial P2 comparison of CD105. The significant loss of a stem-like phenotype in 2D culture can be seen clearly when comparing ASCs in 3D at P6 and P10 versus ASCs in 2D at P2 (Figure 33B). Over the course of five or nine passage equivalents in 3D (i.e., 3 or 6 weeks in the 3D system for P6 or P10, respectively) and only one passage event in 2D (seeded at P1), the ASCs had similar expression patterns for multiple CD markers, with only a significant difference between 3D expression of CD105 at P10 relative to 2D CD105 at P2 (Figure 33B). Both systems maintained a low population (<5%) of positive-staining cells for CD34 and CD45 markers.

5 Delayed induction of senescence in 3D hydrogel over time

 The prevalence of senescent ASC populations over time was evaluated via fluorescent labeling of β -galactosidase activity, a commonly utilized surrogate measurement of cellular senescence (Figure 34A). The baseline expression of β -galactosidase activity was ~5% for both 2D and 3D ASCs at P2. Over the course of multiple passaging events in 2D, senescence
10 was significantly increased in 2D, with 11.7 and 22.5% senescent cells at P6 and P10, respectively (Figure 34B). Conversely, in the 3D system there was no significant change in the prevalence of senescence over time in ASC populations for the time course of this study (6 weeks) (Figure 34B). Similarly, there were notable differences in cellular morphology in the 2D system versus the 3D system. More specifically, 2D ASCs appear to be more
15 flattened, with a more heterogeneous morphological distribution and an apparent increase in cell size over time in culture, whereas the 3D ASCs maintained a more homogeneous morphology with no observable change in cell size or morphology.

Retention of ASC conditioned media wound healing capacity

 Evaluation of the ASC-CM's functional capacity to modulate a secondary cell
20 population was utilized to demonstrate the dynamic interrelationship between ASC population phenotype and ability to promote wound healing activity in KCs (Figure 35D). KCs were treated with either 2D or 3D ASC-CM from each respective time point for up to 24 h. KCs treated with ASC-CM from 2D cultures showed a significant drop in recovered wound area when treated with P6 and P10 ASC-CM, relative to P2. There was also a slight
25 decrease noted in KC migratory activity observed with 3D ASC-CM from P10 relative to P2 (Figures 35A & B). Overall, 3D ASC-CM maintained a significantly higher ability to enhance KC migration and close their respective wounds, relative to their 2D ASC-CM counterparts. The metabolic activity of KCs demonstrated a decreasing trend when treated with ASC-CM from 2D-expanded cells, with a significant decrease noted in P10 relative to
30 P2 ASC-CM (Figure 35C). No significant differences were observed with ASC-CM from 3D cultures over time. Similarly, the proliferative activity of KCs treated with ASC-CM from 2D cultures demonstrated a decreasing trend, with a significant difference between P10 and P2 ASC-CM, but no significant change noted when KCs were treated with ASC-CM from 3D cultures (Figure 35D). Moreover, the proliferative activity of KCs treated with 3D ASC-CM
35 from P6 and P10 was significantly higher than that of their 2D ASC-CM counterparts.

Discussion

 The inherent regenerative properties of MSCs have garnered immense interest for advancing the field of regenerative medicine and tissue engineering. However, MSCs

5 typically must first be removed from a donor tissue source and cultured outside the body within an artificial environment not native to human tissue. To date, commercially available in vitro expansion systems are almost exclusively 2D in nature. Rigid 2D systems are unphysiological for the cells and rapidly result in the loss of MSC multipotent stem-like features, with subsequent loss of viability and induction of senescence. These changes lead to
10 MSC populations with significantly reduced regenerative capabilities, which is compounded by a lack of standardized cell culture conditions, creating a significant bottleneck in the growth and development of regenerative therapeutics. Thus, there is a critical need to develop culture systems for MSC expansion that are 3D and more tissue-mimetic in their mechanical, architectural and substrate composition properties and which can ultimately circumvent many
15 of the limitations of traditional 2D culture, such as the continuous need for subculturing.

Recent advancements in 3D systems have demonstrated progress toward producing MSC populations that are more stem-like. However, 3D systems such as spheroids, organoids, microspheres and many scaffold systems typically do not closely mimic the native mechanics of their cell/tissue source (e.g., adipose mechanics for ASCs) and often require
20 large bioreactor systems and continuous subculturing to achieve large-scale cell numbers for clinical use. However, tissue-engineered hydrogel systems appear to be advantageous toward producing tailorable, tissue-mimetic systems for cell culture systems. More specifically, the mechanotransductive response to the softer substrate of hydrogels is thought to aid in the retention of stem-like characteristics. Unfortunately, most current hydrogel systems are
25 manufactured to promote controlled differentiation of stem cells toward a specific tissue lineage and not to allow the cells to maintain a stem-like phenotype for long-term expansion. Ultimately, an ideal MSC hydrogel expansion system would protect against senescence, while also improving the retention of a regenerative stem-like phenotype and permitting long-term expansion with minimal subculturing or user intervention.

30 Although MSC-based therapies have demonstrated promise, with over 1000 clinical trials to date listed with the US FDA, they have not advanced as quickly as previously thought. This is considered to be due, at least in part, to the detrimental impact senescent MSC populations may have on tissue regeneration. Senescence is a progressive form of cell-cycle arrest, typically due to DNA and/or oxidative damage, which results in MSCs with
35 impaired DNA-repair modalities that no longer proliferate and exhibit a loss of multipotency. Moreover, senescent MSCs have been shown to secrete factors that negatively impact tissue regeneration and wound healing by impairing angiogenesis, increasing oxidative stress and exacerbating inflammation via the secretion of factors known as the senescence-associated

5 secretory phenotype. The composition of this phenotype can be heterogeneous and is dependent on the mechanism of senescence induction and environmental stimuli; therefore, this likely contributes to the heterogeneity in patient outcomes seen in clinical trials with both cell-based and acellular therapies. Thus, developing an in vitro culture expansion system that limits/prevents the induction of senescence in healthy allogeneic or autologous MSC
10 populations intended for patients would improve the efficacy and consistency of MSC-based clinical therapies.

In this current example, culture of ASCs within a traditional 2D culture system resulted in a significant increase in senescence, as previously established in literature. Conversely, the 3D hydrogel system, in accordance with certain embodiments of the
15 invention, resulted in no significant changes of senescence in ASC populations over the course of the 6-week study (i.e., 10 passage equivalents). Moreover, changes in cell morphology and size can be indicative of phenotypic changes; the apparent increase in cell size seen in the 2D ASCs may be associated with the induction of senescence and thus further supports the β -galactosidase imaging data and prior literature. Overall, these data support the
20 previous hypothesis that 3D culture and substrate mechanics maintain a protective role against senescence. However, with the 3D hydrogel system (IPHB), in accordance with certain embodiments of the invention, the need for subculturing is eliminated, and secreted by-products are more readily accessible versus more traditional poured/molded hydrogels that lack a porous microarchitecture.

25 Similarly, the stem-like phenotype of MSCs is critical to their regenerative potential and can rapidly change depending on the culture environment of the cells. MSC populations that differentiate and lose their stem-like characteristics result in variability of cellular phenotype and alterations in secretome composition, ultimately decreasing the consistency of regenerative MSC therapeutics, both cellular and acellular. Thus, MSC populations such as
30 ASCs are often used within only a few passaging events in an attempt to circumvent the loss of regenerative potential. However, as we see in this example, even within one additional passaging event in 2D culture, ASCs significantly alter their expression of stem-like markers. Moreover, ASCs expanded in 3D culture for six or ten passaging equivalents (i.e., P6 or P10) over 6 weeks maintained similar expression levels of several markers relative to the baseline
35 P2 ASCs, and a higher expression relative to their respective 2D counterparts, further highlighting the detrimental effects of 2D culture systems on MSC populations and the potential protective effects of 3D culture. The ability to improve the retention of stem-like properties within MSC populations for longer periods of time is desirable for a multitude of

5 applications, including cell therapies, regenerative tissue engineering, immunotherapy and production of secreted biologics.

As previously mentioned, recent MSC therapies have expanded into investigating biologics as a potential regenerative therapy. MSC populations are highly adaptive in nature, and are known to sense their surrounding environmental stimuli and secrete factors
10 accordingly. Thus, secreted bioactive compounds act to coordinate and bridge a variety of tissue reparative processes in an autocrine, paracrine or endocrine manner. In this study, the conditioned media from ASCs cultured in 2D versus the 3D system were collected over time and utilized as a therapeutic for a secondary cell population, KCs, as a means to functionally assess the phenotype of the ASC secretome toward promoting wound healing activity.
15 Working under the hypothesis that the ASC phenotype deteriorates over time in 2D cultures but is sustained in the 3D system, we expected to see a gradual decline in regenerative capabilities of the ASC-CM from 2D cultures but minimal changes in ASC-CM from 3D cultures. This hypothesis was supported by the metabolic, proliferative and migratory data of KCs treated with ASC-CM. ASC-CM from 2D cultures demonstrated a steady decline in
20 ability to augment KC activity and was consistently outperformed by its 3D counterpart over the course of the example.

The limitations of this example include the formulation of the hydrogel system being intentionally bioinert in order to eliminate any contribution of a bioactive substrate, and thus it was not degradable. As a result, adequate removal of cells from this formulation was not
25 feasible. Therefore, immunofluorescent labeling was utilized as an alternative to flow cytometry or RNA analysis to demonstrate senescence and phenotype of MSC populations. However, the ability to perform in situ visualization of an adherent population such as MSCs without the need to resuspend them is an advantage of immunolabeling over cytometry. Moreover, the relative comparison between 2D and 3D cultures, paired with the functional
30 data of the ASC-CM, helps provide a supportive and holistic perspective that reinforces the immunolabeling data, although a more comprehensive analysis of the secretome is needed to assess both qualitative and quantitative changes.

Conclusion

In this example we illustrate the successful use of a 3D hydrogel system to
35 demonstrate the benefits of culturing MSCs in a system that more closely resembles their native tissue mechanics. The 3D system contains a unique architectural design that does not impede effective mass and fluid transport while also allowing the movement of cells within and between attached hydrogels, in effect providing a continuous 3D culture system that

5 eliminates the need to subculture cells. The continuity is achieved by the addition of supplemental hydrogels to previously seeded hydrogels, much like attaching together two puzzle pieces. The porous microarchitecture creates a ‘tunneling’ system for the cells to interact in the x-, y- and z- planes and to migrate within and between hydrogels, in addition to surface migration at attachment points.

10 These and other modifications and variations to the invention may be practiced by those of ordinary skill in the art without departing from the spirit and scope of the invention, which is more particularly set forth in the appended claims. In addition, it should be understood that aspects of the various embodiments may be interchanged in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is
15 by way of example only, and it is not intended to limit the invention as further described in such appended claims. Therefore, the spirit and scope of the appended claims should not be limited to the exemplary description of the versions contained herein.

5

THAT WHICH IS CLAIMED:

1. A method of growing of tissue *ex vivo* cells, comprising:

10 (i) interlocking together a plurality of interlocking porous hydrogel blocks (IPHB) including a first IPHB and a second IPHB, wherein each of the plurality of IPHBs include a respective three-dimensional (3D) macrostructure defined by a respective continuous polymeric matrix material and a respective network of microporous channels and/or chambers extending throughout the respective continuous polymeric matrix material, and wherein the respective 3D macrostructures each comprise a respective top surface, a
15 respective bottom surface, and a respective thickness defined by at least one respective side edge extending from the respective top surface to the respective bottom surface, and wherein the respective 3D macrostructure structures each include at least one respective interlocking-male component and at least one respective interlocking-female component;

(ii) seeding the first IPHB with a first cell type of interest;

20 (iii) seeding the second IPHB with a second cell type of interest, wherein the first cell type of interest is different than the second cell type of interest;

(iv) feeding the first cell type of interest with a first culture media, and allowing the first cell type of interest to propagate throughout a first network of microporous channels and/or chambers towards the second IPHB;

25 (v) feeding the second cell type of interest with a second culture media, and allowing the second cell type of interest to propagate throughout a second network of microporous channels and/or chambers towards the first IPHB; and

(vi) forming a first interface between the first cell type and the second cell type.

30 2. The method of claim 1, wherein the first network of microporous channels and/or chambers has a first structure and the second network of microporous channels and/or chambers, wherein the first structure is different than the second structure.

5

3. The method of claims 1-2, wherein the first network of microporous channels and/or chambers, the second network of microporous channels and/or chambers, or both have an average diameter comprising from about 100 to about 800 microns, such as at least about any of the following: 100, 120, 150, 180, 200, 220, and 250 microns, and/or at most about any of the following: 800, 780, 750, 720, 700, 680, 650, 620, 600, 580, 550, 520, 500, 480, 450, 420, 400, 380, 350, 320, 300, 280, and 250 microns.

4. The method of claims 1-3, wherein the first network of microporous channels and/or chambers, the second network of microporous channels and/or chambers, or both independently from each other comprises at least about 40% by volume of the respective 3D macrostructure, such as from at least about any of the following: 40, 50, 60, and 70% by volume of the respective 3D macrostructure, and/or at most about any of the following: 90, 85, 80, 75, and 70% by volume of the respective 3D macrostructure.

5. The method of claims 1-4, wherein a first continuous polymeric matrix material of the first IPHB comprises a non-degradable hydrogel material or a or a selectably degradable hydrogel material.

6. The method of claims 1-5, wherein the second continuous polymeric matrix material of the second IPHB comprises a non-degradable hydrogel material or a selectably degradable hydrogel material.

7. The methods of claims 5-6, wherein the first continuous polymeric matrix material of the first IPHB, the second continuous polymeric matrix material of the second IPHB, or both comprise a non-degradable hydrogel material comprising one or more synthetic polymers, such as synthetic polymers derived from petroleum.

- 5 8. The methods of claims 5-6, wherein the first continuous polymeric matrix material of the first IPHB, the second continuous polymeric matrix material of the second IPHB, or both comprise a selectably degradable hydrogel material comprising one or more degradable polymers, such as one or more biopolymers derived from a living organism.
- 10 9. The method of claim 8, wherein the one or more biopolymers derived from a living organism comprises a polynucleotide, polysaccharide, polypeptide, or any combination thereof
- 15 10. The method of claims 8-9, wherein the one or more biopolymers comprises collagen, gelatin, laminin, alginate, glycosaminoglycans, oligonucleotides (e.g., DNA, RNA), carbohydrates, lipids, cellulose, alginate, and proteins that can be gently and degraded, such as with the use of protein specific enzymes, ionic solvents, neutral detergents, weak acids, and peroxides to disrupt the biopolymer chains.
- 20 11. The method of claims 8-10, wherein the one or more biopolymers comprises degradable monomers comprising esters, such as hydroxybutyrate, lactic acid, glycolic acid, and caprolactone; anhydrides, such as adipic acid, and sebacic acid; saccharides, such as cellulose, alginate, pectin, dextrin, chitosan, hyaluronan, Chondroitin sulfate, and heparin; proteins; nucleotides (DNA, RNA); peptides, such as collagen, gelatin, silk, and fibrin; 25 urethanes; phosphates; carbonates; and vinyl chlorides.
- 30 12. The method of claims 8-11, wherein the selectably degradable hydrogel material further comprises a synthetic polymer, such as a polyester, a polyanhydride, a polycarbonate, a polyurethane, a polyphosphate or combinations thereof.
13. The methods of claims 1-12, wherein the first continuous polymeric matrix material of the first IPHB is formed from a non-degradable hydrogel material and the second

5 continuous polymeric matrix material of the second IPHB is formed from a selectably
degradable hydrogel material.

14. The methods of claims 1-13, further comprising interlocking a third IPHB directly to
the second IPHB, and seeding the third IPHB with a third cell type of interest, wherein the
10 third cell type of interest is different that the first cell type of interest and the second cell type
of interest.

15. The method of claim 14, further comprising a step of feeding the third cell type of
interest with a third culture media, and allowing the third cell type of interest to propagate
15 throughout a third network of microporous channels and/or chambers towards the second
IPHB; and forming a second interface between the first cell type and the second cell type.

16. The method of claims 1-15, further comprising a step of harvesting at least a portion
of first cells from the first cell type of interest located throughout the first network of
20 microporous channels and/or chambers of the first IPHB.

17. The method of claim 16, wherein the first continuous polymeric matrix material of the
first IPHB comprise a non-degradable hydrogel material, and the step of harvesting
comprises forming artificial tissue samples by cutting the first IPHB into a plurality of
25 sections exposing at least a portion of the cells of the first cell type of interest at a surface of
the artificial tissue sample.

18. The method of claim 17, further comprises freezing the first IPHB before or after
forming artificial tissue samples.

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5 19. The method of claims 17-18, wherein the second continuous polymeric matrix material of the second IPHB comprise a non-degradable hydrogel material, and the step of harvesting comprises harvesting (i) at least a portion of first cells from the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first IPHB, (ii) at least a portion of second cells from the second cell type of interest located
10 throughout the second network of microporous channels and/or chambers of the second IPHB, and (iii) at least a portion of first interfacing cells forming the first interface by forming multi-cell containing artificial tissue samples by cutting the interlocked first IPHB and second IPHB into a plurality of sections exposing the cells of (i)-(iii) at a surface of each multi-cell containing artificial tissue sample.

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20. The method of claim 19, further comprises freezing the interlocked first IPHB and second IPHB before or after forming each multi-cell containing artificial tissue sample.

21. The method of claim 19, wherein the third continuous polymeric matrix material of
20 the third IPHB comprise a non-degradable hydrogel material, and the step of harvesting comprises additionally harvesting (iv) at least a portion of third cells from the third cell type of interest located throughout the third network of microporous channels and/or chambers of the third IPHB, and (v) at least a portion of second interfacing cells forming the second interface by forming multi-cell containing artificial tissue samples by cutting the interlocked
25 first IPHB, second IPHB, and third IPHB into a plurality of sections exposing the cells of (i)-(v) at the surface of each multi-cell containing artificial tissue sample.

22. The method of claim 21, further comprises freezing the interlocked first IPHB, second IPHB, and third IPHB before or after forming each multi-cell containing artificial tissue sample.

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23. The method of claim 16, wherein the step of harvesting at least a portion of first cells from the first cell type of interest located throughout the first network of microporous

5 channels and/or chambers of the first IPHB comprises flushing them out of the first IPHB with a fluid medium.

24. The method of claim 16, wherein the step of harvesting at least a portion of first cells from the first cell type of interest located throughout the first network of microporous
10 channels and/or chambers of the first IPHB degrading the 3D macrostructure of the first IPHB.

25. The method of claims 1-24, wherein the plurality of IPHBs interlocked together may vary from at least 2 IPBHs, such as at least about any of the following: 2, 3, 5, 10, 20, 30, 40,
15 50, 60, 70, 80, 90, and 100 IPBHs.

26. The method of claims 1-25, wherein the first cell type of interest produces or secretes a first therapeutic of interest, such as a first biologic.

20 27. The method of claim 26, wherein the second cell type of interest produces or secretes a second therapeutic of interest, such as a second biologic.

28. The method of claim 27, wherein the third cell type of interest produces or secretes a third therapeutic of interest, such as a third biologic.

25 29. The method of claims 1-28, further comprising positioning or interlocking a first acellular substrate directly adjacent the first IPHB or directly between the first IPHB and the second IPHB, wherein the first acellular substrate comprises an acellular 3D macrostructure defined by a continuous matrix of extracellular matrix (ECM) material associated with a first cell type of interest and network of microporous channels and/or chambers extending
30 throughout the continuous matrix of ECM material associated with a first cell type of interest, and wherein the acellular 3D macrostructure comprises a top surface, a bottom surface, and a

5 thickness defined by at least one side edge extending from the top surface to the bottom surface.

30. The method of claim 29, wherein the first acellular substrate further comprises at least one interlocking-male component and at least one interlocking-female component.

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31. The methods of claims 29-30, further comprising seeding the first acellular substrate with a fourth cell type of interest, wherein the fourth cell type of interest is different than the first cell type of interest and the second cell type of interest.

15 32. The method of claim 31, further comprising feeding the fourth cell type of interest with a fourth culture media, and allowing the fourth cell type of interest to propagate throughout the network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material towards the first IPHB.

20 33. The method of claim 32, further comprising forming a third interface between the first cell type of interest and the fourth cell type of interest.

34. The method of claims 29-33, further comprising a step of harvesting at least a portion of fourth cells from the fourth cell type of interest located throughout the network of
25 microporous channels and/or chambers extending throughout the continuous matrix of ECM material.

35. The method of claim 34, wherein the step of harvesting comprises forming artificial tissue samples by cutting the first acellular substrate into a plurality of sections exposing at
30 least a portion of the fourth cells of the fourth cell type of interest at a surface of the artificial tissue sample.

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36. The method of claim 35, further comprises freezing the first acellular substrate before or after forming artificial tissue samples.

37. The method of claims 35-36, wherein the step of harvesting comprises harvesting (i) at least a portion of first cells from the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first IPHB, (ii) at least a portion of fourth cells from the fourth cell type of interest located throughout network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material of the first acellular substrate, and (iii) at least a portion of third interfacing cells forming the third interface by forming multi-cell containing artificial tissue samples by cutting the interlocked or adjacent first IPHB and first acellular substrate into a plurality of sections exposing the cells of (i)-(iii) at a surface of each multi-cell containing artificial tissue sample.

38. The methods of claims 29-37, wherein the fourth cell type of interest comprises a native cell from a living organism, such as a mammal or plant.

39. The methods of claims 29-38, a plurality of acellular substrates, including the first acellular substrate, that are interfaced with one or more IPHBs may vary from at least 1 acellular substrate, such as at least about any of the following: 2, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 acellular substrates.

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