BIOLOGICAL CARTRIDGE AND CELL CULTIVATION METHODS USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/312,254, filed February 21, 2022, which is expressly incorporated by reference herein in its entirety.

TECHNICAL FIELD

Embodiments of the presently-disclosed invention relate generally to biological cartridges comprising a housing chamber defining an interior region that houses one or more porous hydrogel block (PHB) seeded with at least one cell type of interest are provided. The biological cartridges include an inlet orifice and an outlet orifice to enable culture media, other fluids, and gases to enter into the PHB(s) to cultivate cell types seeded within the PHB(s). The biological cartridges can be interconnected in series and/or parallel to define a biological cartridge network (BCN) that can mass produce a particular cell type of interest and/or cultivate multiple cell types of interest simultaneously, and/or produce one or more biologics of interest.

BACKGROUND

One of the challenges in mass-producing biological products such as recombinant antibodies, growth factors, exosomes, extracellular vesicles, proteins, and oligonucleotides is that replicating physiological systems outside of nature at scale is difficult from a logistics, economics, and accuracy perspective. The reason being is that most physiological systems are formed to create biological resources for a single individual for a lifetime. These systems are carefully balanced, and up-scaling or down-scaling such systems can disrupt homeostasis within an individual. However, when it comes to replicating these systems outside of the body for the purpose of producing raw materials such as biologics, the systems lack the physiological characteristics and proper scaling to sufficiently work with the resources that are currently available.

Therefore, that remains a need in the art for systems and/or methods that replicate the internal physiological systems of a living organism (e.g., a mammal) outside of the body of the living organism, and operated for example in parallel, such systems and/or methods may produce biologics at scale and economically.

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 biological cartridge including a housing chamber defining an interior region, wherein the housing chamber includes an inlet orifice and an outlet orifice. The biological cartridge also comprises at least one porous hydrogel block (PHB), such as a plurality of PHBs vertically stacked upon each other, located within the interior region of the housing chamber.

In another aspect, certain embodiments of the invention provide a system comprising a plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series to define a biological cartridge network (BCN). The BCN may include at least (i) a first biological cartridge having a first inlet and a first outlet, and (ii) a second biological cartridge having a second inlet in operative communication with the first outlet, and a second outlet. In accordance with certain embodiments of the invention, each of the plurality of biological cartridges include a respective plurality of PHBs seeded with a first cell type of interest housed therein.

In another aspect, certain embodiments of the invention provide a system comprising a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series. The first set of biological cartridges and the second set of biological cartridges may be aligned in a parallel relationship with respect to each other and a common feed source.

In another aspect, certain embodiments of the invention provide a system comprising a biological cartridge network (BCN) comprising a plurality of biological cartridges, such as those described and disclosed herein, wherein the plurality of biological cartridges include (i) a first plurality of biological cartridges that are operatively connected in series, and (ii) a second plurality of biological cartridges are operatively connected in parallel. In this regard, the BCN may comprise a nearly unlimited number of individual biological cartridges in a nearly unlimited number of different overall configurations (e.g., a variety of series and/or parallel interconnections between biological cartridges with a variety of different cell types of interest being seeded in any number of biological cartridges).

In another aspect, certain embodiments of the invention provide a method of cultivating one or more cell types of interest, comprising: (i) providing a first biological

cartridge, such as those described and disclosed herein, wherein the first biological cartridge includes a first plurality of PHBs housed therein; (ii) seeding the first plurality of PHBs with at least a first cell type of interest; (iii) feeding the at least a first cell type of interest with a first culture media, and allowing the at least a first cell type of interest to propagate throughout the first network of microporous channels and/or chambers of the first plurality of PHBs; and (iv) harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the plurality of PHBs.

In another aspect, certain embodiments of the invention provide a method of cultivating one or more cell types of interest, comprising: (a) providing or forming a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series; wherein the first set of biological cartridges and the second set of biological cartridges are aligned in a parallel relationship with respect to each other and a common feed source; (b-1) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding respective immediately subsequent biological cartridges with a second cell type of interest, wherein the first and second cell types are different; or (b-2) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding an immediately subsequent biological cartridge of the first set of biological cartridges with a second cell type of interest, and seeding an immediately subsequent biological cartridge of the second set of biological cartridges with a third cell type of interest, wherein the first cell type, the second cell type, and the third cell type are all different from each other; and (c) harvesting at least a portion of the first cell type of interest and/or at least a portion of the second cell type of interest and/or at least a portion of the third cell type of interest.

BRIEF DESCRIPTION OF THE DRAWING(S)

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:

Figure 1 illustrates, in accordance with certain embodiments of the invention, a biological cartridge including a plurality of PHBs housed vertically therein;

Figure 2 illustrates the biological cartridge of Figure 1 with the PHBs removed so that the an alignment column is viewable;

Figure 3 illustrates a top view of a rendering of a biological cartridge illustrating a top plate and multiple inlet ports for the introduction of different liquids and/or gases in accordance with certain embodiments of the invention;

Figure 4 illustrates an inside surface of the top plate of Figure 3;

Figure 5 is a schematic of a PHB in accordance with certain embodiments of the invention;

Figure 6 is a schematic of another PHB in accordance with certain embodiments of the invention;

Figure 7-10 each illustrate a system including multiple biological cartridges connected in series and/or parallel in accordance with certain embodiments of the invention.

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 systems and/or methods that replicate the internal physiological systems of a living organism (e.g., a mammal) outside of the body of the living organism, and operated for example in parallel, such systems and/or methods may produce biologics at scale and economically. In accordance with certain embodiments of the invention provide a device in which human, animal, plant, bacterial, yeast, and fungus cells can grow naturally, and be regulated at an industrial level that mimics physiological conditions while maintaining an economic edge over other manufacturing systems. In accordance with certain embodiments of the invention, the present invention may provide the ability to combine multiple physiological systems both in sequence and in

parallel to simulate proper physiological and biological function as well as to produce biological products. Replicating the systems correctly outside of the body and pairing the systems together is no small feat as evidenced by the remaining need in the art. Yet, the ability to build an apparatus that appropriately incorporates and combines physiological systems may be particularly important in producing biological products and cells that may be used for therapeutic applications or for physiological modeling to examine and possibly diagnose diseases, cancers, and injuries in organisms. Accordingly, certain embodiments of the invention provide an extraordinary advancement in personalized medicine. For instance, certain embodiments of the invention provide biological cartridges, which may be also be referred to a as "Biological Capsules" (Bio-Capsules), "Biological Cassettes" (Bio-Cassettes), "Biological Pods" (Bio-Pods), and "Biological Chambers" (Bio-Chambers), in which the biological cartridges house or contain porous hydrogel blocks (PHB) that are configured to mimic various environments in the organism of interest (e.g., a human body). When the biological cartridges containing one or more porous hydrogel blocks are perfused with liquid media using, for example, a pump apparatus, the biological cartridges can be connected in sequence or in parallel to mimic the flow of blood, lymph, and other biological fluids through various biological environments to mimic physiological tissues or organs.

Utilizing one or more PHBs comprising synthetically, biologically, or a composite of synthetically and biologically defined hydrogels that contain a network of microporous channels and/or chambers emulating arteries and arterials running the entire length of the hydrogel may be disposed with in the biological cartridges. The biological cartridges may also include additional support porous microstructures that run adjacent to the one or more PHBs each having a respective network of microporous channels and/or chambers to direct airflow and gas exchange within the biological cartridge. Within a PHB, arginine, glycine, and aspartate (RGD) residues or other integrin binding residues may permeate the surface of the PHB for cell attachment. The PHB(s) may be encased or housed within a cartridge housing, such as a cylindrical structure having a circular cross-section or a polyhedral structure having a non-circular cross-section (e.g., cube, prism, etc.). In accordance with certain embodiments of the invention, openings at the top of the cartridge housing for inlet of liquid and/or gas, and an outlet at the bottom of for outlet of liquid, gas, and/or other material (e.g., one or more cells of interest, one or more biologics, etc.). The PHBs may be stacked in the z-direction and run the length of the cartridge housing. When connected to a fluid delivery device, such as a peristaltic pump, different liquids can be perfused through the PHBs inside the cartridge housing. These liquids may contain cells, nutrients, degradative

enzymes, or defined chemical substances that can permeate or run through the stacked PHB structure. In accordance with certain embodiments of the invention, the biological cartridge or cartridge housing alone may be encased inside a closed container where the environmental temperature may be adjusted as desired, such as between 4°C and 47°C, and gas containing, for example, from 1 to 10% CO_2 may be provided at, for example, 1 to 10 PSI. The biological cartridge may be sealed under sterile conditions prior to encasement in an environmental container (e.g., for temperature control). Encasement, for example, creates a closed environment, in which ends of the biological cartridge or cartridge housing alone may be punctured for the movement of fluid and gas. The biological cartridge may be oriented vertically, so fluid flows with gravity though the device, and may generate a vortex through the respective network of microporous channels and/or chambers of each of the PHBs housed therein. In accordance with certain embodiments of the invention, a carousel may be positioned below the biological cartridge and used to sterile filter, separate components, and collect contents of liquid flowing out from biological cartridge. Cylindrical devices may be arranged in a circular or polygonal array to allow for sequential or parallel processing of liquids run through the PHBs. As noted in greater detail below, the PHBs may be engineered to match the mechanical properties of different biological tissues of interest. In accordance with certain embodiments of the invention, specific cells may be run or passed through the PHB(s) for the purpose of attachment, expansion, and production of biologics. Once the surface area (e.g., internal surface area associated with the network of microporous channels and/or chambers) of the PHB(s) is fully covered with cells, the PHB(s) may optionally be dissolved to release cells and biological contents for collection. Liquids, gases, electromagnetic fields, mechanical gradients, osmotic gradients, mechanical gradients, chemical gradients, and temperature gradients may be permeated through hydrogels to achieve physiological conditions mimicking native tissue.

In accordance with certain embodiments of the invention, the biological cartridge provides the first closed device in the industry, in which cell culturing can be automated by eliminating the need for direct manipulation by an end-user. Adherent cells of any type can be perfused into the biological cartridge containing the specified PHBs. Cells of interest can be cultivated with media and nutrient perfusion. Once the cells have reached the desired maturity and number, the PHBs may, in accordance with certain embodiments of the invention, be degraded with, for example, an enzymatic reagent that releases cells and biological contents. Released cells and biological contents can be collected through standard filtration and isolation techniques and used for different research, diagnostic, and

therapeutical applications. Beneficially, the biological cartridges and systems thereof allow for hands-free growth of human, animal, bacterial, yeast, and fungus cells in a standardize format. The outcome of biological cartridge, in accordance with certain embodiments of the invention, is that cells or biological factors secreted by cells can easily be collected and isolated for use as raw materials in the production of personalized therapeutics, personalized diagnostics, personalized autografts, personalized cosmetics, personalized fillers, personalized implants, cultivated plants, cultivated feed stock, and cultivated meat products that include but are not limited to hamburger, steak, rib, fish, and crustacean products. The composition of the hydrogel material defining or forming the PHB(s) may be varied to enable further control for creating a pH gradient, electromagnetic gradient, osmotic gradient, mechanical gradient for the purpose of stimulating the growth and differentiation of cells as well as the secretion of specific biological factors which include but are not limited to: growth factors, exosomes, extracellular vesicles, nucleic acids, proteins, monoclonal antibodies, enzymes, peptides, and organelles.

As noted above the PHB(s) provide a platform that may mimic discrete microenvironments for specific cell types of interest. By joining the PHBs in a vertical configuration, the respective networks of microporous channels and/or chambers of each of the PHBs housed therein align so that fluid, gas, and other aqueous media can flow through the PHBs. The respective PHBs may be formed from a fully or partially synthetic hydrogel material. Without knowing the internal microarchitecture of a particular native tissue of interest, which forms the basis (e.g., produced by an additive manufacturing technique) for the respective networks of microporous channels and/or chambers of each of the PHBs, it is not obvious that the PHBs can be combined vertically to enable continuous fluid flow. Hence, creating a closed device that can house the PHBs in a vertical configuration, enables the ability to control multiple aspects of the environment in which cells grow. For example, each of the PHBs may have a top surface and a bottom surface. The respective top and bottom surfaces, for example, may be identical in terms of the number and/or location of openings at the surfaces that serve as micro-inlets to the internal network of microporous channels and/or chambers. In this regard, each PHB may be loaded into the biological cartridge in a vertically stacked manner, in which the respective networks of microporous channels and/or chambers of each of the PHBs are purposely aligned at interfaces between the respective PHBs to provide a continuous aggregate network of microporous channels and/or chambers.

As will be more clear below, certain embodiments of the invention provide a variety of applications for the culturing and/or production of a variety of biological materials. Non-limiting examples of applications and/or method of producing biological materials include a variety of cell culture, stem cells (e.g., human or animal), hematopoietic cells (e.g., human or animal), somatic cells (e.g., human or animal), plant cells, bacterial cells, fungi, biologics, recombinant antibodies, engineered antibodies, extracellular vesicles, exosomes, growth factors, nucleic acids, DNA, mRNA, tRNA, rRNA, siRNA, miRNA, shRNA, ncRNA, oligonucleotides, proteins, and enzymes. Further yet, embodiments of the present invention may be utilized to provide, for example, personalized diagnostics, personalized therapeutics, personalized cosmetics, cosmetics, fillers, implants, plant production, organic materials, organic waste, meat production (e.g., beef products, ham products, poultry products, fish products), crustacean products, bacteria production, protein production, and plasmid production.

Unlike traditional cell culture vessels or bioreactors, the multiple applications and configurations of a plurality of biological cartridges may be realized, at least in part, due to the ability of configuring a plurality of PHBs within a closed environment where multiple parameters of the environment can be controlled such nutrient diffusion, gas exchange, temperature, pH, viscosity, osmotic pressure, electromagnetic conductivity, mechanical stiffness, permeability, and turbidity. In this regard, traditional cell culture vessels or bioreactors fail to provide such an increased environmental control in comparison to the biological cartridges in accordance with certain embodiments of the invention. As noted herein, multiple biological cartridges may be connected in series/sequence and/or parallel to introduce biological programs through exposure of conditioned media from one biological cartridge to a subsequent biological cartridge for the purpose of manipulating cell growth or stimulating biological secretion.

The invention allows for multiple biological cartridges to be connected in series/sequence or parallel to stimulate mass production of target cell types. Additionally, certain embodiments of the invention allows for a scaling up or scaling down of biological products to be produced, which can include for example cells, biological factors secreted from cells, or organelles, for different applications. Further yet, certain embodiments of the invention

allows for the testing of new therapeutics to permeate through one set of cells within a biological cartridge containing one set of cells and then a second biological cartridge containing a different set of cells to measure efficacy and pharmacokinetics. An additional

benefit realized by certain embodiments of the present invention include, for example, the elimination of the need for cellular sub-culture. For example, cell culturing methods may be devoid of a cellular sub-culturing step or operation.

Additional benefits realized by certain embodiments of the invention include, for example, the release of cells from PHB(s) housed in a biological cartridge for downstream analyses and diagnostics or downstream manufacturing of therapeutics, physiological modeling or disease modeling, mass production of an individual's own cells for diagnostic purposes, bioengineering a custom tissue, bioengineering a custom therapeutic, or bioengineering a custom organ. Certain embodiments of the invention may also include simulating and treating a patient disease or ailment in a closed environment. Moreover, certain embodiments of the invention can be used to pool whole donor population cells together for the purpose of diversifying the production biological products. In accordance with certain embodiments of the invention, the biological cartridge loaded with one or more PHBs enables cells to grow in a more native physiological-like environment compared to culture in a traditional 2D plastic cell culture vessel or other bioreactors. In this regard and discussed herein, the PHBs may be considered to define substrates that can be tuned and to mimic original tissue environment (e.g., native tissue *in vivo*) 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 provides for different cell types to be cultured in sequence or parallel to produce different desired outcomes for production of cellular biological factors. This flexibility cannot be provided by any other technology at the current time.

As noted above, certain embodiments of the invention provide a biological cartridge including a housing chamber defining an interior region, wherein the housing chamber includes an inlet orifice and an outlet orifice. The biological cartridge also comprises at least one porous hydrogel block (PHB), such as a plurality of PHBs vertically stacked upon each other, located within the interior region of the housing chamber. In accordance with certain embodiments of the invention, the housing chamber may have a cross-section in an x-y plane, in which the cross-section comprises a non-round cross-section, such as a polygon having from 3 to 12 sides, in the x-y plane. Such non-round cross-section embodiments may, for example, facilitate secure positioning a plurality of PHBs having a corresponding geometry housed therein, since the non-round cross-sections may prevent independent rotation or movement of the individual PHBs relative to each other. As such, the respective network of microporous channels and/or chambers of each of the PHBs housed therein may remain

purposely aligned with each other to mitigate discontinuities of the continuous aggregate network of microporous channels and/or chambers as noted above. Stated somewhat differently, the non-round geometries of the PHBs and the housing chamber cross-section restricts the relative rotational movement of the PHBs housed within the housing chamber to beneficially facilitate the lack of disturbance of the continuous aggregate network of microporous channels and/or chambers defined by the respective PHBs.

Alternatively, the housing chamber may have a cross-section in an x-y plane, in which the cross-section comprises a round cross-section in the x-y plane. In accordance with certain embodiments of the invention, the cross-section of the housing chamber may be identical along an entire operating length of the housing chamber, wherein the operating length is the section along a z-direction that is perpendicular to the x-y plane and is associated with regions housing the PHB(s).

In accordance with certain embodiments of the invention, the at least one PHB comprises a plurality of PHBs vertically stacked upon each other in a z-direction that is perpendicular to the x-y plane. By way of example only, the plurality of PHBs may comprise from 2 to about 40 individual PHBs, such as at least about any of the following: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18, and 20, and/or at most about any of the following: 40, 35, 30, 25, and 20 individual PHBs.

Figure 1, for example, illustrate a biological cartridge 1 including a chamber housing 1 having a hexagonal prism geometry. Figure 1 illustrates, for example only, that the hexagonal cross-section may have a diameter of about 2.5 cm and a length of 15 cm. These values are merely illustrative, and non-limiting. The chamber housing 1 includes an inlet orifice 20 and an outlet orifice 30. In the particular embodiment illustrated by Figure 1, the biological cartridge 1 also includes an inlet distribution filter 29 located immediately above a vertically stacked plurality of PHBs 50, and a drain filter 39 located immediately below the vertically stacked plurality of PHBs.

In accordance with certain embodiments of the invention, the biological cartridge may also comprise at least one mounting element extending through the interior region. The at least one mounting element may comprises a single mounting element aligned along a central axis of the housing chamber and extending along at least a majority or the entire operating length of the housing chamber. The biological cartridge may also comprise a top plate and/or a bottom plate releasably engaged directly or indirectly to the housing chamber, and the single mounting element may optionally be directly or indirectly attached to the top plate and/or bottom plate. The top plate and/or bottom plate, for example, may form part of a

respective inlet or outlet manifold to facilitate the flow of fluids in and out of the biological cartridge. Figure 2, for example, illustrates the biological cartridge of Figure 1 with the PHBs removed so that the an alignment column 15 is viewable. In this regard, the plurality of PHBs vertically stacked upon each other may include a respective female mounting component comprising a respective PHB orifice extending completely through a respective thickness in the z-direction of the respective PHBs, in which the respective female mounting components configured to allow the at least one mounting element to extend through the respective PHB orifices. Figure 5, for example, illustrates a PHB 50 including a top surface 51, a bottom surface 52, and thickness 53.

The PHB 50 may also include a female mounting component 54 comprising an orifice extending completely through the entirety of the thickness 53 of the PHB, with a top opening 55 and a bottom opening 56 being aligned with each other along the z-direction. In this regard, the PHBs 50 may be mounted on the alignment column 15, in which the alignment column extends into and/or through the respective female mounting components 54 of the respective PHBs. In accordance with certain embodiments of the invention, the at least one mounting element may have a non-round cross-section and the respective PHB orifices have a corresponding non-round cross-section. In this regard, the corresponding non-round geometries of the mounting element and the female mounting components prevent the respective PHBs from rotating about the z-direction relative to each other. The vertically stacked respective PHBs, as such, are restrained from rotating in respective x-y planes relative to each other.

Alternatively, the at least one mounting element may comprise at least one fin component attached to the housing chamber and projecting inwardly into the interior region of the housing chamber. The at least one fin component, for example, may extend along at least a majority of the entire operating length of the housing chamber, such as at least about 55, 60, 65 and 75% of the entire operating length of the housing chamber, and/or at most about any of the following: 100, 98, 95, 90, 85, 80, and 75% of the entire operating length of the housing chamber. In such embodiments, the plurality of PHBs vertically stacked upon each other may include a respective female mounting component comprising a respective PHB cutout portion extending completely through a respective thickness in the z-direction of the respective PHBs, in which the respective female mounting components are configured to allow the at least one mounting element to extend through the respective PHB orifices. Figure 6, for example, illustrates a PHB 50 including a top surface 51, a bottom surface 52, and thickness 53. The PHB 50 may also include one or more female mounting components

57 comprising a cutout portion extending completely through the entirety of the thickness 53 of the PHB. In this regard, the at least one fin components may act as guides for the loading and alignment of the PHBs in the chamber housing. Additionally, the male-female coupling of the at least one fin components and the at least one cutout portions of the respective PHBs prevent the respective PHBs from rotating about the z-direction relative to each other. The vertically stacked respective PHBs, as such, are restrained from rotating in respective x-y planes relative to each other.

In accordance with certain embodiments of the invention, the at least one mounting element may comprise a variety of different materials of construction. In accordance with certain embodiments of the invention, for example, the at least one mounting element may comprise a fiber optic column that may also be used for the purpose of imaging the interiors of the PHBs from within the biological cartridge. In such example embodiments, the at least one mounting element may comprise a durable glass like a borosilicate (Pyrex) or a polystyrene (Plastic) as both are transparent and stiff. In accordance with certain embodiments of the invention, however, the at least one mounting element may comprise a conducting metal such as, for example, copper, silver, and gold for carrying a weak current or an iron material for inducing electromagnetic fields through the PHBs in the biological cartridge. The metals, in accordance with such embodiments, may also be used to help regulate temperature. The at least one mounting element may also comprise a differing hydrogel material from the PHBs that is synthetic or biologic for creating more sophisticated osmotic, diffusion, or mechanical (e.g. silicone) gradients through the PHBs. The hydrogel material forming or defining the at least one mounting element may also be used as a delivery vehicle to release growth factors and other agents into the PHBs following or opposing the direction of fluid flow through the PHBs.

As noted above and illustrated by Figure 1, the biological cartridge may also include an inlet distribution filter located directly or indirectly above all of the at least one PHBs. In this regard, an incoming fluid passes through the inlet distribution filter prior to passing into any of the at least one PHBs. The inlet distribution filter may be formed from a variety of materials, such as nonwoven membranes and woven membranes that have an average pore size is no less than 0.1 microns and no greater than 20 microns (e.g., a 0.2 micron pore-size is used for sterile filtering of contents.) Common, and non-limiting, membrane materials include: Polyethersulfone, Cellulose Esters (Cellulose Acetate and Cellulose Nitrate), Polyvinylidene Fluoride (PVDF), Polypropylene, and Polytetrafluoroethylene (PTFE). Additionally or alternatively, the biological cartridge may also include a drain filter located

directly or indirectly below all of the at least one PHBs. In this regard, an exiting fluid passes through the drain filter prior exiting the biological cartridge. The drain filter may be also comprise a nonwoven or woven membrane and formed from the same materials as the inlet distribution filter. The primary difference between the inlet distribution filter and the drain filter, in accordance with certain embodiments, may be that the filter inlet distribution filter may have a smaller average pore size, such as 0.2 microns, for sterilization purposes, whereas the drain filter may have a larger average pore size to allow the passage of cells or biological factors to easily drain out of the biological cartridge to the next biological cartridge or two a final filtration system downstream of the biological cartridge, depending on location of the biological cartridge and application.

As briefly noted above, the biological cartridge may also include an inlet manifold including at least one inlet port in operative communication with the inlet orifice of the housing chamber. The at least one inlet port, for example, may comprise from 1 to about 5 individual inlet ports each being in operative communication with the inlet orifice of the housing chamber. Each of the inlet ports may also include a one-way or so-called "check valve" to prevent back flow through the respective inlet ports. The inlet ports, for example, may be operatively connected to respective cell cultures, gases, pH adjusting solutions, buffers, etc. In this regard, a wide variety of fluids may be easily flowed into and through the chamber housing and the respective PHBs. The inlet manifold, for example, may further comprises a top plate that is removably engaged directly or indirectly to a portion of the inlet manifold. In this regard, the top plate may be engaged to provide a "closed" or "sealed" state or removed to provide an "open" state. Figure 3, for example, illustrates a top view of a biological cartridge showing the inlet manifold with a top plate 25 in a sealed state by being engaged directly with a non-operating (e.g., no PHB is present in this section) top end of the chamber housing 10. The inlet manifold includes three separate inlet ports 21,22,23. Figure 4 illustrates an inside surface 26 of the top plate of Figure 3 and illustrated the mounting element 15 attached thereto. As noted above, the mounting element may alternatively attached to an inside surface of a bottom plate. In accordance with certain embodiments of the invention, the biological cartridge may also include

an outlet manifold including at least one outlet port in operative communication with the outlet orifice. For example, the at least one outlet port may comprise from 1 to about 5 individual outlet ports each being in operative communication with the outlet orifice of the housing chamber. The outlet manifold may further comprises a bottom plate that that is

removably engaged directly or indirectly to a portion of the outlet manifold in the same manner as that described for the top plate.

In accordance with certain embodiments of the invention, the PHBs can be provide continuous 3D growth of a variety of cells and/or 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 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 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. 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 purpose of expanding the hydrogels to form a continuous substrate. Hydrogels, as noted above, 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.

The PHBs (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 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 PHBs allow for cells to grow in a more native physiological-like environment compared to culture in a 2D plastic cell culture vessel. For instance, the PHBs 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. Moreover, the PHBs can allow for the formation of spheroids and organoids without developing a necrotic core inside the PHB's network of microchannels. For example, the PHBs, 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 PHB (e.g., hydrogel). Beneficially, for instance, the PHBs may permit even nutrient and gas exchange for healthy cell growth unlike other technologies that claim to mass produce cells. In accordance with certain embodiments of the invention, the PHBs may be modified to suite a wide variety of different cell types. In accordance with certain embodiments of the invention, the PHBs enables cells to secrete extracellular matrix and create natural microenvironments that promote cell growth, migration, viability, and function. Still further, the PHBs beneficially eliminate the need to sub-culture cells. Moreover, the use of the PHBs is easy to use as they can be provided in a pre-formed format for loading into the housing chamber, and does not require sophisticated changes in temperature, pH, or chemical exposure to use. The PHBs, for example, may enable users to achieve one or more of the following: grow custom cell 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 points in the production of cells. In accordance with certain embodiments of the invention, the biological cartridges housing PHBs provide a modular platform for any of the above-referenced applications.

In accordance with certain embodiments of the invention, the PHBs have 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. 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. The at least one PHB has a PHB cross-section in an x-y plane, in which the PHB cross-section may comprise a non-round cross-section, such as a polygon having from 3 to 12 sides, in the x-y plane. Alternatively, the at least one PHB has a PHB cross-section in an x-y plane, in which the PHB cross-section comprises a round cross-section in the x-y plane. Additionally, the at least one PHB has a PHB thickness in a z-direction that is

perpendicular to the x-y plane. In accordance with certain embodiments of the invention, the at least one PHB has a dimensional ratio between a longest cross-sectional width in the x-y plane to the thickness from about 1 : 5 to about 1: 0.1, such as at least about any of the following: 1 : 5, 1 : 2, 1 : 1.75. 1 : 1.5, 1 : 1.25, and 1 : 1, and/or at most about any of the following: 1 : 0.1, 1 : 0.25, 1 : 0.5, 1 : 0.75, and 1 : 1. Additionally or alternatively, the 3D macrostructure defines a cylinder, a square prism, or a triangular prism. Additionally or alternatively, the 3D macrostructure defines 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 and as noted above, the 3D macrostructure comprises or defines a female mounting component comprising the PHB orifice extending completely through the thickness of the PHB, and wherein the female mounting component is larger than the average diameter of the network of microporous channels and/or chambers. Additionally or alternatively, the 3D macrostructure comprises or defines the female mounting component comprising a PHB cutout portion extending completely through the thickness of the PHB, and wherein the female mounting component is larger than the average diameter of the network of microporous channels and/or chambers. In accordance with certain embodiments of the invention, the average diameter of the network of microporous channels and/or chambers comprises 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.

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 PHBs housed within the housing chamber may need to be flushed out of the interior network of the network of microporous 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, 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 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 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 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.

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 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 functionality (e.g., —$O(C_2H_4O)_nH$; where n has a value from 1 to 100, polypropylene glycol functionality (e.g., —$O(C_3H_6O)_nH$; 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 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., 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, 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 incorporated 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.

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 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 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 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 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 PHBs can 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 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 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 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 PHB having a network of microporous channels and/or chambers that 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, 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 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 comprises a coating of a compatibilizer selected to promote adhesion of a primary cell of 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 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 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, 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, certain embodiments of the invention provide a system comprising a plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series to define a biological cartridge network (BCN). The BCN may include at least (i) a first biological cartridge having a first inlet and a first outlet, and (ii) a second biological cartridge having a second inlet in operative communication with the first outlet, and a second outlet. In accordance with certain embodiments of the invention, each of the plurality of biological cartridges include a respective plurality of PHBs seeded with a first cell type of interest housed therein. By way of example only, the BCN may include from 2 to about 50 biological cartridges, such as at least about any of the following: 2, 5, 8, 10, 12, 15, 18, 20, 22, and 25 biological cartridges, and/or at most about any of the following: 50, 45, 40, 35, 30, and 25 biological cartridges.

In accordance with certain embodiments of the invention, the BCN may comprises a fresh feed inlet and a product outlet. The fresh feed inlet may comprises a fresh feed-manifold having one or more ports in operative communication with the first biological cartridge, such as described above. The first biological cartridge may be the initial biological cartridge of the BCN to receive fluid from the fresh-feed manifold. Additionally or alternatively, one or more of the plurality of biological cartridges includes a respective inlet manifold operatively connected to an immediately preceding biological cartridge via a respective conduit. The respective inlet manifold, for example, may include one or more supplemental ports in operative configuration with the respective plurality of PHBs for the

supply of supplemental fluids. For example, supplemental fluids (e.g., gases, nutrients, pH adjusting reagents, etc.) may be selectively added directly to one or more biological cartridges of the BCN without the need of passing these supplemental fluids through every preceding biological cartridge.

In accordance with certain embodiments of the invention, the BCN also comprises a shroud, wherein the BNC is located within the shroud. The shroud, for example, may comprise a temperature control mechanism configured to control a temperature of air space located within the shroud and outside of the plurality of biological cartridges.

Figure 7, for instance, illustrates a system 100 including a BCN 90 illustrating a fresh feed entering a first biological cartridge 1a, flowing in series to a second biological cartridge 1b, and next to a third biological cartridge 1c, and exiting the system. Biological cartridges 1a, 1b, 1c may each be seeded with the same cell type of interest. Such a system utilizing a series interconnected configuration may be particularly suitable for the mass cultivation of the cell type of interest. Although Figure 7 illustrates only three biological cartridges in series, certain embodiments of the invention may be comprise from about 2 to about 100 biological cartridges interconnected in series, such as at least about any of the following: 2, 5, 8, 10, 12, 15, 18, 20, 30, 40, and 50 biological cartridges interconnected in series, and/or at most about any of the following: 100, 90, 80, 70, 60, and 50 biological cartridges interconnected in series.

In another aspect, certain embodiments of the invention provide a system comprising a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series. The first set of biological cartridges and the second set of biological cartridges may be aligned in a parallel relationship with respect to each other and a common feed source. In accordance with certain embodiments of the invention, respective initial biological cartridges of the first and second sets of biological cartridges may each be seeded with a first cell type of interest, and respective immediately subsequent biological cartridges may each be seeded with a second cell type of interest, wherein the first and second cell types are different.

Figure 8, for instance, illustrates a system 105 including a BCN 90 having first and second sets of biological cartridges (e.g., first set = 1a, 2a, 3a, and second set = 1b, 2b, 3b) that are aligned in a parallel relationship with respect to each other and a common feed

source. As shown in Figure 8, respective initial biological cartridges of the first and second sets of biological cartridges 1a,1b may each be seeded with a first cell type of interest, respective immediately subsequent biological cartridges 2a,2b may each be seeded with a second cell type of interest, and respective immediately subsequent biological cartridges 3a,3b may each be seeded with a third cell type of interest. The first cell type, the second cell type, and the third cell type may all be different from each other.

In accordance with certain embodiments of the invention, the BCN may include at least 3 sets of biological cartridges connected to each other in series, such as at least about any of the following: 3, 5, 8, 10, 15 and 20, and/or at most about any of the following: 100, 90, 80, 70, 60, 50, 40, 30, and 20.

In accordance with certain embodiments of the invention, one or more of the first plurality and/or second plurality of biological cartridges includes a respective inlet manifold operatively connected to an immediately preceding biological cartridge via a respective conduit. The respective inlet manifold, for example, may include one or more supplemental ports in operative configuration with the respective plurality of PHBs for the supply of supplemental fluids as described previously. The system may also include a shroud, in which the BNC is located within the shroud as previously described.

Figure 9 illustrates another example system 107 including a BCN 90 in which the first set of biological cartridges 1a, 2 (in series configuration) are configured in parallel to the second set of biological cartridges 1b,3 (in series configuration). In this regard, the respective initial biological cartridges of the first and second sets of biological cartridges 1a,1b may each be seeded with a first cell type of interest, while respective immediately subsequent biological cartridges 2,3 may each be seeded with different cell types of interest. Biological cartridge 2 may be seeded with a second cell type of interest, and biological cartridge 3 may be seeded with a third cell type of interest, in which the first cell type, the second cell type, and the third cell type may all be different from each other.

In another aspect, certain embodiments of the invention provide a system comprising a biological cartridge network (BCN) comprising a plurality of biological cartridges, such as those described and disclosed herein, wherein the plurality of biological cartridges include (i) a first plurality of biological cartridges that are operatively connected in series, and (ii) a second plurality of biological cartridges are operatively connected in parallel. In this regard, the BCN may comprise a nearly unlimited number of individual biological cartridges in a nearly unlimited number of different overall configurations (e.g., a variety of series and/or parallel interconnections between biological cartridges with a variety of different cell types of

interest being seeded in any number of biological cartridges). In accordance with certain embodiments of the invention, the BCN may include at least one fresh feed-inlet, such as from 1 to about 10 fresh feed-inlets, and at least one product outlet, such as from 1 to about 10 product outlets.

Figure 10, for example, illustrates a system 103 including a BCN 90 comprising a plurality of individual biological cartridges 1, 2a, 2b, 3a, 3b, 4a, 4b, 5, 6 having an overall configuration utilizing both series interconnections between a portion of the biological cartridges and parallel alignments between a portion of the biological cartridges. The system 103 of Figure 10 shows one fresh feed and three product outlets. In accordance with certain embodiments of the invention, biological cartridge 1 is seeded with a first cell type, biological cartridges 2a,2b (aligned in parallel) may are seeded with a second cell type, biological cartridges 3a,3b (aligned in series) are seeded with a third cell type, biological cartridges 4a,4b are seeded with a fourth cell type, biological cartridge 5 is seeded with a fifth cell type, and biological cartridge 6 is seeded with a sixth cell type.

The foregoing example configurations of a plurality of biological cartridges are merely illustrative of a few possible systems according to certain embodiments of the invention. That is, the foregoing example configurations and figures of a plurality of biological cartridges are merely illustrative and non-limiting.

In another aspect, certain embodiments of the invention provide a method of cultivating one or more cell types of interest, comprising: (i) providing a first biological cartridge, such as those described and disclosed herein, wherein the first biological cartridge includes a first plurality of PHBs housed therein; (ii) seeding the first plurality of PHBs with at least a first cell type of interest; (iii) feeding the at least a first cell type of interest with a first culture media, and allowing the at least a first cell type of interest to propagate throughout the first network of microporous channels and/or chambers of the first plurality of PHBs; and (iv) harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the plurality of PHBs.

In accordance with certain embodiments of the invention, the step of harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first plurality of PHBs comprises flushing them out of the first plurality of PHBs with a fluid medium. Alternatively, the step of harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first plurality of PHBs comprises degrading the respective 3D macrostructure of the respective first plurality of PHBs.

In accordance with certain embodiments of the invention, the method may further comprise providing a second biological cartridge, such as those described and disclosed herein, in which the second biological cartridge includes a second plurality of PHBs seeded with the first cell type of interest housed therein; and operatively connecting the first biological cartridge and the second biological cartridge in series. In this regard, an exiting fluid stream from the first biological cartridge may feed the second biological cartridge. For example, a culture media fed to the first biological cartridge may pass through the first biological cartridge and then enter and flow through the second biological cartridge.

In accordance with certain embodiments of the invention, the method may further comprise providing a second biological cartridge, such as those described and disclosed herein, in which the second biological cartridge includes a second plurality of PHBs seeded with the first cell type of interest housed therein; and operatively connecting the first biological cartridge and the second biological cartridge in series, and optionally feeding the second cell type of interest with a second culture media, and allowing the second cell type of interest to propagate throughout the second network of microporous channels and/or chambers of the second plurality of PHBs. In accordance with certain embodiments of the invention, the method may further comprise harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs. The step of harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs comprises flushing them out of the second plurality of PHBs with a fluid medium. Alternatively,

the step of harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs comprises degrading the respective 3D macrostructure of the respective second plurality of PHBs.

In another aspect, certain embodiments of the invention provide a method of cultivating one or more cell types of interest, comprising: (a) providing or forming a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges, such as those described and disclosed herein, connected to each other in series; wherein the first set of biological cartridges and the second set of biological cartridges are aligned in a parallel relationship with respect to each

other and a common feed source; (b-1) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding respective immediately subsequent biological cartridges with a second cell type of interest, wherein the first and second cell types are different; or (b-2) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding an immediately subsequent biological cartridge of the first set of biological cartridges with a second cell type of interest, and seeding an immediately subsequent biological cartridge of the second set of biological cartridges with a third cell type of interest, wherein the first cell type, the second cell type, and the third cell type are all different from each other; and (c) harvesting at least a portion of the first cell type of interest and/or at least a portion of the second cell type of interest and/or at least a portion of the third cell type of interest. In accordance with certain embodiments of the invention, the method may further comprise feeding the second cell type of interest with a second culture media and feeding the third cell type of interest with a third culture media.

In accordance with certain embodiments of the invention and as noted above, the one or more cell types of interest may produce or secrete a therapeutic 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 biological cartridges may be interconnected in a variety of configurations to provide a BCN, in which multiple cell lines, such as those that may produce or secrete a therapeutic agent, are seeded in different PHBs housed in different biological cartridges.

In accordance with certain embodiments of the invention, the PHBs seeded with a first cell type of interest housed within a first biological cartridge(s) may have a first network of microporous channels and/or chambers having a first structure and PHBs seeded with a second cell type of interest housed within a second biological cartridge(s) may have a second network of microporous channels and/or chambers, wherein the first structure is different than the second structure. As noted above, the PHBs of respective biological cartridges seeded for a particular cell type of interest is structured physically to mimic the native tissue of interest. 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 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 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.

In accordance with certain embodiments of the invention, the first PHB(s) (housed in a first biological cartridge) may comprise a first continuous polymeric matrix material of the first PHB comprising a non-degradable hydrogel material or a or a selectably degradable hydrogel material, such as described above. Additionally or alternatively, the second PHB(s) (housed in a second biological cartridge) may comprise a second continuous polymeric matrix material of the second PHB comprising a non-degradable hydrogel material or a selectably degradable hydrogel material, such as described above. In accordance with certain embodiments of the invention, the first continuous polymeric matrix material of the first PHB, the second continuous polymeric matrix material of the second IPHB, or both comprise a selectably degradable hydrogel material comprising degradable polymers, such as naturally occurring biopolymers like 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 gelatins),dispase, pepsin, hyaluronase, and Dnase/Rnase. The selectably degradable hydrogel material, for example, may comprise one or more degradable polymers, such as one or more biopolymers derived from a living organism (as described above). For example, the one or more biopolymers derived from a living organism may comprise a polynucleotide, polysaccharide, polypeptide, or any combination thereof. In accordance with certain embodiments of the invention, the one or 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 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, Chondoitin

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 polyurethane, a polyphosphate or combinations thereof. In accordance with certain embodiments of the invention, the hydrogel material comprises at least 50% by weight of the one or more degradable polymers, such as one or more biopolymers derived from a living organism, such as at least about 50, 60, 70, and 75% by weight, and/or at most about any of the following: 100, 95, 90, 85, 80, and 75% by weight. In this regard, the cells cultivated in accordance with certain methods of the invention may be readily collected by degrading or dissolving the hydrogel material of the PHBs and flushed out of the respective biological cartridges.

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 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.

THAT WHICH IS CLAIMED:

1. A biological cartridge, comprising:

a housing chamber defining an interior region, wherein the housing chamber includes an inlet orifice and an outlet orifice;

at least one porous hydrogel block (PHB) located within the interior region of the housing chamber.

2. The biological cartridge of claim 1, wherein the housing chamber has a cross-section in an x-y plane, the cross-section comprises a non-round cross-section, such as a polygon having from 3 to 12 sides, in the x-y plane.

3. The biological cartridge of claim 1, wherein the housing chamber has a cross-section in an x-y plane, the cross-section comprises a round cross-section in the x-y plane.

4. The biological cartridge of claims 2-3, wherein the cross-section is identical along an entire operating length of the housing chamber.

5. The biological cartridge of claims 1-4, wherein the at least one PHB comprises a plurality of PHBs vertically stacked upon each other in a z-direction that is perpendicular to the x-y plane.

6. The biological cartridge of claim 5, wherein the plurality of PHBs comprise from 2 to about 40 individual PHBs, such as at least about any of the following: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18, and 20, and/or at most about any of the following: 40, 35, 30, 25, and 20 individual PHBs.

7. The biological cartridge of claims 5-6, further comprising at least one mounting element extending through the interior region.

8. The biological cartridge of claim 7, wherein the at least one mounting element comprises a single mounting element aligned along a central axis of the housing chamber and extending along the entire operating length of the housing chamber.

9. The biological cartridge of claim 7, further comprising a top plate and/or a bottom plate releasably engaged directly or indirectly to the housing chamber, and the single mounting element may optionally be directly or indirectly attached to the top plate and/or bottom plate.

10. The biological cartridge of claims 8-9, wherein the plurality of PHBs vertically stacked upon each other include a respective female mounting component comprising a respective PHB orifice extending completely through a respective thickness in the z-direction of the respective PHBs, the respective female mounting components are configured to allow the at least one mounting element to extend through the respective PHB orifices.

11. The biological cartridge of claim 10, wherein the at least one mounting element may have a non-round cross-section and the respective PHB orifices have a corresponding non-round cross-section, wherein the vertically stacked respective PHBs are restrained from rotating in respective x-y planes relative to each other.

12. The biological cartridge of claim 7, wherein the at least one mounting element comprises at least one fin component attached to the housing chamber and projecting inwardly into the interior region of the housing chamber.

13. The biological cartridge of claim 12, wherein at least one fin component extends along a at least a majority of the entire operating length of the housing chamber, such as at least about 55, 60, 65 and 75% of the entire operating length of the housing chamber, and/or at most about any of the following: 100, 98, 95, 90, 85, 80, and 75% of the entire operating length of the housing chamber.

14. The biological cartridge of claims 12-13, wherein the plurality of PHBs vertically stacked upon each other include a respective female mounting component comprising a respective PHB cutout portion extending completely through a respective thickness in the z-direction of the respective PHBs, the respective female mounting components configured to allow the at least one mounting element to extend through the respective PHB orifices.

15. The biological cartridge of claims 7-14, wherein the at least one mounting element comprises a transparent and rigid substrate, such as glass or plastic, a conducting metal, such as copper, silver, or gold, or a different hydrogel material than that of the PHBs.

16. The biological cartridge of claims 1-15, further comprising an inlet distribution filter located directly or indirectly above all of the at least one PHBs, wherein an incoming fluid passes through the inlet distribution filter prior to passing into any of the at least one PHBs.

17. The biological cartridge of claims 1-16, further comprising a drain filter located directly or indirectly below all of the at least one PHBs, wherein an exiting fluid passes through the drain filter prior exiting the biological cartridge.

18. The biological cartridge of claims 1-17, further comprising an inlet manifold including at least one inlet port in operative communication with the inlet orifice of the housing chamber.

19. The biological cartridge of claim 18, wherein the at least one inlet port comprises from 1 to about 5 individual inlet ports each being in operative communication with the inlet orifice of the housing chamber.

20. The biological cartridge of claims 18-19, wherein the inlet manifold further comprises a top plate that that is removably engaged directly or indirectly to a portion of the inlet manifold.

21. The biological cartridge of claims 1-20, further comprising an outlet manifold including at least one outlet port in operative communication with the outlet orifice.

22. The biological cartridge of claim 21, wherein the at least one outlet port comprises from 1 to about 5 individual outlet ports each being in operative communication with the outlet orifice of the housing chamber.

23. The biological cartridge of claims 21-22, wherein the outlet manifold further comprises a bottom plate that that is removably engaged directly or indirectly to a portion of the outlet manifold.

24. The biological cartridge of claims 1-24, wherein the at least one PHB has a three-dimensional (3D) macrostructure defined by a continuous polymeric matrix material and the network of microporous channels and/or chambers extending throughout the continuous polymeric matrix material.

25. The biological cartridge of claim 24, wherein the at least one PHB has a PHB cross-section in an x-y plane, the PHB cross-section comprises a non-round cross-section, such as a polygon having from 3 to 12 sides, in the x-y plane.

26. The biological cartridge of claim 24, wherein the at least one PHB has a PHB cross-section in an x-y plane, the PHB cross-section comprises a round cross-section in the x-y plane.

27. The biological cartridge of claims 24-25, wherein the at least one PHB has a PHB thickness in a z-direction that is perpendicular to the x-y plane.

28. The biological cartridge of claim 27, wherein the at least one PHB has a dimensional ratio between a longest cross-sectional width in the x-y plane to the thickness from about 1 : 5 to about 1: 0.1, such as at least about any of the following: 1 : 5, 1 : 2, 1 : 1.75. 1 : 1.5, 1 : 1.25, and 1 : 1, and/or at most about any of the following: 1 : 0.1, 1 : 0.25, 1 : 0.5, 1 : 0.75, and 1 : 1.

29. The biological cartridge of claims 24-28, wherein the 3D macrostructure defines a cylinder, a square prism, or a triangular prism.

30. The biological cartridge of claims 24-28, wherein the 3D macrostructure defines 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.

31. The biological cartridge of claims 24-30, wherein the 3D macrostructure comprises defines the female mounting component comprising the PHB orifice extending completely

through the thickness of the PHB, and wherein the female mounting component is larger than the average diameter of the network of microporous channels and/or chambers.

32. The biological cartridge of claims 24-30, wherein the 3D macrostructure comprises defines the female mounting component comprising the PHB cutout portion extending completely through the thickness of the PHB, and wherein the female mounting component is larger than the average diameter of the network of microporous channels and/or chambers.

33. The biological cartridge of claims 24-32, wherein the continuous polymeric matrix material is non-degradable.

34. The biological cartridge of claims 24-32, wherein the continuous polymeric matrix material is selectably degradable.

35. The biological cartridge of claims 24-34, wherein continuous polymeric matrix material comprises a 3D cross-linked polymer network, a non-crosslinked polymer network, or a combination thereof.

36. The biological cartridge of claims 24-35, wherein the continuous polymeric matrix material comprises a swellable hydrogel.

37. The biological cartridge of claim 36, wherein the swellable hydrogel comprises 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.

38. The biological cartridge of claim 37, wherein the at 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.

39. The biological cartridge of claims 37-38, wherein at least one of the monomer or second monomer comprises polyethylene glycol functionality (e.g., —$O(C_2H_4O)_nH$; where n has a value from 1 to 100, polypropylene glycol functionality (e.g., —$O(C_3H_6O)_nH$; 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.

40. The biological cartridge of claims 37-39, wherein at least one of the 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).

41. The biological cartridge of claim 40, wherein the swellable hydrogel comprises one or more natural polymers, such as plant-derived polymers and animal-derived polymers.

42. The biological cartridge of claims 24-41, wherein the continuous polymeric matrix material mimics 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.

43. The biological cartridge of claims 24-42, wherein the continuous polymeric matrix material is formed via an additive manufacturing technique, such as 3D printing of digital light synthesis printing.

44. The biological cartridge of claims 24-43, wherein the network of microporous channels and/or chambers is structured to mimic the morphology of a natural tissue of interest, such as by varying the geometry and dimensions of the network of microporous channels and/or chambers to mirror the morphology of the natural tissue of interest.

45. The biological cartridge of claims 24-44, wherein the average diameter comprises 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.

46. The biological cartridge of claims 24-45, wherein the network of microporous channels and/or chambers comprises 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 macrostructure.

47. The biological cartridge of claims 24-46, wherein an interface between the network of microporous channels and/or chambers and continuous polymeric matrix material comprises a coating of a compatibilizer selected to promote adhesion of a primary cell of interest.

48. A system, comprising:

a plurality of biological cartridges according to any one of claims 1-47 connected to each other in series to define a biological cartridge network (BCN), wherein the BCN includes at least (i) a first biological cartridge having a first inlet and a first outlet, and (ii) a second biological cartridge having a second inlet in operative communication with the first outlet, and a second outlet;

wherein each of the plurality of biological cartridges include a respective plurality of PHBs seeded with a first cell type of interest housed therein.

49. The system of claim 48, wherein the BCN comprises a fresh feed inlet and a product outlet.

50. The system of claims 48-49, wherein the BCN includes from 2 to about 50 biological cartridges, such as at least about any of the following: 2, 5, 8, 10, 12, 15, 18, 20, 22, and 25 biological cartridges, and/or at most about any of the following: 50, 45, 40, 35, 30, and 25 biological cartridges.

51. The system of claims 48-50, wherein fresh feed inlet comprises a fresh feed-manifold having one or more ports in operative communication with the first biological cartridge, wherein the first biological cartridge is the initial biological cartridge of the BCN to receive fluid from the fresh-feed manifold.

52. The system of claims 48-51, wherein one or more of the plurality of biological cartridges includes a respective inlet manifold operatively connected to an immediately preceding biological cartridge via a respective conduit.

53. The system of claim 52, wherein the respective inlet manifold includes one or more supplemental ports in operative configuration with the respective plurality of PHBs for the supply of supplemental fluids.

54. The system of claims 48-53, further comprising a shroud, wherein the BNC is located within the shroud.

55. The system of claim 54, wherein the shroud comprises a temperature control mechanism configured to control temperature of air space located within the shroud and outside of the plurality of biological cartridges.

56. A system, comprising:

a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges according to any one of claims 1-47 connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges according to any one of claims 1-47 connected to each other in series; wherein the first set of biological cartridges and the second set of biological cartridges are aligned in a parallel relationship with respect to each other and a common feed source.

57. The system of claim 56, wherein respective initial biological cartridges of the first and second sets of biological cartridges are each seeded with a first cell type of interest, and respective immediately subsequent biological cartridges are each seeded with a second cell type of interest, wherein the first and second cell types are different.

58. The system of claim 56, wherein respective initial biological cartridges of the first and second sets of biological cartridges are each seeded with a first cell type of interest, and an immediately subsequent biological cartridge of the first set of biological cartridges is seeded with a second cell type of interest, and an immediately subsequent biological cartridge of the second set of biological cartridges is seeded with a third cell type of interest, wherein the first cell type, the second cell type, and the third cell type are all different from each other.

59. The system of claims 56-58, wherein the BNC includes at least 3 sets of biological cartridges connected to each other in series, such as at least about any of the following: 3, 5,

8, 10, 15 and 20, and/or at most about any of the following: 100, 90, 80, 70, 60, 50, 40, 30, and 20.

60. The system of claims 56-59, wherein one or more of the first plurality and/or second plurality of biological cartridges includes a respective inlet manifold operatively connected to an immediately preceding biological cartridge via a respective conduit.

61. The system of claim 60, wherein the respective inlet manifold includes one or more supplemental ports in operative configuration with the respective plurality of PHBs for the supply of supplemental fluids.

62. The system of claims 56-61, further comprising a shroud, wherein the BNC is located within the shroud.

63. The system of claim 62, wherein the shroud comprises a temperature control mechanism configured to control temperature of air space located within the shroud and outside of the plurality of biological cartridges.

64. A system, comprising: a biological cartridge network (BCN) comprising a plurality of biological cartridges according to any one of claims 1-47, wherein the plurality of biological cartridges include (i) a first plurality of biological cartridges that are operatively connected in series, and (ii) a second plurality of biological cartridges are operatively connected in parallel.

65. The system of claim 64, wherein the BCN includes at least one fresh feed-inlet, such as from 1 to about 10 fresh feed-inlets, and at least one product outlet, such as from 1 to about 10 product outlets.

66. A method of cultivating one or more cell types of interest, comprising:

(i) providing a first biological cartridge according to any one of claims 1-47, wherein the first biological cartridge includes a first plurality of PHBs seeded with a first cell type of interest housed therein;

(ii) seeding the first plurality of PHBs with at least a first cell type of interest;

(iii) feeding the at least a first cell type of interest with a first culture media, and allowing the at least a first cell type of interest to propagate throughout the first network of microporous channels and/or chambers of the first plurality of PHBs;

(iv) harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the plurality of PHBs.

67. The method of claim 66, wherein the step of harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first plurality of PHBs comprises flushing them out of the first plurality of PHBs with a fluid medium.

68. The method of claim 66, wherein the step of harvesting at least a portion of the first cell type of interest located throughout the first network of microporous channels and/or chambers of the first plurality of PHBs comprises degrading the respective 3D macrostructure of the respective first plurality of PHBs.

69. The method of claims 66-68, further comprising providing a second biological cartridge according to any one of claims 1-47, wherein the second biological cartridge includes a second plurality of PHBs seeded with the first cell type of interest housed therein; and operatively connecting the first biological cartridge and the second biological cartridge in series.

70. The method of claims 66-68, further comprising providing a second biological cartridge according to any one of claims 1-47, wherein the second biological cartridge includes a second plurality of PHBs seeded with a second cell type of interest housed therein; and operatively connecting the first biological cartridge and the second biological cartridge in series, and optionally feeding the second cell type of interest with a second culture media, and allowing the second cell type of interest to propagate throughout the second network of microporous channels and/or chambers of the second plurality of PHBs.

71. The method of claim 70, further comprising harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs.

72. The method of claim 71, wherein the step of harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs comprises flushing them out of the second plurality of PHBs with a fluid medium.

73. The method of claim 71, wherein the step of harvesting at least a portion of the second cell type of interest located throughout the second network of microporous channels and/or chambers of the second plurality of PHBs comprises degrading the respective 3D macrostructure of the respective second plurality of PHBs.

74. A method of cultivating one or more cell types of interest, comprising:

(a) providing or forming a biological cartridge network (BCN) comprising (i) a first set of biological cartridges comprising a first plurality of biological cartridges according to any one of claims 1-47 connected to each other in series, and (ii) a second set of biological cartridges comprising a second plurality of biological cartridges according to any one of

claims 1-47 connected to each other in series; wherein the first set of biological cartridges and the second set of biological cartridges are aligned in a parallel relationship with respect to each other and a common feed source;

(b-1) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding respective immediately subsequent biological cartridges with a second cell type of interest, wherein the first and second cell types are different; or

(b-2) seeding the respective PHBs within the respective initial biological cartridges of the first and second sets of biological cartridges with a first cell type of interest, and seeding an immediately subsequent biological cartridge of the first set of biological cartridges with a second cell type of interest, and seeding an immediately subsequent biological cartridge of the second set of biological cartridges with a third cell type of interest, wherein the first cell type, the second cell type, and the third cell type are all different from each other; and

(c) harvesting at least a portion of the first cell type of interest and/or at least a portion of the second cell type of interest and/or at least a portion of the third cell type of interest.

75. The method of claim 74, further comprising feeding the second cell type of interest with a second culture media.

76. The method of claims 74-75, further comprising feeding the third cell type of interest with a third culture media.