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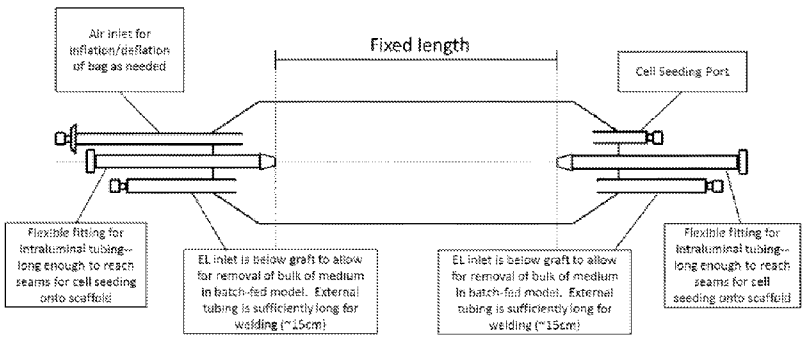
(54) **Title**: APPARATUSES FOR TISSUE AND ORGAN PRODUCTION AND STORAGE

FIGURE 1



(57) **Abstract**: The present invention is directed culture bags comprising a biocompatible material which is suitable to contain and support the long-term culture of cells, tissues, or organs; tolerates sterilization by standard means, and permits the supply of oxygen from outside said culture bag to the cells, tissues or organs within said culture bag and removal of carbon dioxide from within said culture bag.

APPARATUSES FOR TISSUE AND ORGAN PRODUCTION AND STORAGE

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/495,749, filed June 10, 2011, the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] Cell growth is an essential step in manufacturing tissues and organs. The process may begin with the addition of a small number of cells (the inoculum) to a biologic tissue, to a biologic organ, or to a synthetic scaffold that forms the shape of the intended tissue or organ. The tissue, organ, or scaffold is then submerged in liquid culture medium that delivers nutrients and facilitates growth of the cells. The growth process takes place in a container. Growth containers for tissues and organs have been made of rigid plastic and glass. The container may be placed within a gas and temperature controlled incubator, or may be linked to a bioreactor that controls and/or monitors temperature, pH, oxygen and carbon dioxide concentrations to promote tissue or organ growth or maturation.

[0003] Glass and rigid plastic tissue and organ growth containers are designed to facilitate the three dimensional growth of various cell types that frequently are seeded on a polymeric scaffold or acellular biologic scaffold and maintained under controlled environmental conditions (e.g., pH, temperature, pO_2).

[0004] In addition, many reactor containers are designed to allow mechanical stress (e.g., mechanical compression, hydrodynamic pressure, fluid flow) to be imparted on the cultured cells to modulate cell physiology, leading to the formation of engineered tissues with desired characteristics. Such containers have been used to generate blood vessels, heart valve leaflets and cardiac tissue, cartilage, urinary bladders, bone tissue, lung, biohybrid liver, kidney, and other tissues and organs.

[0005] Existing containers are often placed within a carbon-dioxide and temperature controlled incubator, with passive gas exchange. An adult human uses 7.0 mL oxygen per kg body weight per minute. This may be considered to be a "basal" level of oxygen consumption. Rapidly growing tissues conservatively utilize three times as much oxygen, yielding an estimated oxygen requirement of 21 mL oxygen/kg tissue/minute.

[0006] Glass and rigid plastic growth containers often incorporate sophisticated designs and engineering to address the unique culture requirements of each tissue or organ construct. These containers are often custom made and difficult to produce in large numbers. The containers often consist of many parts that may require assembly under aseptic conditions, which requires a significant amount of labor and very specialized technical expertise. Therefore, specialized glass and rigid plastic containers have high costs associated with tissue production.

[0007] Development of a disposable bag for the growth of tissues and organs could eliminate the many aseptic assembly steps that are currently associated with glass and rigid plastic bioreactors. Reduction of aseptic assembly reduces the level of technical expertise required for growth of tissues and organs, and decreases manpower requirements, which both decrease production costs for tissues and organs. In addition, the ability to mass produce bags with the desired shape and desired connectors eliminates variation between custom-made individual containers.

[0008] There is a considerable clinical need for tissue and organ replacements. Development of tissue and organ growth bags could facilitate cost-effective production of tissues and organs. As an example, there is a clinical need for vascular grafts when the patient's own vasculature is either unavailable because of prior harvest or unsuitable secondary to disease. Instances when a vascular graft might be needed include peripheral arterial disease, coronary artery disease, and hemodialysis access for patients with end stage renal disease. Tubular tissues are needed for many other clinical applications, including deep vein replacement, urinary diversions, trachea replacement, esophageal replacement, and others.

SUMMARY OF THE INVENTION

[0009] The present invention provides a culture bag including a biocompatible material wherein the bag is suitable to contain and support the long-term culture of cells, tissues, or organs; tolerates sterilization by standard means, and permits the supply of oxygen from outside the bag to the cells, tissues or organs within the bag and removal of carbon dioxide from within the culture bag.

[00010] The biocompatible material can be flexible. Preferably, the biocompatible material can be ultra low density polyethylene, linear low density polyethylene, fluorinated ethylene polymer, polyvinylidene fluoride or ethyl vinyl acetate.

[00011] The biocompatible material can be minimally gas permeable. The minimally gas permeable material can alter the oxygen and/or carbon dioxide saturation of culture medium within the culture bag by 0-10% within a 3 hour period.

[00012] The biocompatible material can be gas permeable. The gas permeable material can alter the oxygen and/or carbon dioxide saturation of culture medium within the culture bag by more than 10% within a 3 hour period.

[00013] Oxygen delivery and carbon dioxide removal can occur through passive diffusion or through culture medium exchange or circulation. As used herein, culture medium circulation refers to the flow of culture medium from a source exterior to the culture bag through the interior of the culture bag and back out of the culture bag.

[00014] The biocompatible material can tolerate exposure to pH 14. The biocompatible material can tolerate exposure to detergents, sterilizing agents and other stringent agents. The culture bag can be sterilized by treatment with gamma irradiation, ethylene oxide or supercritical CO_2.

[00015] The biocompatible material is transparent or contains a transparent section. The culture bag can further include a light blocking material or a section of light-blocking material.

[00016] The culture bag may contain one or more layers. For example, the innermost layer may be biocompatible, and external layers may provide other properties including opacity, gas impermeability, etc.

[00017] The culture bag can further include one or more anchors. The one or more anchors can support or maintain the shape of the tissues or organs within the culture bag. The one or more anchors can affix the tissues, tubular tissue structures and/or organs to said culture bag. The one or more anchors can prevent the extension, bending, kinking, or folding of the tissues or organs within the culture bag. The one or more anchors can be within the culture bag, attached to the exterior culture bag or present between layers of a multi-layer culture bag.

[00018] The culture bag can further include one or more ports. The one or more ports can serve as one or more anchors to support or maintain the shape of the tissues or organs within the culture bag. Fluid can flow through the one or more ports. The fluid can be a liquid or a gas. The fluid can be culture medium, a cell inoculum, a sterile gas, a decellularization solution, a wash solution, a storage solution or a surface treatment solution to improve polymeric scaffold wettability. Preferably, the surface treatment solution

comprises sodium hydroxide. The flow can be in any direction, unidirectional, bidirectional, circulatory or reversible. As used herein, the term "circulatory flow" refers to the flow of a fluid from a source exterior to the culture bag through the interior of the culture bag and back out of the culture bag. As used herein, the term "reversible flow" refers to the flow a fluid through an inlet and/or port into the interior of the culture bag and then coming out of the same inlet and/or port in the opposite direction. Fluid flow can introduce a fluid into the culture bag or into the tissues or organs within the culture bag or remove a fluid from the culture bag or from the tissues or organs within the culture bag. The one or more ports can contain one or more sterile filters. The one or more ports can permit sampling the contents of the culture bag.

[00019] The one or more ports can include at least a first portion that extends within the interior of the culture bag, referred to herein as the interior portion of the port, and a second portion that extends outside of the culture bag, referred to herein as the exterior portion of the port. In some embodiments, the one or more ports can include a barb or barb-like structure that is attached or coupled to, integrally formed with or otherwise associated with the port. The barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with the interior portion of the port, the barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with the exterior portion of the port, or the barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with both the interior portion and the exterior portion of the of the port. In other embodiments, the one or more ports can be blunt ends or connectors to facilitate attachment of, for example, tubing, other connectors, polymeric scaffolds, sensors, tissues, tubular tissue structures and/or organs.

[00020] In some embodiments, the ports are incorporated within or otherwise associated with an inlet or an outlet. In these embodiments, the ports are referred to as inlet ports and/or outlet ports. Inlet and outlet ports can have the same structure or different structures. Inlet and outlet ports are designed to allow fluid to flow through the port in one direction only, e.g., into the culture bag (also referred to herein as ingress) or out of the culture bag (also referred to herein as egress).

[00021] The port can be coupled or attached to, integrally formed with or otherwise associated with tubing or other fluid conduits. The tubing can be any material such as, for example, silicone.

[00022] In one embodiment where the culture bag includes more than one port and is designed to contain and support the long-term culture of three dimensional tissues and tissue structures having an interior lumen, the culture bag contains at least three inlet ports and at least three outlet ports. The inlet ports include (i) an "ExtraLuminal (EL) Port" that is designed to convey or otherwise allow fluid access to the exterior portion of the tissue structure; (ii) an "IntraLuminal (IL) Port" that is designed to convey or otherwise allow fluid to access the interior lumen portion of the tissue structure; and (iii) an air or other gaseous fluid inlet that is designed to allow gas access to the interior of the culture bag. The outlet ports include (i) an EL port that is designed to convey or otherwise allow fluid from the exterior portion of the tissue structure to exit the culture bag; (ii) an IL port that is designed to convey or otherwise allow fluid from the interior lumen of the tissue structure to exit the culture bag; and (iii) a cell seeding port that is designed to convey or otherwise allow the cell seeding suspension access to the interior of the culture bag. The cell seeding port can also be used to deliver other tissue culture solutions such as culture medium and to allow access to the interior of the bag for sample acquisition.

[00023] At least one of the inlet ports and/or outlet ports includes at least a first portion that extends within the interior of the culture bag, referred to herein as the interior portion of the inlet or outlet port, and a second portion that extends outside of the culture bag, referred to herein as the exterior portion of the inlet or outlet port. The interior portion of the inlet or outlet port can be any length, and the length of the interior portion of the inlet or outlet port that extends into the interior of the culture bag can vary as needed. The exterior portion of the inlet or outlet port can be any length, and the length of the exterior portion of the inlet or outlet port that extends beyond the exterior of the culture bag can vary as needed. In some embodiments, at least one of the inlet ports and at least one of the outlet ports include a first portion that extends within the interior of the culture bag and a second portion that extends outside of the culture bag. In some embodiments where there are multiple inlet ports and/or outlet ports, only one of the inlet ports and/or only one of the outlet ports include a first portion that extends within the interior of the culture bag and a second portion that extends outside of the culture bag. For example, in some embodiments where there are multiple inlet ports and multiple outlet ports, only one of the inlet ports and only one of the outlet ports include a first portion that extends within the interior of the culture bag and a second portion that extends outside of the culture bag.

[00024] In some embodiments, the inlet port and/or outlet port can include a barb or barb-like structure that is attached or coupled to, integrally formed with or otherwise associated with the port. The barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with the interior portion of the port, the barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with the exterior portion of the port, or the barb or barb-like structure can be attached or coupled to, integrally formed with or otherwise associated with both the interior portion and the exterior portion of the of the port.

[00025] The culture bag can further include a flexible mandrel inside of a tissue, tubular tissue structure and/or organ within the culture bag. The flexible mandrel can stretch to impart mechanical strain and/or stress to the tissue, tubular tissue structure and/or organ within the culture bag. For example, the flexible mandrel could be silicone.

[00026] The inlet ports and outlet ports are designed to allow for flow of fluids or air, for example in pulsatile cycles that impart strain to the tissue within the bag, the latter creating an effect referred to herein as the intraluminal pulse.

[00027] The culture bag can be coupled to at least one reservoir or at least one flow system suitable to deliver a fluid to the culture bag.

[00028] The culture bag can further include a seeding trough. The seeding trough can be a seam in the culture bag or an internal fixture that facilitates seeding of the particular shape of the construct to be seeded. The seeding trough can be formed by shaping the flexible biocompatible material. Preferably, the flexible biocompatible material is shaped by manual manipulation, compression or vacuum.

[00029] The culture bag can resist over-pressurization when filled with a fluid.

[00030] The culture bag can further include an inner liner and an outer liner, wherein the outer liner maintains the sterility of the outer surface of the inner liner to allow the culture bag to be opened for aseptic removal of the tissue, tubular tissue structure and/or organ.

[00031] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications,

patents, and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

[00032] Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[00033] Figure 1 is a schematic illustrating the side view of the single-use bioreactor shows 6 ports, 3 on either end. Two of these ports (one on each end) are to be used to deliver the intraluminal pulse. Two other ports (one on either end) will be connected to an external culture medium reservoir in order to supply fresh medium and remove spent medium. The final two ports will be utilized for delivering the cell seeding suspension and adding sterile air.

[00034] Figure 2 is a schematic illustrating the top view of the single-use bioreactor shows seams on either end of the bag to serve as troughs in which the cell inoculum will be pooled for seeding into a mesh construct. This view also shows the ports to be used for delivering the intraluminal pulse.

[00035] Figure 3 is a schematic illustrating the end view of the single-use bioreactor shows the intraluminal inlet, intraluminal outlet, the extraluminal inlet, extraluminal outlet, the cell inoculum delivery port, and the sterile air delivery port.

[00036] Figure 4 is a schematic illustrating that the ports presented in Figures 1, 2, and 3 could be fitted with tubing and connectors according to the above schematic.

[00037] Figure 5 is a schematic illustrating that the framework could be attached to the single-use bioreactor to provide structure. This could be an internal attachment, external attachment, or an embedding between film layers in the bag material.

[00038] Figure 6 is a schematic of a culture bag designed with 5 ports and a magnetic impeller for continuous mixing of solutions in the bag.

[00039] Figure 7 is a schematic of a compression apparatus designed to direct cell suspension into scaffold during cell seeding.

[00040] Figure 8 is a schematic culture bag for culturing tissue engineered vascular grafts is designed with 3 ports. Culture medium is batch-fed at regular intervals.

[00041] Figure 9 is a schematic of a culture bag designed with 5 ports for growth of a tissue engineered vascular graft. A rigid plastic trough is included for cell seeding.

[00042] Figure 10 is a schematic of culture bags for culturing tissue engineered vascular grafts are arranged in parallel in a continuous flow loop connected to an external culture medium reservoir that is monitored and controlled.

[00043] Figure 11 is a schematic of a culture bag designed with 6 ports for growth of an organ shaped construct.

[00044] Throughout the drawings the culture bag of the present invention is identified as a bioreactor.

DETAILED DESCRIPTION OF THE INVENTION

[00045] The present invention provides a culture bag comprising a biocompatible material which is suitable to contain and support the long-term culture of cells, tissues, tissue structures or organs; tolerates sterilization by standard means, and permits the supply of oxygen from outside said culture bag to the cells, tissues, tissue structures or organs within said culture bag and removal of carbon dioxide from within said culture bag.

[00046] The culture bags provided herein contain working monitoring probes or other sensors, maintain seal integrity and sterility when exposed to perfusion pressures, are stable after contact with a variety of tissue culture media, additives, solutions such as, for example, decellularization solutions.

[00047] The culture bag can be sized appropriately to contain the tissue, tubular tissue structure and/or organ and any anchors or other components needed for support, flow, or mechanical stress. The culture bag can also be sized for an appropriate volume of culture medium for culture of the cells, tissues or organs.

[00048] Oxygen delivery and carbon dioxide removal can be achieved either by passive diffusion through the biocompatible material (if gas permeable) or by a constant or intermittent supply of oxygenated culture medium and removal of deoxygenated culture medium via medium circulation or exchange. Any biocompatible material may be chosen to accommodate the desired gas diffusion rate.

[00049] The culture bag can comprise a flexible material. The culture bag permits long term culture of the cells, tissues or organs for at least two months, more preferably for at least three months. The culture bag can tolerate exposure to pH 14. The culture bag can tolerate exposure to stringent agents such as detergents and sterilization agents.

[00050] Sterilization of the culture bag can include any means known in the art, including but not limited to, treatment with gamma irradiation, ethylene oxide or supercritical CO_2.

[00051] The culture bag can be utilized as a container for surface treatment of a shape forming scaffold prior to growth of tissues and organs. Surface treatment can include sodium hydroxide treatment of polymeric scaffolds to improve scaffold wettability. Surface treatment can also include the attachment of proteins or chemistries to the surface of a polymeric scaffold, an acellular tissue, or and acellular organ.

[00052] The culture bag can also be utilized as a container for decellularization of tissues and organs. The culture bag can tolerate exposure to at least pH 12.5 and detergents during this decellularization process, and can thereafter be used for long-term storage of tissues or organs.

[00053] The culture bag can be transparent, or contain a transparent section, to allow visual monitoring of the cells, tissues or organs during culture. The culture bag can be constructed to block light. Preferably, the culture bag can contain an additional layer or can be attached to an external device to block light. Alternatively, the culture bag can be stored in a dark area.

[00054] The present invention also provides a culture bag comprising one or more anchors capable of supporting and maintaining the shape of the tissue, tubular tissue structure and/or organ within said culture bag, and methods to maintain the culture bag dimensions in appropriate directions.

[00055] The culture bag can further comprise one or more fixture(s) or skeleton(s) capable of fixing length or shape of the culture bag in order to maintain the shape of the tissue, tubular tissue structure and/or organ and to avoid unwanted kinks, folds, stretching or other unwanted tensile forces in or on any portion of the culture bag.

[00056] In some embodiments, a rigid skeleton structure can be inserted within the culture bag, between layers of a multi-layer culture bag, or attached to the outside of the culture bag via anchors. An external fixture can be used to hold the culture bag at a fixed length and/or in a fixed shape. A skeleton structure can be connected to a fixture to maintain length and/or shape. In one embodiment, the culture bag is maintained at a fixed length for tubular tissues in order to maintain the length of the tissue, and to prevent extension, bending, kinking, or folding of the tissue.

[00057] The present invention also provides a culture bag comprising one or more ports. In one embodiment, the port can be a flow through port. Flow may occur through ports that double as anchors for the tissue, tubular tissue structure and/or organ, or through non-anchoring ports. If flow through the tissue is required, the anchors can be flow ports at 1 or more orifices of the tissue, tubular tissue structure and/or organ. Flow can be delivered through anchoring ports in a circulatory fashion (*i.e.*, from a source exterior to the culture bag through the interior of the culture bag and back out of the culture bag) with 1 or more inflow ports and 1 or more outflow ports. Flow can be delivered through one or more anchoring ports in a reversible fashion, with flow going in the port and then coming out of the same port in the opposite direction. One or more flow paths may be created in each culture bag using the circulatory flow approach in which fluid from a source exterior to the culture bag flows through the interior of the culture bag and back out of the culture bag, the reversible flow approach, or a combination of the circulatory and reversible flow approaches. Circulatory and reversible flow paths can also be used to add, remove, or circulate solutions or gases through anchoring or non-anchoring ports. Gas or liquid may be used as the flowing material. Flow can be achieved by any means known in the art, including but not limited to, mechanical means (compression, pumping, etc.), gravity, agitation, magnetic force. Ports can be sized appropriately for the dimensions of the tissue, tubular tissue structure and/or organ.

[00058] The culture bag can comprise at least 1 inflow port for liquid (culture medium, decellularization solutions, storage solutions, surface treatment solutions, etc.) addition, which could be an independent port, or a multi-purpose port. The culture bag can comprise at least 1 outflow port for liquid removal, which could be an independent port, or a multi-purpose port. The culture bag can comprise at least 1 port for delivery of the cell inoculum, which could be an independent port, or a multi-purpose port. The culture bag can comprise at least 1 port for delivery of sterile gas, which could be an independent port, or a multi-purpose port. This port can contain a sterile filter to remove contaminants upon delivery of the gas. The culture bag can comprise at least 1 port for removal of gas, which could be an independent port, or a multi-purpose port. The culture bag can comprise 1 or more ports for sampling the contents of the bag, which could be an independent port, or a multi-purpose port. The culture bag can comprise 1 or more ports to impart flow through the tissue, tubular tissue structure and/or organ. For example, for growth of a tubular tissue, 2 anchoring ports are required, with a preference for flow through one or both ports.

Depending on the number of ports targeted for multi-purpose use, and depending on the circulation strategy for culture medium and/or gas (circulatory gas flow in which gas from a source exterior to the culture bag flows through the interior of the culture bag and back out of the culture bag, reversible gas flow in which gas flows into the culture bag via an inlet and/or port in one direction and then comes out of the same inlet and/or port in the opposite direction, or a combination), 1-6 non-anchoring ports are required to facilitate (1) liquid addition, (2) gas addition, (3) liquid removal, (4) gas removal, (5) delivery of the cell inoculum, and (6) sampling. Ports on the culture bag allow for attachment of tubing or connectors where necessary to facilitate flow or anchoring. Ports are sized appropriately for the dimensions of the tissue, tubular tissue structure and/or organ.

[00059] The culture bag of the present invention can also comprise a flexible mandrel inside of a tissue, tubular tissue structure and/or organ within said culture bag. The flexible mandrel can serve as an inner container for liquid or gas that stretches (expands and contracts) to impart mechanical strain and/or stress to the tissue, tubular tissue structure and/or organ.

[00060] The culture bag of the present invention can be coupled to at least one flow system that brings fluid or air into the culture bag at one or more ports. The fluid or air can be directed into the tissue, tubular tissue structure and/or organ, or may be directed to bathe the outside of the tissue, tubular tissue structure and/or organ.

[00061] The culture bag allows insertion of the tissue, tubular tissue structure, organ, or polymeric scaffold used for tissue growth, along with any association flow path supports (e.g., internal tubing), prior to bag closure.

[00062] The culture bag allows the tissue, tubular tissue structure, organ, or polymeric scaffold used for tissue growth to be positioned for saturation with small or large volumes of cell inoculum. Anchoring connectors attached to a flexible culture bag can allow movement of the construct to enable positioning of the graft in a seeding trough. A seeding trough can be a seam in the culture bag or an internal fixture that facilitates seeding of the particular shape of the construct to be seeded. In the absence or presence of a physical seeding trough, the flexible culture bag may be shaped during seeding to create a seeding trough. Shaping can occur by any means known in the art, including without limitation, manual manipulation, with a mechanical device that imparts compression, or via air removal by compression or vacuum. The culture bag allows manual manipulation of the polymeric scaffold during cell inoculation to aid in cell seeding. Manual manipulation is

defined as rubbing or massage to aid in getting a nearby cell suspension into the scaffold and wetting the scaffold.

[00063] The culture bag can resist over-pressurization when filled with liquid or gas. In one embodiment, the culture bag can be coupled to an external system to aid in culture bag resistance to over-pressurization. Such a system may be an external support to fix the culture bag shape, or use of flow regulators.

[00064] The culture bag can comprise a minimally gas permeable material, which alters the oxygen and/or carbon dioxide saturation of culture medium within the culture bag by 0-10% within a 3 hour period. In one embodiment, the culture bag can be coupled to an external system (such as a bioreactor system) that controls oxygen and carbon dioxide levels of the culture medium within the culture bag during tissue, tubular tissue structure and/or organ growth.

[00065] The culture bag can also comprise a gas permeable material, which allows oxygen and/or carbon dioxide to permeate the culture bag, such that the saturation of any gas in the culture medium within the culture bag is altered by more than 10% within a 3 hour period. In one embodiment, passive gas diffusion through the culture bag may be used to regulate the gas saturation in culture medium within the culture bag during tissue, tubular tissue structure and/or organ growth. The culture bag can be placed in a gas chamber, such as a gas-controlled incubator, to control the composition of gas that diffuses into the culture bag during tissue, tubular tissue structure and/or organ growth. In another embodiment, the culture bag can be coupled to an external system (such as a bioreactor system) that controls oxygen and carbon dioxide levels of the culture medium entering the culture bag during tissue, tubular tissue structure and/or organ growth.

[00066] Examples of suggested, but not limited to, minimally gas permeable or gas permeable materials, along with their O_2/CO_2 transmission rate, are presented in Table 1.

Table 1

Bag Material (contact layer)	**O_2 Transmission Rate**	**CO_2 Transmission Rate**
Ultra Low Density Polyethylene	0.4 cc/m^2/day	< 1 cc/m^2/day
Linear Low Density Polyethylene	0.3 cc/m^2/day	0.15 cc/m^2/day
Fluorinated Ethylene Polymer	3100 cc/m^2/day	7100 cc/m^2/day
Polyvinylidene fluoride	20 cc/m^2/day/bar	100 cc/m^2/day/bar
Ethyl Vinyl Acetate	0.11 cc/100in^2/day/atm O2 @23C	3.4 cc/100in^2/day @23C

[00067] The culture bag of the present invention allows aseptic removal of the tissue, tubular tissue structure and/or organ. In one embodiment, the culture bag can be wrapped in an outer liner to maintain sterility of the outer surface of the inner liner to allow the culture bag to be opened for aseptic removal of the tissue, tubular tissue structure and/ or organ. The outer liner can encompass the entire culture bag, or may cover only a portion of the culture bag. An easy-open closure may be utilized to provide aseptic access to the tissue, tubular tissue structure and/or organ instead of using an outer liner.

[00068] The culture bag of the present invention can be a single use culture bag or can be a multiple use culture bag.

[00069] The culture bag of the present invention can be coupled to one or more reservoirs containing culture medium. Preferably, the reservoir is monitored and controlled. The culture medium can flow from the reservoir to the culture bag. The flow can be continuous or pulsatile. A reservoir can be coupled to one culture bag or coupled to more than one culture bag. In embodiments where the reservoir is coupled to more than one

culture bag, the culture bags are coupled to the reservoir in parallel. In other embodiments, the culture bag can be designed to operate independently in a batch-fed manner.

[00070] The culture bag may be inflated and deflated with air.

[00071] The culture bag can be any size or shape capable of comprising the shape and size of a tissue, tubular tissue structure and/or organ.

[00072] The culture bag can include monitoring probes or other sensors for determining one or more tissue culture characteristics such as, for example, dissolved oxygen (pO_2), glucose, glutamine, pH, lactate, ammonia, temperature.

[00073] All percentages and ratios used herein, unless otherwise indicated, are by weight. Other features and advantages of the present invention are apparent from the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

EXAMPLES

Example 1: Culture Bag Size:

[00074] The culture bag can be any size or shape capable of comprising the shape and size of a tissue, tubular tissue structure and/or organ. For example, culture bags for growth of tubular engineered tissues with a diameter of 6mm and a length of 23cm would contain an ideal volume of 300ml per bag, with sizing acceptable in the volume range of 200-800ml per bag. Culture bag volume would adjust accordingly with changes in tissue size. For example, a tubular tissue with a diameter of 6mm and a length of 35cm would contain an ideal volume of 450ml per bag, with sizing acceptable in the volume range of 300-1220ml per bag.

Example 2: Culture Bag Gas Permeability:

[00075] The culture bag can comprise a gas permeable or a minimally gas permeable material. In one example, for a bioreactor containing a single vessel that is 20 cm in length and 6 mm diameter: tissue dimensions can be generously estimated at (20 cm long x 2 cm wide x 0.1 cm thick). This gives a tissue volume of 4 mL. If tissue density is 1, then 1 liter = 1 kg, and 4 mL = 4 g. Assuming a factor of 3 in the increase in oxygen consumption per

weight (see above), this translates into an oxygen utilization rate of 0.09 mL oxygen/minute, or 5 mL oxygen per hour, or 120 mL oxygen per day, for this single vessel.

[00076] In another example, for a bioreactor with 120 vessels, each 40 cm long and 6 mm in diameter, then the oxygen requirements are multiplied by a factor of 240. This means: 20 mL oxygen per minute, 1.2 liters oxygen per hour, and 29 liters of oxygen per day. This assumes perfect oxygen delivery to growing tissues and perfect mixing of oxygen within the bioreactor, with no flow separation of gas in the medium that could lead to lower delivery to growing tissues.

Example 3: Culture of a 6mm diameter x 23 cm length tubular tissue

[00077] A bag with six ports is used for culture of a tubular tissue that is 6mm in diameter and23cm in length (Fig. 1-4). A tubular polymeric scaffold with a diameter of six millimeters and a length of twenty three centimeters was placed onto a flexible thin walled silicone tube and was inserted into the bag. The silicone tubing and tubular scaffold were tightly secured to anchoring ports in a manner that prevented leakage of intra luminal fluid or gas and also prevented contracting of the growing tubular tissue. The bag was then sealed and prepared for tubular scaffold surface treatment. The bag was filled with a sodium hydroxide solution that chemically treated the surface of the tubular scaffold and allowed it to readily absorb culture media. The bag was filled with several wash solutions that removed all residual sodium hydroxide. The bag was filled with air to dry the tubular scaffold and remove all moisture from the inner surface of the bag.

[00078] Medical grade air was injected into the bag via pressurized cylinders through a sterile filter to inflate the bag so the tubular scaffold would be protected during shipping to and from the site of gamma sterilization. The bag was sterilized via gamma sterilization and stored in a desiccated environment to prevent degradation of the tubular scaffold material.

[00079] Human smooth muscle cells were isolated from aortas. Cells from multiple donors were pooled for culture of pooled donor grafts. Human cells were used at passage 2 to produce tubular engineered tissues. Cells were expanded, harvested, and suspended in culture media and the bag was inoculated through the cell seeding port via syringe. The cell suspension was carefully directed into the lower seam of the bag creating a trough filled with cells suspended in culture media. The flexible tubing and anchoring ports attached to the tubular scaffold were manipulated to force the tubular scaffold into the trough. The

outer layers of the bag were manipulated near the lower seam to gently mechanically massage the cell suspension into the tubular scaffold. Special attention was paid to massaging the cell suspension into the tubular scaffold near the anchoring ports. The bag was rotated one hundred and eighty degrees circumferentially and the cell suspension was carefully guided to the top seam of the bag so no cell suspension entered any other ports of the bag. The tubular scaffold was forced into this second seam and the remaining cell suspension was massaged into the unseeded side of the tubular scaffold.

[00080] The bag was attached to an external framework that allowed the tubular scaffold to maintain a fixed length for the entirety of culture (Fig. 5). The external framework prevented the bag from folding and collapsing on itself and damaging the tubular tissue. The external framework prevented the bag from moving and kinking or damaging the pathways or connections to the extraluminal and intraluminal flow systems.

[00081] Tubing coming from the external culture medium reservoir was connected to the extraluminal (EL) inlets and outlets of the bag. These ports were placed in a position where culture media circulation would not create a significant amount of shear force that would detach cells from the tubular scaffold. These ports were also placed in a position below the graft near the lower seam that would facilitate removal of all culture media at any time during culture to remove all residual cells and tissue that were not attached to the tubular scaffold.

[00082] Tubing was connected to the intraluminal flow ports on both sides of the bag to provide pulsatile flow through the lumen of the tubular scaffold. This pulsatile flow was produced by fluid or gas passing through the thin walled flexible silicone tubing within the tubular scaffold. The pulsatile flow served as a mechanical stimulus that prompted the cells to produce the extracellular matrix necessary to grow a strong tubular tissue.

[00083] A tubular tissue was cultured for ten weeks with constant circulation of media that was being supplemented in a controlled and monitored reservoir. Periodically all or a portion of the media was drained from the system including the bags and replaced with fresh culture media. Room air was injected into the bag via syringe through a sterile filter attached to an inlet port to replace volume recently occupied by media during full media exchanges to prevent the surface of the bag from collapsing and making contact with the culturing tubular tissue. Periodically culture media from the bags was sampled for analysis by attaching a syringe to a port on the bag.

[00084] When culture of the tissue was completed, all media was drained from the entire system. Decellularization solutions were circulated through the bags for several days to remove all cellular material from the tubular tissues. Wash solutions were circulated through the bags for several days to remove all residual decellularization solutions and cellular material. The bag was disconnected from the extraluminal and intraluminal flow systems and the bag was stored in the external framework filled with storage liquid. The bag was brought into the operating room and the outer layer of the bag was removed revealing a sterile inner layer that was brought into the sterile field. The sterile bag was opened with a scalpel and the tubular tissue was cut from the anchoring ports and prepared for implantation.

Example 4: Culture of a 6mm diameter x 23 cm length tubular tissue

[00085] A bag made of a highly gas permeable material (e.g., fluorinated ethylene polymer) with five ports is used for culture of a tubular tissue that is 6mm in diameter and23cm in length (Fig. 6). A tubular polymeric scaffold with a diameter of six millimeters and a length of twenty three centimeters was placed onto a flexible thin walled silicone tube and was inserted into the bag. The silicone tubing and tubular scaffold were tightly secured to anchoring ports in a manner that prevented leakage of intra luminal fluid or gas and also prevented contracting of the growing tubular tissue. A small magnetic impeller was placed into the bag that would provide adequate mixing of culture media during the tissue culture period (Fig. 6). The bag was then sealed and prepared for tubular scaffold surface treatment. The bag was filled with a sodium hydroxide solution via the inlet/outlet port to chemically treat the surface of the tubular scaffold and allowed it to readily absorb culture media. The bag was filled with several wash solutions via the inlet/outlet port to remove all residual sodium hydroxide. The bag was filled with air via the air inlet/outlet port to dry the tubular scaffold and remove all moisture from the inner surface of the bag.

[00086] Medical grade air was injected into the bag via pressurized cylinders through a sterile filter to inflate the bag so the tubular scaffold would be protected during shipping to and from the site of gamma sterilization. The bag was sterilized via gamma sterilization and stored in a desiccated environment to prevent degradation of the tubular scaffold material.

[00087] Human SMCs were isolated from aortas. Cells from multiple donors were pooled for culture of pooled donor grafts. Human cells were used at passage 2 to produce

tubular engineered tissues. Cells were expanded, harvested, and suspended in culture media. The bag was compressed to reduce the effective volume of the bag from its operating volume of 200-1200mL to a seeding volume of 10-20mL (Fig. 7). The bag was inoculated through the cell seeding port via syringe, filling the entire compressed volume. The outer layers of the bag were manipulated to gently mechanically massage the cell suspension into the tubular scaffold. Special attention was paid to massaging the cell suspension into the tubular scaffold near the anchoring ports. The bag was rotated one hundred and eighty degrees circumferentially and the remaining cell suspension was massaged into the unseeded side of the tubular scaffold. Once the tubular scaffold was saturated with cell suspension, all unseeded cells were removed through the inoculation port and the compression apparatus was removed from the bag.

[00088] The bag was attached to an external framework that allowed the tubular scaffold to maintain a fixed length for the entirety of culture. The external framework prevented the bag from folding and collapsing on itself and damaging the tubular tissue. The external framework prevented the bag from moving and kinking or damaging the pathways or connections to the intraluminal flow systems.

[00089] The bag was filled with culture media via the flow inlet/outlet port on the bag. This port was placed in a position where the filling and draining of culture media would not create a significant amount of shear force that would detach cells from the tubular scaffold. This port was also placed in a position below the graft near the lower seam of the bag that would facilitate removal of all culture media at any time during culture to remove all residual cells and tissue that were not attached to the tubular scaffold.

[00090] Tubing was connected to the intraluminal flow ports on both sides of the bag to provide pulsatile flow through the lumen of the tubular scaffold. This pulsatile flow was produced by fluid or gas passing through the thin walled flexible silicone tubing within the tubular scaffold. The pulsatile flow served as a mechanical stimulus that prompted the cells to produce the extracellular matrix necessary to grow a strong tubular tissue.

[00091] A tubular tissue was cultured for ten weeks with frequent full volume and partial volume media exchanges via the inlet/outlet port to remove waste products and replenish necessary metabolites and biological molecules. Tubular tissue culture took place in a tissue culture incubator, where temperature, humidity, and gas composition were monitored and controlled. The materials used to produce the bag were deliberately chosen to have high gas diffusion rates in and out of the bag to ensure that the concentrations of

oxygen, nitrogen, and carbon dioxide inside the bag reached equilibrium with the tissue culture incubator to achieve conditions optimal for tubular tissue culture. Bag permeability eliminated the need for a circulating flow loop system to deliver culture medium with gas saturation controlled by a bioreactor system that delivers and monitors gas. A small magnetic impeller controlled by a stirplate provided sufficient mixing of the culture media to prevent any gradients that would cause uneven tissue culture (Fig. 6).

[00092] When culture of the tissue was completed, all media was drained from the entire system via the inlet/outlet port. Decellularization solutions flowed into and out of the bag via the inlet/outlet port on the bag several days to remove all cellular material from the tubular tissues. Wash solutions flowed into and out of the bag via ports in the bag for several days to remove all residual decellularization solutions and cellular material. The bag was disconnected from the intraluminal flow systems and the bag was stored in the external framework filled with storage liquid. The bag was brought into the operating room and the outer layer of the bag was removed revealing a sterile inner layer that was brought into the sterile field. The sterile bag was opened with a scalpel and the tubular tissue was cut from the anchoring ports and prepared for implantation.

Example 5: Culture of a 6mm diameter x 23cm length tubular tissue

[00093] An acellular biologic scaffold with a diameter of six millimeters and a length of twenty three centimeters was placed onto a flexible thin walled silicone tube and was inserted into the bag (Fig. 8). The silicone tubing and acellular biologic scaffold were tightly secured to anchoring ports in a manner that prevented leakage of intraluminal fluid or gas and also prevented contracting of the growing tubular tissue. The bag was then sealed and prepared for growth.

[00094] Medical grade air was injected into the bag via pressurized cylinders through a sterile filter to inflate the bag so the acellular biologic scaffold would be protected during shipping to and from the site of gamma sterilization. The bag was sterilized via supercritical CO_2 and stored in a desiccated environment to prevent degradation of the acellular biologic scaffold material.

[00095] Human smooth muscle cells were isolated from aortas. Human cells were used at passage 2 to produce tubular engineered tissues. Cells were expanded, harvested, and suspended in culture media and the bag was inoculated through the inlet/outlet port via syringe. The cell suspension was carefully directed into the lower seam of the bag creating

a trough filled with cells suspended in culture media. The flexible tubing and anchoring ports attached to the acellular biologic scaffold were manipulated to force the acellular biologic scaffold into the trough. The bag was aseptically deflated via the inlet/outlet port to assist in directing the cell suspension into the acellular biologic scaffold. The outer layers of the bag were manipulated near the lower seam to gently mechanically massage the cell suspension into the acellular biologic scaffold. Special attention was paid to massaging the cell suspension into the acellular biologic scaffold near the anchoring ports. The acellular biologic scaffold was forced into this second seam and the remaining cell suspension was massaged into the unseeded side of the acellular biologic scaffold.

[00096] The bag was fabricated with an embedded framework that allowed the acellular biologic scaffold to maintain a fixed length for the entirety of culture. The embedded framework prevented the bag from folding and collapsing on itself and damaging the tubular tissue. The embedded framework prevented the bag from moving and kinking or damaging the pathways or connections to the extraluminal and intraluminal flow systems.

[00097] Tubing was connected to an intraluminal flow port on either side of the bag to provide pulsatile flow through the lumen of the acellular biologic scaffold. This pulsatile flow was produced by fluid or gas passing through the thin walled flexible silicone tubing within the acellular biologic scaffold. The pulsatile flow served as a mechanical stimulus that prompted the cells to produce the extracellular matrix necessary to grow a strong tubular tissue.

[00098] The inlet/outlet port was used to aseptically remove spent culture medium and feed fresh culture medium. At regular intervals, the bag was aseptically connected to an empty waste reservoir into which the spent medium was pumped. After removing spent medium, the bag was aseptically connected to a reservoir containing warmed fresh medium, which was pumped into the bag. Concentrations of nutrients, metabolites, dissolved gases, and pH were monitored from the waste media. Samples can also be taken aseptically from the inlet/outlet port.

[00099] When culture of the tissue was completed, all media was drained from the entire system. Decellularization solutions were circulated through the bags via the inlet/outlet port for several days to remove all cellular material from the tubular tissues. Wash solutions were circulated through the bags for several days to remove all residual decellularization solutions and cellular material. The decellularized graft was seeded with a second cell suspension. Cells were adhered and cultured for one hour to four days.

[000100] The bag was disconnected from the intraluminal flow systems and filled with storage liquid. The tissue was stored for 12 months in the bag. The sterile bag was opened with a scalpel and the tubular tissue was cut from the anchoring ports and prepared for implantation.

Example 6: Culture of a 6mm diameter x 23 cm length tubular tissue

[000101] A bag made of a minimally gas permeable material (e.g., ultra-low density polyethylene) with five ports is used for culture of a tubular tissue that is 6mm in diameter and 23cm in length (Fig. 9). A tubular polymeric scaffold with a diameter of six millimeters and a length of twenty three centimeters was placed onto a flexible thin walled silicone tube and was inserted into the bag. The silicone tubing and tubular scaffold were tightly secured to anchoring ports in a manner that prevented leakage of intra luminal fluid or gas and also prevented contracting of the growing tubular tissue. The bag was then sealed and prepared for tubular scaffold surface treatment. The bag was filled with a sodium hydroxide solution via the inlet/outlet port to chemically treat the surface of the tubular scaffold and allowed it to readily absorb culture media. The bag was filled with several wash solutions via the inlet/outlet port to remove all residual sodium hydroxide. The bag was filled with air via the air inlet/outlet port to dry the tubular scaffold and remove all moisture from the inner surface of the bag.

[000102] Medical grade air was injected into the bag via pressurized cylinders through a sterile filter to inflate the bag so the tubular scaffold would be protected during shipping to and from the site of gamma sterilization. The bag was sterilized via gamma sterilization and stored in a desiccated environment to prevent degradation of the tubular scaffold material.

[000103] Human smooth muscle cells were isolated from aortas. Cells from multiple donors were pooled for culture of pooled donor grafts. Human cells were used at passage 2 to produce tubular engineered tissues. Cells were expanded, harvested, and suspended in culture media. The bag was constructed with a rigid plastic insert in the area underneath the tubular scaffold that functions as a trough (Fig. 9). The bag was inoculated through the cell seeding port via syringe, filling the rigid plastic trough insert with cell suspension. The flexible tubing and anchoring ports attached to the tubular scaffold were manipulated to force the tubular scaffold into the trough. The outer layers of the bag were manipulated to gently mechanically massage the cell suspension into the tubular scaffold. Special attention

was paid to massaging the cell suspension into the tubular scaffold near the anchoring ports. The flexible tubing and anchoring ports attached to the tubular scaffold were rotated and twisted circumferentially so that the remaining cell suspension could be massaged into the unseeded areas of the tubular scaffold. Once the tubular scaffold was saturated with cell suspension, all unseeded cells were removed from the rigid plastic trough insert through the inoculation port.

[000104] The bag was attached to an external framework that allowed the tubular scaffold to maintain a fixed length for the entirety of culture. The external framework prevented the bag from folding and collapsing on itself and damaging the tubular tissue. The external framework prevented the bag from moving and kinking or damaging the pathways or connections to the intraluminal flow systems.

[000105] Tubing coming from the external culture medium reservoir was connected to the extraluminal inlets and outlets of the bag. These ports were placed in a position where culture media circulation would not create a significant amount of shear force that would detach cells from the tubular scaffold. These ports were also placed in a position below the graft near the lower seam that would facilitate removal of all culture media at any time during culture to remove all residual cells and tissue that were not attached to the tubular scaffold.

[000106] Tubing was connected to the intraluminal flow port of the bag to provide a pulsatile mechanical stimulus from within the scaffold. The pulsatile flow was achieved using an alternating pump that introduced fluid or gas into a flow path that passed through the flexible thin walled silicone tubing and terminated at the internal fluid delivery outlet port. This dead end flow path inflated and deflated the silicone tubing and provided a mechanical stimulus that prompted the cells to produce the extracellular matrix necessary to grow a strong tubular tissue.

[000107] A tubular tissue was cultured for up to ten weeks with constant circulation of media that was being supplemented in a controlled and monitored reservoir (Fig. 10). Periodically all or a portion of the media was drained from the system including the bags and replaced with fresh culture media. Room air was injected into the bag via syringe through a sterile filter attached to an inlet port to replace volume recently occupied by media during full media exchanges to prevent the surface of the bag from collapsing and making contact with the culturing tubular tissue. Periodically culture media from the bags was sampled for analysis by attaching a syringe to a port on the bag.

[000108] When culture of the tissue was completed, all media was drained from the entire system via the inlet/outlet port. Decellularization solutions flowed into and out of the bag via the inlet/outlet port on the bag several days to remove all cellular material from the tubular tissues. Wash solutions flowed into and out of the bag via ports in the bag for several days to remove all residual decellularization solutions and cellular material. The bag was disconnected from the intraluminal flow systems and the bag was stored in the external framework filled with storage liquid. The bag was brought into the operating room and the outer layer of the bag was removed revealing a sterile inner layer that was brought into the sterile field. The sterile bag was opened with a scalpel and the tubular tissue was cut from the anchoring ports and prepared for implantation.

Example 7: Culture of an organ shaped tissue

[000109] A bag with six ports is used for culture of an organ shaped tissue (Fig. 11). An organ shaped scaffold was placed onto a flexible thin walled silicone bladder and was inserted into the bag. The silicone bladder and organ shaped scaffold were tightly secured to anchoring ports in a manner that prevented leakage of internal fluid or gas and also prevented contracting of the growing organ shaped tissue. The bag was then sealed and prepared for scaffold surface treatment. The bag was filled with a sodium hydroxide solution via the inlet and outlet ports for external fluid delivery that chemically treated the surface of the organ shaped scaffold and allowed it to readily absorb culture media. The bag was filled with several wash solutions via the inlet and outlet ports for external fluid delivery that removed all residual sodium hydroxide. The bag was filled with air via the air inlet/outlet to dry the organ shaped scaffold and remove all moisture from the inner surface of the bag.

[000110] Medical grade air was injected into the bag via pressurized cylinders through a sterile filter to inflate the bag so the organ shaped scaffold would be protected during shipping to and from the site of gamma sterilization. The bag was sterilized via gamma sterilization and stored in a desiccated environment to prevent degradation of the organ shaped scaffold material.

[000111] Human donor cells were isolated and used to produce organ shaped tissues. Cells were suspended in culture media and the bag was inoculated through the cell seeding port via syringe. The effective bag volume was reduced for seeding by vacuum. The bag was inoculated through the cell seeding port via syringe, filling the entire volume. The

outer layers of the bag were manipulated to gently mechanically massage the cell suspension into the organ shaped scaffold. Special attention was paid to massaging the cell suspension into the organ shaped scaffold near the anchoring ports. The bag was rotated one hundred and eighty degrees circumferentially and the remaining cell suspension was massaged into the unseeded side of the organ shaped scaffold. Once the organ shaped scaffold was saturated with cell suspension, all unseeded cells were removed through the inoculation port and the compression apparatus was removed from the bag.

[000112] The bag was attached to an external framework that allowed the organ shaped scaffold to maintain a fixed position for the entirety of culture. The external framework prevented the bag from folding and collapsing on itself and damaging the organ shaped tissue. The external framework prevented the bag from moving and kinking or damaging the pathways or connections to the external and internal fluid delivery systems.

[000113] Tubing coming from the external culture medium reservoir was connected to the external fluid delivery inlets and outlets of the bag. These ports were placed in a position where culture media circulation would not create a significant amount of shear force that would detach cells from the organ shaped scaffold. These ports were also placed in a position below the tissue near the lower seam that would facilitate removal of all culture media at any time during culture to remove all residual cells and tissue that were not attached to the organ shaped scaffold. Tubing was connected to an intraluminal flow port on either side of the bag to provide flow through the orifice of the organ shaped scaffold.

[000114] An organ shaped tissue was cultured with constant circulation of media via the external fluid delivery inlet and outlet ports that was being supplemented in a controlled and monitored reservoir. Periodically all or a portion of the media was drained from the system including the bags and replaced with fresh culture media. Gas was injected into the bag through a sterile filter attached to the air inlet/outlet port to replace volume recently occupied by media during full media exchanges to prevent the surface of the bag from collapsing and making contact with the culturing organ shaped tissue. Periodically culture media from the bags was sampled for analysis by attaching a syringe to the sampling port on the bag.

WHAT IS CLAIMED IS:

1. A culture bag comprising a biocompatible material wherein said bag is suitable to contain and support the long-term culture of cells, tissues, or organs; tolerates sterilization by standard means, and permits the supply of oxygen from outside said bag to the cells, tissues or organs within said bag and removal of carbon dioxide from within said culture bag.

2. The culture bag of claim 1, wherein the biocompatible material is flexible.

3. The culture bag of claim 1, wherein the biocompatible material is ultra low density polyethylene, linear low density polyethylene, fluorinated ethylene polymer, polyvinylidene fluoride or ethyl vinyl acetate.

4. The culture bag of claim 1, wherein the biocompatible material is minimally gas permeable.

5. The culture bag of claim 4, wherein the minimally gas permeable material alters the oxygen and/or carbon dioxide saturation of culture medium within the culture bag by 0-10% within a 3 hour period.

6. The culture bag of claim 1, wherein the biocompatible material is gas permeable.

7. The culture bag of claim 6, wherein the gas permeable material alters the oxygen and/or carbon dioxide saturation of culture medium within the culture bag by more than 10% within a 3 hour period.

8. The culture bag of claim 1, wherein oxygen delivery and carbon dioxide removal occurs through passive diffusion.

9. The culture bag of claim 1, wherein oxygen delivery and carbon dioxide removal occurs through culture medium exchange or circulation.

10. The culture bag of claim 1, wherein the biocompatible material tolerates exposure to pH 14.

11. The culture bag of claim 1, wherein said biocompatible material is transparent or contains a transparent section.

12. The culture bag of claim 1, wherein said bag further comprises a light blocking material.

13. The culture bag of claim 1, further comprising one or more anchors.

14. The culture bag of claim 13, wherein said one or more anchors support or maintain the shape of the tissues or organs within said culture bag.

15. The culture bag of claim 13, wherein said one or more anchors affixes said tissues or organs to said culture bag.

16. The culture bag of claim 13, wherein said one or more anchors prevent the extension, bending, kinking, or folding of the tissues or organs within said culture bag.

17. The culture bag of claim 13, wherein said one or more anchors are within the culture bag.

18. The culture bag of claim 13, wherein said one or more anchors are attached to the exterior culture bag.

19. The culture bag of claim 13, wherein said one or more anchors are present between layers of a multi-layer culture bag.

20. The culture bag of claim 1, further comprising one or more ports.

21. The culture bag of claim 20, wherein said one or more ports serve as one or more anchors to support or maintain the shape of the tissues or organs within said culture bag.

22. The culture bag of claim 20, wherein a fluid flows through said one or more ports.

23. The culture bag of claim 22, wherein fluid is a liquid or a gas.

24. The culture bag of claim 22, wherein said flow is unidirectional.

25. The culture bag of claim 22, wherein said flow is bidirectional.

26. The culture bag of claim 22, wherein said flow is circulatory.

27. The culture bag of claim 22, wherein said flow is reversible.

28. The culture bag of claim 22, wherein said fluid is culture medium.

29. The culture bag of claim 22, wherein said fluid is a cell inoculum.

30. The culture bag of claim 22, wherein said fluid is a sterile gas.

31. The culture bag of claim 22, wherein said fluid is a decellularization solution.

32. The culture bag of claim 22, wherein said fluid is a wash solution.

33. The culture bag of claim 22, wherein said fluid is a storage solution.

34. The culture bag of claim 22, wherein said fluid is a surface treatment solution to improve polymeric scaffold wettability.

35. The culture bag of claim 34, wherein said surface treatment solution comprises sodium hydroxide.

36. The culture bag of claim 22, wherein said flow introduces a fluid into said culture bag.

37. The culture bag of claim 22, wherein said flow introduces a fluid into said tissues or organs within said culture bag.

38. The culture bag of claim 22, wherein said flow removes a fluid from said culture bag.

39. The culture bag of claim 22, wherein said flow removes a fluid from said tissues or organs within said culture bag.

40. The culture bag of claim 20, wherein said one or more ports contains one or more sterile filters.

41. The culture bag of claim 20, wherein said one or more ports permits sampling the contents of said culture bag.

42. The culture bag of claim 1, further comprising a flexible mandrel inside of a tissue or organ within said culture bag.

43. The culture bag of claim 42, wherein flexible mandrel stretches to impart mechanical strain and/or stress to the tissue or organ within said culture bag.

44. The culture bag of claim 1, wherein said culture bag is coupled to at least one reservoir or at least one flow system suitable to deliver a fluid to said culture bag.

45. The culture bag of claim 1, further comprising a seeding trough.

46. The culture bag of claim 45, wherein the seeding trough is a seam in the culture bag or an internal fixture that facilitates seeding of the particular shape of the construct to be seeded.

47. The culture bag of claim 45, wherein the seeding trough is formed by shaping the flexible biocompatible material.

48. The culture bag of claim 47, wherein the flexible biocompatible material is shaped by manual manipulation.

49. The culture bag of claim 47, wherein the flexible biocompatible material is shaped by compression.

50. The culture bag of claim 47, wherein the flexible biocompatible material is shaped by vacuum.

51. The culture bag of claim 1, wherein said culture bag resists over-pressurization when filled with a fluid.

52. The culture bag of claim 1, further comprising an inner liner and an outer liner, wherein the outer liner maintains the sterility of the outer surface of the inner liner to allow the culture bag to be opened for aseptic removal of the tissue or organ.

FIGURE 1

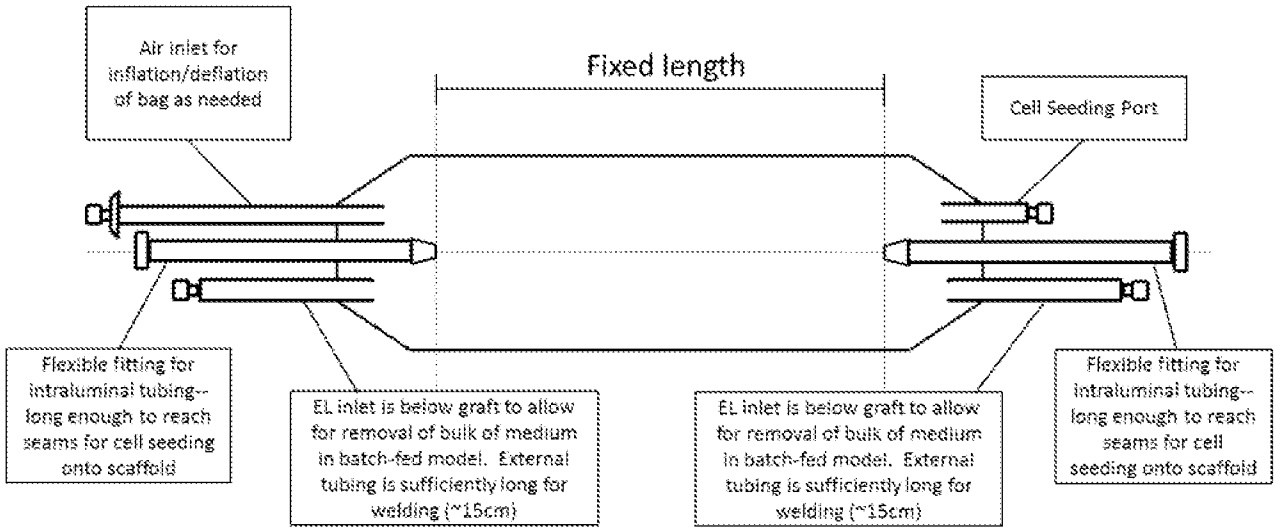


FIGURE 2

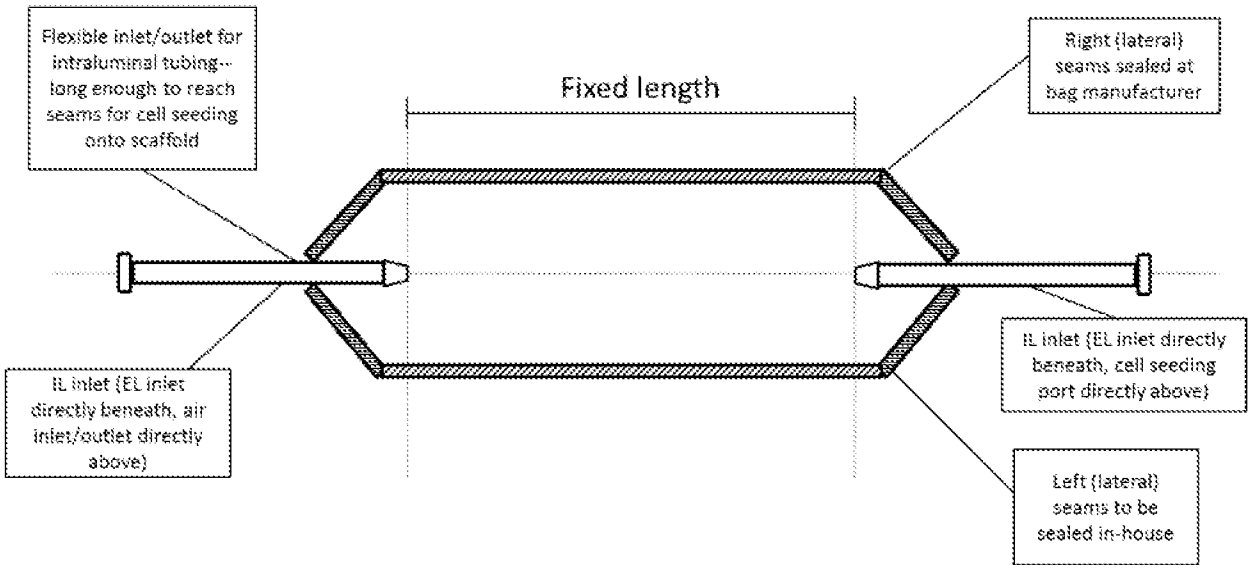


FIGURE 3

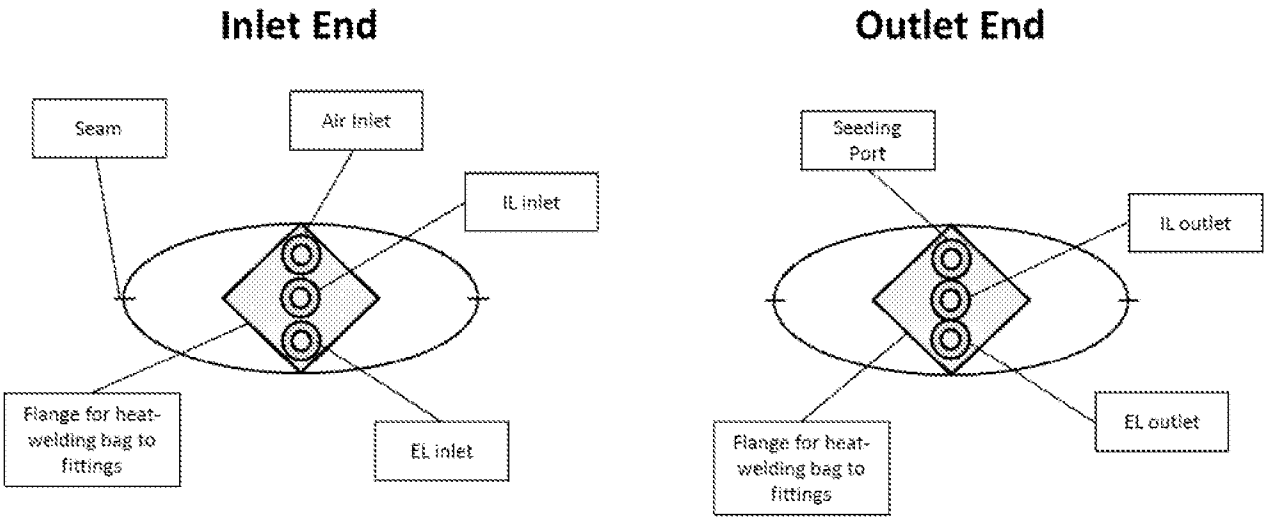


FIGURE 4

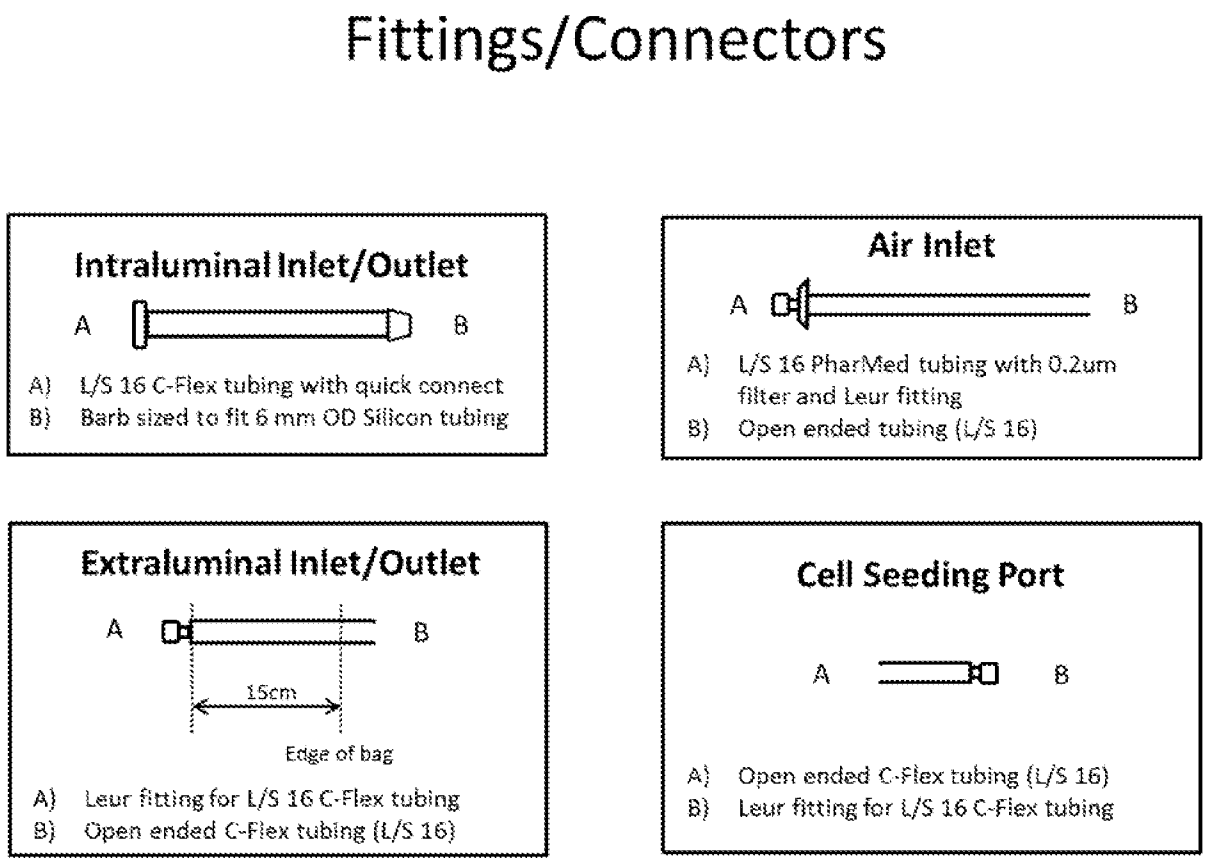


FIGURE 5

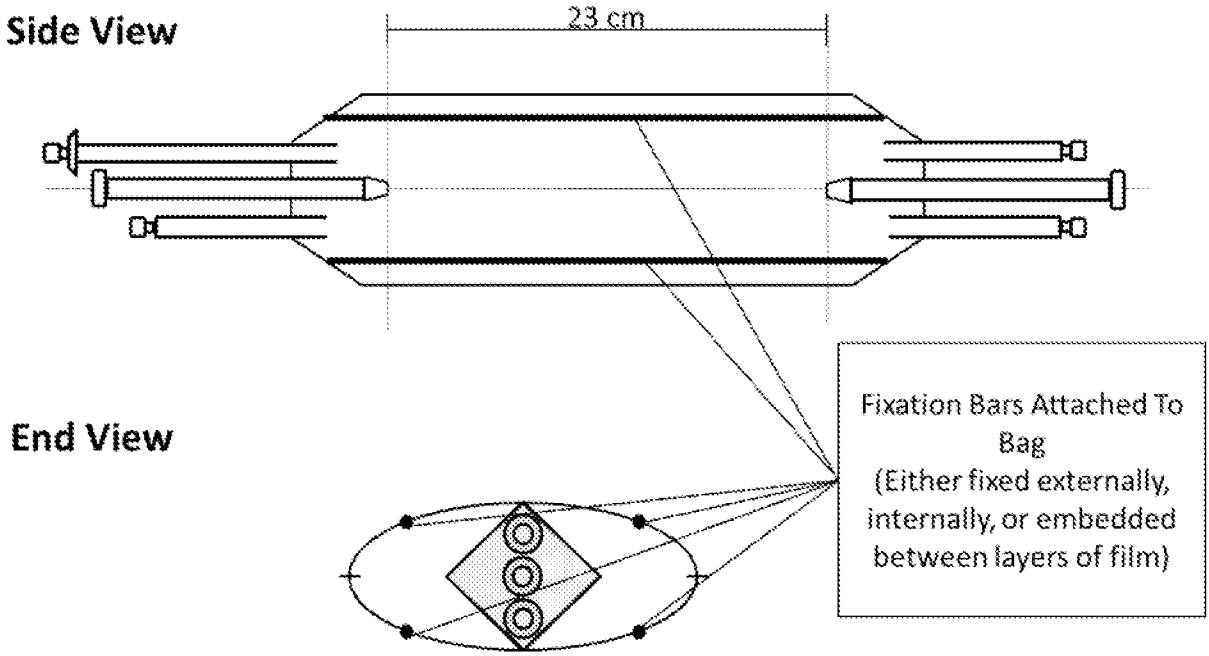


FIGURE 6

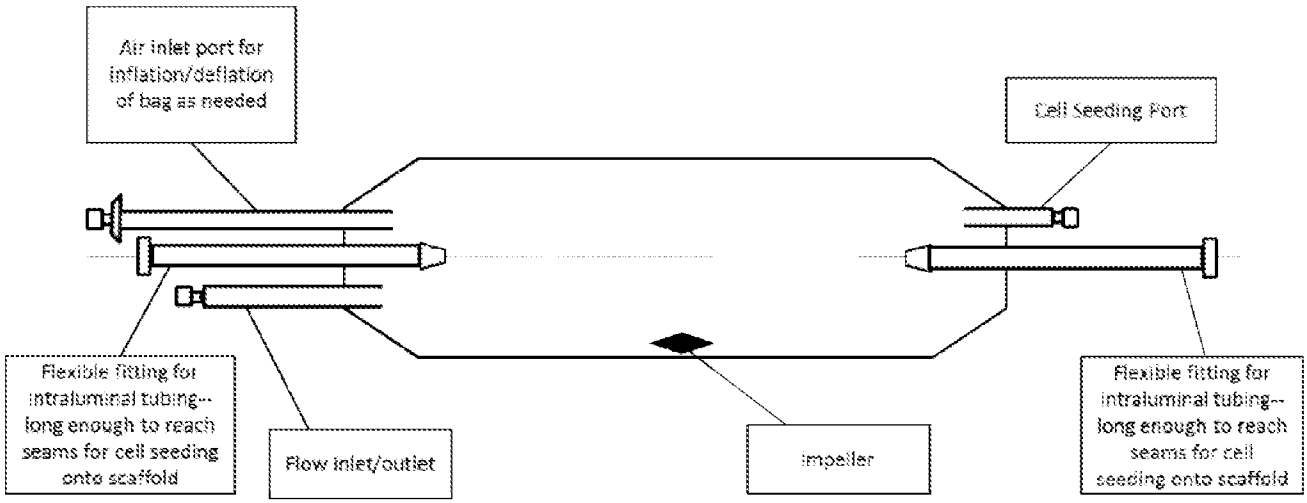


FIGURE 7

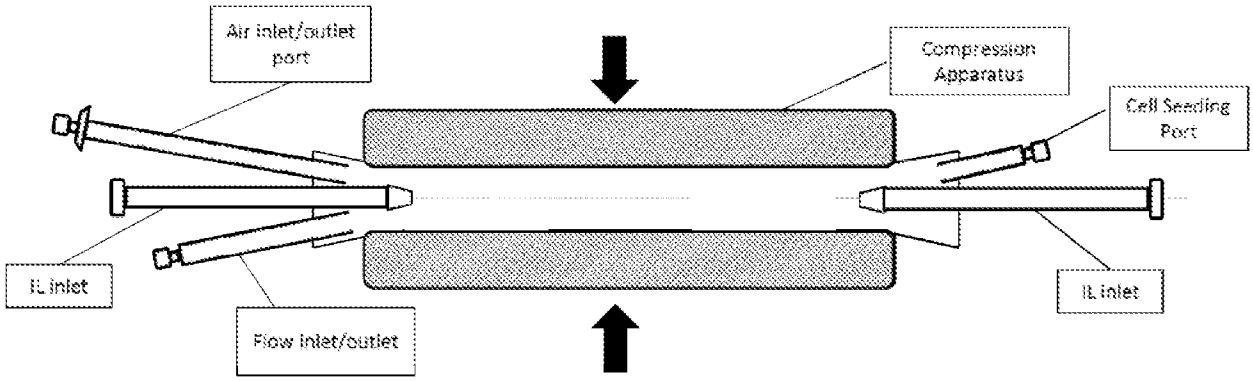


FIGURE 8

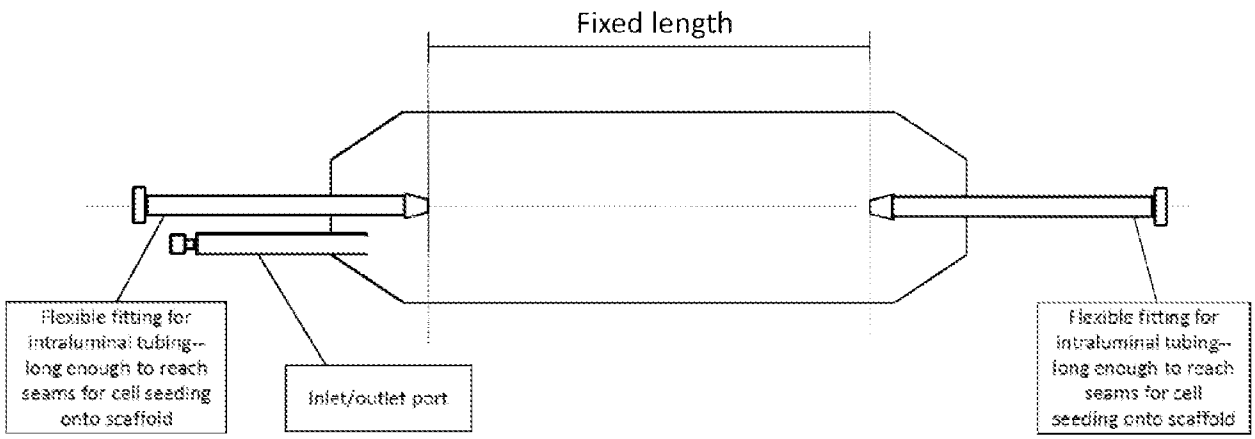


FIGURE 9

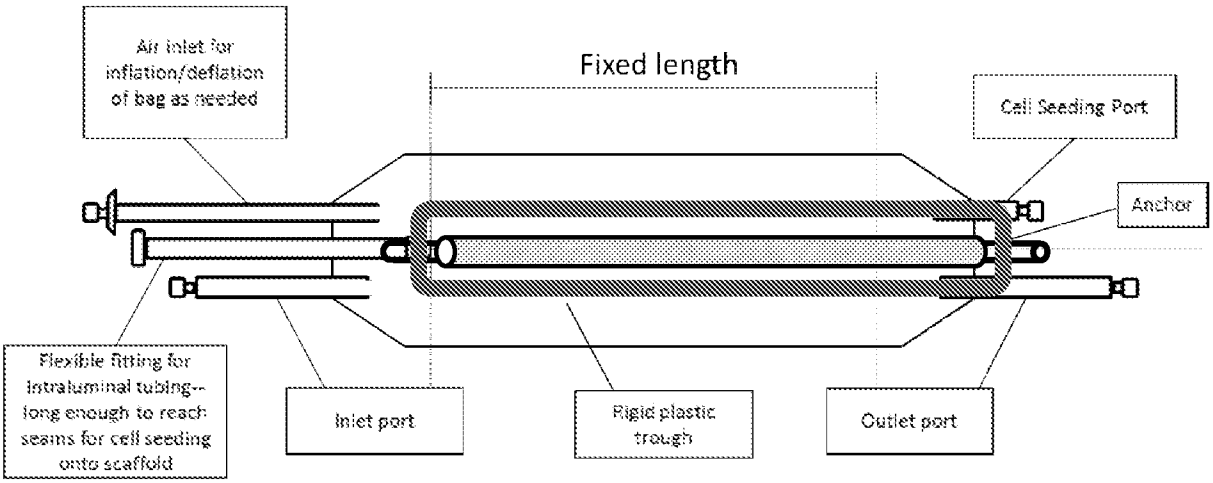


FIGURE 10

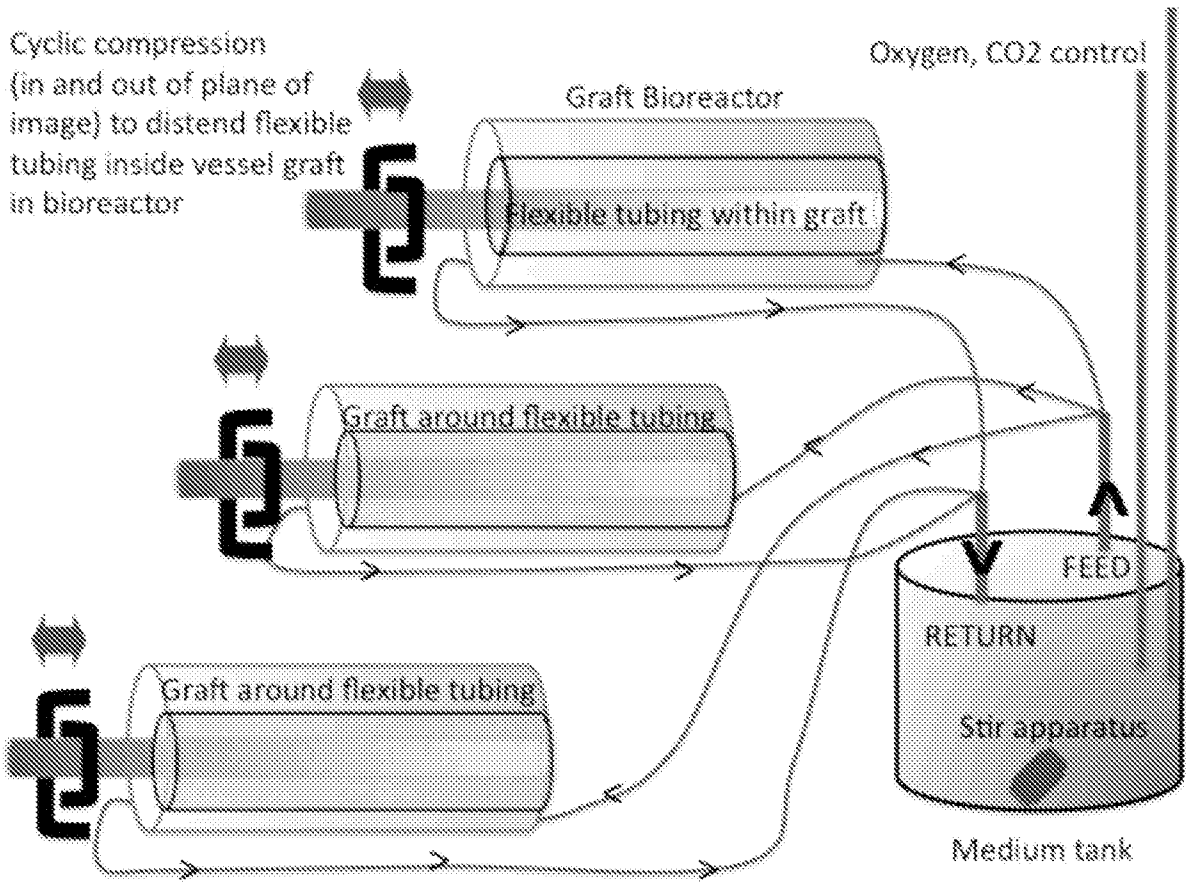
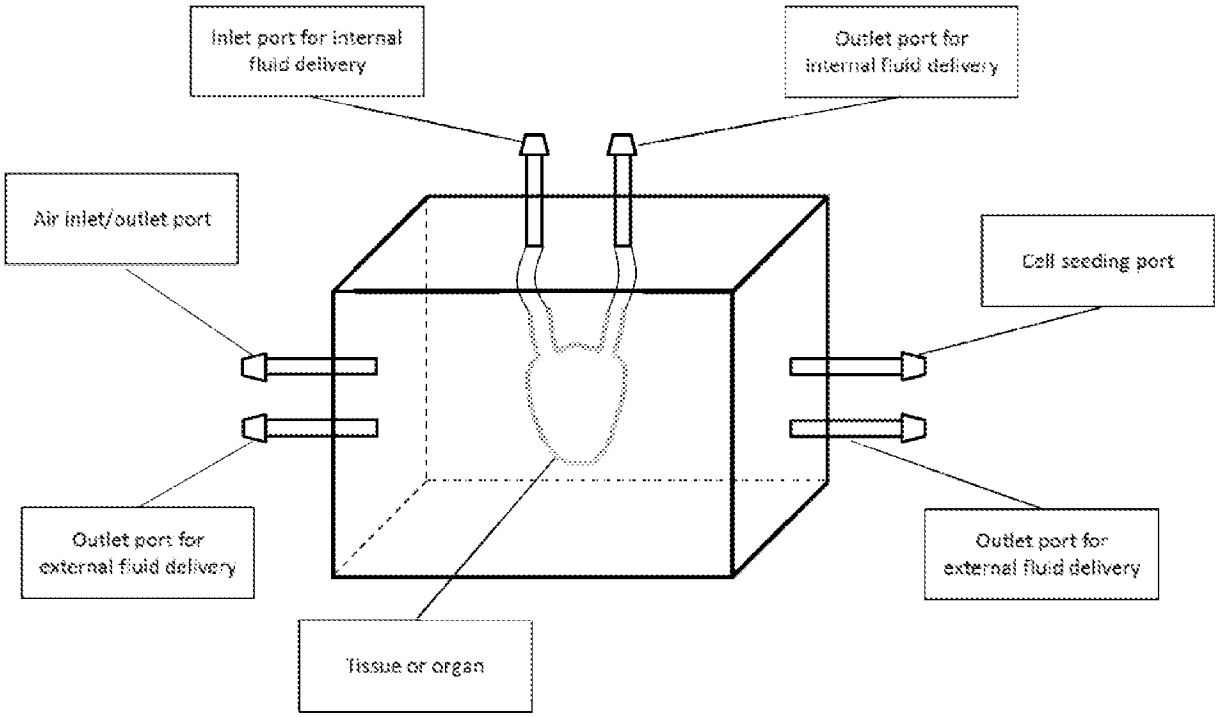


FIGURE 11



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