

Design of Bioreactors for Cardiovascular Applications

E. Lyons and A. Pandit*

Summary

Systems are being developed in the field of reparative medicine to generate cell and tissue-based constructs. Currently available substitutes for failing cardiac tissue have serious limitations. Mechanical heart valves provide effective structural durability and mechanical function, but they are associated with a substantial risk of thromboembolism, thus requiring lifelong anticoagulation. Currently used biological heart valves are known to have superior hemodynamics, but durability is limited. Autografts, allografts and xenografts, which are used as vascular grafts in the surgical treatment of atherosclerosis, have seen limited success, with the vessels failing due to dimensional restrictions, mechanical property mismatch and disjunction at the anastomoses. Synthetic vascular grafts implanted as medium diameter vessel replacements exhibit an acute loss of patency within several months after implantation primarily due to thrombosis. The common trend and major drawback of these cardiac prostheses is that they are composed of foreign body material without the ability to grow, to remodel or to repair. The field of tissue engineering may be the solution to the problem as it offers the possibility of developing a biological substitute material in vitro with the inherent mechanical and morphological properties required in vivo. A major challenge is to design an in vitro system that produces these clinically effective cardiovascular tissue-based constructs. Such a system, that attempts to simulate a physiological environment for the creating and preconditioning of cells and

tissues in vitro, is known as a bioreactor. This chapter outlines the requirements and current designs of bioreactors for the tissue engineering of cardiovascular constructs, such as heart valve and vascular grafts.

Keywords: bioreactor, cardiovascular, tissue engineering, heart valve, vascular grafts, cell culture



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Introduction

Current *in vitro* and *in vivo* studies have shown limited regeneration properties of the heart in adult mammals (1-3). The functional deficits caused by heart failure can only be partly ameliorated by transplantation due to shortages of donor tissues (4). These limitations have fuelled research in the areas of cardiac tissue and organ replacement. Tissue engineering may provide an alternative to organ and tissue transplantation to counteract the severe shortage of donor tissue available (5-12). Tissue engineering seeks to address some of these problems by developing strategies to culture functional tissues *in vitro* for subsequent implantation (4, 13). Engineering tissue is generally achieved by seeding autologous cells onto a three-dimensional scaffold/matrix followed by *in vitro* culture (14-17). Once implanted, the seeded scaffold starts to remodel and integrate with the surrounding tissue (12, 18-21). Although the feasibility of *in vitro* and *in vivo* formation of cardiovascular tissue has been demonstrated in previous experiments, the fabrication of viable tissue *in vitro* remains a significant problem for tissue engineering autologous cardiovascular structures (18, 21, 22). The development of suitable dynamic systems for *in vitro* investigation of three-dimensional cardiovascular cell growth (22-28) is an essential aspect of tissue engineering. Advancement in the design of these systems will support the ambitious goal of engineering viable heart tissue.

The ideal *in vitro* conditions for the formation of tissue constructs are not known, but several studies have shown that mechanical preconditioning benefits the generation of tissue-engineered cardiovascular constructs (29-35). For both two-dimensional (36) and three-dimensional (37) culture systems, periodic stretch increases the contractile apparatus of smooth muscle cells, enhances synthesis and secretion of proteins (17), potentiates cellular proliferation (38) and effects the level of gene expression. Other studies suggest that pulsatile fluid flow exposure might be important to guide the development of tissue-engineered heart valves or vessels *in vitro* before implantation into an *in vivo* model (4, 29, 39-41). It is also believed that cardiovascular constructs

perform better in laminar flows with uniform distribution of moderate shear stresses and laminar convective mass transfer (42-46).

The main advantage of a dynamic cell culture environment is that experimental parameters can be carefully controlled, providing a well-characterized biochemical and biomechanical environment. In vivo animal models allow for a high degree of physiological relevance. Unfortunately, the ability to control the experimental parameters beyond the physiological range, or the physio-chemical environment, is restricted. An additional drawback is the high cost of animal studies. To overcome the limitations of the in vivo conditioning of tissue-engineered constructs, various forms of bioreactors have been developed. A bioreactor is a system that simulates the physiological environments for the creation, physical conditioning and testing of cells, tissues, support structures and organs in vitro (47).

Bioreactor design

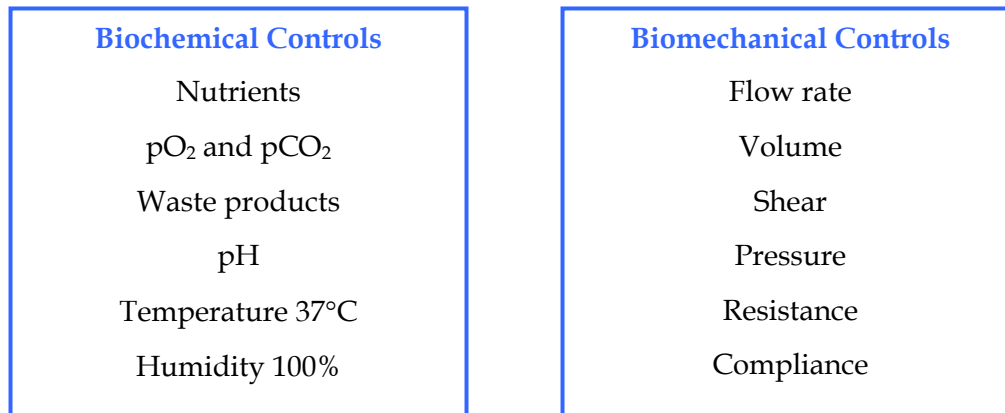
The design of a bioreactor is a complex task as an understanding of both engineering and scientific backgrounds are required in order to develop such a mechanically controlled environment for the growth of animal tissue. A number of criteria establish a blueprint for the design of a bioreactor. The criteria may change for various tissue types under development, but in general, a bioreactor must be designed to meet the following requirements:

- control the physiochemical environment
- facilitate monitoring of cell/tissue quality
- ensure the culture of tissue samples occurs under sterile conditions
- establish a substantial level of cellular distribution and attachment to developing scaffolds

- ensure tissues have sufficient nutrient and waste exchange with their surroundings (i.e. provide efficient mass transfer to the tissue)
- expose the developing tissue to mechanical forces such as compression and expansion, as well as hydrodynamic forces such as shear stress and pressure
- maintain a high degree of reproducibility
- control the flow of media whether it be steady or pulsatile
- reduce excessive turbulence in the fluid flow
- provide a low volume capacity
- make effective use of growth factors and medium components
- ensure that the materials from which the bioreactor is fabricated are compatible with cells/tissues
- be easy to clean and maintain
- enable the user to easily fix the seeded scaffold in place
- ensure the culture of tissue samples under physiological conditions
- be compact in size to fit in a standard size incubator
- avoid the accumulation of metabolites

The design and functional requirements of the tissue to be engineered determine the specific design requirements in the bioreactor. In the design of a bioreactor, both the biomechanical and biochemical controls are essential in the creation of a simulated physiological environment for cell and tissue growth. Pulsatile forces, pressure, flow rate, compression, expansion, shear stress, frequency, stroke rate and stroke volume are extremely important design considerations. The biochemical environment is equally important, with the transfer of nutrients and the removal of waste products essential aspects of cellular proliferation and healthy tissue development.

Fundamentally, a bioreactor for cardiovascular applications attempts to mimic the parameters that exist in vivo such as cardiac flow rates and pressures. The following is a summation of the ideal physiological parameters required in the design of such a bioreactor:



Biochemical controls

Nutrient and waste transport is the primary function of the cardiovascular system. Direct diffusion acts as the driving physical force for the distribution of nutrients and oxygen to tissue both *in vivo* and *in vitro* (42, 48-51).

Oxygen is a sparingly soluble gas that is essential for cell cultivation. Blood needs to deliver about 10mmol of O₂ per minute to the body. The gross circulation rate is about 5L/min. Therefore, blood has to deliver about 2mmol oxygen per litre during each pass through the circulation. Oxygen must be provided at the same rate as being consumed. If oxygen delivery is too slow compared to consumption, the local concentration drops to unacceptably low levels, which will affect cell viability and enzymatic activities (52). Cells exposed to hypoxic conditions undergo apoptosis. *In vivo* these cells would be digested by phagocytes; however, *in vitro* these cells may leak proteases, DNA and other cellular components, which may have a detrimental effect on the surrounding tissue. Therefore, it is important that the oxygen requirement of the tissue is met to preserve the integrity and reliability of the organ culture system. Conversely, if oxygen

is being supplied at a rate that is too high, then its concentration can build up to inhibitory levels. A study by Carrier et al. investigated the effects of medium perfusion rate and pO₂ on the in vitro reconstruction of engineered cardiac muscle. It was found that a higher perfusion rate and higher pO₂ correlated with more aerobic cell metabolism and higher DNA and protein contents (53).

A bioreactor must possess the ability to supply media to penetrate the most profound sections of the developing tissue and prevent necrosis (54-57). Lack of perfusion, which would guarantee nutrient support, greatly limits the thickness of engineered tissue (58). In contrast to static culture, active continuous transport of nutrients and gases to the cells and tissue by the act of medium perfusion promotes a more sufficient aerobic metabolism. In static culture, diffusional transfer results in anaerobic cell metabolism (58, 59). Mass transfer boundary layers, within bioreactors, occur at slow flowing regions. There will always be slower flowing regions close to any side, or tubing, walls within a bioreactor.

To ensure homeostasis, intracellular and extracellular fluids must contain almost balanced quantities of acids and bases. Homeostatic mechanisms maintain the pH of blood between 7.35 and 7.45. In blood as acidity increases (pH decreases), the affinity of hemoglobin for O₂ decreases and O₂ dissociates more readily from hemoglobin. Thus, lowered pH drives O₂ off hemoglobin, making more O₂ available for tissue cells. By contrast, elevated pH increases the affinity of hemoglobin for O₂.

Metabolically active cells liberate heat. If the body's heat production equals heat loss, a constant core temperature is maintained. Body temperature is normally around 37°C. It may rise as high as 43°C or fall to 32°C in various conditions, but the risk to life is only serious above 41°C or below 35°C. Many bioreactors are designed to fit entirely or partially in an incubator. The incubator has the ability to control and maintain the physiological temperature while the bioreactor is functioning. An alternative method of

achieving 37°C is by means of an added heat exchanger component within the bioreactor's flow system.

Biomechanical controls

Cardiac output equals the stroke volume, the volume of blood ejected by the ventricle with each contraction, times the heart rate, the number of heart beats per minute. In a typical resting adult, stroke volume averages 70 ml/beat and the heart rate is about 75 bpm. Therefore, cardiac output is approximately 5.25 L/min. During mild exercise, the stroke volume may increase to 100 ml/beat and the heart rate to 100 beats per minute, resulting in a cardiac output of 10 L/min. In an adult, the cross-sectional area of the aorta is only 3-5 cm², and the average velocity of blood there is 40 cm/s.

Pumps that are most frequently employed to emulate pulsatile flow rates include piston, peristaltic, centrifugal and diaphragm pumps. Piston pumps can achieve a high volume output while accurately mimicking the cardiac cycle. However, these pumps are cumbersome in a laboratory environment and are associated with a high incidence of apoptosis when used in direct contact with cells due to the mechanical compression and force involved. The piston pump may be indirectly applied by means of a diaphragm. A flexible diaphragm or bladder can be displaced by the action of the piston or by respirator fluctuations to force media through a bioreactor (35, 50). Peristaltic and centrifugal pumps have been used successfully in heart and lung machines. Peristaltic pumps offer sterile pumping and are associated with low shear, while centrifugal pumps exhibit higher shear, which is linked to the denaturing of mammalian cells.

Hemodynamics plays an important role in the development of intimal hyperplasia and thrombus formation *in vivo*. The wall shear rate, wall shear stress, flow separation, recirculation and turbulence are suspected of being the major fluid dynamic factors

influencing the development of intimal hyperplasia. Wall shear stress is known to be the most important factor. Shear stress is defined as the force per unit area acting on the parallel direction to the surface. The shear strain rate and viscosity determine the shear stress induced by fluid motion. Shear strain rate is defined by the time rate of shear deformation and is the velocity gradient (du/dy) in pure shear flow. Shear stress (τ) is proportional to the strain rate, and the proportional constant (μ) is the viscosity for the Newtonian fluid.

It is natural for endothelial cells to sense and respond to shear stress. Arteries adapt to long-term increases or decreases in wall shear stress. Decreased flow rates will cause a thickening of the intimal layer to re-establish a normal wall shear stress. Arteries will adapt to maintain a wall shear stress of 15 dyn/cm². Typically, most intimal thickening is found where the average wall shear stress is <10 dyn/cm² (60, 61). However, excessively high shear stress or turbulence can damage adherent, mammalian cells. Weston et al. investigated the shear stress on the surface of an aortic valve leaflet at steady flow rates up to 22.5 L/min in a trileaflet biomechanical valve and recorded a maximum shear stress of 79 dyn/cm² (62).

Studies performed in vitro have also shown that changes in the mechanical environment cause alterations in the biosynthetic activity of valvular cells (63). Cusp tissue exposed to pulsatile and steady flow and laminar shear stresses between 1 and 22 dyn/cm² for 48 hours showed significantly different levels of protein, GAG and DNA synthesis when compared to statically incubated tissue (64). However, when the synthesis activity was compared to freshly excised tissue, the only difference seen was in the protein synthesis of cusp tissue exposed to shear stress. These results indicate that fluid flow maintains the synthetic activity of leaflets at normal levels.

Numerical models have been developed to examine the affect of shear stress and mass transfer on constructs in a bioreactor (62, 65, 66). Numerical simulation is of critical

importance since it can be used to improve bioreactor design, and hence, cell and tissue growth within bioreactors.

Besides heart rate and stroke volume, two other factors influence cardiac output: pressure and resistance. In vivo, cardiac output equals mean arterial blood pressure divided by resistance. If cardiac output rises, then blood pressure rises, so long as resistance remains steady (67). The left ventricle delivers blood to the body with considerable force. It raises the pressure to about 120mmHg with contraction, the same as the pressure in the arteries of the body. Between beats, the flow of blood into the capillaries lowers the blood in the arteries to about 80mmHg. The mean arterial pressure in a person whose blood pressure is 120/80mmHg is about 93mmHg.

The effect of constant pressure on the biological properties of porcine aortic valve leaflets was studied in vitro by Xing, et al (68). Results showed that collagen synthesis was significantly increased at 140 and 170 mmHg when compared to static controls, whereas no change was seen when leaflet tissue was exposed to 100 mmHg. No significant difference was seen in GAG or DNA synthesis. This study demonstrated that leaflets will remodel in response to changes in the mechanical environment. However, the leaflets were unable to preserve α -smooth muscle actin immunoreactive cells suggesting that other mechanical forces, such as bending or stretch caused by back pressure, are required to preserve cell phenotype.

A common method of introducing resistance in a bioreactor set-up is to clamp the fluid line distal to the developing tissue. The extent to which the clamp occludes the fluid line determines the pressure within the system. Resistance refers to the opposition to blood flow as a result of friction between blood and the walls of the vessel. The friction, and thus the resistance, depends on the media viscosity, the total vessel/tubing length and the average vessel/tubing radius.

When bioreactors are continuously pulsing, the issue of dynamic compliance in these in vitro systems becomes extremely important. An air filled chamber is often used as compliance, representing the elastic function of the large arteries (50, 69). The pressure in the air space can be varied to adjust the compliance through a range of values. A compliant volume of roughly 900cm³ should adequately duplicate the physiological capacitance of 0.008cm³/mmHg.

Current bioreactor designs

Bioreactors evolved in response to the limitations of static cell and tissue culture. Static culture, the traditional method of seeding scaffolds (22, 29, 70), posed many problems such as an uneven cell distribution over the construct, cell death attributable to a lack of nutrients and oxygen and insufficient extracellular matrix production (71). To overcome these limitations, bioreactors were created to effectively seed cells onto polymers (59), to improve subsequent tissue formation and to enhance the mechanical strength of the tissue-engineered constructs (29, 72).

There are currently a number of bioreactor designs for cardiovascular tissue engineering applications that attempt to create some form of a biochemical and biomechanical environment. These bioreactor designs may be categorized into two groups:

1. Bioreactors for general tissue engineering applications
2. Bioreactors “tailor” designed to engineer cardiovascular tissue

1. Bioreactors designed for general tissue engineering applications

There are a number of commercially available bioreactor designs that provide a substantial form of stimulus and an effective means of seeding cells to developing tissues. These bioreactors can potentially be used to engineer cartilage, liver or cardiac

tissue in a dynamic environment that is not tailored to mimic the actual physiological environment in relation to the tissue that is being created.

The simplest of these bioreactor designs is a spinner flask (73). Typically, seeded scaffolds are attached to needles hanging from the stopper of the flask. These flasks are mixed at 50-80 rpm using a magnetic stir bar in the bottom of the flasks, and the medium is changed every 2-3 days to insure high nutrient concentration (74). Gas exchange is provided by surface aeration of the culture medium through fitted ports and filters (75).

In the spinner flasks, mass transfer occurs by convection, and fluid flow at the surface is unsteady and turbulent. The smallest turbulent eddies have sizes of 250 μm and velocities of 0.4 cm/sec (76). Because of the high level of agitation occurring near the stir bar at the bottom of the flask, the mean shear stress is at a maximum in the vicinity of the stir bar and decreases progressively moving away from the bar. Studies by Freed et al. (77) showed that constructs cultured in spinner flasks were larger and contained more cells after five weeks than those grown in petri dishes.

The wave bioreactor is a flexible plastic pre-sterilized disposable cell culture bag. The bag is filled with culture media, inflated to rigidity and maintained on a rocking platform. The headspace in the bag is continuously aerated. The wave induced agitation increases the air-liquid surface area for oxygen transfer, mixes the fluid in the bioreactor and suspends the cells with low shear.

The National Aeronautics and Space Administration (NASA) designed the first bioreactor to simultaneously integrate co-cultivation, low shear, high mass transfer and three-dimensional growth (78-80). This bioreactor was referred to as a rotating-wall vessel bioreactor, wherein cells are grown in a microgravity environment and are available in two different geometries: the slow lateral turning vessel (STLV) and the high aspect ratio vessel (HARV) (76, 81, 82).

The slow lateral turning vessel (STLV) is a non-perfused, horizontally-rotating bioreactor comprised of two concentric cylinders. The stationary inner cylinder has a silicone membrane that allows gas exchange while the outer cylinder, made from a non-permeable material, rotates. The vessel rotational speed (15-40 rpm) is adjusted such that the constructs remain suspended close to a stationary point within the vessel, relative to an observer on the ground, due to dynamic equilibrium between the acting gravitational, centrifugal and drag forces. Mixing occurs due to the small amount of unavoidable settling which creates movement of the scaffold relative to the media.

The High Aspect Ratio Vessel (HARV) is a non-perfused, horizontally rotating bioreactor with a large radius and a short length. The STL is configured as the annular space between two concentric cylinders, the inner of which is a silicone "gas exchange" membrane, whereas the HARV is a cylindrical vessel with a gas exchange membrane at its base. Because of size and design differences, the HARV has five times as much surface area for gas exchange per unit of reactor volume as the STL.

The perfusion of fresh media into the vessel at rates sufficient to support nutrient delivery, metabolic gas exchange and waste product removal was an alteration to the rotating-wall vessel design. This bioreactor is known as a rotating-wall perfused vessel and is configured as an annular space between two concentric cylinders. The device is connected to two variable-rate motors that independently control the rotation of the vessel's outer wall and the hollow inner centreline spin filter. Rotation rates for the vessel's outer wall and spin filter can be varied in order to create different levels of fluid shear and turbulence.

2. Bioreactors designed for cardiovascular tissue engineering applications

Recently, a number of systems have been developed which emulate the cardiovascular condition in vitro. The designs of these bioreactors are primarily concerned with the tissue engineering of 1) heart valves and 2) blood vessels.

1) Bioreactors designed for the tissue engineering of heart valves

The option of creating heart valves from autologous cells offers many potential advantages (83) compared to other commercially available heart valve substitutes, such as mechanical prostheses and heterograft/homograft tissue valves. These include the elimination of unfavourable side effects associated with anticoagulation therapy, the elimination of immune rejection and the potential for growth, repair, and remodelling.

Mechanical heart valve prostheses provide effective structural durability (84, 85) and mechanical function but are associated with a substantial risk of thromboembolism, thus requiring life-long anticoagulation (50, 86, 87). It can be argued that the ideal treatment for repair or replacement of diseased human heart valves should be based on tissue technology rather than on the use of mechanical devices (88). Commercially available tissue valves include heterografts and homografts (85, 89). The most important advantages of these valves are the lower incidence of valvular thrombosis and a lower risk of infection. However, heterograft and homograft valves are subject to gradual deterioration from a process known as calcific degeneration, which limits their long-term benefits in children and young adults (90-96). Tissue valves fail over time either because of excessive stiffening of the tissue, leading to stenosis and regurgitation (97), or from primary tissue failure with tearing (88). For the engineering of the potentially advantageous autologous heart valves, a number of bioreactors have been specifically designed by exposing the developing tissue to bending stresses and shear stresses associated with blood flow *in vivo* (98). The aim is to create a heart valve substitute that exhibits the mechanical integrity of native tissue.

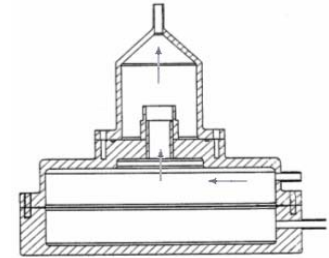
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Description of Bioreactor Design

Image

Hoerstrup
(35, 72, 99, 100)

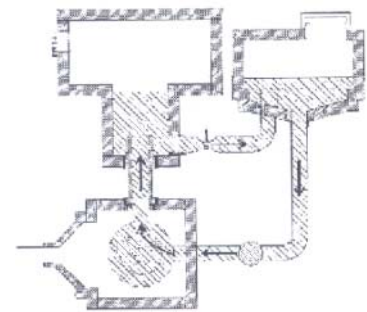
This bioreactor consists of two chambers separated by a flexible silicone diaphragm. The chamber below the diaphragm is periodically filled with air by a respirator pump in order to displace the silicone diaphragm. This displacement forces the media in the chamber above the diaphragm through the heart valve construct. By adjusting the stroke volume and ventilation rate of the pump, pulsatile flow rates from 50 to 2L/min and pressures ranging from 10 to 240mmHg are possible.



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Dumont
(50)

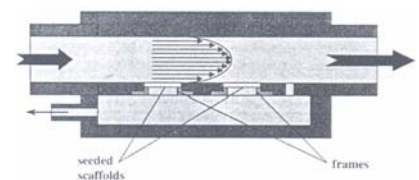
This design is based on a mock pulsatile flow loop of the left ventricle and a two-element windkessel model. A bladder is compressed and decompressed by the movement of a piston forcing medium through the developing tissue. A mechanical valve is added to ensure flow is in one direction. A chamber filled with a known quantity of air provides a form of compliance, which represents the elastic function of the large arteries. Distal to the compliance chamber is a non-linear resistance to simulate the peripheral resistance of the circulation. With such a system (controllable resistance, compliance, stroke volume and frequency), a wide physiological range of hydrodynamic conditions can be simulated.



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Jockenhoevel
(46)

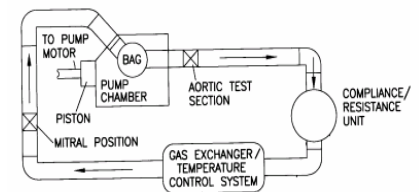
This bioreactor consists of a peristaltic pump producing a laminar flow rate of 250-500 ml/min through a chamber that houses developing PGA scaffolds. The flow chamber is divided into two sections: one section above and one below the developing constructs. In the upper section, a relatively high flow rate applies shear stress to the construct surface. The lower section provides nutritional support to the tissue sample under a lower flow rate to facilitate a higher level of mass transfer.



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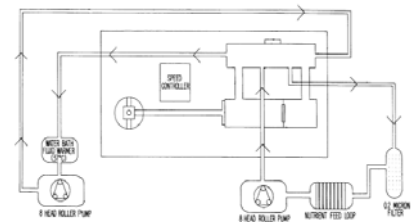
Goldstein
(69)

This patented bioreactor consists of a mechanical mitral valve, a bladder simulating the left ventricle, a test section with aortic valve samples mounted in parallel, a downstream compliance and resistance element, an oxygenator and a heat exchanger. The flexible rubber bladder is mounted inside a chamber filled with water, which approximates the left ventricle. The piston displaces the water; this in turn squeezes the flexible bladder and propels the media out through the engineered valve. The piston motion is programmed to produce a flow that simulates the cardiac cycle with a variable frequency from 60-120 beats per minute and a cardiac output of 2-7.5L/min.



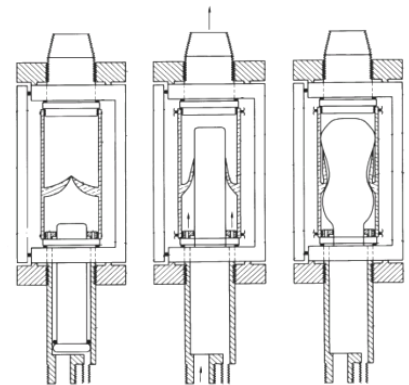
Elizondo
(101)

This patented bioreactor includes a processing chamber to house the heart valve, an environmental control circuit, a nutrient supply circuit and a pulse evaluation unit. The environmental control circuit provides temperature control to the system. The nutrient supply circuit pumps nutrients through the system. The pulse evaluation circuit consists of pump and a timing circuit for producing pressure and a vacuum in order to displace a diaphragm and in turn create pulsatile flow in the system.



Wolfinbarger
(102)

An inflatable piston is utilized in this design to extend into the luminal volume of a developing heart valve during various stages of processing in order to control/change the volume of media flow in the lumen of the tissue. The piston is inflated using air pressure, controlled by a pressure regulator and applied at the air pressure inlet, such that the air moves into the pressure chamber forcing the piston out its shaft and into the lumen of the developing heart valve. For tissue conditioning, a peristaltic pump provides pulsatile flow through the remaining volume of the lumen.



The bioreactor designs described above typically rely on pulsatile flow to generate a complex biomechanical environment resembling *in vivo* conditions (16, 29, 35, 103, 104), to promote both the development of mechanical strength (29, 72, 104) and the modulation of cellular function (105) within the engineered tissue. Jockenhoevel (46) developed a flow system to investigate the impact of laminar flow on extracellular matrix formation and tissue development. It has been reported that the application of laminar flow showed a significant increase in mechanical properties when compared with static and turbulent culture conditions (59, 106). Jockenhoevel (46), whose bioreactor is designed to use collagen as a matrix material (15, 56, 107-109) states that the peaks of a pulsatile flow pattern lead to high distension forces at the fixation area which might destroy new tissue.

An alternative to the application of pulsatile and laminar flow in the engineering of heart valves is put forth by Engelmayer (110, 111) who designed a bioreactor to provide a simple mode of mechanical stimulation through cyclic flexure on tissue engineering scaffolds. Few bioreactors have been designed to study the individual effect of specific modes of mechanical stimulation on engineered cardiovascular tissues (112-116). Since flexure is a major mode of deformation in heart valve leaflets (117, 118), Engelmayer sought to develop a device to study the independent effect of flexure on tissue heart valve scaffolds and constructs.

2) Bioreactors designed for the tissue engineering of blood vessels

Tissue engineering endeavours to produce responsive and self-renewing vascular tissue with the inherent potential to repair and remodel according to the needs of the specific environment (119-121). Current treatments for atherosclerosis include the insertion of autografts, allografts, xenografts and synthetic grafts. However, the use of natural vessels such as saphenous veins or mammary arteries has seen limited success, with patency rates generally ranging between 50-70%. These vessels fail due to dimension restrictions, mechanical property mismatch and disjunction at the anastomoses (122-124). Transplanted allogeneic vessels suffer very high rates of allograft vasculopathy

(125, 126), approaching 100% in autopsy series of cardiac transplants. Modified bovine xenografts have also been employed with less success compared to autologous vein grafts (127). Synthetic vascular grafts implanted as a large vessel replacement have achieved a reasonable degree of success. However, in medium and small diameter prostheses loss of patency within several months after implantation is more acute. Graft failure due to thrombosis or intima hyperplasia with thrombosis is primarily responsible in failures within 30 days after implantation, and intimal hyperplasia formation is the reason for failures within 6 months after surgery (127). The major drawback of these vascular prostheses is that they consist of foreign body material without the ability to grow or to repair.

In attempting to engineer viable vascular tissue with the potential to repair and remodel, recent studies have focused on cell and tissue culture under pulsatile flow conditions to produce vessels with mechanical properties and histological organization similar to native tissue.

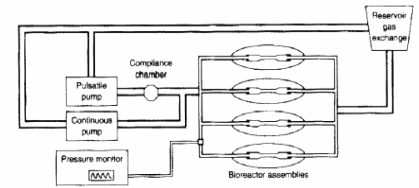
Researchers

Description of Bioreactor Design

Image

Niklason
(4, 13, 29)

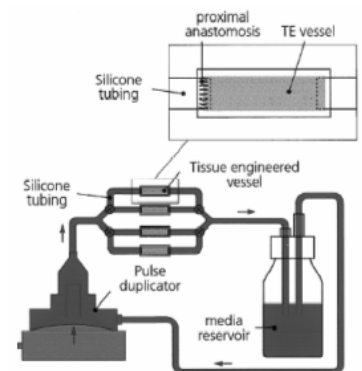
This bioreactor design incorporates a number of seeded vascular scaffolds which are conditioned in parallel under pulsatile flow. Two pumps are employed in the system to provide a continuous flow through each vascular construct with a superimposed pulsatile flow. Cyclic stretch, as a form of mechanical conditioning, is applied to each construct by means of the superimposed pulsatile flow.



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Hoerstrup
(35)

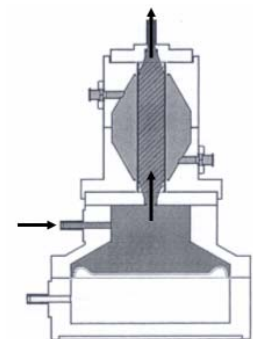
This bioreactor, developed for the conditioning of heart valves, is also used by Hoerstrup to tissue engineer small-caliber vascular grafts. A modification to the flow system incorporates multiple seeded vascular constructs in parallel. The pulsed flow of media (125mL/min at 30mmHg to 750mL/min at 55mmHg) is directed immediately through the vascular lumen, thereby generating direct shear stress to the luminal surface as well as periodical radial distension of the vessel wall.



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Sodian
(128)

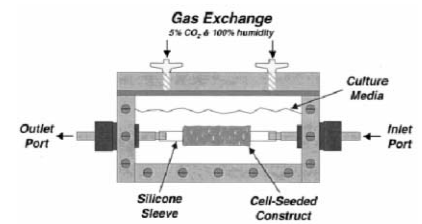
Further advancement on the Hoerstrup bioreactor design was reported by Sodian. The modified design consists of three chambers as opposed to the previous two chamber set-up used to engineer heart valves. The added chamber is for the cell-seeding and perfusion of a vascular conduit. The construct to be engineered is surrounded and continuously perfused by media with flow rates ranging from 100mL/min to 3L/min. Pressures in the range of 3 to 150mmHg and a shear stress of 1.12 to 32.45dyn/cm² have also been achieved.



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Seliktar
(29, 72, 104)

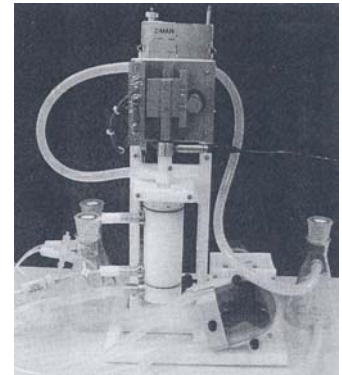
This bioreactor is designed to impart cyclic strain on developing vascular tissue. The tubular constructs are cultured over thin-walled silicone tubes, which are inflated with culture medium under pneumatic control to produce a cyclic distension of 10%. The tissue engineered vessel is exposed to dynamic conditioning through inflation and deflation of the conduits at a frequency of 1Hz.



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McCulloch
(129)

This bioreactor is designed to apply physiological conditions to tubular cardiovascular samples by emulating the in vivo pressure and straining environment. Circumferential strains can be provided by a pulsatile flow, created by a peristaltic pump through the construct lumen, and axial strains are imparted by elongating the construct by means of a strain induction mechanism. Throughout experimentation, the flow rates are maintained at 282mL/min with pressures ranging from approximately 70mmHg to 120mmHg. The strain induction mechanism creates a force that oscillates at amplitudes of 9, 25, 40 and 60 grams, at 1Hz, over a series of time periods.



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Williams
(130)

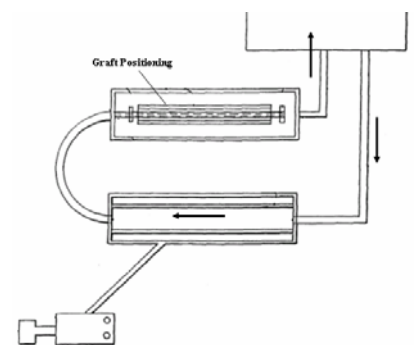
This cylindrical glass bioreactor was designed to house small arterial constructs. The scaffold is mounted between glass tubes and held in place by cable ties. Media is pumped through the lumen of the arterial construct (40mL/min at 1Hz) and simultaneously over its external surface by means of peristaltic pumps. The primary purpose of the external flow is to provide nutrients to the surface of the construct.



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Peterson
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A system was patented that primarily consists of a reservoir, pump, processing chamber and an alternating pressure source. The processing chamber houses an expandable tube on which vascular graft scaffolding is placed. The tube is expanded and contracted by an alternating pressure source. By expanding and contracting the tube, it places varying radial stress on the vascular graft scaffolding simulating physiological conditions. An alternative to Peterson's concept of housing the vascular graft in an expandable tube was devised and patented by Dunkelman. In this alternative set-up, the vascular graft is connected directly to the cap of the processing chamber using a luer. This cap provides a means for evenly diffusing fluid to multiple vascular grafts undergoing treatment within the processing chamber.



Conclusion

A current trend in the field of cardiovascular tissue engineering is the design of a stimulating environment in which to condition developing tissue. By controlling and monitoring of cardiovascular environmental conditions *in vitro*, bioreactors provide a method of achieving defined and reproducible studies that determine the specific biomechanical and biochemical parameters that play an important role in engineering tissue. The use of bioreactors has resulted in the development of cardiovascular constructs with better mechanical properties and morphological characteristics compared to those cultured statically.

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