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### IV.3. Bioreactors in tissue engineering.

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**Biomaterials and Tissue Engineering**  
Part 3 Bioreactors in Tissue Engineering

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## **I. Introduction**

### **a. What is a bioreactor?**

Bioreactors have been used for many years in areas other than tissue engineering. They have been used in diverse areas such as in fermentation, in water treatment, in food processing and in the production of pharmaceuticals [1]. All of these bioreactors are devices in which biological or biochemical processes develop under a closely monitored and tightly controlled environment. Bioreactors have been used in animal cell culture since the 1980s in order to produce vaccines and other drugs and to culture large cell populations. Bioreactors for use in tissue engineering have progressed from such devices. A tissue engineering bioreactor can be defined as a device that uses mechanical means to influence biological processes [2]. In tissue engineering, this generally means that bioreactors are used to stimulate cells and encourage them to produce extra-cellular matrix (ECM). There are numerous types of bioreactor which can be classified by the means they use to stimulate cells. A number of these will be discussed below.

### **b. Why are bioreactors needed in tissue engineering?**

Tissue engineering technologies are based on the biological triad of cells, signalling mechanisms and extracellular matrix. To simulate the development of tissues *in vitro*, tissue engineering aims to optimise cell growth, by providing regulatory signals in the form of growth factors and a regeneration template in the form of scaffold. Bioreactors may be used as an alternative to or in conjunction with growth factors in the signalling part of the triad. Defects requiring tissue-engineering solutions are typically many millimetres in size [3]. Scaffolds in such a size range are easily fabricated. However, problems arise when culturing cells on these scaffolds. Static culture conditions result in scaffolds with few cells in the centre of the construct [4]. This heterogeneous cell distribution is a major obstacle to developing any three-dimensional tissue or organ *in vitro*. It has been shown that despite homogeneous cell seeding, after long periods in culture, more cells are found on the periphery of demineralised trabecular bone constructs [4]. This is due to cell necrosis and cell chemotaxis. Necrosis occurs at the centre of the

scaffold due to a lack of nutrient delivery to, and waste removal from that area. The only mechanism by which nutrients and waste can move when a scaffold is in static culture is by diffusion. As the size of the scaffold increases, diffusion to the centre of the construct becomes more difficult. In addition, as cells on the periphery grow and secrete extracellular matrix, movement of fluid to the interior of the scaffold is further impeded. Chemotaxis of the cells from the interior towards the periphery occurs because of the concentration gradient in nutrients that has been set up [3]. Nutrient concentration is greater at the periphery so cells move along this gradient towards the periphery in order to obtain the nutrients they require. It has been found that mineralised bone matrix reaches a maximum penetration depth of 240  $\mu\text{m}$  on poly( DL-lactic-*co*-glycolic acid) scaffolds seeded with stromal osteoblasts, which is far thinner than an ideal bone graft replacement [5].

Figure 5.3.1 shows the distribution of smooth muscle cells in tissue engineered vascular tissue. Homogeneous distribution has been achieved by using a bioreactor [6]. In order to increase cell viability throughout a scaffold, fluid transport needs to be enhanced. A bioreactor may be used to achieve this aim. In addition to enhancing cell distribution, another important aspect of bioreactor use is cell stimulation. Cells respond to mechanical stimulation and bioreactors can be used to apply such stimulation. This can encourage cells to produce ECM in a shorter time period and in a more homogeneous manner than would be the case with static culture. For example, in comparisons between ECM protein levels after 5 weeks in culture, scaffolds cultured under hydrostatic pressure showed significant improvements over scaffolds cultured in static medium [7]. A benefit of ECM production is the increase in mechanical stiffness that it provides to the construct. A six-fold increase in equilibrium aggregate modulus (an intrinsic property of cartilage which is a measure of stiffness) was found after 28 days of culture in a compression bioreactor compared to free swelling controls [8].

Another important application of bioreactors is in cellular differentiation. Mechanical stimulation can be used to encourage stem cells down a particular path and hence provide the cell phenotype required. Bioreactors can provide biochemical and physical regulatory

signals that guide differentiation [9]. There is great potential for using mesenchymal stem cells and other multipotent cells to generate different cell types and bioreactors can play an important role in this process.

### **c. Bioreactor design requirements**

In general, bioreactors are designed to perform at least one of the following five functions: 1) providing uniform cell distribution, 2) maintaining the desired concentration of gases and nutrients in the medium, 3) providing mass transport to the tissue, 4) exposing tissue to physical stimuli, or 5) providing information about the formation of 3D tissue [10].

While the detailed requirements for bioreactor design are tissue- and/or application-specific, there are a few general principles which have to be adhered to when developing a bioreactor. The material selection is very important as it is vital to ensure that the materials used to create the bioreactor do not elicit any adverse reaction from the cultured tissue. Any material which is in contact with media must be biocompatible or bioinert. This eliminates the use of most metals, although stainless steel can be used if it is treated so that chromium ions do not leach out into the medium. Numerous plastics comply with this constraint but there are further limitations on material selection that must also be kept in mind. Materials must be usable at 37°C in a humid atmosphere. They must be sterilisable if they are to be re-used. Bioreactor parts can be sterilised by autoclaving or disinfected by submersion in alcohol. If they are to be autoclaved, materials that can withstand numerous cycles of high temperature and pressure must be used in bioreactor manufacture. Alternatively, some non-sterilisable disposable bioreactor parts may be used which can be replaced after each use of the bioreactor. Other material choices are between transparent or opaque and flexible or inflexible materials. Materials with different properties are needed for various components in the bioreactor. For example, transparent materials can be of benefit in allowing the construct to be monitored in the bioreactor during culture while flexible tubing can help with assembly of the bioreactor.

The design of the bioreactor should be as simple as possible, avoiding the introduction of, for example, machined recesses which could collect condensed steam during autoclaving and become breeding grounds for micro-organisms. Simplicity in design should also mean that the bioreactor is quick to assemble and disassemble. Apart from being more efficient, this ensures that cell-seeded constructs inserted into the bioreactor are out of the incubator for the minimum amount of time possible. This minimises the risk to the cells and the experiment being undertaken.

The specific application of the bioreactor must be kept in mind during the design process to ensure that all the design constraints are met. If various parameters such as pH, nutrient concentration or oxygen levels are to be monitored, these sensors should be incorporated into the design. If a pump or motor is to be used, it must be small enough to fit into an incubator and also be usable at 37°C and in a humid environment. The forces needed for cellular stimulation are very small so it is important to ensure that the pump/motor has the capability to apply small forces accurately. In any design involving fluids, problems can arise with leaking fluid seals and, if possible, the need for seals should be removed. However, in most cases, fluid seals are necessary and good design should decrease the problems with them. If a prototype bioreactor is being designed, it is worthwhile thinking about scale up opportunities for the bioreactor from the outset. This may mean designing a device that is relatively easy to enlarge without changing its characteristics or designing a simple device of which many more can be made so that numerous scaffolds can be cultured at one time.

## **II. Bioreactors in tissue engineering**

### **a. Spinner flask bioreactor**

Continuous stirred-tank reactors are commonly used in bioprocessing, for example in solid-state fermentation of organisms such as yeast [11] and spinner flask bioreactors for use in tissue engineering progressed from these devices. In a spinner flask (Figure 5.3.2), scaffolds are suspended at the end of needles in a flask of culture media. A magnetic stirrer mixes the media and the scaffolds are fixed in place with respect to the moving

fluid. Flow across the surface of the scaffolds results in eddies in the scaffolds' superficial pores. Eddies are turbulent instabilities consisting of clumps of fluid particles that have a rotational structure superimposed on the mean linear motion of the fluid particles. They are associated with transitional and turbulent flow. It is via these eddies that fluid transport to the centre of the scaffold is thought to be enhanced [3]. Typically, spinner flasks are around 120 ml in volume (although much larger flasks of up to 8 litres have been used), are run at 50-80 rpm and 50% of the medium used in them is changed every two days [12]. Cartilage constructs have been grown in spinner flasks to thicknesses of 0.5 mm [13]. While this is an improvement on cartilage grown in static medium, it is still too thin for clinical use. Mass transfer in the flasks is not good enough to deliver homogeneous cell distribution throughout scaffolds and cells predominantly reside on the construct periphery [3].

#### **b. Rotating wall bioreactor**

The rotating wall bioreactor was developed by NASA [14]. It was originally designed with a view to protecting cell culture experiments from high forces during space shuttle take off and landing. However, the device has proved useful in tissue engineering here on earth. In a rotating wall bioreactor (Figure 5.3.3), scaffolds are free to move in media in a vessel. The wall of the vessel rotates, providing an upward hydrodynamic drag force that balances with the downward gravitational force, resulting in the scaffold remaining suspended in the media. Fluid transport is enhanced in a similar fashion to the mechanism in spinner flasks and the devices also provide more homogeneous cell distribution than static culture [3]. Gas exchange occurs through a gas exchange membrane and the bioreactor is rotated at speeds of 15-30 rpm. Cartilage tissue of 5 mm thickness has been grown in this type of bioreactor after seven months of culture [15]. As tissue grows in the bioreactor, the rotational speed must be increased in order to balance the gravitational force and ensure the scaffold remains in suspension.

### **c. Compression bioreactors**

Another widely used type of bioreactor is the compression bioreactor. This class of bioreactor is generally used in cartilage engineering and can be designed so that both static loading and dynamic loading can be applied. This is because static loading has been found to have a negative effect on cartilage formation while dynamic loading, which is more representative of physiological loading, has provided better results than many other stimuli [16].

In general, compression bioreactors consist of a motor, a system providing linear motion and a controlling mechanism. An example of such a system is shown in Figure 5.3.5, where a cam-follower system is used to provide displacements of different magnitudes and frequencies. A signal generator can be used to control the system and load cells and linear variable differential transformers can be used to measure the load response and imposed displacement respectively [8, 17]. The load can be transferred to the cell-seeded constructs via flat platens which distribute the load evenly, however in a device for stimulating multiple scaffolds simultaneously, care must be taken that the constructs are of similar height or the compressive strain applied will vary as the scaffold height does. Mass transfer is improved in dynamic compression bioreactors over static culture (as compression causes fluid flow in the scaffold) and dynamic compression can also improve the aggregate modulus of the resulting cartilage tissue to levels approaching those of native articular cartilage [8].

### **d. Strain bioreactors**

Tensile strain bioreactors have been used in an attempt to engineer a number of different types of tissue including tendon, ligament, bone, cartilage and cardiovascular tissue. Some designs are very similar to compression bioreactors, only differing in the way the force is transferred to the construct. Instead of flat platens as in a compression bioreactor, a way of clamping the scaffold into the device is needed so that a tensile force can be applied. Tensile strain has been used to differentiate mesenchymal stem cells along the chondrogenic lineage. A multistation bioreactor was used in which cell-seeded collagen-

glycosaminoglycan scaffolds were clamped and loaded in uniaxial tension [18]. Alternatively, tensile strain can also be applied to a construct by attaching the construct to anchors on a rubber membrane and then deforming the membrane. This system has been used in the culture of bioartificial tendons with a resulting increase in Young's modulus over non-loaded controls [19].

#### **e. Hydrostatic pressure bioreactors:**

In cartilage tissue engineering, hydrostatic pressure bioreactors can be used to apply mechanical stimulus to cell-seeded constructs. Scaffolds are usually cultured statically and then moved to a hydrostatic chamber for a specified time for loading. Hydrostatic pressure bioreactors consist of a chamber which can withstand the pressures applied and a means of applying that pressure (Figure 5.3.6). For example, a media-filled pressure chamber can be pressurised using a piston controlled by an actuator [16]. For sterility, the piston can apply pressure via an impermeable membrane so that the piston itself does not come into contact with the culture media. Variations on this design include a water-filled pressure chamber which pressurises a media-filled chamber via an impermeable film and is controlled using a variable backpressure valve and an actuator [20].

#### **f. Flow perfusion bioreactor**

Flow perfusion bioreactors generally consist of a pump and a scaffold chamber joined together by tubing. A media reservoir may also be present. The scaffold is kept in position across the flow path of the device (Figure 5.3.4). Media is perfused through the scaffold, thus enhancing fluid transport.

Culture using flow perfusion bioreactors has been shown to provide more homogeneous cell distribution throughout scaffolds. Collagen sponges have been seeded with bone marrow stromal cells and perfused with flow. This has resulted in greater cellularity throughout the scaffold in comparison to static controls, implying that better nutrient exchange occurs due to flow [21]. Using a biphasic calcium-phosphate scaffold, abundant ECM with nodules of CaP was noted after 19 days in steady flow culture [22].

In comparisons between flow perfusion, spinner flask and rotating wall bioreactors, flow perfusion bioreactors have proved to be the best for fluid transport. Using the same flow rate and the same scaffold type, while cell densities remained the same using all three bioreactors, the distribution of the cells changed dramatically depending on which bioreactor was used. Histological analysis showed that spinner flask and static culture resulted in the majority of viable cells being on the periphery of the scaffold. In contrast, the rotating wall vessel and flow perfusion bioreactor culture resulted in uniform cell distribution throughout the scaffolds [3, 23]. After 14 days in culture, the perfusion bioreactor had higher cell density than all other culture methods [3].

In our laboratory, a flow perfusion bioreactor has been developed to examine the effects of different flow profiles on cell-seeded collagen-glycosaminoglycan scaffolds [24]. The scaffold chamber was specifically designed to ensure that the compliant scaffold was under perfusive flow. This involved using a scaffold of larger diameter than the flow path and using spacers to ensure the scaffold was under 10% compression during culture. A programmable syringe pump was used in order to stimulate the cell-seeded constructs using different flow profiles. As discussed below, this device demonstrated that intermittent flow perfusion is advantageous for mechanically stimulating osteoblasts while maintaining cell viability.

#### **g. Combined bioreactors**

In addition to the bioreactors mentioned thus far, numerous combinations of the different types of bioreactor have been used in order to better mimic the *in vivo* environment *in vitro*. In most cases, these more complicated bioreactors involve adding a perfusion loop on top of a standard bioreactor. Examples include compression, tensile strain or hydrostatic bioreactors with added perfusion [7, 20, 25]. These different bioreactors allow for nutrient exchange to take place due to perfusion while stimulation occurs due to a different mechanical stimulus. Bioreactors for engineering very specialised tissues have also been developed. One example of such a bioreactor is a combined tensile and vibrational bioreactor for engineering vocal fold tissue [26]. This bioreactor mimics the

physiological conditions that human vocal folds experience in an attempt to develop the tissue in the laboratory (Figure 5.3.7). Vocal fold tissue experiences vibrations of 100-1000 Hz at an amplitude of approximately 1 mm, which is a unique stimulus in the human body [27].

### **III. Tissue formation in bioreactor systems**

Tissue engineering of all three dimensional tissues require homogeneous cell distribution in order for homogeneous tissue to develop. Therefore, there is a need for bioreactor culture in numerous different disciplines in tissue engineering. Bioreactor culture has been used in a diverse range of applications including skin, bladder and liver tissue engineering [1, 28]. Here, the use of bioreactor culture in bone, cartilage and cardiovascular tissue engineering will be discussed.

#### **a. Bone**

Bone is comprised of a mineral phase, collagen and cells. The mineral phase of bone is a hard, brittle material (hydroxyapatite) that is strong in compression but cannot withstand large shear or tensile loads. In contrast, collagen fibres are strong in tension and flexible in bending but weak in compression. Bone grafts are required to aid bone defect and non-union healing. The aim in using them is to restore function to the damaged area as quickly and completely as possible [29]. They are required in a number of procedures including, for example, replacing diseased bone, filling bone voids after non-unions or cyst removal, reconstructive surgery and in spinal fusion operations. The most commonly used graft in bone replacement is the autograft. Autografts are grafts taken from the patients themselves, usually from the iliac crest, although the distal femur, the greater trochanter, and the proximal tibia can also be used [29]. This process is expensive and the size of graft that can be obtained is limited. Morbidity of the site the graft is acquired from is another problem and complications can arise due to infection and chronic pain [29]. An alternative to the autograft is the allograft. The term allograft is used for bone grafts which are obtained from an organ donor. A drawback to this option

is the danger of infection. Xenografts, acquired from animal bone, are another alternative. However, the morphology of the bone is different to that of humans and the risk of cross-species infection exists. Coral has also been used but its structure is very different to that of bone and hence osteointegration of this type of graft has proved difficult [30].

The use of natural bone grafts has proved problematic and therefore attention has turned to tissue engineering. Engineered tissue must be strong enough to allow load bearing after implantation. Resorbability of the scaffold is another important issue. Ideally, as the scaffold is resorbed, bone should be deposited in its place, thus ensuring no loss in mechanical strength. A morphology that allows movement of cells and supports vascularisation is important for a scaffold material. A high porosity and controllable pore size can provide this. Cells must be able to penetrate into the core of the scaffold so that they are homogeneously distributed throughout the graft. There must be space to allow for transport of nutrients to and waste removal from cells, so the pores must be interconnected [31]. Thus, a balance must be struck between mechanical strength and porosity to develop an ideal scaffold for bone tissue engineering. The use of a bioreactor with such a scaffold should provide a homogeneous distribution of stimulated cells.

It has been shown that fluid flow can stimulate bone cells to increase levels of bone formation markers [4, 32-37] and its use could improve mineralisation of the scaffold on which cells are seeded. Flow perfusion bioreactors increase alkaline phosphatase (ALP) expression after 7 and 14 days more than spinner flasks or rotating wall vessels [3] and are more commonly used than any other bioreactor for use in 3-D stimulation studies. In one study, MC3T3-E1 cells were seeded on decalcified human trabecular bone, the flow rate of perfusion altered and the mRNA expression of Runx2, OC and ALP measured [4]. It was found that using a steady flow rate of only 1 mL/min killed nearly all the cells on the scaffold after 7 days in culture. However, a flow rate of 0.01 mL/min led to a high proportion of viable cells both on the surface and inside the scaffold. This compared favourably to static culture, where cells were predominantly on the periphery [4].

Using a CaP scaffold and a flow rate of 0.025 mL/min in a flow perfusion bioreactor, PGE<sub>2</sub> levels were found to increase over static controls. When a stimulus of 30 minutes

of oscillatory flow at 1 Hz with a 40 mL/min peak was superimposed on the steady flow, PGE<sub>2</sub> levels increased further. The number of cells left residing on the scaffolds decreased due to this large dynamic stimulus but this decrease was not found to be statistically significant [38]. When different flow profiles were used intermittently to stimulate cells on a highly porous collagen-glycosaminoglycan scaffold in our laboratory, it was found that intermittent flow caused greater stimulation than a continuous low flow rate without a loss in cell number [39]. Cyclooxygenase-2 and osteopontin expression increased due to culture in the bioreactor, as did prostaglandin E<sub>2</sub> production. This lends further backing to the hypothesis that the combination of a perfusion period (for nutrient delivery and waste removal) and a stimulation period may deliver enhanced fluid transport with enhanced stimulation of cells and may yet prove to be the optimum regime for bioreactor culture of bone cells.

## **b. Cartilage**

Cartilage is a supporting tissue containing chondroitin sulphates, collagen and elastic fibres and cells. The cells present in cartilage are known as chondrocytes and they are situated in lacunae in the cartilage matrix. Cartilage is avascular and nutrient and waste product exchange occurs purely by diffusion through the cartilage matrix. When minor damage occurs to cartilage, it can repair by appositional growth, but when severe damage occurs, the body cannot replace the cartilage [40]. Cartilage tissue engineering may offer the solution to this problem. There are three types of cartilage: hyaline cartilage, elastic cartilage and fibrocartilage. Joints can contain both hyaline cartilage and fibrocartilage, with the more flexible hyaline cartilage covering the bone and the more durable fibrocartilage acting as a shock-absorber between bones. As a joint moves, there is motion between two articulating layers of cartilage. This deforms the cartilage, causes fluid flow within it and induces a hydrostatic pressure load on it. These mechanical forces affect the chondrocytes in the cartilage. The force applied, along with the length of time it is applied for and the frequency of application modifies the response of chondrocytes [41]. This is useful in bioreactor design and for use in cartilage tissue engineering; if the

correct stimulation pattern is used, chondrocytes can be induced to produce more extracellular matrix and this can result in more cartilage-like tissue being formed.

After twenty weeks in static culture, the aggregate modulus of tissue-engineered cartilage was  $179 \pm 9$  kPa. This is 40% of the value reported for native cartilage [42]. At twenty five weeks, the modulus had not increased further so this may be the closest approximation to cartilage that can be cultured without the aid of a bioreactor. The most commonly used bioreactors in cartilage tissue engineering are compression bioreactors. When free swelling controls were compared to dynamically loaded agarose gels, after 28 days in culture, there was a six-fold increase in the equilibrium aggregate modulus for the loaded gels [8]. A sinusoidal strain of 10% at 1 Hz was applied to the gels for five days per week for a total of three hours per day with a rest period of one hour between each hour of loading. This complex loading pattern was deemed to be physiological and it resulted in increased glycosaminoglycan content over free swelling controls after 21 days in culture. The combination of increased modulus and increased glycosaminoglycan formation over free swelling controls after only four weeks in culture demonstrates the benefits of bioreactor culture in cartilage tissue engineering.

Compression bioreactors have also been used to examine the effect of loading on the differentiation of bone marrow mesenchymal stem cells down the chondrocytic lineage. Growth factors such as transforming growth factor (TGF- $\beta$ ) can also be used to encourage differentiation. In a study comparing the use of compressive loading, the use of TGF- $\beta$  and the use of a combination of loading and TGF- $\beta$ , it was found that compressive loading alone was just as effective at inducing chondrogenic differentiation as TGF- $\beta$  or TGF- $\beta$  plus loading [17].

### **c. Cardiovascular tissue**

There are three main types of tissue that are encompassed by the heading cardiovascular tissue, namely: vascular, cardiac muscle and heart valve tissue. These different tissues experience different mechanical environments in the body and therefore, in tissue

engineering, they are cultured using different bioreactors and under different loading conditions in an attempt to recreate physiological loading conditions in the laboratory.

### *Vascular tissue*

Blood vessels must be flexible enough to move during physiological activities and yet tough enough to withstand the pressure changes that occur as the heart beats. Blood vessels consist of three layers of tissue: the tunica intima, the tunica media and the tunica externa. The intima (innermost part of the blood vessel) consists of an endothelial lining and a layer of connective tissue containing elastic fibres. The media consists of smooth muscle tissue and loose connective tissue and in arteries, the externa consists of connective tissue and collagen fibres. Arteriosclerosis is associated with half of all deaths in the United States each year [40]. It is a thickening and toughening of the arterial walls and can lead to coronary heart disease if it occurs in the coronary arteries or stroke if it occurs in the arteries supplying the brain with blood. Coronary heart disease can be treated by bypass surgery, when a section of artery or vein is removed from elsewhere in the body and used to bypass the blockage. Drawbacks with this procedure are that it creates another trauma site in the body and often, sufficient tissue for bypass is not available [6]. Research into tissue engineering of vascular tissue is proving promising for growing arteries *in vitro* that may be used instead of the patient's own blood vessels.

The ideal graft for vascular bypass consists of a confluent endothelium at the lumen and a smooth muscle layer surrounding it with sufficient mechanical integrity for suture retention and tolerance of arterial pressures. The endothelial layer is important as a confluent layer of endothelial cells inhibits the proliferation of smooth muscle cells. The smooth muscle cells are therefore more likely to be quiescent and this decreases the risk of thrombosis formation and luminal occlusion [6]. In order to recreate the structure of blood vessels *in vitro*, co-cultures of different cell types must be used. Scaffolds can be cultured with smooth muscle cells initially and then a layer of endothelial cells can be seeded as the culture period nears its end.

The arteries nearest the heart experience the highest pressures and they have resilient walls in order to cope with these changing pressures. They are known as elastic arteries and their tunica media contains a high proportion of elastic fibres. This enables them to expand as the pressure inside the lumen increases and recoil when the pressure decreases, thus allowing them to deal with pressure changes but also to damp the pressure oscillations to make blood flow continuous rather than oscillatory in nature [40]. It is this expansion and recoil of blood vessels that is the inspiration for a number of bioreactors for use in vascular tissue engineering.

Some success has been achieved when culturing vascular tissue in both rotating wall vessels [13] and biomimetic bioreactors [6, 25]. Biomimetic bioreactors use combinations of strain and perfusion to stimulate vascular tissue development. In one such bioreactor, a tube of biodegradable polymer was seeded with smooth muscle cells and silicone tubing placed through the centre of the tube. The construct was maintained in a bath of stirred media and the highly distensible silicone tubing was pulsed at 165 beats per minute so that the scaffold experienced 5% radial strain over an eight week culture period [6]. The silicone tubing was then removed, an endothelial cell lining applied to the inner layer of the construct and culture medium perfused through the centre of the scaffold. This set up resulted in vessels with a rupture strength similar to that of native tissue and with a morphology that showed migration of smooth muscle cells throughout the construct. In contrast, scaffolds kept in static culture over the same culture period showed no migration of smooth muscle cells and therefore an inhomogeneous structure. A bioreactor has also been designed to apply both radial and axial strain to cell-seeded constructs, to mimic the environment in the body more closely [25]. The bioreactor has two perfusion loops that feed the internal and external parts of the scaffold. The internal perfusion can be pulsatile in nature and is of variable frequency.

### *Cardiac muscle tissue*

Cardiac muscle cells are called cardiocytes or cardiomyocytes. They form extensive connections with each other and are almost totally dependent on aerobic metabolism for energy. When a myocardial infarction (heart attack) occurs, part of the blood supply to

the heart muscle is blocked and cardiac muscle cells die due to lack of oxygen delivery [40]. Cardiac muscle only has a limited ability to regenerate and scar tissue formed may create additional problems by constricting other vessels in the damaged region [40, 43]. Cardiac muscle tissue engineering is therefore of interest in developing a method for myocardial repair. Oxygen delivery to cardiac cells is vital in order that a sufficiently thick layer of tissue can be grown in vitro. This means that the use of bioreactors that increase oxygen delivery to cells is important in cardiac muscle tissue engineering. The ideal tissue-engineered cardiac muscle tissue graft should have a dense, uniform distribution of cardiomyocytes, should contract in response to an electrical stimulus and have the mechanical integrity to allow implantation [43].

Polyglycolic acid (PGA) scaffolds have been seeded with cardiomyocytes and cultured in spinner flasks and rotating wall bioreactors [44, 45]. In both these culturing systems, a peripheral region densely populated with cells and a central region with a sparse population of cells was apparent. Spontaneous contractions of the tissue occurred during culture using the spinner flask and impulse propagation occurred due to electrical stimulation, but only in the peripheral region [44]. Use of a perfusion system, however, delivered a much more uniform distribution of cells with higher cell number on perfused scaffolds than scaffolds cultured in an orbitally mixed dish at all time points up to 7 days [43]. Spontaneous contractions were also observed in the perfusion system but only up to 5 days after seeding. Perfused scaffolds contracted synchronously and at constant frequency upon electrical stimulation after seven days, while dish-cultured scaffolds exhibited an arrhythmic contraction pattern [43].

### *Heart valves*

There are four valves in the heart that prevent backflow of blood from the ventricles to the atria or from the pulmonary trunk and aorta into the ventricles. Problems with these valves decrease the heart's ability to maintain circulatory flow [40]. In severe cases, valve replacement is the only option. However, thromboembolism is a substantial risk when mechanical valves are used to replace native valves and xenografts or other non-viable tissue-based grafts often fail due to calcification [46, 47]. Patients with congenital

malformations of the valves often require a durable replacement that can grow as the patient does, and a tissue-engineered valve may therefore be the best option.

Heart valves experience a complex mechanical environment including high bending stress and high shear stress *in vivo* [10]. When grown in static culture, valve leaflets are fragile, have a rough surface and a low suture retention strength. In bioreactor culture under pulsatile flow, however, leaflets that are intact, mobile, pliable, competent during closure and have a tensile strength greater than that of native tissue are formed after 14 days [46]. In this case, the pulse was applied to the scaffolds by pumping air into a chamber connected to the scaffold chamber via a silicone diaphragm. Scaffolds were seeded with myofibroblasts initially and after a four day culture period, with endothelial cells. The pressure at which the pulse was applied was increased over time, as was the flow rate of the media. Thus, as the scaffold became stronger, the forces applied to it were increased. The resulting valve leaflets were implanted into lambs and there was no evidence of thrombus formation up to 20 weeks after valve replacement. There was some evidence of pulmonary regurgitation at 16 and 20 weeks, however. Optimisation of many aspects of the culturing process remains to be completed but bioreactor culture of valve leaflets has enabled a functioning heart valve to be developed *in vitro* [46].

#### **IV. Bioreactors: state of the art and future directions**

The use of bioreactors has brought us a step closer to engineering numerous tissue types in the laboratory. At present, most bioreactors are specialised devices with low volume output. Their assembly is often time consuming and labour intensive. Many also exhibit operator dependent variability. While scaled-up versions of some devices may be useful for developing larger amounts of tissue, problems with process consistency and process contamination may persist. A better understanding of the different effects of mechanical stimulation on cell signalling and mechanotransduction is also needed. This can be achieved through the use of existing simple bioreactors in conjunction with numerical simulation of culture conditions to minimise the number of experiments needed.

In the future, ways of minimising the time and effort needed in order to form tissue must be found if costs are to be minimised and the use of engineered tissue is to become routine clinically. One way to do this is to automate the process. The ideal bioreactor would need autologous material and a scaffold as inputs and, after a defined culture period, would output the required amount of the required tissue. Automated systems for culturing cells already exist and work has begun on extending them to incorporate mechanical stimulation into the culturing process. Aastrom has developed a system that takes bone marrow as an input and expands the stem and progenitor cell population. The Automation Partnership has numerous systems for expanding cells. If systems such as these are used in conjunction with monitoring systems and a feedback loop, so that factors such as the temperature, oxygen level and pH can be regulated, cell culture can be optimised. Closed bioreactor systems for seeding and culturing skin grafts under perfusion were developed by Advanced Tissue Sciences before the company's liquidation in 2002. As this system demonstrated, the technology exists to enable the incorporation of mechanical stimulus into an automated cell culture system and this may be the future for bioreactors in tissue engineering.

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Figure 5.3.3. A rotating wall vessel bioreactor. Scaffolds are suspended in medium due to opposing gravitational and drag forces  
([www.che.msstate.edu/research/MDERL/demos/DEMOBioRctrExperiment\\_files/image002.jpg](http://www.che.msstate.edu/research/MDERL/demos/DEMOBioRctrExperiment_files/image002.jpg))

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## Figures



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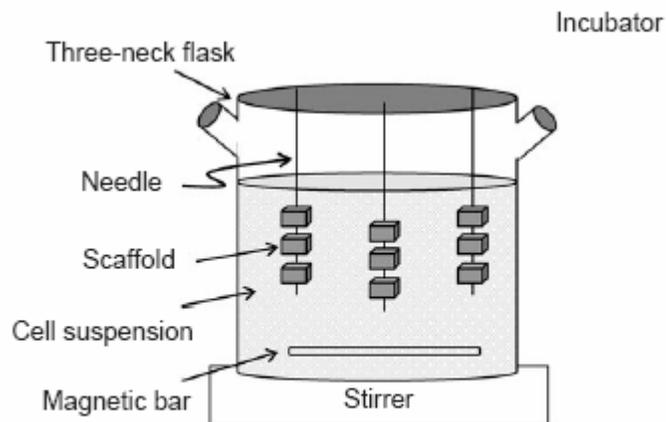


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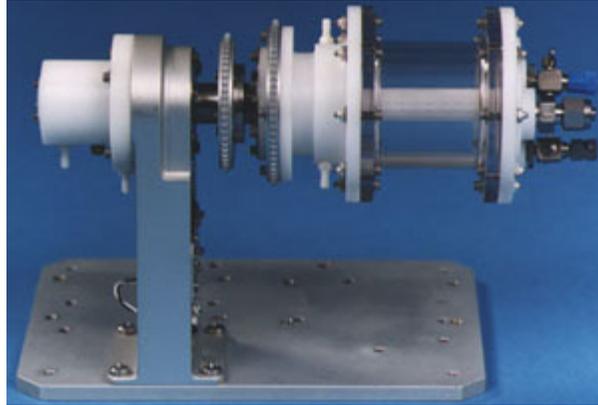


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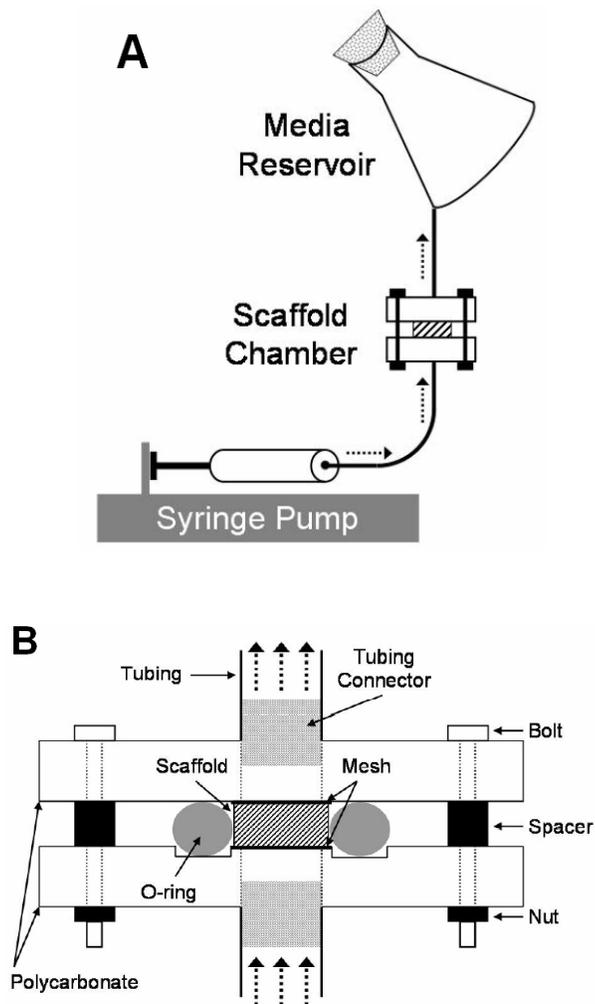


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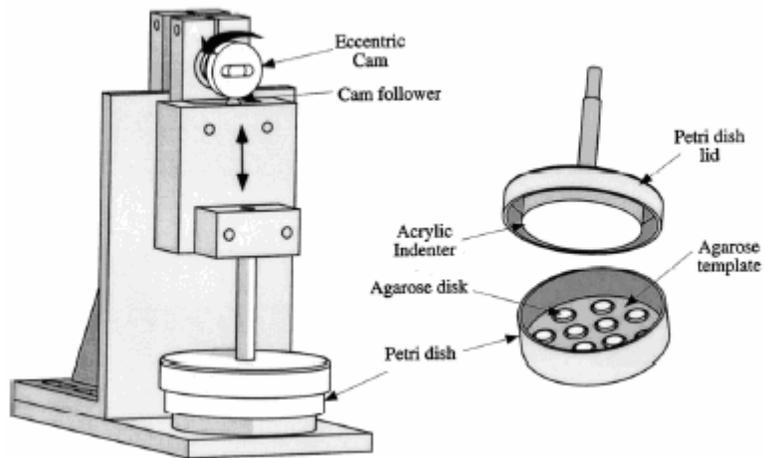


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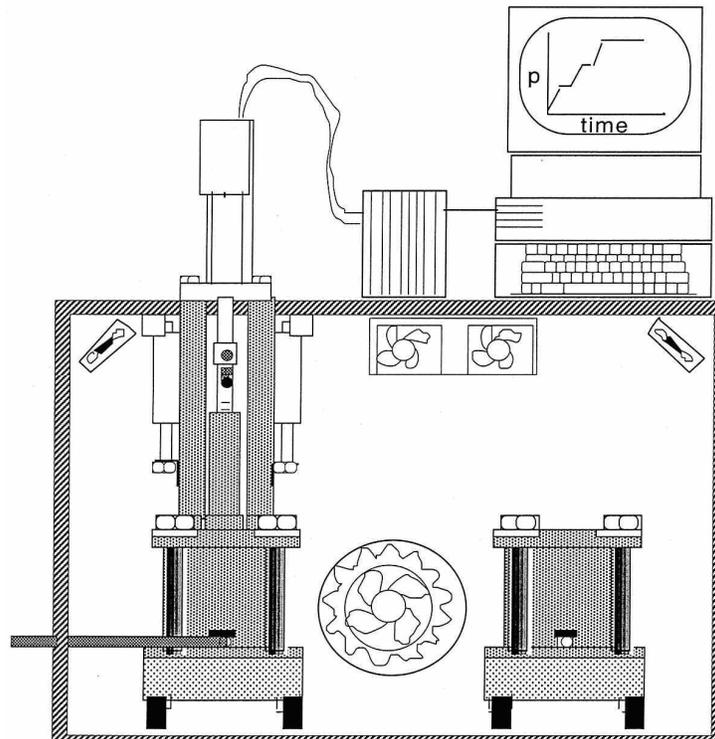


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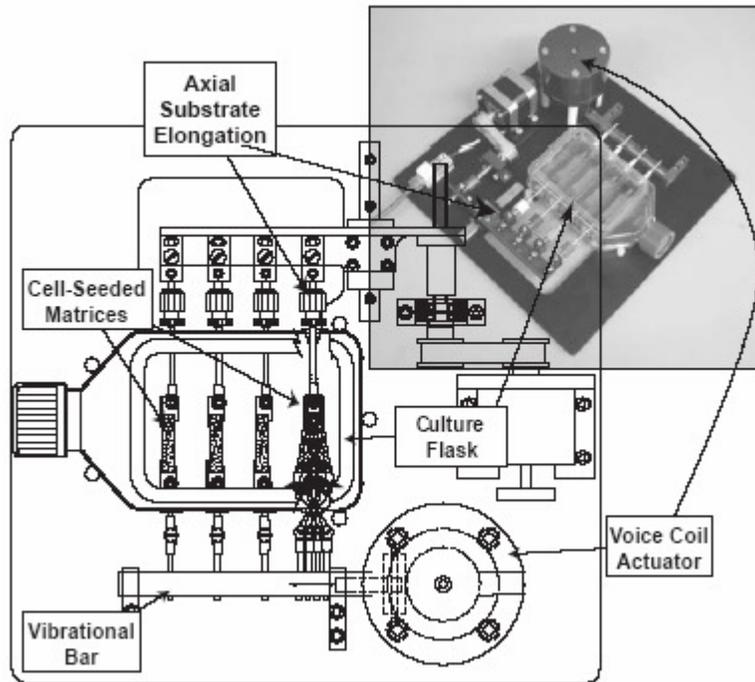


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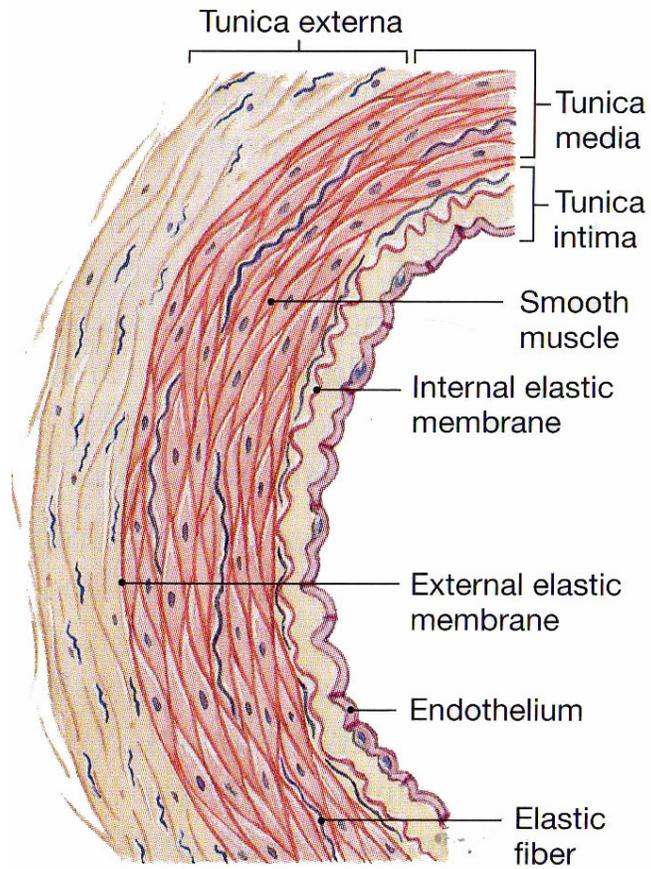


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