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1. Introduction

1.1 Tissue Engineering

The field of tissue engineering (regenerative medicine) aims to repair and regenerate damaged tissues by developing biological substitutes that mimic the natural extracellular matrix to help guide the growth of new functional tissue in vitro or in vivo to restore, maintain or improve tissue function. Tissue engineering technologies are based on a biological triad and involve the successful interaction between three components: (1) the scaffold that holds the cells together to create the tissue’s physical form, (2) the cells that synthesise the tissue and (3) signalling mechanisms (i.e., mechanical and chemical signals) that direct the cells to express the desired tissue phenotype (Langer & Vacanti, 1993). Bioreactors can be used to provide the signals in this latter area in order to influence biological processes by the application of a mechanical stimulus, and may also be used as an alternative to, or in conjunction with growth factors.

![Tissue Engineering Triad](image)

Fig. 1. The tissue engineering triad; factors that need to be considered when designing a suitable structure for tissue engineering applications (Lyons, et al., 2008).
1.2 Use of Bioreactors in Tissue Engineering

A tissue engineering bioreactor can be defined as a device that uses mechanical means to influence biological processes (Darling & Athanasiou, 2003). Bioreactors can be used to aid in the in vitro development of new tissue by providing biochemical and physical regulatory signals to cells and encouraging them to undergo differentiation and/or to produce extracellular matrix prior to in vivo implantation. Bioreactors are devices in which biological or biochemical processes develop under a closely monitored and tightly controlled environment.

Cells respond to mechanical stimulation and bioreactors can be used to apply mechanical stimulation to cells. This can encourage cells to produce extracellular matrix (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 of equine articular chondrocytes cultured on polyglycolic acid scaffolds after 5 weeks in culture, constructs cultured under hydrostatic pressure showed significant improvements over constructs cultured in static medium (Carver & Heath, 1999). 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 (Mauck, et al., 2000). 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 (Altman, et al., 2002). 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.

As well as providing mechanical stimulation, bioreactors can also be used to improve cellular spatial distribution. A heterogeneous cell distribution is a major obstacle to developing any three-dimensional tissue or organ in vitro. Defects requiring tissue engineering solutions are typically many millimetres in size (Goldstein, et al., 2001). Scaffolds in such a size range are easily fabricated, however, problems arise when culturing cells on these scaffolds. As the size of the scaffold increases, diffusion of nutrients to the centre of the construct becomes more difficult. Static culture conditions result in scaffolds with few cells in the centre of the construct (Cartmell, et al., 2003). It is hypothesised that this is due to limited cell penetration during seeding, cell migration to the scaffold periphery during culture, or cell death in the centre of the scaffold (Glowacki, et al., 1998, Ishaug-Riley, et al., 1998, Goldstein, et al., 2001, Yu, et al., 2004, Gomes, et al., 2005). The only mechanism by which nutrients and waste can move when a scaffold is in static culture is by diffusion. It has been shown that despite homogeneous cell seeding, after long periods in culture, more cells are found on the periphery of constructs (Cartmell, et al., 2003) leading to peripheral encapsulation which hinders nutrient and waste exchange from the centre, resulting in core degradation of tissue engineered constructs. This is of major concern in the field of tissue engineering, and is a major obstacle in the formation of a viable tissue in vitro. For this reason, for a number of tissue types, the move towards clinical trials has been slow and progress to date in engineering significant quantities of functional tissue in vitro for implantation in humans in vivo has been somewhat disappointing.

Thus, bioreactors can be used in tissue engineering applications to overcome problems associated with traditional static culture conditions, improve cellular distribution and
accelerate construct maturation (Freed, et al., 2006) whilst applying biophysical signals to constructs to improve tissue formation in vitro prior to in vivo implantation. In general, bioreactors are designed to perform at least one of the following five functions, to (1) provide a spatially uniform cell distribution, (2) maintain the desired concentration of gases and nutrients in culture medium, (3) facilitate mass transport to the tissue, (4) expose the construct to physical stimuli and/or (5) provide information about the formation of 3D tissue (Vunjak-Novakovic, et al., 1998, Bancroft, et al., 2003).

1.3 Bioreactor Design Requirements
The design of the bioreactor should be as simple as possible e.g. avoiding the introduction of machined recesses which could 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 detailed requirements for bioreactor design are tissue- and/or application-specific, however, 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 able to be sterilised 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 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 reduced. 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.

2. Types of Bioreactors in Tissue Engineering

Bioreactors are laboratory tissue culture devices that provide a controllable, mechanically active environment that can be used to study and potentially improve engineered tissue structure, properties and integration. Many tissue engineering studies employ conventional methods (static seeding) that result in constructs comprising a thin tissue-like layer at the base of the scaffold due to gravitational settling of the cells. In contrast, convective mixing (spinner flasks) and convective flow (flow perfusion) can improve initial cell seeding and homogeneity, and thereby improve tissue architecture (Freed, et al., 2006). Numerous types of bioreactors including spinner flasks, rotating wall, compression, strain and flow perfusion bioreactors are used in various tissue engineering applications, which will all be discussed below. All of these bioreactors rely on forced media flow through and/or around the scaffold to provide nutrient and waste exchange within the scaffold.

2.1 Spinner Flask Bioreactors

One of the most basic bioreactors, a spinner flask induces mixing of oxygen and nutrients throughout the medium and reduces the concentration boundary layer at the construct surface. In a spinner flask, 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 (figure 2). Flow across the surface of the scaffolds results in eddies which 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 (Goldstein, et al., 2001). 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 (Freshney, 2000). Cartilage constructs have been grown in spinner flasks to thicknesses of 0.5 mm (Freed & Vunjak-Novakovic, 2000). While this is an improvement on cartilage grown in static culture, 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.
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![Fig. 2. A spinner flask bioreactor. Scaffolds are suspended in medium and the medium is stirred using a magnetic stirrer to improve nutrient delivery to the scaffold](image)

2.2 Rotating Wall Bioreactors

The rotating wall bioreactor was developed by NASA (Schwarz, et al., 1992). 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, scaffolds are free to move in media in a vessel. A rotating wall vessel bioreactor consists of a cylindrical chamber in which the outer wall, inner wall, or both are capable of rotating at a constant angular speed (figure 3). The vessel wall is then rotated at a speed such that a balance is reached between the downward gravitational force and the upward hydrodynamic drag force acting on each scaffold. 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. Dynamic laminar flow generated by a rotating fluid environment is an alternative and efficient way to reduce diffusional limitations of nutrients and wastes while producing low levels of shear. Media can be exchanged by stopping the rotation temporarily or by adding a fluid pump whereby media is constantly pumped through the vessel. Fluid transport is enhanced in a similar fashion to the mechanism in spinner flasks and the devices also provide a more homogeneous cell distribution than static culture. 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 (Freed, et al., 1997). 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.
2.3 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 and dynamic loading can be applied. This is because static loading has been found to have a negative effect on cartilage formation in comparison to dynamic loading, which is more representative of physiological loading (Darling & Athanasiou, 2003). In general, compression bioreactors consist of a motor, a system providing linear motion and a controlling mechanism used to provide displacements of different magnitudes and frequencies. A signal generator can be used to control the system including load cells while transformers can be used to measure the load response and imposed displacement (Huang, et al., 2004). The load can be transferred to the cell-seeded constructs via flat platens which distribute the load evenly (Thorpe, et al., 2008), 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) which results in the improvement of the aggregate modulus of the resulting cartilage tissue to levels approaching those of native articular cartilage (Mauck, et al., 2000).

2.4 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 mesechymal 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 (McMahon, et al., 2008). 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 (Garvin, et al., 2003).
2.5 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. For example, a media-filled pressure chamber can be pressurised using a piston controlled by an actuator (Darling & Athanasiou, 2003). 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 (Watanabe, et al., 2005).

2.6 Flow Perfusion Bioreactors

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 (Glowacki, et al., 1998). Using a calcium phosphate scaffold, abundant extracellular matrix (ECM) with nodules of calcium phosphate was noted after 19 days in steady flow culture (Janssen, et al., 2006). 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 (Goldstein, et al., 2001, Yu, et al., 2004).

Flow perfusion bioreactors generally consist of a pump and a scaffold chamber joined together by tubing. A fluid pump is used to force media flow through the cell-seeded scaffold. The scaffold is placed in a chamber that is designed to direct flow through the interior of the scaffold. The scaffold is kept in position across the flow path of the device and media is perfused through the scaffold, thus enhancing fluid transport. Media can easily be replaced in the media reservoir (figure 4). However, the effects of direct perfusion can be highly dependent on the medium flow rate. Therefore optimising a perfusion bioreactor for the engineering of a 3D tissue must address the careful balance between the mass transfer of nutrients and waste products to and from cells, the retention of newly synthesised ECM components within the construct and the fluid induced shear stresses within the scaffold pores.
3. Tissue Formation in Bioreactor Systems

3.1 Flow Perfusion Bioreactors for Bone Tissue Engineering

Bone grafts are required to aid bone defect and non-union healing, and restore function to the damaged area as quickly and completely as possible (Perry, 1999). They are required in a number of procedures e.g. replacing diseased bone, filling bone voids, reconstructive surgery and in spinal fusion operations. The use of bone grafts substitutes (autografts, allografts and xenografts) are associated with donor site morbidity, small volume of donor tissue harvested, disease transmission, infection and chronic pain (Perry, 1999, Langer, 2000). Therefore, attention has turned to bone tissue engineering.

It has been shown that fluid flow can stimulate bone cells to increase levels of bone formation markers (Reich & Frangos, 1991, Klein-Nulend, et al., 1997, You, et al., 2001, Cartmell, et al., 2003, Li, et al., 2004, Batra, et al., 2005, Kreke, et al., 2005, Jaasma & O’Brien, 2008) and its use could improve mineralisation of the scaffold on which cells are seeded. Flow perfusion bioreactors increased alkaline phosphatase (ALP) expression after 7 and 14 days of culture compared to constructs cultured in spinner flasks or rotating wall vessels (Goldstein, et al., 2001), and are more commonly used than any other bioreactor for use in 3-D stimulation studies. In one study, pre-osteoblastic MC3T3-E1 cells were seeded on decalcified human trabecular bone, the flow rate of perfusion altered and the mRNA expression of Runx2, Osteocalcin (OC) and ALP measured (Cartmell, et al., 2003). It was found that using a steady flow rate of 1 mL/min killed a majority of 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 (Cartmell, et al., 2003).

During locomotion, bone cells are primarily subjected to oscillatory and pulsatile flow in vivo (Jacobs, et al., 1998). Results from 2D cultures show that osteoblasts are more responsive to pulsatile than steady and oscillatory flow (Jacobs, et al., 1998). In our laboratory, a flow perfusion bioreactor has been developed to examine the effects of different flow profiles on
It has been shown that fluid flow can stimulate bone cells to increase levels of bone. Therefore, attention has turned to bone tissue engineering. Bone grafts are required to aid bone defect and non-union healing, and restore function to the damaged area as quickly and completely as possible (Perry, 1999). They are required in a number of procedures for use in spinal fusion operations. The use of bone grafts substitutes (autografts, allografts, xenografts and synthetic substitutes) is limited due to the high number of procedures and the potential for complications. In addition, these methods require the removal of intact bone from one part of the body to another. Although this can work well, it is not a cure-all solution. Due to this limitation, tissue engineering may offer a solution to this problem. There are three types of cartilage: hyaline, fibrocartilage and elastic cartilage. Current treatment options for cartilage defects are limited. Currently, autologous chondrocyte implantation (ACI), osteochondral grafting and bone marrow stimulation techniques are used to stimulate the regeneration of native cartilage. However, these techniques generally result in the formation of fibrocartilage which has poor mechanical properties and does not perform as well as native cartilage. Cartilage tissue engineering may offer a solution to this problem. There are three types of cartilage: hyaline,
elastic 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 (Mauck, et al., 2007). 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±9 kPa. This is 40% of the value reported for native cartilage (Ma & Langer, 1999). 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 (Mauck, et al., 2000). 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-β) can also be used to encourage differentiation. In a study comparing the use of compressive loading, the use of TGF-β and the use of a combination of loading and TGF-β, it was found that compressive loading alone was just as effective at inducing chondrogenic differentiation as TGF-β or TGF-β plus loading (Huang, et al., 2004).

4. Bioreactors: State of the Art and Future Directions
The use of bioreactors has brought us a step closer to engineering numerous tissue types. Recently, a bioreactor was used to culture a tissue engineered trachea that was successfully implanted into a patient. A donor trachea was decellularized and subsequently seeded with the patients own cells. The bioreactor was specifically designed to seed and culture different cell types on either side of the donor tissue to allow for nutrient supply and waste removal, to provide biomechanical cues in the form of a hydrodynamic shear stress, as well as being designed to be autoclaved and handled in a sterile manner. The bioreactor rotated the trachea around its longitudinal axis so that a shear stress was applied to the cells to stimulate them and to ensure the even distribution of nutrients and waste. In addition to the rotation of the graft, the culture medium was continuously mixed to increase oxygenation and the exchange of waste and nutrients in the bioreactor chamber (Macchiarini, et al., 2008).
However, most bioreactors at present are specialised devices with a low volume output. Their assembly is often time consuming and labour intensive. Many also exhibit operator dependent variability. Ways of minimizing the time and effort needed to form tissue must be sought if costs are to be reduced so that engineered tissues can be routinely used in clinical environments. In the future, scaled up versions of some devices (which may be automated) could potentially supply larger amounts of tissue. The ideal bioreactor would generate the required amount of tissue after a defined culture period. In addition, development of tissue could be monitored throughout the culture period through the incorporation of for example video microscopy and microcomputed tomography (μCT) for observing the structural properties of the growing tissue. 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. This may be the future for bioreactors in tissue engineering.

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6. References


The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient’s needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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