Bioreactors for bone tissue engineering

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Abstract: Engineering bone tissue for use in orthopaedics poses multiple challenges. Providing the appropriate growth environment that will allow complex tissues such as bone to grow is one of these challenges. There are multiple design factors that must be considered in order to generate a functional tissue *in vitro* for replacement surgery in the clinic. Complex bioreactors have been designed that allow different stress regimes such as compressive, shear, and rotational forces to be applied to three-dimensional (3D) engineered constructs. This review addresses these considerations and outlines the types of bioreactor that have been developed and are currently in use.

Keywords: bioreactor, tissue enigineering, bone, MSC

1 INTRODUCTION

There is an increasing need for new therapies to treat bone defects that arise from trauma and disease. Tissue engineering offers a solution to the limitations that are present in current treatments such as poor implant integration and long-term mechanical integrity. The standard tissue engineering concept of placing autologous cells onto a degradable three-dimensional (3D) scaffold and culturing *in vitro* prior to patient implantation sounds relatively simple at first. However, the reality is far more complex, as several factors that affect the *in vitro* culture environment have to be considered in order rapidly to generate functional bone tissue for clinical applications. These factors or generic principles are consistent for a variety of organs across a range of *in vitro* tissue-engineered implants.

1.1 Design considerations

The first of these factors is the size of the tissue-engineered construct to be created. It is important for all the cells within a 3D tissue to receive sufficient nutrients and to have effective waste product removal. In static culture, cells seeded on the periphery of the 3D constructs grow readily; however, those that reside in the centre of a construct tend to undergo necrosis [**1**, **2**]. Constructs as small as 10 mm cubes can suffer from this phenomenon. In constructs with lower levels of porosity and reduced perfusion, these effects are observed more rapidly in culture with even smaller constructs. *In vivo*, physiological tissues do not suffer from low PO_2 or mass transfer owing to the presence of blood vessels that transport the nutrients and waste products.

In order to address this problem, several different bioreactors have been designed that improve mass transfer into larger tissue constructs. It was shown by Ishaug *et al.* [**3**] that only cells within 200–800 μm of the surface of the scaffolds are viable during static culture. Systems that address this issue (such as the spinner flask, rotatory bioreactors, and perfusion systems) are discussed in more detail below.

1.2 Structure and form

The shape of the created tissue-engineered bone is another important factor to be considered. Many bioreactors are designed to create reproducible simple shapes of tissue such as cylinders or cubes. However, it may be beneficial for clinical applications (such as maxillofacial surgery) to create specific shapes of tissue. The magnetic particle bioreactor, as described below, has this capability.

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1.3 Cell integration

Another factor that has to be considered regarding tissue-engineered bone constructs (especially when considering scale-up of the tissue size) is the method of placing (seeding) cells effectively onto the construct. Typically, optimal constructs require a homogeneous single cell seeding throughout the entire construct. Perfusion systems have been utilized to deliver cells throughout an entire construct [**4**]. In some circumstances, however, variable placement of the cells may be required (for example, a larger number of cells in one portion of a construct and no cells seeded in another portion of the construct). Bioreactors have the potential to control the cell seeding strategy when culturing these constructs.

1.4 Cell populations

There is a variety of different cell types in bone, and typically the bone-matrix-producing cell (the osteoblast) or the osteoblast precursors such as mesenchymal stem cells (MSCs) derived from the bone marrow stroma are utilized to create a bone-tissue-engineered construct. Other approaches to this technique include cocultures of cell types or various sources of bone-derived cells from adipose or cartilage tissues. Osteoblasts and osteoclasts have been cultured together on materials in bioreactors to enable the engineered tissue to be remodelled to extrinsic and intrinsic factors, with a view to create a more functional construct [**5**]. This concept hypothesizes that the time of culture *in vitro* will be shortened, as growth factor communication between the two cell types will increase the extracellular matrix production as well as improving the host site integration of the construct. Other coculture systems such as for osteochondral grafts, combine bone and chondrogenic progenitor cells in the same construct. Designs of these coculture bioreactors focused on orthopaedic applications of more complex functional structures pose specialized challenges.

1.5 Mechanical forces

When bone-forming cells are grown *in vitro*, given the correct chemical supplementation, they will produce both soft and hard extracellular matrix. However, if mechanical forces are applied to the cells, extracellular matrix production may be increased and laid down in mechanically defined local areas [**6**]. This increase in matrix production and potential reduction in culture time often results in a more functional tissue-engineered construct. Thus, many designs of bioreactor include a potential mechanical force stimulus – either by applying the forces directly to the cell or to be transferred to the cell via forces applied to the scaffold on which the cells reside [**7**, **8**].

The following text describes past and present bioreactors that have been used to grow bone segments either as experimental test systems for pharma or for clinical applications with a view to producing more functional tissue that improves integration and long-term repair.

2 SPINNER FLASK

The spinner flask is the simplest design of bioreactor discussed in this paper, consisting of cells seeded onto 3D scaffolds that are suspended via a wire in a large volume of culture medium. The culture medium is stirred using a magnetic bar at a typical rotation of 50 r/min. Many tissue types have been researched using this type of bioreactor. Bone-tissue-engineered constructs cultured using this system have been demonstrated to show improved cell viability, proliferation, and distribution throughout the construct in comparison with static culture [**8**]. However, although reproducible and easy to use, the spinner flask configuration has its limitations. The size of the tissue to be grown using this method is limited owing to the trade-off between nutrient diffusion and applied shear stresses. To provide effective nutrient delivery and waste removal, the magnetic bar rotation needs to be increased in order for nutrients to reach the cells in the centre of larger constructs. This turbulent flow increases a shear stress that is then delivered to the periphery of the housed constructs and leads to cell necrosis in these areas.

3 ROTATING VESSEL

A fluid force bioreactor is the rotating-wall vessel (RWV) bioreactor originally designed by Schwarz and colleagues at NASA's Johnson space centre. They based the bioreactor on two basic design principles: (1) solid body rotation and (2) a silicone rubber membrane for oxygenation. The solid body rotation is a vessel that rotates horizontally and is filled with culture medium. This method simulates some aspects of microgravity by reducing shear and turbulence associated with stirred bioreactors. Oxygen can diffuse through a silicone membrane, which

is central in this vessel [**9**]. This system is now commercially available from Synthecon (Houston, Texas) and from Cellon (Luxembourg) in Europe, who describe the product as a horizontally rotating, bubble-free vessel with diffusion gas exchange. The system minimizes the shear forces and damage that can occur in the cells, as the cells in the system establish an environment of uniform, low-shear, fluid suspension orbit within the vessel.

There are two main types of RWV: the slow-turning lateral vessel (STLV) and the high-aspect-ratio vessel (HARV). The STLV has been used on the space station MIR, as well as for Earth-based experiments. Its function is to control the culture conditions such as the supply of oxygen, the pH, and temperature, and it is rotated at such a speed that the constructs are in a continuous state of free fall, or 'stationary'. The HARV vessel is very similar to the STLV; however, the speed required to keep the constructs 'stationary' is reduced, and enhances gaseous exchange [**4**].

The rotating-wall vessel was initially designed to reduce the shear stresses generated during launch and landing of the space shuttles. However, when the system was tested on Earth it was observed that cells aggregated and formed structures that resembled tissues. Previous studies have shown that the use of a rotating bioreactor increases the number of cells present in constructs after 28 days of culture compared with static and perfusion cultures [**10**]. This is thought to be a result of an improvement in mass transport between the cells seeded within the constructs and the culture media [**11**].

Using the rotating bioreactor, Granet *et al.* [**12**] reported that the osteoblast-like cells were able to proliferate and differentiate, reinforcing the potential for use in bone tissue engineering. Sikavitsas *et al.* [**13**] compared three different culture conditions – static, spinner flask, and rotating-wall bioreactor – in terms of their ability to promote stromal cell proliferation and differentiation when seeded onto a PLGA porous scaffold. They reported that the spinner flask increased osteoblastic activity after 21 days in culture, and established that the increase in activity was due to the superior mixing in the spinner flask compared with the static and rotating-wall bioreactor. Botchwey *et al.* [**14**] have shown that, when osteoblasts are cultured on polymer microcarriers in the RWV bioreactor for 7 days, the osteoblasts retain an osteoblastic phenotype and an increased alkaline phosphatase activity by comparison with static culture. MSCs cultured in these bioreactors on silk scaffolds have shown enhanced calcium accumulation compared with static cultures and have resulted in constructs that resemble trabecular bone with respect to structure and mineralized tissue [**15**].

In previous work by the present authors, biomimetic-calcium-phosphate-coated poly(caprolactone) nanofibre meshes (BCP-NM) were demonstrated to be more effective in supporting cell attachment and proliferation under static conditions than poly (caprolactone) nanofibre meshes (PCL-NM). Under dynamic conditions, the production of proteins associated with the ECM of bone was higher on BCP-NM constructs than in PCL-NM constructs, which indicates that coated samples may provide cells with a better environment for tissue growth. It is suggested that improved mass transfer in the bioreactor in combination with the appropriate substrate were decisive factors for this highly positive outcome for generating bone [**16**].

When creating 3D bone tissue using the rotating vessel system, the method of seeding of cells onto the scaffold is important. Jones and Cartmell [**17**] have investigated whether seeding the scaffolds with osteoblasts prior to placing into a rotating bioreactor or placing the scaffold into the bioreactor with the cell suspension to allow seeding is optimal. Their results showed that, in comparison with static seeding conditions, the optimal cell seeding occurred when the scaffold was placed into the vessel separately to the cells in suspension.

4 PERFUSION SYSTEM

Perfusion bioreactors have been used to deliver cells to a 3D engineered construct via controlled flow, which reverses back and forth within the construct [**18**]. In addition, direct perfusion of 3D tissue-engineered constructs is known to enhance osteogenesis, which can be partly attributed to enhanced nutrient and waste transport. In addition, flow-mediated shear stresses are known to upregulate osteogenic differentiation and mineralization [**9**]. A quantification of the hydrodynamic environment is therefore crucial to interpret and compare results of *in vitro* bioreactor experiments. Mathematical modelling has been carried out to analyse the effects of mechanical force perfusion on 3D tissue-engineered constructs [**19**]. These studies have assisted in optimal design of scaffold configuration and the mechanical environment for growth.

The strength of evidence on the effects of fluid shear on bone cell behaviour comes from *in vitro* planar studies, which emulate the canicular flows

present in the osteocytic network in bone [**6**]. Evidence with planar cell cultures has shown that dynamic flow regimens elicit an enhanced cellular response over steady flow regimens. Results from recent studies testing a variety of differing flow regimes in perfusion bone-engineered systems have demonstrated that all flow conditions can enhance osteopontin expression and alkaline phosphatase activity. Additionally, these markers are preferentially enhanced by pulsatile flow over continuous flow, suggesting that cells are sensitive to frequency of pulsatile flow [**20**].

Other factors affecting the design of a perfusion culture environment are the frequency of flow and the flow rate. Cartmell *et al.* [**21**] have described the culture of an osteoblast-seeded 3D construct (using human trabecular bone as a scaffold) in a perfusion system. After 7 days of culture, it was shown that varying the flow rate from 0.01 to 2 mL/min had dramatic effects on the cell viability and cell activity. Keeping the flow rate low pertained to increases in cell viability and proliferation. However, increasing the flow rate, although some cell necrosis was observed, led to increased levels of osteogenic gene expression (normalized to cell number).

5 COMPRESSION SYSTEMS

The *in vivo* environment of bone and cartilage is such that they receive a combination of different types of mechanical loading, including tension, compression, bending, shear, and torsion. In order to replicate this more closely, recent approaches to bioreactors enable the application of several different types of physical stimulation to the constructs. Recently, bioreactors have been developed that apply mechanical forces via piston/compression systems, substrate bending, hydrodynamic compression, and fluid shear (for a review, see reference [**8**]). The perfusion compression bioreactor, first designed in 1991 by El Haj *et al.*, has been demonstrated to maintain viable bone explants *ex vivo*. The bioreactor has subsequently been adapted to enable the growth of cell-seeded constructs [**22**]. In 2005, El Haj *et al.* [**23**] cultured poly-L-lactic acid (PLLA) scaffolds seeded with MG63 (osteosarcoma cell line) cells in this adapted system. After 3 weeks of culture under perfusion, the cell-seeded constructs were submitted to a loading regime of 0.1 per cent strain for 1 h per day at a frequency of 1 Hz for 7 days, with media being perfused through the system at a rate of 0.1 mL/min. A combination of compression and perfusion resulted in a significant increase in the expression of osteogenic markers compared with static and perfusion-only samples. Recent work by Bölgen *et al.* has demonstrated the usefulness of this bioreactor for testing tissue-engineered constructs – in these studies, dynamic conditions (perfusion and/or compression) greatly improved cell ingrowth and extracellular matrix (ECM) synthesis. Alkaline phosphatase activity results confirmed the positive effect of dynamic conditions on bone cells [**24**].

In addition, this bioreactor has also been used for the culture of tissue-engineered cartilage. It has been demonstrated that *in vivo* physiological loads are sometimes too excessive for engineered constructs, and low levels of strain can have greater growth-promoting effects [**2**]. Perfusion/tensile/compression systems have been commercialized by companies such as BOSE Ltd and are becoming increasingly used for bone tissue engineering research. These commercial systems combine bioreactors with mechanical conditioning environments and measurement systems that enable tissue maturation and mechanical integrity to be monitored with time. Technical design improvements and evaluation with these systems are moving these bioreactors more rapidly towards regulatory acceptance for use in the clinical environment.

These studies demonstrate how the ability to understand cell behaviour relative to strain profile will allow the optimization of mechanical conditioning regimes in bone tissue engineering. The authors have designed a model system to investigate the effects of strain profile on bone cell behaviour within a pore. This simplified model has been designed with a view to providing insight into the types of strain distribution occurring across a single pore of a scaffold subjected to perfusion compression conditioning [**25**]. These studies demonstrated a direct correlation between stress applied and the mineralization of the matrix surrounding the cells within a pore. 3D studies have gone further to investigate across a whole multiporous construct. Using a similar perfusion compression system as described above, Baas *et al.* [**26**] have shown, using FE modelling and uCT analysis, a correlation between areas of high strain and matrix mineralization in a bone-tissue-engineered construct (Fig. 1).

Rotation compression bioreactors have also been used in cartilage tissue engineering. In 2006, Grad *et al.* [**27**] applied dynamic compression and fluid flow to chondrocyte-seeded 3D scaffolds using an oscillating ceramic hip ball to represent *in vivo* condi-

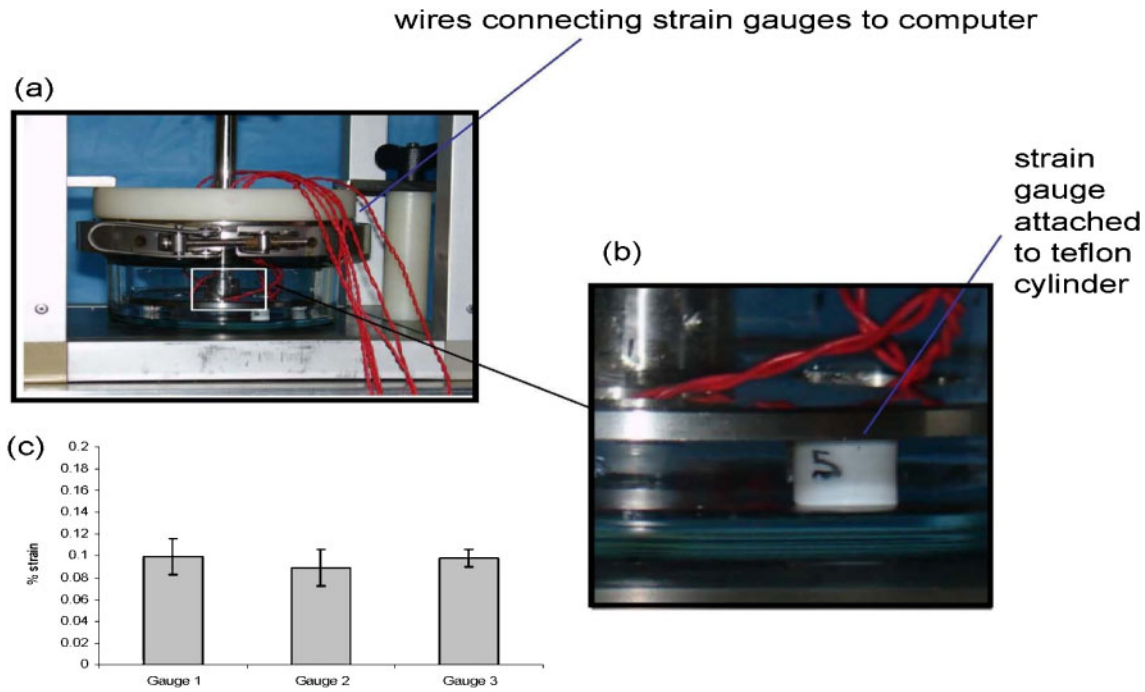

Fig. 1 Modelling of load profiles to matrix deposition. (a) FE modelling of bone-cell-seeded scaffolds that have been subjected to cyclical compression in a perfusion compression bioreactor over one load compressive cycle. The chart denotes the level of stress throughout the porous scaffold. (b) uCT image of a PLLA porous scaffold without cells. (c) uCT analysis of a porous bone-cell-seeded scaffold grown in a perfusion compression bioreactor.

tions present in the hip joint. Although all the samples underwent dynamic compression, the samples that were exposed to both fluid flow and compression showed a marked increase in the expression levels of cartilage-related genes, demonstrating the advantages of combining stimuli to recreate an environment more representative of that found *in vivo*. Therefore, the combination of compression and rotation may enhance the differentiation of mesenchymal stem cells.

There are, however, problems associated with these types of bioreactor. Any force-producing mechanism that invades the bioreactor (such as in piston/compression systems) may cause infection. The scaffold materials must transmit the force to the cells, and, in order to withstand the loads in a compression perfusion bioreactor, the scaffolds must be strong. This often results in long degradation times. In order to generate new biological matrices, the support scaffolds are often rapidly degrading, which compromises their mechanical properties and makes them weak. These mechanically weak scaffolds therefore may not be capable of transmitting the required forces and may be unsuitable for a large range of available bioreactors. Although the forces required to activate MS ion channels via cell membrane deformation are small (5–100 pN) [**28**], this requirement may present technical problems for bioreactor and scaffold design.

Among the most important aspects of bioreactor design are validation and monitoring. Systems that apply mechanical strain to constructs through the application of direct force rely on three important aspects of the loading system:

(a) direct contact between the loading plate and the samples;
(b) reproducible movement of the loading plate;
(c) even distribution of the load being applied to samples.

The amount of error incorporated in each of these aspects needs to be taken into account when determining the amount of strain being applied to the tissue-engineered constructs. Figure 2 shows strain measurements of three constructs in a multiple compression bioreactor. This ensures accuracy and consistency across the loading plate, but relies upon uniform dimensions and precision moulding for scaffold implants to be grown in the bioreactor.

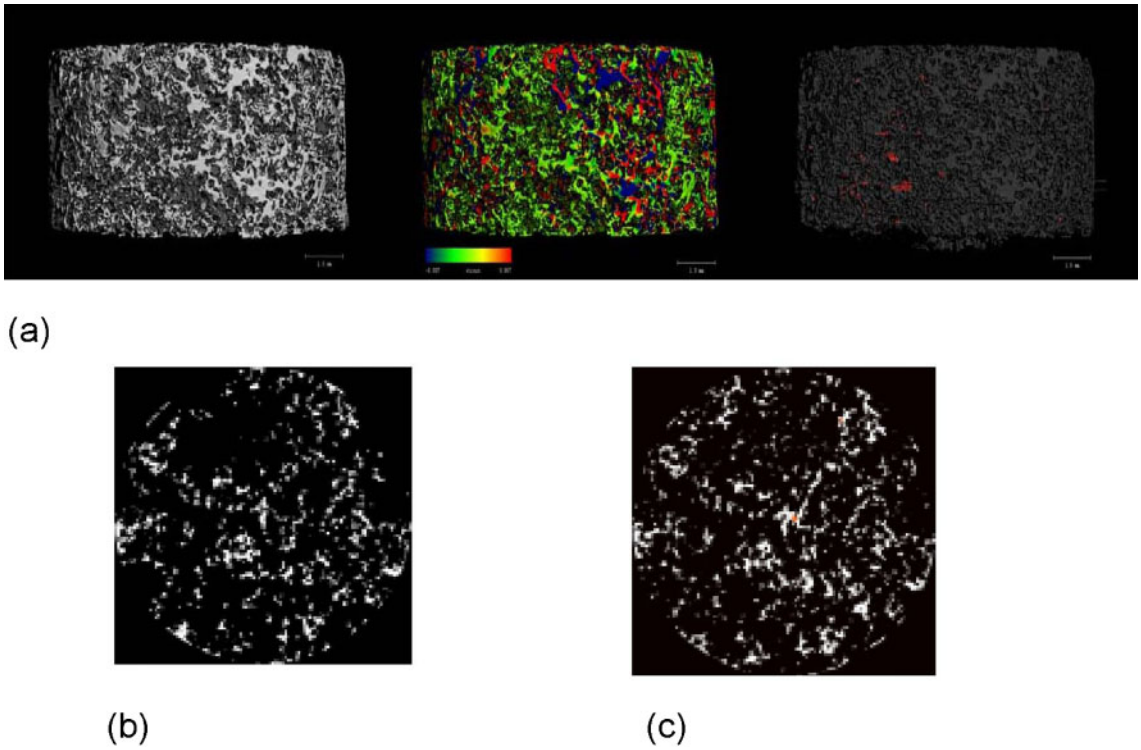

Fig. 2 (a) Photograph showing the bioreactor with the strain gauges attached to Teflon cylinders and placed between the base of the bioreactor and the loading plate. (b) Enlarged image (from white box) of the Teflon phantom scaffold with attached strain gauges. (c) Graph showing the percentage strain applied to the three cylinders subjected to mechanical compression in the multiple-sample bioreactor

6 MAGNETIC FORCE BIOREACTOR

Although these mechanical conditioning systems can support rudimentary cartilage and bone development, they still face a number of problems. For example, the perfusion of cell nutrients may be interrupted, long-term sterility is often a problem, and, in the case of compression systems, gels must infiltrate the matrix in order to transmit the applied forces through the scaffold matrix to the cells within the pores. In most bioreactor designs, the forces are applied to the scaffold rather than directly to the cell membrane or cytoskeleton where they are required. In addition, it is not possible to apply spatially varying stresses in three dimensions in order to form complex tissue structures such as a complete joint with a cartilage/bone interface. There are also serious problems with scale-up when multiple compression systems are applied to large numbers of samples, for example in the case of high-throughput screening applications.

A new development in bioreactor design is based on the theoretical principles and prototype design of a novel mechanical conditioning system – a magnetic force bioreactor (MFB), which is designed to apply stress directly to the cell membrane using forces acting on magnetic nanoparticles (Fig. 3) [**29**]. In this system, biocompatible magnetic particles are attached to the cell membrane (e.g. via RGD, collagen, or integrin receptors) or directly to an ion channel membrane protein. The cells may be in two-dimensional (2D) culture or seeded into porous, bioresorbable scaffolds and introduced into a bioreactor.

The applied force simulates mechanical loading of the cell membrane without requiring direct access to the cells inside the bioreactor and without requiring the stress to be transmitted from the scaffold to the cells. Loads can be varied easily by changing the magnetic field strength and gradient or the magnetic properties of the nanoparticles. Cells carrying particles with different magnetic properties can be seeded into different regions within the 3D scaffold, producing a spatial variation in force using the same magnetic field geometry.

The application of cyclical external magnetic fields (at physiological frequencies such as 1–3 Hz) applies either a translational force (due to the attraction of magnetic nanoparticles along the magnetic field

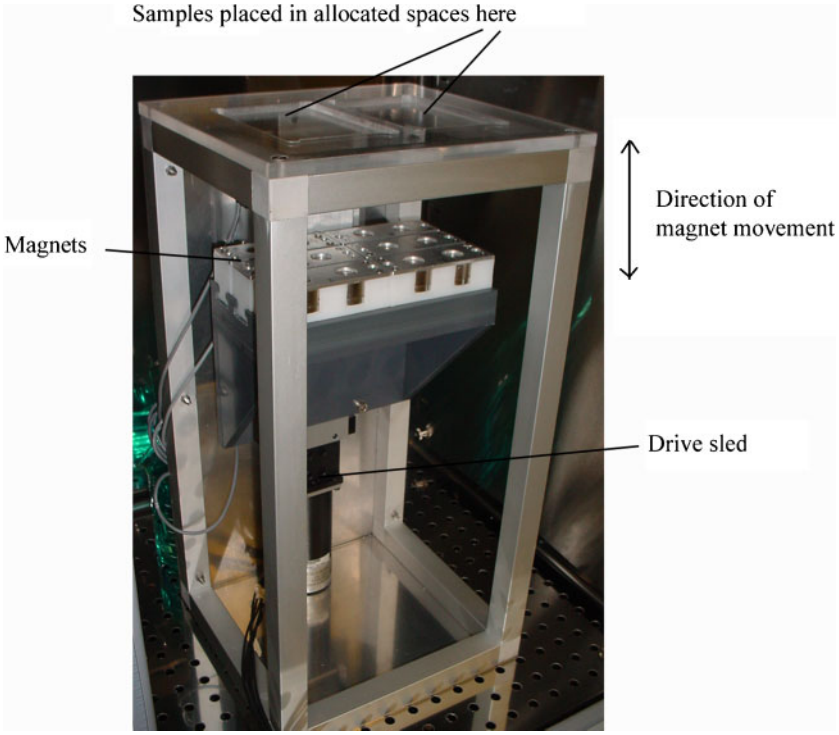

Fig. 3 Photograph of the magnetic force bioreactor (MFB), which applies cyclical magnetic fields to monolayer cultures of cells grown in standard tissue culture flasks. The magnetic particles are attached to the cells, and cyclical stress can then be applied to the membranes of the cells or specific targeted receptors

gradient) or a combination of translational force and torque (for larger, magnetically blocked nanoparticles and microparticles) that is transmitted directly to the cell membrane or the cytoskeleton and can be varied in three dimensions within a scaffold. Hughes *et al.* [**30**] have demonstrated how magnetic microparticles attached to ion channels through His-tagged clones can be remotely activated by time-varying magnetic fields.

Initial results from static 3D and 2D cultures using these systems demonstrated significant upregulation of bone matrix proteins [**29**, **31**]. After 1 week in culture, osteocalcin, osteopontin, and alkaline phosphatase showed upregulation when compared with controls. Work is now progressing towards building complexity in the biological models with cocultures and multiple tissues. The system is also alignable to a perfusion bioreactor allowing mass transfer to be addressed. The MFB systems should provide several advantages over current mechanical stimulation systems, such as:

1. High-precision control of the physical stress parameters is possible through variation of the magnetic force.
2. As the stress is applied directly to the cell membrane, strong scaffold materials are no longer required.
3. Particles are remotely coupled to the magnet array, with no components connected into the bioreactor, which leads to a reduction in infection.
4. The system is scalable and presents the potential to apply a spatially varying load profile via seeding with particles of differing magnetic properties.

In addition to cell culture and 3D-scaffold-based culture work, the MFB has been used to investigate the magnetic activation of calcium pathways in human bone marrow stem cells. Observation of Ca^{2+} fluorescence activity in these cells showed significant levels of baseline calcium activity in 30–50 per cent of cells, with many of these demonstrating oscillating intracellular calcium levels. The application of 600 G static magnetic fields did not greatly influence the behaviour of cells already undergoing Ca^{2+} oscillations; however, cells with steady baseline Ca^{2+} levels showed clear characteristic transients in response to magnetic stimulation, indicating activation of mechanosensitive calcium pathways [**30**].

7 COCULTURE BIOREACTORS

As mentioned above, osteoblast and osteoclast cells have been grown together in a Synthecon rotatory bioreactor [**5**]. A 1:100 ratio of osteoblast:osteoclasts was cocultured for a 10 day period in 50 mL disposable Synthecon vessels. These cells were cultured with films of dentine, chitosan, PLLA, or silk fibroin. Comparison with static culture was made, and it was found that bioreactor culture did not improve cell adherence or proliferation. The same authors also performed rotatory bioreactor culture using these two cell types on 3D scaffolds rather than films, and saw similar results [**12**]. Lower concentrations of DNA were observed in the bioreactor groups, compared with the static cultures, which showed that the cells had adhered to the scaffolds in both culture conditions, but the cells in bioreactor cultures did not proliferate to the same extent as cells in the static cultures. This has also been reported by Botchwey *et al.* [**14**], who reported a significant decrease in cell number by day 7 in scaffolds that were in rotating culture, compared with non-rotating static cultures. It has been reported, as outlined above, that seeding cells in a rotatory bioreactor may be more successful if cells are suspended in the media and then migrate to the scaffold, rather than by seeding the cells onto a scaffold and then transferring into the vessel [**17**].

Coculture bioreactors have been designed to grow both cartilage and bone together [**32**, **33**]. These two tissues reside next to each other in the human body and communicate via protein production in an endocrine fashion. Cartilage plugs that have been tissue engineered often have poor integration into the host tissue. Anchoring the cartilage into the bone using an attached piece of engineered bone tissue may assist in the integration of the cartilage tissue. As these two tissue types require different culture conditions (such as optimal chemical factors, oxygen tensions, mechanical force application, and nutrient diffusion), separate chambers in the bioreactor may be necessary to provide the separate tissue environments while maintaining a bone/cartilage interface. Michael *et al.* [**32**] describe a modified perfusion system to allow the growth of these two tissue types. Different flow rates are utilized for the cartilage and bone perfusion, and visualization of the flow path has been characterized using an iodine contrast agent and CT scanning. Typically, the chondrocytes are suspended in a gel system that has been cast in the cartilage section of the bioreactor chamber (a cylindrical chamber of 20 mm diameter and 5 mm height) onto a 'capped' porous 3D scaffold onto which osteoblasts have been preseeded (in the bone chamber section of cylindrical dimensions 10 mm height × 15 mm diameter). Further investigations into controlling the mixing of the cartilage and bone culture media is under way by this research group with the intention of creating a hypertrophic region in the lower cartilage segment and thus producing increased anchoring of the tissue-engineered cartilage to the tissue-engineered bone section. Other research groups have also analysed perfusion bioreactors in a similar way to produce osteochondral plugs [**33**, **34**].

8 MONITORING

Another important aspect of validation of the bioreactor design is the capability to perform online monitoring of the culture environment. Specialized sensing and measurement instruments are under development to aid in the controlled culture of cells in bioreactors. Cell and tissue culture processes are dynamic, and therefore optimal control requires monitoring of the key process variables. When cells are given the appropriate conditions for survival and growth, the cell number will increase in a predictable way, with simultaneous consumption of O_2 and glucose and production of CO_2, together with shifts in culture medium pH. Studies have shown that these factors can affect the proliferation and differentiation of cells in culture [**35**], and therefore the monitoring of these variables would be beneficial in optimizing the bioreactor system.

In terms of bioreactor process monitoring, there are two possible strategies available using biosensors. One strategy involves the use of sensors being placed inside the bioreactor. This invasive method, where the biosensor can be located either in the culture fluid or in direct contact with the cells or tissue/scaffold construct, can be advantageous in situations where sample extraction and transport could cause difficulties. However, the biosensor must be capable of withstanding sterilization, and recalibration of the device during use is difficult. Microelectrodes can be used in a bioreactor; microelectrodes are essentially used to measure the concentration of O_2 when a change in oxidation potential occurs. However, there are several disadvantages with this strategy. The electrode must be introduced into the sample and at different locations, calibration is lengthy and difficult, and problems can arise with sterilization [**36**]. Invasive fibre-optic sensors have also been used for PO_2 and pH measurements.

Non-invasive sensing can be carried out using optical methods such as spectrophotometry or

fluorimetry. This approach obviously avoids the difficulties of needing to sterilize sensors, but there is more of a challenge in achieving high specificity and high sensitivity for target molecules such as glucose. Reporter patches fixed to the inside of an optical window in the bioreactor wall are being investigated. Such patches contain ion-sensitive dyes or O_2-quenchable fluorophores [**37**]. The use of biosensors outside the bioreactor offers many advantages. The problem of sensor sterilization is completely avoided, and any sample preparation required, such as dilution to within the linear range of the sensor, pH adjustment by the addition of an appropriate buffer, and temperature measurements, can be accomplished easily.

9 MONOLAYER CULTURES

It may be noted that other devices exist, such as the parallel-plate bioreactor, four-point bending models, and flexcell tissue train systems, that have been used to test bone cell cultures in monolayers in standard tissue culture plastic formats. However, these instruments are not covered here because they are for basic science testing purposes only, rather than for producing functional tissue that could possibly be used for clinical use.

10 CONCLUSION

Bioreactors enable the culture of cells in a 3D environment and can be used to apply reproducible and accurate regimes of mechanical forces to cell-seeded constructs. Bioreactors can therefore be used to investigate the effects of mechanical stimulation on cells in a 3D environment, which has previously been shown to enhance the differentiation of mesenchymal stem cells along numerous lineages and the ability for bone cells to generate increased matrix production in a mechanically directed manner. The aim would be to generate more functional constructs that have been conditioned to the loads they will encounter *in vivo*.

The next challenge is to scale up these bioreactors into the production of high numbers of constructs in a clinically relevant environment. Commercialization of bone tissue engineering will rely on GMP manufacturing processes for large-scale production of bone tissue, which can compete with existing allograft bone bank sources.

REFERENCES

1 **Cartmell, S., Huynh, K., Lin, A., Nagaraja, S.,** and **Guldberg, R.** Quantitative microcomputed tomography analysis of mineralization within three-dimensional scaffolds in vitro. *J. Biomed. Mater. Res. A*, 2004, **69**(1), 97–104.

2 **Freyria, A.-M., Yang, Y., Chajra, H., Rousseau, C. F., Ronziere, M.-C., Herbage, D.,** and **El Haj, A. J.** Optimisation of dynamic culture conditions: effects on biosynthetic activities of chondrocytes grown in collagen sponges. *Tissue Engng*, 2005, **11**, 5–6.

3 **Ishaug, S. L., Crane, G. M., Miller, M. J., Yasko, A. W., Yaszemski, M. J.,** and **Mikos, A. G.** Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. *J. Biomed. Mater. Res.*, 1997, **36**(1), 17–28.

4 **Martin, Y.** and **Vermette, P.** Bioreactors for tissue mass culture: design, characterization, and recent advances. *Biomaterials*, 2005, **26**(35), 7481–7503.

5 **Jones, G. L., Motta, A., Marshall, M., El Haj, A. J.,** and **Cartmell, S. H.** Osteoblast: osteoclast co-cultures on silk fibroin, chitosan and PLLA films. *Biomaterials*, 2009, **30**(29), 5376–5384.

6 **Mullender, M., El Haj, A. J., Yang, Y., Van Duin, M. A., Burger, E. H.,** and **Klein-Nulend, J.** Mechanotransduction of bone cells in vitro: mechanobiology of bone tissue. *Med. Biol. Engng Comput.*, 2004, **42**(1), 14–21.

7 **Sharma, P., Cartmell, S.,** and **El Haj, A. J.** Tissue engineered bone grafts. In *Applications of cell immobilisation biotechnology* (Eds V. Nedovic and R. Willaert), 2005, pp. 153–166 (Springer, Dordrecht, The Netherlands).

8 **Cartmell, S.** and **El Haj, A. J.** Mechanical bioreactors for tissue engineering. In *Bioreactors for tissue engineering* (Eds J. B. Chaudhuri and M. Al Rubeai), 2006 (Kluwer Academic Publishers).

9 **Schwarz, R. P., Goodwin, T. J.,** and **Wolf, D. A.** Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. *J. Tissue Cult. Meth.*, 1992, **14**(2), 51–57.

10 **Pound, J. C., Green, D. W., Chaudhuri, J. B.,** *et al.* Bioreactor culture of cartilage from mesenchymal populations. *J. Bone Joint Surg. Br.*, 2006, **88B** (Suppl. III), 405.

11 **Yu, X., Bothway, E. A., Levine, E. M.,** *et al.* Bioreactor based bone tissue engineering – the influence of dynamic flow on osteoblast phenotypic expression and matrix mineralization. *Proc. Natl Acad. Sci. USA*, 2004, **101**(31), 11 203–11 208.

12 **Granet, C., Laroche, N., Vico, L.,** *et al.* Rotating-wall vessels, promising bioreactors for osteoblastic cell culture: comparison with other 3D conditions. *Med. Biol. Engng Comput.*, 1998, **36**(4), 513–519.

13 **Sikavitsas, V. I., Bancroft, G. N., Holtorf, H. L., Jansen, J. A.,** and **Mikos, A. G.** Mineralized matrix deposition by marrow stromal osteoblasts in 3D perfusion culture increases with increasing fluid

shear forces. *Proc. Natl Acad. Sci. USA*, 2003, **100**(25), 14 683–14 688.
14 **Botchwey, E. A., Pollack, S. R., Levine, E. M.,** and **Laurencin, C. T.** Bone tissue engineering in a rotating bioreactor using a microcarrier matrix system. *J. Biomed. Mater. Res.*, 2001, **55**(2), 242–253.
15 **Marolt, D., Augst, A., Freed, L. E.,** *et al.* Bone and cartilage tissue constructs grown using human bone marrow stromal cells, silk scaffolds and rotating bioreactors. *Biomaterials*, 2006, **27**(36), 6138–6149.
16 **Araujo, J. V., Cunha-Reis, C., Rada, T., Alves da Silva, M., Gomes, M., Yang, Y., Ashammakhi, N., Reis, R. L., El Haj, A. J.,** and **Neves, N. M.** Dynamic culture of osteogenic cells in biomimetically coated poly(caprolactone) nanofibre mesh constructs. *Tissue Engng Part A*, 2010, **16**(2), 557–563.
17 **Jones, G.** and **Cartmell, S. H.** Optimization of cell seeding efficiencies on a three-dimensional gelatin scaffold for bone tissue engineering. *J. Appl. Biomater. Biomechanics*, 2006, **4**(3), 172–180.
18 **Martin, I., Smith, T.,** and **Wendt, D.** Bioreactor-based roadmap for the translation of tissue engineering strategies into clinical products. *Trends Biotechnol.*, 2009, **27**(9), 495–502.
19 **Milan, J. L., Planell, J. A.,** and **Lacroix, D.** Computational modelling of the mechanical environment of osteogenesis within a polylactic acid–calcium phosphate glass scaffold. *Biomaterials*, 2009, **30**(25), 4219–4226.
20 **Kavlock, K. D.** and **Goldstein, A. S.** Effect of pulsatile flow on the osteogenic differentiation of bone marrow stromal cells in porous scaffolds. *Biomed. Sci. Instrum.*, 2008, **44**, 471–476.
21 **Cartmell, S. H., Porter, B. D., Garcia, A. J.,** and **Guldberg, R. E.** Effects of medium perfusion rate on cell-seeded three-dimensional bone constructs in vitro. *Tissue Engng*, 2003, **9**(6), 1197–1203.
22 **Shelton, R. M.** and **El Haj, A. J.** A novel microcarrier bead model to investigate bone cell responses to mechanical compression. *J. Bone Miner. Res.*, 1992, **7**(2), 403–405.
23 **El Haj, A. J., Wood, M., Thomas, P.,** and **Yang, Y.** Controlling cell biomechanics in orthopaedic tissue engineering and repair. *Pathologie*, 2005, **53**, 581–589.
24 **Bölgen, N., Yang, Y., Korkusuz, P., Güzel, E., El Haj, A. J.,** and **Pişkin, E.** Three-dimensional ingrowth of bone cells within biodegradable cryogel scaffolds in bioreactors at different regimes. *Tissue Engng Part A*, 2008, **14**(10), 1743–1750.
25 **Wood, M. A., Yang, Y., Baas, E., Meredith, D. O., Richards, R. G., Kuiper, J. H.,** and **El Haj, A. J.** Correlating cell morphology and osteoid mineralization relative to strain profile for bone tissue engineering applications. *J. R. Soc. Interface*, 2008, **5**(25), 899–907.
26 **Baas, E., Kuiper, J. H., Yang, Y., Wood, M. A.,** and **El Haj, A. J.** In vitro bone growth responds to local mechanical strain in three-dimensional polymer scaffolds. *J. Biomechanics*, 2010, **43**(4), 733–739.
27 **Grad, S., Gogolewski, S., Alini, M.,** and **Wimmer, M. A.** Effects of simple and complex motion patterns on gene expression of chondrocytes seeded in 3D scaffolds. *Tissue Engng*, 2006, **12**(11), 3171–3179.
28 **Walker, L. M., Holm, A., Cooling, L., Maxwell, L., Oberg, P. A., Sundqvist, T.,** and **El Haj, A. J.** Mechanical manipulation of bone and cartilage cells with optical tweezers. *FEBS Lett.*, 1999, **459**(1), 39–42.
29 **Dobson, J., Cartmell, S. H., Keramane, A.,** and **El Haj, A. J.** Principles and design of a novel magnetic force mechanical conditioning bioreactor for tissue engineering, stem cell conditioning, and dynamic in vitro screening. *IEEE Trans. Nanobiosci.*, 2007, **5**(3), 173–177.
30 **Hughes, S., Dobson, J.,** and **El Haj, A. J.** Magnetic targeting of mechanosensors in bone cells for tissue engineering applications. *J. Biomechanics*, 2007, **40**, S96–S104.
31 **Cartmell, S., Dobson, J., Verschueren, S. B.,** and **El Haj, A. J.** Development of magnetic particle techniques for long-term culture of bone cells with intermittent mechanical activation. *IEEE Trans., NanoBiosci.*, 2002, **1**, 92–97.
32 **Michael, V., Gittings, J., Turner, I., Chaudhuri, J., Ellis, M., Waters, S., Cummings, L., Goodstone, N.,** and **Cartmell, S.** Co-culture bioreactor design for skeletal tissue engineering. *Tissue Engng*, 2007, **13**, 1657–1658.
33 **Whittaker, R. J., Booth, R., Dyson, R., Bailey, C., Parsons-Chini, L., Naire, S., Payvandi, S., Rong, Z., Woollard, H., Cummings, L. J., Waters, S. L., Mawasse, L., Chaudhuri, J. B., Ellis, M. J., Michael, V., Kuiper, N. J.,** and **Cartmell, S.** Mathematical modelling of fibre-enhanced perfusion inside a tissue-engineering bioreactor. *J. Theor. Biol.*, 2009, **256**(4), 533–546.
34 **Mahmoudifar, N.** and **Doran, P. M.** Tissue engineering of human cartilage and osteochondral composites using recirculation bioreactors. *Biomaterials*, 2005, **26**(34), 7012–7024.
35 **Chang, C.-H., Lin, C.-C., Chou, C.-H., Lin, F.-H.,** and **Liu, H.-C.** Novel bioreactors for osteochondral tissue engineering. *Biomed. Engng – Applic., Basis Commun.*, 2005, **17**(1), 38–43.
36 **Malda, J., Woodfield, T. B. F., Van Der Vloodt, F.,** *et al.* The effect of PEGT/PBT scaffold architecture on oxygen gradients in tissue engineered cartilaginous constructs. *Biomaterials*, 2004, **25**(26), 5773–5780.
37 **Rolfe, P.** Sensing in tissue bioreactors. *Measmt Sci. Technol.*, 2006, **17**(3), 578–583.