The Role of Bioreactors in Tissue Engineering for Musculoskeletal Applications

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Abstract: Tissue engineering involves using the principles of biology, chemistry and engineering to design a 'neotissue' that augments a malfunctioning *in vivo* tissue. The main requirements for functional engineered tissue include reparative cellular components that proliferate on a biocompatible scaffold grown within a bioreactor that provides specific biochemical and physical signals to regulate cell differentiation and tissue assembly. We discuss the role of bioreactors in tissue engineering and evaluate the principles of bioreactor design. We evaluate the methods of cell stimulation and review the bioreactors in common use today.

Keywords: Bioreactors, design, scaffolds, stimulation, tissue engineering.

Cells of the haematopoeitic system, musculoskeletal tissues and visceral organs can malfunction either due to developmental disorders, degeneration or trauma. A number of conservative, medical or surgical techniques may be employed to enhance the natural regeneration of these tissues but all of these techniques have limitations and do not necessarily ensure the regeneration of tissue with the biochemical and biomechanical properties of the native tissue.

Tissue engineering theoretically overcomes the limitations of these traditional curing techniques by repairing or replacing damaged tissue with a *de novo* tissue that resembles the native tissue. The basic principle involves the harvesting of cells with the potential to proliferate and differentiate into the desired tissue, and implanting them into an appropriate scaffold which provides mechanical stability and a template for the organizing tissue [1, 2]. A bioreactor then applies any combination of chemical, mechanical, electrical or magnetic stimulation to enhance mass transfer and nutrient transport within the seeded cells and to facilitate the correct development of the tissue [3]. Bioreactors can theoretically be used to support the expansion of diseased cells such as those of the haematopoietic system, the growth of three dimensional tissues such as bone and cartilage prior to implantation, and as an organ support device prior to organ transplantation [4]

In this article we discuss the important principles of bioreactor design and discuss the stimulation techniques employed by bioreactors in the augmentation of neotissues. We describe commonly used bioreactors and evaluate their effectiveness.

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ASPECTS OF BIOREACTOR DESIGN

A number of basic design principles need to be fulfilled for a successful bioreactor to function [5] and these are discussed below.

First and foremost a bioreactor should allow the precise control of the physiological environment of the culture. This means controlling the temperature, oxygen concentrations, pH values, nutrients, media flow rate, metabolite concentrations and specific tissue markers within close limits. Tissue culture is a continuous, non steady state process and the cultivation and tissue specific parameters are changing with time. At the moment it is not possible to easily measure all of these variables 'real-time' and there is a requirement to develop sensors for 'real-time' measurement, or alternatively to remove samples for as close as possible 'real-time' analysis.

The provision of essential nutrients and gases to the culture is of fundamental importance in bioreactor design and needs to emulate the rich and extensive vascular network of the human body. Porous scaffolds which allow high nutrient fluxes to cells have been employed in order to optimize the mass transfer processes. An overview of scaffold materials for tissue engineering is provided by Yang *et al.* [1].

Bioreactors should also be able to support the culture of two or more cell types simultaneously when used to regenerate complex tissues. This usually involves first maintaining the various cell types under different culture conditions to expand cell numbers and then at appropriate time, switching to a common cultivation protocol in one bioreactor.

A bioreactor should also be designed to operate under strict aseptic conditions to prevent any influx of microorganisms that may contaminate the tissue. This necessitates pre sterilization of the equipment, preparation of sterile media and maintenance of sterility during the tissue

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engineering process. The manufacturing requirements of a bioreactor must also be considered and designs must follow the appropriate manufacturing practice and quality assurance guidelines of the regulatory bodies in the appropriate locality.

BIOREACTOR STIMULATION

The bioreactor uses various chemical, mechanical or electro-magnetic stimulation techniques to obtain *de novo* tissue with biomechanical properties comparable to the damaged or desired tissue.

Chemical stimulation techniques are employed by using chemicals such as growth factors, which are polypeptides that support various terminal phenotypes and regulate stem cell differentiation, and proliferation. Commonly used growth factors are transforming growth factor-β, bone morphogenic proteins and fibroblast growth factors [5]. Mechanical stimulation techniques involve subjecting a scaffold to mechanical stresses resembling the *in vivo* environment. Suckosky *et al.* [6] have shown that applying mechanical stimulation by subjecting a scaffold to dynamic flow provides a uniform cell distribution throughout the three dimensional seeded construct resulting in a homogenous matrix deposition, whereas Altman *et al.* [7] have been able to show that direct mechanical strain applied on seeded silicone scaffolds induces the differentiation of cells into a ligament-like cell lineage in preference to bone or cartilage cell lineages. Electric and magnetic stimuli have also been used experimentally with encouraging results. In one trial a single application of low energy laser therapy on the middle cruciate ligament of a rat model significantly increased the collagen fibril size [8]. In another trial, an increase in osteoblast proliferation and alkaline phosphatase activity was reported when rabbit bone marrow was electrically stimulated [9].

IN VITRO BIOREACTORS

1. Static Culture

Static culture bioreactors have been widely used in the past and involve the deposition of cells on a scaffold, supplied with the appropriate growth media, and cultured in an incubator. These bioreactors have severe limitations and studies have shown that static culture results in a non-homogenous cell distribution that does not resemble the native tissue [10]. Furthermore the failure of static cultures to recreate the mechanical environment of *in vivo* tissue and to achieve mass transport of nutrients into large scaffolds result in the preferential growth of cells at the periphery of the scaffold which lack the biomechanical and histological properties of the native tissue [11].

2. Rotating Wall Vessel

This bioreactor was developed at NASA and consist of two concentric cylinders, within which lies an annular space containing the scaffold [12]. The outside wall is capable of rotating, and gravitational forces are balanced with centrifugal forces, establishing microgravity-like culturing conditions within the annular space and subjecting the scaffold to dynamic laminar flow [10]. Saini *et al.* [13] have shown that this technique is preferably used in cartilage tissue engineering as it provides a favorable hydrodynamic environment conductive to cartilage phenotype differentiation and cartilage tissue growth. In their experiment, porous poly-lactic acid constructs, seeded dynamically in the bioreactor using isolated bovine chondrocytes, were cultured for four weeks at three seeding densities and three different shear stresses to characterize the effect of chondrocyte density and hydrodynamic loading on construct growth. Construct seeding efficiency with chondrocytes was greater than 95% within 24 hours. Extensive chondrocyte proliferation and matrix deposition were achieved so that after 28 days in culture, constructs from bioreactors seeded at the highest cell densities contained up to 15 x 10^6 cells, 2 mg glycosaminoglycan, and 3.5 mg collagen per construct and exhibited morphology similar to that of native cartilage.

Marlovits *et al.* [14] inoculated differentiated chondrocytes in a rotating wall vessel without the use of any scaffolding material. After 90 days of cultivation, cartilage-like neotissue was formed, encapsulated by fibrous tissue that closely resembled the perichondrium. Overall the rotating wall vessel bioreactor has currently been shown to optimize nutrient transport and promote cartilage growth and differentiation superior to other currently tested culturing techniques [15].

3. Spinner Flask

In this type, cell seeded scaffolds attached to needles are suspended from the top cover of a flask in culture medium. Mixing of the medium is sustained with a magnetic stir bar placed at the bottom of the flask [16]. Constructs cultured in spinner flasks have a higher cell seeding density and more uniform distribution of cells when compared to a static culture model [17]. Vunjak-Novakovic *et al.* [18] have shown that turbulent mixing of nutrients in a spinner flask bioreactor significantly enhances the biochemical compositions and alters morphologies of engineered constructs.

The spinner flask bioreactor seems to support osteogenesis more than the rotating wall vessel bioreactor. Sikavitsas *et al.* [19] in their study compared the outcomes of rat mesenchymal stem cells cultured on polymeric scaffolds for a period of 21 days using static, spinner flask and rotating wall vessel systems. Results showed that cells cultured in the spinner flask had the highest alkaline phosphatase activity and osteocalcin secretion among the three culturing systems. Additionally spinner flask constructs had higher proliferation rate and calcium content than statically cultured constructs.

4. Flow Perfusion

Flow perfusion bioreactors utilize a pump to percolate medium continuously through the scaffold's interconnected pores and eliminate the internal transport limitations of the spinner flask and rotating wall vessel [20]. The enhanced nutrient transfer has been shown to result in improved mass transfer [16], homogeneous cell distribution and high seeding efficiency [11] throughout the thickness of the scaffold. Fluid shear forces in flow perfusion systems also causes mechanical stimulation of the culture and has been shown to enhance the expression of the osteoblastic phenotype [21].

It has also been widely reported that fluid shear enhances *in vitro* osteogenesis. In the study conducted by Gomes *et al.* [22] bone marrow derived mesenchymal stem cells were cultured statically and in a perfusion system. Only a surface layer of cells was observed for statically cultured constructs as opposed to a homogeneous cell distribution throughout the scaffold for perfused constructs. The proliferation rate and alkaline phosphatase activity patterns were similar for both types of culture techniques, while scaffolds that were cultured under perfused conditions showed a significant increase in calcium deposition.

Goldstein *et al.* [23] used osteoblastic cells, which were seeded on PLGA foams and cultured for two weeks in three different bioreactors: rotating wall vessel, spinner flask and flow perfusion. Cell seeding efficiencies and osteocalcin content were similar for the three systems. However, the spinner flask produced the least uniform cell distribution throughout the foams and the rotating wall vessel system resulted in the lowest levels of alkaline phosphatase activity. Consequently, the flow perfusion system appears to be a very attractive culturing technique for bone constructs.

IN VIVO BIOREACTORS

This is a bioengineering approach that depends on the conductive properties of the implanted scaffold to recruit mesenchymal stem cells from neighboring tissue and takes advantage of the physiological environment to supply the necessary growth factors and nutrients to the construct. The main challenge is to find the appropriate scaffolding material that would induce the differentiation of mesenchymal stem cells into an adequate lineage. Several attempts have been made to develop *in vivo* bioreactors which can generate vascularised host bone tissue.

Stevens *et al.* [24] hypothesized that by inhibiting angiogenesis and promoting a more hypoxic environment within the bioreactor space, cartilage formation could be exclusively promoted. They incorporated an *in vivo* bioreactor, where artificial space created between the tibia and the periosteum using alginate gel, into the tibia of New Zealand white rabbits. Preosteoblastic cells were recruited from the inner layer of the periosteum. The engineered bone tissue was found to be biomechanically identical to native bone. When harvested and implanted into contralateral tibial defects, the engineered bone completely integrated within the native bone tissue after six weeks with no apparent morbidity at the donor site.

In the design of another *in vivo* bioreactor used by Holt *et al.* [25] the scaffold was composed of coralline cylinders and supplemented with the growth factor BMP-2. A vascular pedicle was incorporated into the scaffold to supply a channel for the mesenchymal stem cells recruited by BMP-2 from the blood circulation into the bioreactor and silicone was used to isolate the bioreactor from the surrounding environment. This closed system ensured that bone formation would depend solely on the internal scaffold and the invading cells with osteogenic potential. The designed *in vivo* bioreactors were implanted into 12 male rats and harvested after six weeks. The extracted implants demonstrated neovascular ingrowth and new bone formation of 11.3%. This unique design can be used in future tissue engineering applications and can potentially help in conditions of skeletal defects e.g. nonunion and tumor post-resection reconstruction. It may also serve as a model in which to study primary and metastatic cancers of bone.

CONCLUSION

Tissue Engineering is a pioneering field and bioreactors have an important role in creating the ideal environment for the generation of a particular neotissue. The principles of bioreactors have been defined and advances in the understanding of stimulation techniques and appropriate scaffolds will lead to these goals being met *in vitro* and *in vivo*. The role of growth factors in the differentiation and proliferation of stem cells is clear, and mechanical stimulation has been shown to be important especially in musculoskeletal tissue engineering. Early generation static bioreactors are associated with non-homogenous cell distributions and sub-optimal mechanical tissue properties, whereas flow perfusion and rotating wall vessels are associated with more efficient nutrient transport, cell distribution and more ideal cell and tissue characteristics. Despite this, there is a need to further elucidate the specific biochemical and biomechanical factors necessary for cell, tissue or organ development and to modify the designs of bioreactors if needed, to result in neotissues with optimal features for clinically successful tissue engineering.

DISCLOSURE

Part of information included in this manuscript has been previously published in Tissue Engineering: Advances in Experimental Medicine and Biology, 2007, Volume 585, Section 4, 243-259.

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CONFLICT OF INTEREST

None declared.

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