CHAPTER 6

ENGINEERING DESIGN ASPECTS OF TISSUE ENGINEERING

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INTRODUCTION

Tissue engineering is rapidly evolving from the initial proof-of-principle demonstrations of feasibility to the development of products intended for widespread clinical use. A number of critical engineering design issues (Figure 6.1) must be addressed during this transition to enable large-scale manufacture.

Fig. 6.1. The typical tissue engineering approach demonstrates multiple engineering design issues that must be addressed. Cells are expanded from a tissue source, requiring bioreactor technology. Following combination with a biomaterial, three-dimensional engineered tissues are often cultured for a period of time \textit{in vitro}, again requiring bioreactor systems. Storage of cells and tissues prior to transplantation requires appropriate preservation, and the survival and function of tissues following implantation requires vascularization from the host in most situations. In addition, the mechanical properties of the engineered tissue (e.g., cartilage, blood vessels) must be appropriate if it is to suitably replace tissue function (M.C. Peters, U. Michigan; used by permission).

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and use of a variety of engineered tissues. These challenges include elements of mass transport, biomechanics, biomaterials, and bioelectronics. Biomaterials and bioelectronic issues are covered in other chapters of this report. Important engineering design issues addressed in this chapter include

- Adaptation of existing bioreactor technology for large-scale cell expansion and three-dimensional tissue production
- Identification of appropriate techniques (e.g., cryopreservation) for preserving both cells and engineered tissues (cytopreservation)
- Development of strategies to promote vascularization of engineered tissues (mass transport issues)
- Evaluation of the critical mechanical properties of the tissues that need to be replaced
- Determination of the minimum values of native tissue mechanical properties required of an engineered tissue
- Exploitation of externally applied mechanical stimuli to regulate the development and function of engineered tissues

Significant progress has been made in the United States, as compared to Europe and Japan, in addressing many of the bioreactor issues. However, significant progress will be required in both the cryopreservation and vascularization areas to achieve the full potential of tissue engineering products. The importance of the biomechanics issues are just now being fully recognized, and this is an underdeveloped area. Significant progress in all of these areas is critical to efforts to engineer functional tissues that can exist in a mechanically dynamic environment (e.g., bone cartilage, blood vessels).

A brief review of each topic is given in the following sections. More information regarding specific efforts at different sites can be found in the site reports (see Appendices B and C). A tabular summary is given in the final section. For the tissue engineering field to reach its potential, there are clearly critical requirements for advances in several areas.

**BIOREACTOR TECHNOLOGY**

Bioreactors are utilized in tissue engineering for a variety of diverse applications (Miller 2000):

- Cell production on both a small, individual patient and a large, multipatient scale
- Production of three-dimensional tissues in vitro
- Directly as organ support devices

**Cell Expansion**

Many tissue engineering strategies rely on multiplying cells from a small biopsy or initial tissue source and subsequently harvesting these cells for transplantation directly or on a polymeric scaffold. Currently, efforts in both Japan and Europe are focused on the use of autologous cell therapies, and a large number of their academic centers and companies are developing
autologous tissue engineering products. These include the Japan Tissue Engineering Co. and Riken Cell Bank in Japan; Cell Lining GmbH (Germany); Imperial College (UK); Valley Tissue Engineering Center (Germany); and Biomaterials and Tissue Repair Inserm-U.443 (France). In contrast, both autologous and allogeneic therapies are being pursued in the United States. Representative U.S. companies that have commercialized allogeneic cell-based products include Advanced Tissue Sciences (La Jolla, CA), and Organogenesis (Canton, MA). U.S. companies that utilize autologous cell therapies include Genzyme Tissue Repair (Cambridge, MA), Curis (Cambridge, MA), and Aastrom (Ann Arbor, MI).

Allogeneic products are amenable to large-scale manufacturing at a single central site, while autologous therapies will likely lead to more of a service industry, with a heavy emphasis on local or regional cell banking/expansion. Previous bioreactor technologies, which focused on growing single cells or small cell clusters, provide a suitable starting point for both the autologous and allogeneic types of cell expansion work. However, different technologies will likely be optimal for the two approaches. The local or regional cell expansion required for autologous therapies will require robust, mobile cell multiplication systems. However, European and Japanese sites do not appear to be focused on developing new bioreactor technologies, but are adapting established processes. Only one company known to the panelists (Aastrom) has focused on this issue.

**Three-Dimensional Tissue Culture**

Production of three-dimensional engineered tissues in vitro for subsequent transplantation is a major emphasis in many tissue engineering companies and academic laboratories. This process typically involves culture of cell-biomaterial constructs following seeding of previously expanded cells (see previous paragraphs) onto the three-dimensional scaffold. Engineered skin products, some of which are available for clinical use and others in late-stage clinical trials (Naughton 1999; Parenteau 1999), are an example of this approach. Several U.S. companies have developed large-scale tissue production facilities, with the goal of reproducibly producing large numbers of individually packaged tissues.

**Bioreactors as Organ Support Devices**

Cell-containing bioreactors are also used directly as support devices for liver (bioartificial liver, BAL) (Figure 6.2) or kidney (bioartificial kidney, BAK) function. The BAK is proposed as an adjunct or replacement to dialysis for
patients with kidney failure. The BAL devices may be useful as a bridge to transplantation in cases of irreversible liver failure or as a bridge to restoration of liver function in situations of acute liver toxicity (Tabata 2000). This concept has been pursued for several years by a number of U.S. academic groups and companies (e.g., Circe Biomedical of Lexington, MA and Hepatix of La Jolla, CA). Due to societal limitations on liver transplantation, this technology is of great interest in Japan. Several research groups have active programs in this area, including Dr. Oshima’s group at the University of Tsukuba, Dr. Iwata’s group at Kyoto University, and Dr. Akaike’s work at the Tokyo Institute of Technology. In Germany the Virchow/Hybrid Organ GmbH is also attempting to develop and commercialize a BAL system. For both the BAL and BAK, transport between the cells in the device and fluids flowing through or in partial contact with the contained cells must be optimized (McLaughlin et al. 1999; Nikolovski et al. 1999), as the utility of these devices is completely dependent on this function (e.g., clearance of toxic metabolites in blood). A large number of BAL designs have been developed in an effort to optimize this process while minimizing the device volume (McLaughlin et al. 1999), while a lesser amount of work has been done to date with the BAK.

Summary

Many different types of bioreactors have been developed for the diverse bioreactor applications in tissue engineering. Ideally they must all allow for control over the physicochemical environment (e.g., pO2, pH, PCO2, shear rate), allow aseptic feeding and sampling to follow tissue development, and maximize use of automated processing steps to increase reproducibility. Standard bioreactor technologies are well suited to address many of these issues for cell expansion, but they have limitations when used for the other tissue engineering applications (Miller 2000). In particular, the cultivation of three-dimensional tissue constructs and use of bioreactors for BAL and BAK applications place great demands on the mass transport function (e.g., nutrient distribution), and this is the basis for significant research (Obradovic et al. 1999). In addition, it may be necessary to simultaneously culture multiple cell types for certain applications, and this may require more complex bioreactor designs (Emerson et al. 1991).

PRESERVATION OF CELLS AND ENGINEERED TISSUES

Cells, macromolecular biologically active drugs, and three-dimensional tissues grown in bioreactors will all likely be important tissue-engineering products. In all three cases, it will be critical to develop technologies for the stable storage of these products following production and prior to clinical utilization. Cryopreservation, as compared to cold storage, potentially affords long shelf life, low risk of microbial contamination, and cost effectiveness (Karlsson and Toner 2000). This type of storage typically involves reducing or removing water (e.g., lyophilization of protein solutions). The controlled transport of water from the proteins, cells, and tissues is a complex mass transfer problem. Long-term storage of protein products is an important issue that has received extensive attention in the biotechnology and pharmaceutical industries
MASS TRANSPORT ISSUES FOLLOWING IMPLANTATION

There are at least two critical mass transport issues following implantation of an engineered tissue. First, it is critical that transplanted cells or engineered tissues have sufficient nutrient and waste exchange with their surroundings in order to survive, function appropriately, and become integrated with host tissue following implantation. Oxygen transport is typically considered the limiting factor for nutrient exchange (Colton 1995) (Figure 6.3). Secondly, in immunoisolated cell therapies the membrane must not be a barrier to diffusion of desirable molecules (e.g., oxygen, therapeutic molecules secreted by...
transplanted cells) while blocking diffusion of undesirable species (e.g., elements of the host immune response).

**Vascularization**

Tissues in the body overcome issues of oxygen and nutrient distribution by containing closely spaced capillaries that provide conduits for convective transport of nutrients and waste products to and from the tissues. It is similarly considered critical for any engineered tissue of significant size to become vascularized, with the exception of cartilage. Several approaches are currently being investigated to promote vascularization of engineered tissues. First, scaffolds utilized for cell transplantation are designed to promote invasion of host fibrovascular tissue by the inclusion of large, interconnected pores (Mikos et al. 1993). However, fibrovascular ingrowth into the scaffolds occurs at a rate less than 1 mm/day and typically takes one to two weeks to completely penetrate even relatively thin (e.g., 3 mm thick) scaffolds. The second, more active, approach to promote vascularization of engineered tissues is the delivery of angiogenic growth factors (e.g., VEGF, bFGF) to the implant site. It has recently been demonstrated that these factors may be directly included within the tissue engineering scaffolds for a sustained delivery at the desired site (Tabata 2000; Sheridan et al. 2000).

Other vascularization strategies are being explored as well. It may be possible to utilize local gene therapy to promote vascularization by delivery of plasmid DNA, which encodes the growth factors from the tissue-engineering scaffold (Fang et al. 1996; Shea et al. 1999; Ochiya et al. 2000). The majority of protein and DNA delivery strategies focus on release of the factors from polymeric scaffolds to allow for their diffusion into surrounding tissues. In contrast, some groups (e.g., A. Goepferich’s group at Regensburg University in Germany) are instead covalently coupling these factors to the polymer scaffold. This approach will specifically target cells in contact with the scaffold. Another approach to promote vascularization is to transfect the cells to be transplanted with genes encoding for angiogenic molecules (Ajioka et al. 1999). A third approach to enhance angiogenesis in engineered tissues is to co-transplant endothelial cells along with the primary cell type of interest. The endothelial cells seeded into a tissue engineering scaffold form capillaries that can merge with capillaries growing into the scaffold from the host tissue (Nor et al. 1999). This may increase the rate and extent of vascularization of engineered tissues.

A long-term goal of tissue engineering is to grow large-three dimensional tissues (e.g., a complete liver) in culture for subsequent transplantation. To be successful in this approach it will be necessary to develop a pseudo-vascular network in the tissue. This network would be perfused with medium in culture to enable appropriate nutrient distribution throughout the tissue volume, and anastomosed to the native blood supply following implantation to meet the same requirement *in vivo*. This is clearly an ambitious goal, but several research groups (e.g., J.P. Vacanti at Harvard Medical School and H. Iwata at Kyoto University in Japan) have begun efforts to address this possibility.
Immunoisolation

In certain tissue-engineering applications the function of transplanted cells is purely biochemical (e.g., secretion of a protein for local or systemic distribution). In this situation it may be possible to transplant xenogeneic or allogeneic cells without host immunosuppression, if the cells can be isolated from the host immune system. Polymeric membranes are often utilized in these situations (Lysaght et al. 1994). However, cells in the devices must survive by diffusion of nutrients from the surrounding host tissue, and this limits the maximum size of these devices to diameters less than 1 mm (Colton 1995). The constraint imposed by mass transfer limitations has led to several device designs that attempt to balance maximum diffusional transport potential without compromising other functions of the device such as mechanical stability (Lysaght et al. 1994). In any design, however, the numbers of cells that can be delivered in any practical system are limited, and this approach is only appropriate when relatively few cells (e.g., millions) need to be delivered. However, this may not be a limitation for many important clinical applications, potentially including diabetes and central nervous system applications (Sun et al. 1996; Bachoud-Levi et al. 2000). A critical engineering design issue in this area is the lack of data regarding the relationship between barrier permeation properties and immunoisolation effectiveness. Furthermore, widely differing degrees of success have been reported by various groups, perhaps relating to immunological or mass transport issues specific to each application and device design (Colton 2000).

BIOMECHANICS ISSUES

Many of the tissues for which one may desire to engineer a replacement have a mechanical function(s), including blood vessels, bone, and cartilage. However, the mechanical properties of many tissues engineered to date are inferior to those of native tissues (Cao et al. 1994; Carver and Heath 1999; Kim et al. 1999; Niklason et al. 1999; Mauck et al. 2000; Seliktar et al. 2000). This finding clearly leads to two key biomechanics questions. First, what is the relevance of the mechanical properties of the engineered tissues to their function in vivo? Second, assuming the mechanical properties will be important, how can one control these properties of the engineered tissues? To address the first question, there will likely be several biomechanics aspects of native tissues that must be targeted. However, the mechanical properties of many of these tissues have not yet been precisely defined, and it is unclear which of the properties are important to use as design parameters for the engineered replacement tissues, and to what degree. It is relevant to the second question that externally applied mechanical signals are clearly regulators in the development and function of a variety of tissues. Increasing evidence from basic biology studies indicate cells mediate the response of tissues to mechanical signals, and the increasing amount of information available from these studies is now beginning to find utility in the design of engineered tissues.
Minimum Mechanical Properties Required of Engineered Tissues

In order to develop appropriate standards for the mechanical properties of engineered tissues it will be necessary both to understand the in vivo stress/strain in normal tissues in a variety of states, and to determine the complete mechanical properties of native tissues. There is considerable information available for certain tissues such as blood vessels and bone in the normal in vivo mechanical environment. However, for other tissues such as cartilage, there is a lack of data (Guilak 2000). Similarly, while there has been considerable effort to determine the mechanical properties of various tissue types, most biological tissues can be considered to be inhomogeneous, viscoelastic, nonlinear, and anisotropic materials (Guilak 2000). This complicates analysis of tissues, and the relationships between composition, structure, and mechanical properties of tissue are not completely defined.

At the current time it is unclear which of the many measurable tissue properties would be most important for specific engineered tissues, nor is it clear what minimum values for these properties would be appropriate for functional replacement. This issue is further complicated by the potential adaptation of engineered tissues to their mechanical environment following implantation. The limitations in the current knowledge base have been recognized by U.S. National Committee on Biomechanics, which has formed a subcommittee to provide an organized framework for addressing these issues. The principles underlying this endeavor have recently been outlined (Butler et al. 2000).

Mechanical Signals Regulating Cell Function

It has long been recognized that mechanical signals regulate the development of normal tissues, and a large number of investigators worldwide have been working to delineate the molecular mechanisms responsible for the response of individual cells to mechanical signals. For example, hemodynamic influences on the vascular system have been extensively studied (Konstantopoulos and McIntire 1997; Nerem 1993; Ando et al. 2000). There has been significant interest in identifying the role of specific cell-adhesion receptors in conveying this mechanical information into the cell (Ingber 1991; Shyy and Chien 1997), and in the complementary interactions between typical chemical-mediated (e.g., growth factors) signaling pathways and mechanical-mediated pathways (Giancotti and Ruoslahti 1999). These studies will likely define specific regimens of mechanical stimulation that optimally regulate gene expression in culture, and they may provide valuable input for mechanical stimulation of engineered tissues (see next section). In addition, delineation of the mechanisms by which mechanical signals regulate gene expression may ultimately provide new targets for intervention to regulate the structure and mechanical properties of engineered tissues.

Mechanical Signals Regulating Engineered Tissue Properties

A number of research groups, mainly in the United States, have recently begun to mechanically stimulate engineered tissues during in vitro development to determine if their mechanical properties may be modified with this type of input. The development of engineered skeletal muscle is clearly regulated by
mechanical signals (Vandenburgh et al. 1991; Dennis and Kosnik 2000). The organization, composition, and function of engineered smooth muscle tissues and blood vessels can be readily modulated by application of physiologic regimens of cyclic strain (Niklason et al. 1999; Kim et al. 1999; Seliktar et al. 2000). For example, application of continuous cyclic strain (7% amplitude; 1 Hz) leads to significant increase in the ultimate strength of engineered smooth muscle tissue, as compared to static cultured control tissues (Figure 6.4).

Similarly, the mechanical properties of engineered cartilage can be improved by appropriate mechanical stimulation (Carver and Heath 1999; Mauck et al. 2000). These results are promising, but the properties of the engineered tissues still fall short of native tissues. Significant additional work is clearly required to identify the types of mechanical stimulation required to optimize the formation of mechanically competent engineered tissues. A limitation to date has been the lack of suitable experimental systems that can readily provide a range of relevant mechanical, and possibly magnetic or electrical, stimulation to three-dimensional engineered tissues in sufficient numbers to allow large-scale screening studies to be performed. A new device (Figure 6.5) has recently been developed in the laboratory of Robert Dennis at the University of Michigan that meets these criterion for engineered muscle tissue, and the development of similar systems will be key to accelerating progress with other tissues as well.
SUMMARY

Clearly, a large number of design aspects must be considered to engineer tissues for clinical applications. There has been considerable work recently in many of these areas, with promising results. However, significant work remains in each of these areas. Table 6.1 provides an estimation of both the current knowledge base in each of the areas discussed in this chapter, as well as an indication of the amount of work done to date in each area.

Table 6.1
Current Levels of Knowledge and Research in the Engineering Design Aspects of Tissue Engineering

<table>
<thead>
<tr>
<th>Area</th>
<th>Knowledge base</th>
<th>Work to date</th>
</tr>
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<tbody>
<tr>
<td>Bioreactors for 2D cell expansion</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>Bioreactors for 3D tissue growth</td>
<td>Modest</td>
<td>Modest</td>
</tr>
<tr>
<td>Liver and kidney assist bioreactors</td>
<td>Modest</td>
<td>Modest</td>
</tr>
<tr>
<td>Promoting vascularization of engineered tissues</td>
<td>Modest</td>
<td>Little</td>
</tr>
<tr>
<td>Cell storage technology</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>Storage of three-dimensional engineered tissues</td>
<td>Modest</td>
<td>Little</td>
</tr>
<tr>
<td>Identifying mechanical properties of native tissues</td>
<td>Modest</td>
<td>Extensive</td>
</tr>
<tr>
<td>Identifying the minimum properties required of engineered tissues</td>
<td>Little</td>
<td>Little</td>
</tr>
<tr>
<td>Mechanical signals regulating cell function</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>Mechanical signals regulating engineered tissues</td>
<td>Little</td>
<td>Little</td>
</tr>
</tbody>
</table>

Fig. 6.5. Novel device for applying specific regimens of mechanical and/or electrical stimulation to engineered tissues in vitro developed in the laboratory of R. Dennis (University of Michigan). Left. The system is modular and is designed to operate in stacks of 6 units per tower in an incubator. Center. The system uses standard cell-culture disposable plastic dishes. Individual tissue constructs are grown in 35 mm-diameter culture dishes. A 100 mm-diameter culture dish houses the tissue in the 35 mm culture dish, a servo motor, a force transducer, a stepper driver, a high-voltage bipolar stimulator, and two microcontrollers. The units are interchangeable and connect with the main power and data bus via a 25-pin D-sub connector. Right. A close up view of the prototype device, showing the force transducer in the foreground in the 100 mm dish, the servomotor in the background, and the electronics module to the left of the 35 mm culture dish. The mounting fixtures for the tissue construct and the electrodes are not shown. Micropower techniques have been employed to minimize power dissipation and heat accumulation (R. Dennis; used by permission).
REFERENCES


