Tissue Engineering: Challenges and Opportunities

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Abstract: This article reviews the key developments in the tissue engineering field over the past several years. The issues related to the development of the components of tissue-engineered products including cells, biomaterials, and biomolecules, and their integration into safe and effective products are presented. Moreover, the article outlines the challenges to the commercialization of tissue-engineered products, and highlights the ongoing efforts by the American Society for Testing and Materials (ASTM) in developing standards for tissue-engineered medical products. Furthermore, funding opportunities at the Advanced Technology Program at NIST are presented. © 2000 John Wiley & Sons, Inc.* J Biomed Mater Res (Appl Biomater) 53: 617–620, 2000

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INTRODUCTION

Tissue loss or end-stage organ failure resulting from an injury or a disease is a major health care problem in the U.S. as the transplantation of tissues or organs in these patients is severely limited by availability of compatible donors.[1] The currently used alternatives such as mechanical devices or artificial prostheses do not repair the tissue or organ function and are not intended to integrate into the host tissue. Additionally, mechanical devices or artificial prostheses may be subjected to wear upon long-term implantation, and could induce inflammatory response in the host.[2]

The term *tissue engineering* was initially defined by the attendees of the first NSF sponsored meeting in 1988 as "application of the principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationship in normal and pathological mammalian tissues and the development of biological substitutes for the repair or regeneration of tissue or organ function."[3] In 1993, Langer and Vacanti summarized the early developments in this field and defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue or organ function."[4]

Tissue engineering has now emerged as a potential alternative to tissue or organ transplantation. With this technology, tissue loss or organ failure can be treated either by implantation of an engineered biological substitute or alternatively with *ex vivo* perfusion systems. The tissue-engineered products may be fully functional at the time of treatment (e.g., liver assist devices, encapsulated islets), or have potential to integrate and form the expected functional tissue upon implantation (e.g., chondrocytes embedded in a matrix carrier). In some cases, biomaterials are modified to enhance migration and attachment of the specific cell populations, which repair or replace the damaged tissue.

During the 1990s, the tissue-engineering field has progressed rapidly and biological substitutes are in development for several tissues in the body. Tissue-engineered products such as bioartificial skins with viable and nonviable cells (Apligraf from Organogenesis and TranCyte from Advanced Tissue Sciences) and autologous cultured chondrocytes (Carticel from Genzyme Tissue Repairs) have reached the market.[†] Scientists are now engineering cardiovascular tissues such as heart valves[5,6] and blood vessels.[7,8] Encapsulated pancreatic islets have been implanted in the patients for the treatment of diabetes[9] and liver assist systems containing encapsulated hepatocytes have been used clinically to provide extracorporeal support to the patients with liver failure.[10] A kidney support system with encapsulated urothelial cells is in development for the treatment of patients with kidney failure[11] and a bioartificial bladder has been developed as a replacement engineered organ.[12] Additionally, investigators have attempted to engineer the nervous system by encapsu-

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[†]Certain commercial equipment, instruments, and materials are identified in this article in order to specify the experimental procedure as completely as possible. In no case does this identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the material, instrument, or equipment identified is necessarily the best available for the purpose.

lation of genetically modified ciliary neurotrophic factor (CNTF) secreting neural cells to treat amytrophic lateral sclerosis,[13] and nerve guidance channels have been developed for peripheral nerve regeneration[14] and spinal cord repair.[15,16] Ophthalmological engineering efforts are targeted to develop cornea and retina[17,18] and lens tissue.[19] Significant progress has been made in orthopedic tissue engineering for the repair of bones,[20] tendons,[21] cartilage,[22,23] and ligaments.[24] Moreover, Landis and coworkers[25] demonstrated the formation of small phalanges and whole joints using bovine chondrocytes and tenocytes, and bovine periosteum on biodegradable polymer matrices. Scientists have also attempted to generate dental pulp using dental fibroblasts and synthetic matrix.[26] Novel approaches have been pursued to develop vascularized network inside a tissue-engineered skin for grafting on deep wounds to improve vascularization of the skin graft.[27] In the U.S., a Multi-Agency Tissue Engineering Sciences (MATES) working group was recently formed to increase cross-agency communications across federal agencies that are supporting research in tissue engineering. Importantly, an international collaboration among scientists, engineers, and clinicians from academia, industry, and the government (The Life Initiative) began in 1998 in Toronto, Canada, and is targeted to creating an unlimited supply of engineered vital organs for transplantation within a decade with the heart as the initial challenge.[28]

COMPONENTS OF TISSUE ENGINEERED PRODUCTS

Cells are a key to tissue regeneration and repair due to their proliferation and differentiation, cell-to-cell signaling, biomolecule production, and formation of extracellular matrix. The functionality of an engineered tissue may be structural (e.g., bone, cartilage, and skin) or metabolic (e.g., liver, pancreas), or both. Cells may be a part of an engineered tissue, or alternatively, these cells may be recruited *in vivo* with the help of biomaterials and/or biomolecules. When selecting the cellular component of an engineered product, it is important to identify appropriate cells and to be able to isolate them from the primary source. In addition, expansion of these cells without permanently altering the phenotype and function during the expansion phase and without introduction of any adventitious and species-specific bacterial/viral agents poses significant challenges. The issues with contaminating viruses and bacterial agents are even more significant when xenogenic cells and/or culture components are used, because potentially infectious xenogeneic agents may be introduced into the human population with this vehicle. Finally, when genetically modified cells are used in a tissue-engineered product, there are additional concerns such as cell transformation by the vector, vector stability, and optimal function of the inserted gene. For additional information on the concerns related to the cellular component, the publication by Chapekar[29] is helpful.

Recent developments in the stem cell field have impacted significantly on the progress of tissue engineering. Isolation of several adult stem cells including mesenchymal,[30] hematopoietic,[31] neural,[32] and hepatic[33] stem cells have opened a novel avenue for obtaining an unlimited supply of cells. However, full understanding of the factors involved in the differentiation of these stem cells and the regulation of lineage formation is critical to control and development of normal tissue. Along these lines, the molecules such as transforming growth factor, insulin, and dexamethasone have been shown to differentiate the mesenchymal stem cells along the chondrocytic and astrocytic lineages.[30] The host immune response to allogenic and xenogeneic cells poses a major challenge, and investigators are working on altering the cell surface molecules to reduce antigenicity of these cells. Recently, human embryonic stem cells[34] and embryonic germ cells[35] have been isolated, and the human embryonic cells have been shown to differentiate into multiple lineages.[34] The potential of these cells in tissue engineering has yet to be investigated.

Development of biomaterials also poses significant challenges. Formation of implanted tissue is greatly influenced by the composition, architecture, and three-dimensional environment of the scaffold, and biocompatibility of the biomaterial. Moreover, incorporation of signal peptides such as RGD into the material has been attempted to effectively mimic the extracellular matrix and induce cell migration.[36,37] The mechanical strength of the scaffold material needs to mimic the mechanical properties of the tissue it is intended to repair or replace. Moreover, material porosity and pore size distribution and continuity greatly influence the attachment of specific cell types and interaction of the biomaterials with the host. Investigators have also applied mechanical strain to smooth muscle cells on polymeric scaffolds to enhance the mechanical properties of engineered smooth muscle tissue.[38] It is also preferable that the biomaterial degrades *in vivo* to minimize the long-term biocompatibility concerns, with the material degradation rate matching the regeneration rate of the tissue, and that the resulting degradation products are nontoxic to the host. Premature degradation of the material combined with lack of timely *in vivo* development of replacement tissue may result in reduced mechanical strength of an engineered tissue over time, which may lead to its failure. The inflammatory response to biomaterials has been a major safety problem. Many of the currently utilized biomaterials elicit inflammatory responses upon implantation.[39] The fibrotic capsule around the implanted engineered tissue generated by the inflammatory biomaterials may further inhibit the tissue remodeling and function by forming a barrier to the nutrient transport and angiogenesis. Vascularization is critical more specifically for three-dimensional engineered tissues greater than 1 mm^3 to meet their nutritional and metabolic requirements. Investigators have incorporated angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) or their genes into the implants to stimulate angiogenesis in engineered tissues.[40,41] Naturally derived materials such as acellular por-

cine heart valves and small intestinal submucosal tissue have also been used by some investigators to minimize the bioreactivity to materials.

MANUFACTURE AND ASSESSMENT OF AN ENGINEERED TISSUE

Consistent manufacture of an engineered tissue under good manufacturing practices and its preservation and shipping pose additional challenges. For full commercialization, these products need to be universal rather than patient specific and will require long-term storage without loss of function. Novel test methods need to be developed to evaluate the structure and function of engineered tissues during their manufacture and after preservation and shipping. Comparison of an engineered tissue with native tissue is also important and will involve creating databases and enhancing the bioinformatics capabilities. Engineered tissue, however, need not fully resemble a normal tissue as long as it is functionally comparable.

For further clinical and commercial advancements in this area, appropriate standards are needed to ensure consistency and quality of tissue-engineered products. Towards this end, a voluntary standards development effort was initiated in 1997 under the auspices of the American Society for Testing and Materials (ASTM). Division IV under the ASTM committee F4 was charged with developing standards for tissue-engineered medical products (TEMP) . However, it is important that all interested parties become involved in this process to ensure the development of consistent TEMP standards. Further information on the ASTM standards development effort can be found on the following web sites: (1) http://www.fda.gov/CDRH/Tisseng/TEMPS, for introduction and links to other sites; (2) http://lindacuster.com/temps, for meeting information and working documents for Division IV members; (3) http://www.astm.org, for general information and meeting minutes and meeting schedules; and (4) http://astmforums20.micronexx.com, for ASTM online forums for task group members to comment on draft documents.

FUNDING OPPORTUNITIES AT THE ADVANCED TECHNOLOGY PROGRAM AT NIST

The National Institute of Standards and Technology (NIST) is one of the federal agencies that fund extramural research. The Advanced Technology Program (ATP) at the NIST is a rigorously competitive cost-sharing program. Initiated in 1990, ATP awards funds to the industry applicants for developing high-risk technologies that promise significant payoffs and widespread national benefits. A single U.S. incorporated company may receive up to 2 million dollars over a maximum period of 3 years. Large, Fortune-500 companies participating as a single firm need to share at least 60% of total project costs. For a joint venture (JV) between a minimum of two for-profit companies, there is no upper funding limit and these awards can extend up to 5 years. However, the JV needs to cost share greater than 50% of the project cost. Importantly, university researchers contribute substantially to the ATP-funded research as subcontractors to the industry applicants or as full JV partners with two for-profit companies. In 1997, ATP sponsored a specific competition in Tissue Engineering, awarding funds to several industry applicants. In 1999, the ATP changed the competition structure to an open competition for all technology areas using technology specific review boards. The FY 2000 Open Competition followed the same structure. For additional information on the ATP mission and its current status, the competition structure, program eligibility, award selection criteria, and process, instructions for writing a preproposal or full proposal, the ATP web site (URL: www.ATP.nist.gov) is useful. Preproposals are accepted on a year-round basis to provide immediate feedback on whether the project idea is potentially competitive for cost-shared funding from ATP and is on track with respect to information essential to developing a successful full proposal. However, full proposals are accepted only in response to an announced competition, with proposals typically due in the spring of each year.

CONCLUSION

Although significant progress has been made in the tissue-engineering field, many challenges remain and further development in this area will require ongoing interactions and collaborations among the scientists from multiple disciplines, and in partnership with the regulatory and the funding agencies.

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