A COURSE ON TISSUE ENGINEERING

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Tissue engineering is a new and emerging interdisciplinary field that combines engineering, materials science, and cellular biology knowledge to solve the critical problems of tissue loss and organ failure. Approaches involve using biological and synthetic materials, together with mammalian cells, to create new tissues or biological substitutes for functional replacement. Materials are used as supportive matrices for functional cells, as necessary barriers between transplanted cells and host tissues, or as stimulants for a desired cellular response.

Chemical engineers are uniquely qualified to make significant contributions to this new field since they can apply the engineering principles of transport and reaction phenomena to understand the biological processes occurring in the human body. The undergraduate chemical engineering curriculum requires courses in organic chemistry, which are usually prerequisites for biochemistry and cellular biology courses, and also offers electives in materials science and engineering. This basic training gives chemical engineers an advantage over other engineering disciplines in communicating with life scientists and clinicians to investigate problems in medicine and to respond to challenges for new technological developments.

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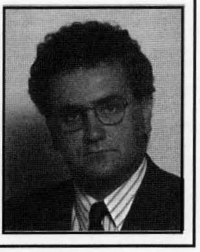

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Engineering, materials science, and cellular biology are distinct disciplines, and their courses do not usually cover a specialized subject such as tissue engineering in sufficient depth. This course aims to not only teach aspects of engineering and cellular biology in the same semester, but also to present them in such a way that the student learns the cellular phenomena involved in tissue development and growth and gains an appreciation of the role of biochemical and mechanical environment in regenerating tissues.

Classes are held in a traditional lecture format, but emphasis is placed on class discussions and question/answer sessions. It is ultimately a design course in which students use fundamental concepts and principles to develop ways to regenerate tissues and methods to replace the function of diseased organs. The three-credit-hour course meets two days a week during the semester. It is offered as an elective to graduate students of any discipline, but it appears to be predominately of interest to science and engineering majors. The course was first offered in the spring of 1993 and was attended by seventeen students, including two students of the Baylor College of Medicine/Rice University MD/PhD program.

COURSE CONTENT

The course content includes background information on biomaterials, cell/tissue interactions with materials, tissue development and growth, and new approaches to tissue regeneration and replacement of function (see Table 1). We devote approximately half of the semester to covering background information since this knowledge is essential for discussing the design of biological substitutes.

In their natural environment, cells are surrounded by extracellular matrix (ECM). Biomaterials used in tissue-engineering constructs are generally designed to simulate the environment experienced by cells and can therefore be referred to as ECM analogs. In attempting to regenerate tissues, some form of ECM is required to either organize transplanted cells or recruit cells from the surrounding tissue. We review the role of natural ECM found in the body to inform the students of its importance in holding together and compartmentalizing tissues. These ECM molecules can be isolated and used in tissue-engineering constructs, or synthetic materials can be manufactured for the same purpose. A majority of these ECM analogs are polymers of synthetic or biological origin; therefore, we present a brief review of polymer chemistry, covering topics such as polymer chain types, kinetics, thermodynamics, morphology, mechanical properties, synthesis, and fabrication techniques. We also discuss the chemical structure and morphology of the most common synthetic and natural polymers used in tissue engineering, with emphasis on structure-property relationships.

The surface, biochemical, and mechanical environment encountered by cells has a direct effect on their function and thus determines the success of a cell-based therapy. The environment created by the materials used in tissue-engineering approaches must be appropriate to promote the desired response from transplanted or recruited cells. In order to understand how the environment should be altered to achieve a desired cellular response, we give lectures on the variety of factors that influence the function and survival of cells, such as cellular adhesion, cell-biomaterial interactions, and migration, as well as metabolism.

TABLE 1
Course Outline

Extracellular Matrix Analogs (3 lectures)
- Extracellular Matrix
- Synthetic Polymers
- Natural Polymers

Regulation of Cell Function (4 lectures)
- Cell Adhesion
- Cell-Biomaterial Interactions
- Cell Migration
- Cell Metabolism

Tissue Development and Growth (6 lectures)
- Tissue Remodeling
- Tissue Repair
- Angiogenesis
- Inflammatory Response
- Immunoprotection
- Drug Delivery

Tissue Engineering Approaches (10 lectures)
- Tissue Induction
  - ▷ *Skin, Nerve, Esophagus, Blood Vessel*
  - ▷ *Tendon, Ligament, Bone*
- Cell Transplantation
  - ▷ *Skin, Cartilage, Bone*
  - ▷ *Endothelium, Urothelium, Intestine, Nervous System*
  - ▷ *Liver, Kidney*
- Biohybrid Organs
  - ▷ *Liver*
  - ▷ *Pancreas*
- Blood Substitutes
- Gene Therapy
  - ▷ *Cardiovascular System*
  - ▷ *Other Systems*

Tissue Engineering Products (2 lectures)
- Patents
- Regulations

We present an example that demonstrates the importance of cellular adhesion in anchorage-dependent mammalian cells which need to adhere to a substrate in order to grow and retain their phenotypic expression. The involvement of adhesion molecules and cell-surface receptors in cellular phenomena suggests that biomaterials used in tissue engineering should mimic their natural counterparts, the ECM of the body. We discuss cellular adhesion, with emphasis on the role of adhesive receptors and adhesion recognition sequences. We also consider the engineering of intelligent biomaterials with immobilized adhesion recognition sequences for targeted cellular adhesion, using illustrated examples of the effects of substratum chemistry on cellular physiology.

Cellular migration is also regulated by receptor/ligand adhesive interactions. We review the mechanisms of cellular motility and locomotion and discuss contact inhibition. We analyze the cellular binding and trafficking processes and present mathematical models to better understand receptor-mediated cellular functions and the effects of cytokines and growth factors on cellular migration.

Cellular adhesion, migration, and metabolism are all interrelated. The vascular and skeletal systems provide excellent examples of this interplay, and we discuss them along with the influence of the biochemical and mechanical environment on cellular metabolism. We cover the stress and strain effects on vascular gene expression as related to the production of therapeutic molecules for vascular proliferative diseases. We also analyze the mechanical load effects on osteoblast proliferation and migration and the production of bone matrix proteins, which are all important in the bone remodeling process.

Tissue development and growth is a complex process involving many cells and components. Understanding the process of natural tissue development and growth is necessary to appreciate the cellular or mechanical components of the different tissue-engineering approaches presented in later lectures. We teach tissue development and growth processes in separate lectures that focus on tissue remodeling, tissue repair, angiogenesis, inflammatory response, immunoprotection, and drug delivery, all of which are described below.

Dynamic processes occur constantly in healthy as well as in injured tissue. Bone is an excellent example of a tissue that continuously remodels itself. In one lecture we discuss the steps involved in bone remodeling so that students can understand the role of the cellular, biochemical, and mechanical environments in tissue remodeling. We devote another lecture to wound healing as an example of tissue repair because this naturally occurring process has been extensively studied and offers the chance to observe the actual repair and regeneration of tissue. We review in detail the roles of the cells and ECM components involved in the different steps of wound healing.

Many metabolic organs, including the liver, are highly vascularized. A high frequency of blood vessels throughout the liver is necessary for the survival of the cells housed within, as well as for their vascular, secretory, and metabolic functions. Therefore, while creating a new construct to regenerate such a vascularized organ, the mass transport principles specific for nutrient diffusion and waste removal must be considered. We address the need for angiogenesis (the formation of new blood vessels) to occur within the construct, as well as the angiogenic factors and inhibitors involved in the vascularization mechanism.

The materials or cells used in organ regeneration or replacement devices may come in direct contact with blood or tissues, and therefore they must be biocompatible. A complete understanding of the immunological response to foreign materials or cells is necessary in order to develop a system that is immunologically "invisible." We present a review of immunology to teach the students about those cells and other components involved in immune and inflammatory responses, including the chemical mediators of inflammation, phagocytosis, and foreign-body reaction. We also outline methods for developing materials that do not elicit an immune response.

Synthetic or natural biodegradable polymers used as cell transplantation and tissue induction scaffolds can also serve as controlled release systems to deliver bioactive molecules. Many tissue-engineering scenarios require the local release of angiogenic, growth, and differentiation factors to facilitate the development and growth of new organoids. Therefore, we present drug delivery systems based on biodegradable polymers, with emphasis on design parameters and drug-release kinetics.

The next section of the course deals with case studies of strategies for the creation of new tissues, including tissue induction, cell transplantation, biohybrid organs, blood substitutes, and gene therapy. We give a brief overview of the existing options to replace human tissue before each case study, stressing their shortcomings and emphasizing the great need for alternative methods.

Tissue induction techniques rely on the recruitment of cells from the tissue surrounding the implant site into biomaterial matrices. These matrices are designed to aid the regeneration process by providing a suitable environment for the organization and function of the recruited cells and tissue. We examine the principles of tissue induction and follow with illustrated examples of how this technique is being explored to regenerate tissues. We cover practices for regenerating skin, nerve, esophagus, and blood vessels in one lecture, whereas those for tendon, ligament, and bone are covered in another lecture.

Transplantation of isolated cells seeded onto polymer matrices provides another method for regenerating organs and tissues. These matrices serve the same purpose as those used in tissue induction techniques, but may have to be designed differently to accommodate the introduction of isolated cell populations. We explore the material requirements of cell transplantation scaffolds, along with case studies for regenerating skin, cartilage, bone, endothelium, urothelium, intestine, nervous system, liver, and kidney.

Biohybrid organs may provide yet another means of replacing functions lost by diseased or injured organs. Biohybrid organs are artificial systems that use cells donated by a different person or animal for functional replacement of metabolic organs. In these systems, the donor cells are isolated from the host by semipermeable membranes which allow for the passage of metabolites but not immunogenic molecules. We analyze the function of biohybrid organs, using chemical engineering principles such as mass transport since multiple nutrient transport barriers exist between the encapsulated cells and the host's blood vessel, all of which take part in the overall diffusion limitation of the devices. In one lecture we present the important membrane parameters and other design parameters for cell immobilization devices, such as intravascular stents, macrocapsules, and microcapsules, whereas biohybrid liver and pancreas are covered in separate lectures.

The prime function of the red blood cells is to transport oxygen to various parts of the body. Blood substitutes are bioartificial constructs designed to replace this function when it is compromised. The development of blood substitutes would be advantageous in circumventing the problems arising from the lack of blood donors, the transfer of blood-born pathogens in transfusions, and long-term storage. We therefore cover the important criteria that must be considered in the design of blood substitutes, along with methods to fabricate blood substitutes and ways to assess their performance.

The advances of the growing field of genetic engineering can be used in tissue engineering. Gene therapy may be defined as the introduction of genetic material into cells to alter selected cellular functions. This technique may be used to deliver enzymes, proteins, or other compounds produced by the genetically modified cells. One lecture focuses on cardiovascular gene transfer and another concentrates on gene transfer applied to other systems, including the blood-clotting, hematopoietic, and hepatic systems.

A lecture on patents is appropriate since one should be aware of the means of protecting intellectual property developments. We therefore discuss the patentability requirements of an invention and the formal requirements of a patent, along with giving examples of tissue-engineering patents.

We conclude the course with an overview of the FDA regulations for tissue-engineering product investigations. We present the guidelines for human biological products and the procedures for submitting an investigational new drug application in order to give the students an appreciation of the series of tests required to bring a tissue-engineering product to market.

COURSE WORK

Students are graded on their participation in class discussions, one in-class exam, and a group project. The project requirements consist of a written paper and a thirty-minute oral presentation. The students are divided into groups of two or three and are allowed to choose an organ or tissue which they investigate and for which they devise a tissue-engineering strategy. The students are encouraged to use the knowledge they gained in the course, together with past engineering and scientific experience, to develop an innovative method to regenerate an organ or improve an existing method. In the spring of 1993, seven design project topics were chosen by the class and involved strategies to regenerate the pancreas, kidney, bone marrow, intestine, breast, blood vessel, and nerve.

RESOURCE MATERIAL

It is difficult to find just one textbook for this course since it covers such a broad range of subjects. Most of the resource material used is from research and review articles in journal publications, proceedings, or books. Two excellent review articles on tissue-engineering accomplishments, challenges, and directions were recently published in *Science*.[1,2] The key chemical engineering principles involved in tissue engineering (mass transport and materials synthesis and fabrication) are presented in a recent article in *Chemical Engineering Progress*.[3] A few of the important journals that publish articles related to tissue engineering include *ASAIO Journal, Annals of Biomedical Engineering, Biomaterials, Biotechnology and Bioengineering, Cell Transplantation, Diabetes, Human Gene Therapy, Journal of Applied Biomaterials, Journal of Biomechanical Engineering, Journal of Biomechanics, Journal of Orthopaedic Research, Journal of Biomedical Materials Research, Transplantation,* and *Transplantation Proceedings.* Surgical journals, such as *Journal of Bone and Joint Surgery, Journal of Craniofacial Surgery, Journal of Pediatric Surgery,* and *Plastic and Reconstructive Surgery,* also publish articles on tissue engineering.

Starting in January of 1995, a new journal, *Tissue Engineering,* will be published. Also, *Biotechnology and Bioengineering* has published two special-issue volumes[4,5] that include only articles related to the subject. The *Journal of Biomechanical Engineering,* as well, had a special issue on tissue engineering.[6]

The Materials Research Society also published two symposium proceedings devoted to tissue-engineering topics.[7,8] Two additional proceedings publications [9,10] contain useful articles—though most of them may now be outdated.

Currently, many organ regeneration and functional replacement methods are either in pre-clinical or clinical trials; therefore, new information is constantly appearing in the literature. We recommend performing literature searches to uncover the most recent work in this new field before teaching a similar class. An edited volume by Hay[11] includes useful review articles on ECM metabolism and regulation of tissue development and growth, and a book by Lauffenburger and Linderman[12] is an excellent reference on receptor-mediated cell function, including cell adhesion, migration, and metabolism. A book by Fung[13] is a valuable reference on cell and tissue mechanics and the role of mechanical environment on tissue remodeling and repair, while a handbook by Culver[14] presents the methods for gene transfer and summarizes the most recent developments in gene therapy for treating non-neoplastic disorders. Finally, the conference proceedings of an annual continuing-education course offered by Rice University[15] may be a helpful reference material.

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