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INTRODUCTION TO TISSUE ENGINEERING

Learning Objectives:

After completing this chapter, students should be able to:

1. Provide examples of tissue and organ systems being developed using tissue engineering strategies.
2. Describe how tissue engineering can help solve the problem of chronic shortage of donor organs.
3. Discuss the tissue engineering paradigm as it applies to cardiovascular tissue engineering.
4. Define tissue engineering.
5. Describe the process of fabricating artificial tissue.
6. Discuss design principles related to tissue engineering.
7. Identify building blocks for the field of tissue engineering.
8. Describe scientific and technological challenges in the field of tissue engineering.
9. Describe strategies for the functional assessment of 3D artificial tissue.
10. Discuss seminal papers in the field of tissue engineering.
11. Describe potential applications for 3D artificial tissue.
12. Explain the relative advantages of 3D culture over 2D monolayer culture.

Introduction to Tissue Engineering: Applications and Challenges, First Edition. Ravi Birla.

13. Describe the collaborative model for tissue engineering research.
14. Discuss the growth in the field of tissue engineering.
15. Discuss the participation rate from different disciplines in tissue engineering.
16. Explain the differences between tissue engineering and other related fields.

CHAPTER OVERVIEW

We begin this chapter by providing a broad overview of tissue engineering research and providing examples of tissue and organ systems that are currently under development using tissue engineering strategies. We next describe the chronic shortage of donor organs and provide a vision of how tissue engineering can help alleviate this problem. In the next section, we describe the tissue engineering paradigm and how it applies to the cardiovascular system. We then provide a formal definition of tissue engineering and describe the process to bioengineer 3-dimensional artificial tissue. In the next section, we describe the design principles related to tissue engineering and identify fundamental building blocks in the field. We then discuss some of the scientific and technological challenges in the field of tissue engineering. Next, we describe strategies for functional assessment of 3D artificial tissue and describe functional, biological and histological metrics. We next discuss seminal publications in the field of tissue engineering and the contribution of these toward the development of the field. We then move on to discuss potential applications of 3D bioengineered artificial tissue. Tissue engineering is a multidisciplinary field, and in the next section, we discuss the multidisciplinary nature of the field and how researchers from many different backgrounds work together. The next section is focused on the growth of tissue engineering as a scientific discipline and some of the drivers of this growth. We end this chapter by providing a description of scientific disciplines that are closely related to tissue engineering.

1.1 INTRODUCTION TO TISSUE ENGINEERING

We begin our discussion of tissue engineering with a broad overview of the field—*what exactly is tissue engineering and why is it important?* While in the next section, we provide a formal definition of tissue engineering, we begin this discussion with a general overview of the field. *Research in the field of tissue engineering is focused on the fabrication of artificial tissue and organs*. The statement of purpose defined for tissue engineering (fabrication of artificial tissue and organs) is very challenging with numerous scientific and technological challenges, many of which we will discuss during the course of this book. However, the important concept to grasp is the simple notion that tissue engineering is equivalent to tissue and organ fabrication, a recurring theme throughout this book.

We have seen that tissue engineering refers to the fabrication of artificial tissue and organ systems; however, this statement requires further clarification. Artificial

organ development using mechanical components is a mature field of research with mechanical hearts and left ventricular assist devices being used in patients. The field of tissue engineering should be differentiated from this area of research, as the objective of tissue engineering is to fabricate biological artificial organs that are similar in form and function to mammalian organs. Cells and biomaterials (which simulate mammalian extracellular matrix) are important components of artificial organs fabricated using tissue engineering strategies.

What is the long-term objective of tissue engineering research? The overarching theme in tissue engineering is artificial tissue and organ development. The potential application of artificial organs is obvious: transplantation in patients with damaged or diseased organs. There is a chronic shortage of donor organs, as the number of waitlisted patients is significantly greater than the number of donor organs available. Tissue engineering has the potential to alleviate this problem by fabricating artificial organs that can be used clinically.

Let us continue our discussion on tissue engineering by looking at some areas where active research is being conducted in the fabrication of artificial tissue and organs. Tissue engineering research has expanded significantly in the last decade with active research programs across the country and worldwide encompassing many different tissue and organ systems. There has been significant interest in cardiovascular tissue engineering, with research devoted to the fabrication of artificial heart muscle, blood vessels, valves, cell based cardiac pumps, ventricles, and entire bioartificial hearts. Another active area of research has been in the musculoskeletal system, encompassing fabrication of bone, cartilage, skeletal muscle, and tendons. A significant amount of research has been invested in tissue engineering of the urinary system, which consists of kidneys, urinary bladder, ureters, and urethras. Tissue engineering of the airway system has focused on fabrication of artificial tracheas and artificial lung tissue. The digestive system has been a very active area of tissue engineering research focused on the development of artificial liver tissue, pancreas, intestine tissue, and esophageal tissue. In addition, there is significant interest in the development of artificial skin and tissue engineering strategies for the central nervous system.

1.2 CHRONIC SHORTAGE OF DONOR ORGANS

There is a chronic shortage of donor organs available for transplantation. This can be illustrated by the case of kidney and liver transplantation (Figure 1.1). As can be seen in the figure, the number of patients on the waiting list is significantly greater than the number of donor organs available (1). This chronic shortage of donor organs is evident in other organ systems as well, and highlights the urgency to develop novel strategies to address this problem. The ability to fabricate artificial organs in the laboratory using tissue engineering strategies can alleviate some of the problems associated with chronic shortage of donor organs. Rather than having a patient on a waiting list for a donor organ, the promise of tissue engineering is that artificial organs can be fabricated under controlled conditions in

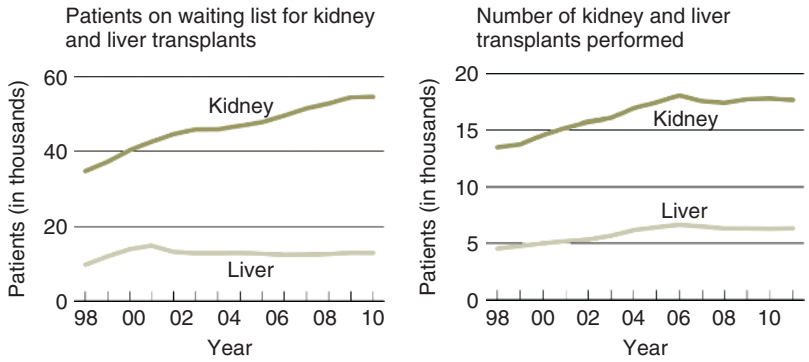


Figure 1.1 Donor Organ Shortage in the US—There is a chronic shortage of donor organs. The number of patients waitlisted for kidney and liver transplants is significantly higher than the number of donor organs available. *Note*–The data and analyses reported in the 2011 Annual Data Report of the Organ Procurement and Transplantation Network and the US Scientific Registry of Transplant Recipients have been supplied by the Minneapolis Medical Research Foundation and UNOS under contract with HHS/HRSA. The authors alone are responsible for reporting and interpreting these data; the views expressed herein are those of the authors and not necessarily those of the US Government.

the laboratory and used for transplantation. This strategy can provide life-saving options for millions of patients around the globe. This is the grand vision of tissue engineering—fabrication of artificial organs that can provide life-saving options for patients around the world.

1.3 THE TISSUE ENGINEERING PARADIGM

In this section, we introduce the tissue engineering paradigm using the cardiovascular system as an example. There are several conditions that can compromise the function of the heart, including acute myocardial failure, atherosclerosis, valve stenosis or hyperplastic left heart syndrome. Several strategies, including pharmacological agents, mechanical devices like pumps, and surgical interventions like heart transplantation, have been developed to help patients with cardiovascular disorders. Undoubtedly, these strategies have helped numerous people and saved many lives. However, heart transplantation is plagued by the chronic shortage of donor hearts, and many of the other treatment strategies also have limitations. The ability to bioengineer artificial hearts and components of the cardiovascular system can provide an alternative treatment modality for many patients; this can lead to an improvement in the quality of life and can also save the lives of many patients.

The field of cardiovascular tissue engineering is focused on the fabrication of artificial heart muscle, blood vessels, tri-leaflet heart valves, cell based cardiac pumps, tissue-engineered ventricles and bioartificial hearts (2). Artificial tissues and organs related to the cardiovascular system can be used in a variety of ways to

help patients with cardiovascular disorders. For example, heart muscle can be used to provide functional support to the left ventricle of compromised hearts, thereby assisting in cardiac function. As another example, bioartificial hearts can be used as transplantable organs for patients with end-stage heart failure, thereby providing a life-saving option for many patients across the globe.

The purpose of the discussion presented in this section was to illustrate the tissue engineering paradigm using the cardiovascular system as an example. As we have seen, tissue engineering strategies can be applied toward the fabrication of artificial hearts and components of the cardiovascular system that can be used to repair, replace or augment the functional performance of comprised hearts. This is the fundamental premise of tissue engineering—fabrication of artificial tissue and organs that can be used clinically to help patients by providing functional recovery of diseased or damaged tissue and organs.

1.4 DEFINITION OF TISSUE ENGINEERING

In this section, we define tissue engineering and discuss different terms (regenerative medicine, reparative medicine), that have been used to describe the field. The definition of tissue engineering has been evolving over the last several years. As with any new discipline, the scope of the definition changes with a better understanding of the scope of the field. In addition, due to the diversity of scientific disciplines of researchers participating in the field, the definition changes to accommodate these differences in training. Before presenting our own definition, we would like to consider various definitions provided by renowned researchers in the field as well as those definitions adopted by major scientific governing bodies.

Tissue Engineering—[definition] (National Science Foundation, 1997) (3)

The production of large amounts of functional tissues for research and applications through the elucidation of basic mechanisms of tissue development combined with fundamental engineering production processes.

The NSF's definition of tissue engineering closely reflects the Foundations mission of understanding and promoting science at a very basic level and applying engineering principles for problem solving. These two fundamentals are reflected in the NSF's definition of tissue engineering.

Tissue Engineering—[definition] (Eugene Bell, 1992) (4)

1. *providing cellular prosthesis or replacement parts for the human body;*
2. *providing formed acellular replacement parts capable of inducing regeneration;*
3. *providing tissue or organ-like model systems populated with cells for basic research and for many applied uses such as the study of disease states using aberrant cells;*
4. *providing vehicles for delivering engineered cells to the organism; and*
5. *surfacing non biological devices.*

The definition provided by Dr. Eugene Bell is very much focused on the delivery of end products and is application based. This definition refers to replacement parts for the human body, tissue- or organ-like model systems, and vehicle delivery of engineered cells.

Tissue Engineering—[definition] (Dr. J. P. Vacanti & Dr. R. Langer, 1993) (5)

Tissue Engineering is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue formation.

The definition provided by Dr. Vacanti and Dr. Langer is the one that is most frequently cited in the tissue engineering literature. The publication in which this definition surfaced is one of the seminal papers in the field and is discussed in detail in a later section. The definition provides several governing principles of the field, including the interdisciplinary nature of the research and reference to the end products to improve tissue function.

Tissue Engineering—[definition] (National Institute of Health, 2001) (6)

Reparative medicine, sometimes referred to as regenerative medicine or tissue engineering, is the regeneration and remodeling of tissue in vivo for the purpose of repairing, replacing, maintaining, or enhancing organ function, and the engineering and growing of functional tissue substitutes in vitro for implantation in vivo as a biological substitute for damaged or diseased tissues and organs.

The first point to note about the definition provided by the National Institute of Health (NIH) is the reference to the terms reparative medicine, regenerative medicine and tissue engineering; NIH refers to these three terms as the same scientific discipline. While NIH refers to all three fields asone, there are subtle differences between the fields; these will be discussed in a later section. The second point about the NIH definition is its emphasis on improving human health by the use of the phrase "implantation *in vivo* as a biological substitute for damaged or diseased tissues and organs"; this is consistent with the mission of the NIH, which is to improve and enhance human health.

Tissue Engineering—[definition] (Dr. M. V. Sefton, 2002) (7)

From working with microencapsulated cells and immunoisolation systems for many years, we have learned that successful implementation of a tissue-engineering construct requires (1) an adequate, viable cell mass; (2) the appropriate behavior of the cells; and (3) sufficient durability of the function in vivo. The specific requirements are determined by the application, the nature of the cells, the implantation site, and the biocompatibility of the device.

This definition refers to encapsulation technology as it relates to tissue engineering. This is due to the nature of Dr. Sefton's work, which is focused on the development

of encapsulation technology. However, these are two distinct fields and the differences will be provided in a later section. The definition provides a list of requirements for tissue engineered constructs.

Tissue Engineering—[definition] (Dr. A. Atala, 2004)(8)

Tissue engineering, one of the major components of regenerative medicine, follows the principles of cell transplantation, materials science, and engineering toward the development of biological substitutes that can restore and maintain normal function.

Similar to the definition provided by NIH, this definition draws a comparison between tissue engineering and regenerative medicine. While NIH considers tissue engineering and regenerative medicine to be the same, the Atala definition refers to tissue engineering as a component or branch of regenerative medicine.

Tissue Engineering—[definition] (Dr. R. Nerem, 2006) (9)

Whether one uses the term bioengineered tissues, tissue engineering, or regenerative medicine, what one means in general is the replacement, repair, and/or regeneration of tissues and organs.

In this definition, tissue engineering and regenerative medicine are considered to be the same, as mentioned earlier.

Tissue Engineering—[definition] (Dr. C. Mason and Dr. P. Dunnill, 2008) (10)

Regenerative Medicine is an emerging interdisciplinary of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma, and aging.

This is our final definition and talks exclusively about regenerative medicine, though the definitions refer to many of the guiding principles of tissue engineering, which have been presented in the earlier definitions.

What have we learned by looking at these definitions? There are many definitions of tissue engineering, often based on the principles of the researcher or the scientific organization. The terms tissue engineering, regenerative medicine, and reparative medicine have been used extensively in the literature and often refer to the same field. The term tissue engineering has been extensive in the literature and has been used more often than regenerative medicine and reparative medicine. The use of the terms regenerative medicine and reparative medicine is fairly new, and their exact definitions are still being developed. Tissue engineering, regenerative medicine and reparative medicine are often used interchangeably while, in other instances, tissue engineering is considered to be a sub-group of the two (regenerative medicine and reparative medicine). Often, tissue engineering has been defined as the ability to generate functional 3D tissue constructs *in vitro*, with potential

clinical applications to replace, restore and/or augment lost tissue function. Regenerative Medicine has been commonly defined as any strategy directed at stimulating the body's own repair mechanisms, e.g., through the use of gene and/or cell transplantation. Certain authors define regenerative medicine as a broader field, with tissue engineering being one branch.

Although a diversity of definitions has evolved in the recent literature, each one provides a novel insight into the field of tissue engineering. Rather than accepting any one given definition, it is a valuable exercise to study the underlying principles that have evolved in the field of tissue engineering. Based on a survey of the definitions of tissue engineering presented earlier, it was seen that the field has been defined based on participating disciplines (engineering, biology, and surgery), building blocks (cells, biomaterials, and bioreactors), and/or end product applications (tissue repair and/or replacement). Based on this, we have formulated a working definition of tissue engineering, which encompasses these concepts and is illustrated in Figure 1.2.

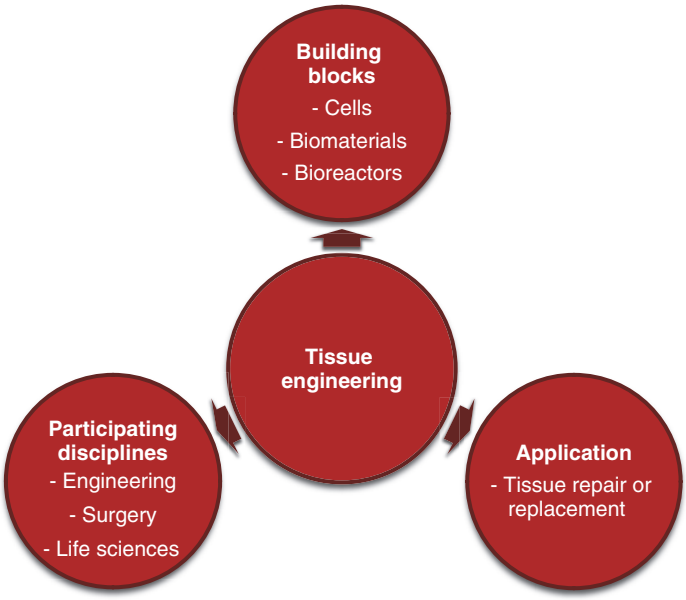


Figure 1.2 Definition of Tissue Engineering—The field of tissue engineering is focused on the development of technologies to support the fabrication of artificial tissue and organs. The building blocks of tissue engineering are cells, biomaterials, and bioreactors. Tissue engineering is a multidisciplinary field with researchers from different backgrounds working together; researchers with training in engineering, medicine, and life sciences have contributed significantly to the development of the field. Potential applications of bioengineered artificial tissue and organs is for repair and/or replacement of damaged or injured tissue.

"*Tissue engineering is a multidisciplinary field bringing together experts from engineering, life sciences and medicine, utilizing the building blocks of cells, biomaterials and bioreactors for the development of 3-dimensional artificial tissue and organs which can be used to augment, repair and/or replace damaged and/or diseased tissue*."

This definition highlights the multidisciplinary nature of tissue engineering as a scientific discipline, provides insight into the building blocks of the field, and provides information about the potential use of artificial tissue and organs. The terms regenerative medicine and reparative medicine have not been included in this definition. We will adapt this definition throughout the book and will refer to it from time to time. In subsequent chapters, we will also provide an in-depth discussion of many of the underlying principles of tissue engineering.

1.5 PROCESS OF BIOENGINEERING 3D ARTIFICIAL TISSUE

Introduction—The process of tissue fabrication has been aggressively debated over the last couple of years, and different researchers use different processing schemes to fabricate artificial tissue and organs. Nonetheless, several general themes have evolved over the years that provide impetus for the development of a process flow sheet that is required for the fabrication of 3D artificial tissue (Figure 1.3). The steps in the process have evolved from studies at different research institutions and represent a general scheme for the fabrication of artificial tissue and organs. The specific process implemented for any given application will vary, and steps may need to be added or eliminated from the process flow sheet.

Eight Step Process for Tissue Fabrication—In this section, we provide a brief overview of the process of bioengineering 3D artificial tissue (Figure 1.3). The process differs based on the specific tissue system as well as any differences in the tissue engineering strategy adopted; however, several common themes have been identified and can be categorized into 8 stages. Depending on the tissue system and the specific technology, the sequence of steps may also need to be changed. The eight-step process of bioengineering 3D artificial tissue involves: (Figure 1.3)

1. *Cell sourcing*—Cells provide the functional component of artificial tissue. Identification, isolation, purification, expansion, and characterization of a suitable cell source are important steps in cell sourcing. During initial stages of technology development and feasibility studies, cells can also be obtained from animal sources, with cell lines being another option. As the research progresses toward the development of artificial tissue for use in humans, researchers need to determine if the cells will be obtained from autologous or allogeneic sources. The recent expansion in the field of stem cell biology has provided researchers with many different options for cell sourcing, some of which include embryonic stem cells, induced pluripotent stem cells, and adult derived stem cells (hematopoietic stem cells and bone marrow derived mesenchymal stem cells).

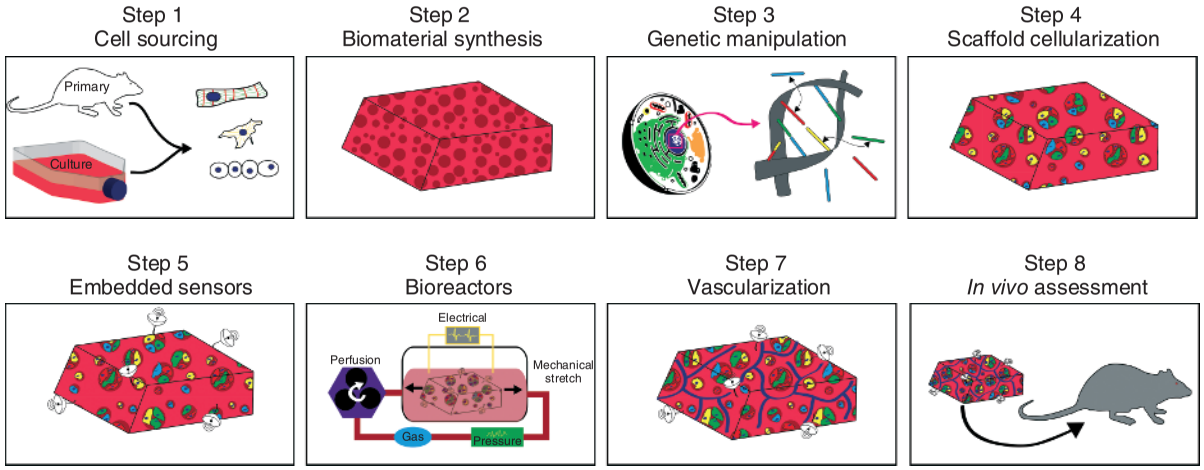


Figure 1.3 Process of Fabricating 3D Artificial Tissue and Organs—**(a) Step 1—Cell Sourcing**—the first step in the process is the isolation and/or expansion of cells, which serve to provide function for artificial tissue and organs. **(b) Step 2—Biomaterial Synthesis**—biomaterials are designed to simulate the properties of the mammalian extracellular matrix and provide structural support during fabrication of artificial tissue and organs. **(c) Step 3—Genetic Manipulation**—genetic properties of cells are modified to improve function and reduce apoptosis and other adverse effects. **(d) Step 4—Scaffold Cellularization**—in this step, cells are coupled with scaffolds. **(e) Step 5—Embedded Sensors**—sensors are embedded to monitor tissue development and maturation. **(f) Step 6—Bioreactors**—bioreactors are used to deliver controlled physiological stimulation to guide development and maturation of artificial tissue and organs. **(g) Step 7—Vascularization**—vascularization is required to support the metabolic activity of 3D artificial tissue and organs. **(h) Step 8—*In vivo* Assessment**—the final step in the process is to test the functional performance of artificial tissue *in vivo*.

2. *Biomaterial synthesis*—Biomaterials provide structural support during 3D tissue fabrication and serve the role provided by mammalian extracellular matrix. During this stage of the tissue fabrication process, biomaterial synthesis and characterization are important variables that require rigorous optimization. The choice of biomaterial depends on the specific tissue application; there are many different biomaterials to choose from, including polymers, metals and ceramics.
3. *Genetic manipulation*—Prior to scaffold cellularization, the genetic profile of the cells can be modified to increase the likelihood of cell survival or functional integration with the host. Specific genes can be manipulated to reduce apoptosis or increase the expression of specific integrins to increase cell-matrix interactions. In addition, functional genes can be upregulated, like myosin heavy chain for heart muscle, to increase the functional performance of 3D artificial tissue.
4. *Scaffold cellularization*—Scaffold cellularization refers to the process by which isolated cells are seeded within a 3D scaffold. An important variable during the scaffold cellularization process is coupling isolated cells with the scaffold to promote functional integration at the cell-cell and cell-material interface. Successful implementation of the scaffold cellularization process is critical to support 3D tissue formation. The cellularization strategy needs to be optimized to ensure uniformity in cell distribution throughout the scaffold.
5. *Sensor technology*—Sensors are necessary to monitor the overall health of the artificial tissue during the formation, development, and maturation stage of the tissue fabrication process. Embedded sensors are necessary to monitor functional performance of artificial tissue, and data obtained from embedded sensors can be utilized in a feedback loop to regulate processing variables for tissue fabrication. Monitoring of cell behavior, cell-cell interaction, cell-matrix interaction, and tissue formation and function is critical during the tissue fabrication process.
6. *Bioreactors for guidance*—During normal physiological function, mammalian tissue is exposed to a wide array of stimuli, which include electromechanical impulses, fluid stresses, and changes in the chemical environment based on changing concentrations of growth factors, hormones, and cytokines. These signals are important in maintaining tissue function. During the fabrication of 3D artificial tissue, it is critical to develop strategies to deliver these signals. Specialized systems known as bioreactors are designed to deliver physiological signals to 3D artificial tissue, which in turn provides guidance to drive tissue development and maturation.
7. *Vascularization*—Incorporation of blood vessels as an integrated component of the artificial tissue is a critical requirement and is required to support the metabolic activity of 3D artificial tissue.
8. *In vivo assessment*—Once functional 3D artificial tissue has been fabricated, the final step in the process is *in vivo* testing. In this case, the effectiveness

of the tissue graft to repair, replace, and/or augment the function of damaged or diseased tissue is assessed.

Brief Discussion of the Tissue Fabrication Process—Now that we have discussed the process flow sheet of bioengineering 3D artificial tissue, we next present a brief description of how this process comes together for the fabrication of artificial tissue. The identification of a suitable cell source remains a formidable challenge, especially for cardiac applications, as adult derived cardiomyocytes are difficult to obtain and non-proliferative *in vitro*, thereby limiting their applicability. There are several areas of opportunity for cell sourcing, including human embryonic stem cells, adult derived stem cells, and autologous cells derived from patients. The choice of cell source will vary significantly depending on the application; autologous derived skeletal muscle cells can be utilized for cardiac regeneration, while autologous derived cardiac cells may not be the most feasible choice. Selection of suitable scaffolding material depends on the ability of the material to simulate properties of the extracellular matrix (ECM), promote cell viability and proliferation, possess easily controllable degradation kinetics, and have a high degree of immune tolerance when implanted *in vivo*. There are several matrices currently available which meet many of these requirements, while new and improved biomaterials with improved functionality are continuously being developed. The cells are then subjected to strategies for genetic manipulation to increase function and reduce cell apoptosis. The next stage requires successful colonization of the scaffold by the cells; the viability of the cells during culture within the scaffold, the ability of the cells to maintain differentiated phenotype, and the ability of the cells to functionally interact with the biomaterial become important considerations. Sensors are embedded to provide real-time noninvasive monitoring of tissue function and development and are used in a feedback loop to guide processing variables during the fabrication of artificial tissue.

Once cellularization of the scaffold is complete, the next step in the tissue fabrication process involves bioreactors to guide the development and maturation of artificial tissue. It becomes necessary to provide mechanical, electrical, and chemical/hormonal cues to support the functional development of 3D artificial tissue. Bioreactors need to be implemented to induce electro-mechanical stimulation of bioengineered tissue, leading to gene expression that closely resembles the gene expression of *in vivo* tissue. The development of microperfusion systems becomes increasing important to replicate the physiological flow conditions observed *in vivo*. As tissue growth and maturation occurs, vascularization of the bioengineered tissue construct is important. Finally, the ability of the tissue-engineered construct to integrate with the host tissue, without immune rejection and the ability of the construct to both survive and elicit a functional benefit, would need to be demonstrated.

1.6 DESIGN PRINCIPLES FOR TISSUE ENGINEERING

The process for the fabrication of artificial tissue is governed by design principles. In simplest of terms, tissue engineering equals tissue fabrication and, like any

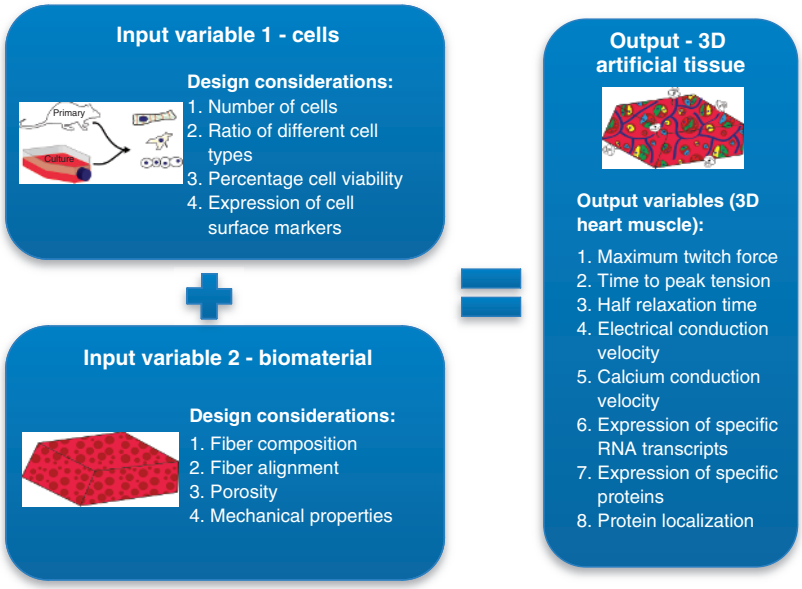


Figure 1.4 Tissue Engineering and Tissue Fabrication—Tissue engineering should be viewed as a process of fabricating artificial tissue; in other words, tissue engineering equates to tissue fabrication. Like any fabrication process, tissue fabrication has inputs (cells and biomaterials) and an output (artificial tissue). At each step of the process, there are critical design variables and design constraints which need to be addressed, some of which are shown in the Figure.

fabrication technology, is based on design principles with critical decision making at every step in the process. Like any other engineering problem, there is an input that feeds into the tissue fabrication process, and there is also an output, 3D artificial tissue. Design considerations for tissue fabrication are shown in Figure 1.4, and, for the sake of simplicity, only two input variables are included in our discussion: cell sourcing and biomaterial synthesis.

For cell sourcing, there are important design considerations that need to be taken into account, some of which include the number and density of cells, relative proportion of different cell types, percentage of viable cells, and expression of specific cell surface markers. All of these variables are under the control of the user and can be changed prior to feeding into the tissue fabrication process. The same argument can be applied toward biomaterial synthesis, and important design considerations include fiber composition and alignment, material porosity, and tensile properties of the materials. Again, all of these variables can be changed by the user prior to feeding into the tissue fabrication process. The input variables just described, cells and biomaterials, are fed into the tissue fabrication process, leading to a specific output—3D artificial tissue. The success of the process is measured by predefined metrics defined by the user, and, depending on the specific application, output criteria will vary.

In the current discussion, we have only provided examples of two steps of the tissue fabrication process (cell souring and biomaterial synthesis), although the same process is valid for all eight steps; for every step in the tissue fabrication process, researchers need to define specific design requirements for artificial tissue fabrication. Process optimization is necessary to achieve predefined values for output variables for any given tissue system. The tissue fabrication process, along with an understanding of input and output variables, is central to the field of tissue engineering.

1.7 BUILDING BLOCKS OF TISSUE ENGINEERING

Earlier in this chapter we described an eight-step process for the fabrication of 3D artificial tissue. All eight steps in the tissue fabrication process are critical and the absence of any one would disrupt the process. However, there are three steps in the tissue fabrication process that are considered to be the building blocks of artificial tissue: cells, biomaterials and bioreactors (Figure 1.5).

Cells are the functional components of artificial tissue; biomaterials are the structural components of artificial tissue while bioreactors provide guidance for tissue development and maturation. In the absence of any one of these three building blocks of tissue engineering, the functional performance of 3D artificial tissue will be significantly compromised. At the start of any tissue fabrication process, the

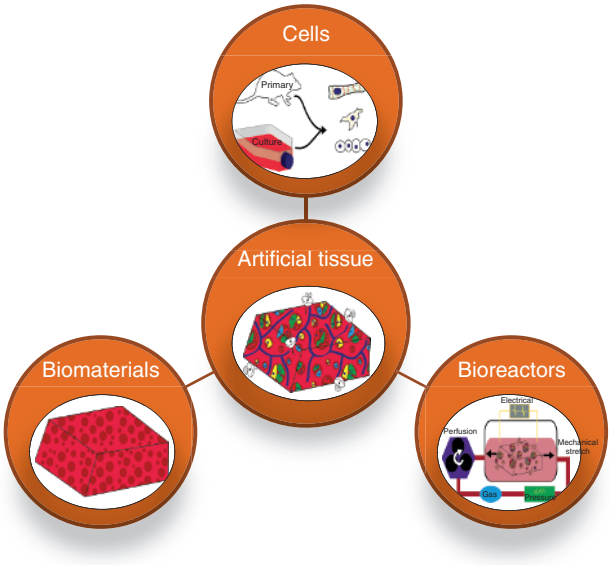


Figure 1.5 Building Blocks of Tissue Engineering—The building blocks of tissue engineering are cells, biomaterials and bioreactors.

researcher must identify the source of cells, the biomaterial to be utilized, and the guidance stimuli to be used; collectively, these three provide the platform to initiate any tissue fabrication study. Using these three building blocks, researchers can build an initial prototype for first-generation artificial tissue. This can be viewed as an entry point into the tissue fabrication process, similar to laying the foundation for building a house.

1.8 SCIENTIFIC AND TECHNOLOGICAL CHALLENGES

The process of tissue fabrication is very convoluted and complex, and at each step of the process, there are numerous scientific and technological hurdles that need to be overcome (Figure 1.6).

1. *Cell sourcing*—Where will the cells come from? Human embryonic stem cells, induced pluripotent stem cells or mesenchymal stem cells? If using stem

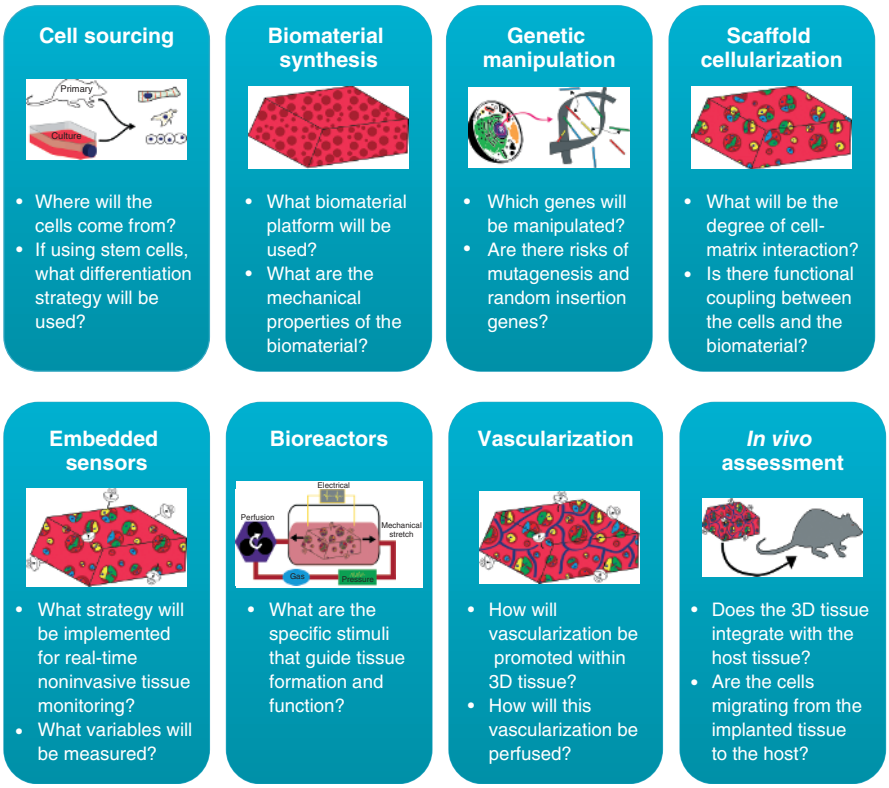


Figure 1.6 Scientific Challenges in Tissue Engineering—At every step of the tissue fabrication process, there are numerous scientific challenges, some of which are illustrated in the Figure.

cells, what differentiation strategy will be implemented? What is the differentiation efficiency? How will the differentiated cells be separated from the non-differentiated cells?

2. *Biomaterial synthesis*—What biomaterial platform will be used? Polymeric scaffolds, biodegradable hydrogels, decellularized scaffolds, or scaffold-free technologies? What are the mechanical properties of the biomaterial? What is the porosity, pore diameter, and pore orientation? Does the biomaterial have attachment sites for cell surface receptors? Is the material degradable and, if so, what are the degradation kinetics? Can the degradation kinetics be modulated?
3. *Genetic manipulation*—Which genes will be manipulated? What delivery mechanism will be implemented? Are there risks of mutagenesis and random insertion of genes?
4. *Scaffold cellularization*—What will be the degree of cell matrix interaction? Will there be functional integration at the cell material interface? Is there functional coupling between the cells and the biomaterial, or are the cells passively resting on the biomaterial? Does the presence of the cells change the properties of the biomaterial? What is the viability of the cells after cellularization, and how does this viability change with time?
5. *Embedded sensors*—What strategy will be implemented for real-time noninvasive monitoring of tissue function? What variables will be measured? How will the data be used in a positive feedback control loop?
6. *Bioreactors*—What are the specific stimuli that guide tissue formation and function? How important is stretch, electrical stimulation, and perfusion? What should be the spatial and temporal variations in physiological stimuli? Will different stimuli be used at different points during the tissue fabrication process?
7. *Vascularization*—How will vascularization be promoted within 3D tissue? Will *in vivo* methods of vascularization be used? Will *in vitro* methods of vascularization be used? How will this vascularization be perfused?
8. *In vivo assessment*—Does the 3D tissue construct integrate with the host tissue? Is there mechanical coupling? Is there electrical coupling? Are the cells migrating from the implanted tissue to the host? Does implantation of the 3D artificial tissue lead to functional improvement of the host tissue or organ?

1.9 FUNCTIONAL ASSESSMENT OF ARTIFICIAL TISSUE

The objective of tissue engineering is to fabricate 3D artificial tissue—after completing the tissue fabrication process, how do we measure performance of the bioengineered tissue? The ability to define performance metrics, which accurately reflect critical functional variables, is extremely important for the tissue fabrication process. Performance metrics need to be carefully defined and must accurately assess the function of artificial tissue.

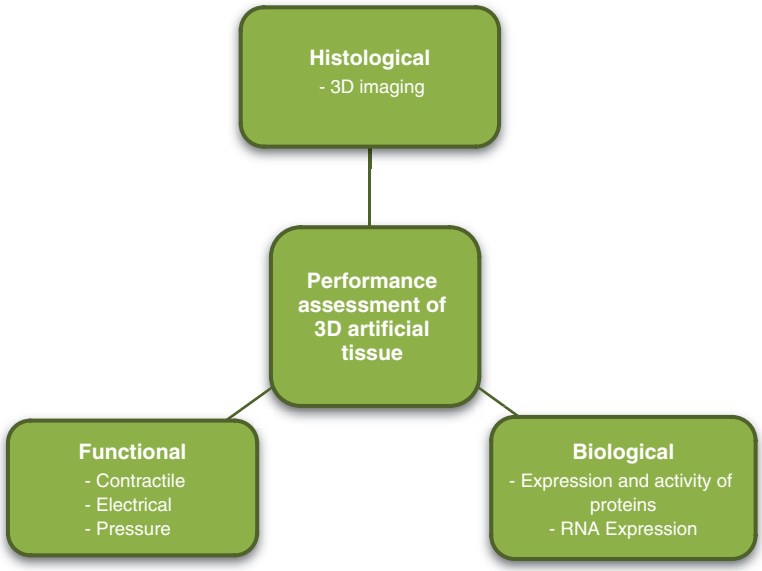


Figure 1.7 Performance Metrics for 3D Artificial Tissue—The success of artificial tissue can be assessed based on functional performance metrics, biological performance metrics, and, finally, based on histological performance metrics.

How exactly do we define performance metrics for 3D artificial tissue? The objective of the tissue engineering process is to bioengineer artificial tissue that is similar in form and function to mammalian tissue equivalents. Therefore, the best way to measure functional performance of 3D artificial tissue is by direct comparison with functional performance of mammalian tissue; the closer the two, the better.

There are three categories of metrics designed to assess the performance of 3D artificial tissue: functional, biological, and histological (Figure 1.7).

Functional performance metrics are designed to assess function of artificial tissue. Some examples include contractile force, intraluminal pressure and electrical properties. Depending on the function of the tissue, different performance metrics are used; the primary function of heart muscle is force generation, therefore, the contractile properties of artificial heart muscle are important functional metrics. Biological metrics refer to the expression and activity of specific proteins using western blotting or the expression of mRNA transcripts using rt-PCR. The cells of all mammalian tissue perform specialized functions, and in order to perform specialized functions, have a characteristic gene/protein expression pattern. For example, in order for heart muscle to generate force, specific proteins like myosin heavy chain (MHC) are expressed; the greater the expression of MHC, the higher the twitch force. Therefore, measurement of MHC expression for 3D artificial heart muscle proves to be an important assessment tool. Histological metrics refer to the localization of specific proteins, either in the extracellular matrix or

intracellular proteins. Histological tools allow visualization of the cells relative to the extracellular matrix; this visualization in turn provides information about cellular organization and tissue level architecture.

None of the three performance metrics (functional, biological, and histological) are more important than the others. The collective information gathered from all three performance metrics provides an accurate assessment of the success of the tissue fabrication process and the quality of bioengineered artificial tissue.

1.10 SEMINAL PAPERS IN TISSUE ENGINEERING

Seminal work in the area of tissue engineering was conducted by Dr. Robert S. Langer, who is widely recognized as the founder and father of the field. The early work in tissue engineering, when the field was unknown to the general public and to other researchers in the field, was conducted in the laboratory of Dr. Langer at Massachusetts Institute of Technology (MIT). Several publications by Dr. Langer are seminal in the field and have provided the foundation for many researchers to build upon. Even today, Dr. Langer leads one of the largest and most respected academic research laboratories in the country. In addition to his scientific endeavors, Dr. Langer has trained numerous scientists who have gone on to hold prominent positions in both academia and industry. In recognition of his vast contribution to the field, this section begins with a brief biography of Dr. Langer, followed by a discussion of his seminal papers in the field. All information presented in Dr. Langer's biography has been obtained from public sources and is cited appropriately. This biography has not been reviewed, validated, or endorsed by Dr. Langer.

Dr. Robert S. Langer was born on August 29th, 1948 in Albany, New York (11). Dr. R. Langer received his undergraduate degree in Chemical Engineering from Cornell University in 1970 and his Ph.D, also in Chemical Engineering, from MIT in 1974, with Dr. Clark Colton serving as his doctoral advisor[1] (12). Dr. Langer did post-doctoral training with Dr. Judah Folkman at the Children's Hospital of Boston from 1974–77[1] (13). Dr. Langer is currently the Germeshausen Professor of Chemical and Biomedical Engineering at MIT (14) and was honored with an Institutional Professorship (the highest honor award to a faculty member) in 2005 (15).

During his professional career, Dr. R. Langer has won more than 150 major scientific awards. Dr. R. Langer was awarded the National Medal of Science, the nation's highest scientific honor, on July 27th 2006; the award was presented by President George W. Bush (16). In 2002, Dr. R. Langer was awarded the Charles Stark Draper award (equivalent of the Nobel Prize for Engineers) by the National Academy of Engineering (17). Also in 2002, Dr. R. Langer was awarded the Lemelson-MIT award, the highest recognition for inventorship (18). In addition, Dr. R. Langer was named as one of the 15 most influential innovators worldwide by Forbes Magazine in 2002 (19) while CNN and Time Magazine (2001) named Dr. R Langer among America's Best in Science and Medicine (20).

Dr. R. Langer is an author of more than 950 scientific papers, many of which describe seminal work in the field of tissue engineering. His work has provided

the foundation for several areas of tissue engineering, including controlled drug delivery, heart valves, heart muscle, lungs, livers, bioreactors and microperfusion. Dr. R. Langer's research has been protected by over 600 patents, with more than 100 of these being licensed to companies and at least 35 products, at the time of publishing, either in the market or in clinical trials. Dr. R. Langer has founded several companies and was instrumental in the commercialization of the first tissue engineering product, skin.

Three papers published by Dr. R. Langer are considered seminal in the field of tissue engineering (5,21–22).

In 1976, Dr. R. Langer provided evidence for the controlled release of large molecules from 3D polymeric matrices, providing the foundation for the field of controlled drug release [21]. In this study, several materials were screened based on inflammatory response, and based on the results, two materials, hrydron-S and ethylene-vinyl acetate copolymer, were tested as controlled release vehicles. Several proteins were embedded into the material architecture, including soybean trypsin inhibitor, alkaline phosphate, and catalase. The release kinetics were monitored for up to 100 days. The release kinetics was shown to approach zero-order kinetics and the activity of proteins after being released from the polymer was confirmed. This study is seminal since it was the first demonstration of the release of large macromolecules from polymer substrates and laid the foundation for the field of controlled release technology (21).

In 1988, Dr. R. Langer published an article providing evidence to support the culture of primary hepatocytes derived from rodent livers within 3D matrices fabricated from different polymers (polyglactin, polyorthoesters, polyanhydride) (22). The cells were cultured within the 3D matrices *in vitro*. Scaffold fabrication was conducted using several different technologies, including solvent casting, compression molding, and filament drawing. Cells were plated onto the polymer scaffold at a concentration of 1×10^5 or 1×10^6 cells/ml, and cellularized scaffolds were maintained in culture for 3 to 4 days in a 10% CO_2 environment. The cellularized scaffolds were then transplanted onto the omentum of recipient animals after a partial hepatectomy. Histological evidence showed engraftment of cells within 3D matrices during *in vitro* culture. Cell survival was also demonstrated *in vivo*, during the implantation period. This paper is seminal as it was the first time primary cells were cultured within a 3D polymeric scaffold and shown to maintain viability during *in vitro* culture (22). The strategy used in this study, one consisting of cellularization of custom fabricated scaffolds followed by 3D culture, has now become a hallmark of the field of tissue engineering, with numerous publications using this strategy. Clearly, this publication had a significant impact on the field and is responsible for significant growth that has occurred within the last two decades.

In 1993, Dr. R. Langer published a review article on tissue engineering (5). In this article, Dr. R. Langer provided a broad overview of the field as well as his visionary goals for the potential impact, in terms of clinical applications, for tissue-engineered products (5). Equally important, a now widely adopted definition of tissue engineering was also proposed. According to Dr. R. Langer (5), "*tissue engineering is an interdisciplinary field that applies the principles of engineering and*

the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue formation." In addition to providing a definition of the field, several examples of potential applications of tissue engineering technologies in different systems were explained, including skin, cartilage, and bone. At the time of his article's publication, the field of tissue engineering was unknown to the general public and to researchers across the country. However, this publication appeared in a journal, *Science*, which is both broad based and very influential, and therefore served in creating awareness for the field. Researchers recognized the importance of the field and the potential impact successful tissue engineering therapies could have on human health. This served to encourage researchers to enter the field and resulted in an increase in the number of scientific publications describing tissue engineering research.

The three papers published by Dr. R. Langer are seminal due to the awareness and recognition provided to the field of tissue engineering and their establishment of scaffold cellularization as a tissue engineering strategy. Collectively, these three publications provided the foundation for the field of tissue engineering as we know it today.

1.11 APPLICATIONS OF 3D ARTIFICIAL TISSUE

During the course of this chapter, we have studied the process of bioengineering 3D artificial tissue. Prior to moving on, it is a good exercise to discuss potential applications of artificial tissue—*how exactly is bioengineered artificial tissue going to be used?* Based on our discussion thus far, it may be fairly obvious that the most significant application of artificial tissue and organs is clinical, with the objective to develop novel treatment modalities that can have an impact on human health. This is indeed the ultimate goal and long term vision for the field of tissue engineering: to fabricate artificial tissue and organs that can be implanted in humans to support, repair, augment and/or replace damaged or diseased tissue and organs.

However, in addition to the potential clinical applications, there are several other areas in which 3D artificial tissue can be used. Some potential applications for artificial tissue are as models for basic research, tools to study the effect of space radiation on human health, tools for high throughput screening assays, and as grafts for the attachment of mechanical devices to host tissue.

Artificial tissue can be used as a model for basic research to gain an understanding of the processes related to tissue formation and development and as an insight into cell-cell and cell-matrix interaction. There are several models that are currently used, with 2D monolayer culture of isolated cells being a commonly used model. Monolayer 2D culture systems are based on the isolation and culture of cells in a 2D environment (23); these cells are then be subjected to different interventions, some of which include controlled exposure to pharmacological agents and environmental toxins. The response of the cells during 2D monolayer culture can be studied in a controlled *in vitro* environment. Monolayer cell culture is a standard technique and is used extensively around the globe for numerous applications. One of the primary

advantages of monolayer cell culture is the ability to study physiological effects of regulated stimuli in the absence of confounding systematic variations from *in vivo* systems and therefore lead to an understanding of cause and effect relationships. While monolayer cell culture techniques have tremendously enhanced our understanding of basic cell biology, monolayer culture systems are conducted in 2D and lack 3D tissue level architecture and therefore do not provide a true representation of mammalian tissue. Artificial tissue, which has been engineered in the laboratory, overcomes this limitation by replicating many of the features found in mammalian tissue; 3-dimensionality is a significant advantage of artificial tissue when compared with cells maintained in monolayer culture. Just as 2D monolayer culture is a standard technique across research laboratories, 3D artificial tissue has the potential to replace these models and become the staple for basic research across the country.

Artificial tissue can prove to be a powerful tool to study the effect of space radiation on human health. When astronauts travel to space, they are exposed to harsh environments consisting of space radiation, microgravity, and oxidative stress, all of which have adverse effects on human health (24–27). The specific effects of space radiation and other stimuli on human tissue are not known due to the lack of models to undertake systematic studies. It is critical to gain an understanding of the dose–response behavior of specific stimuli observed in space to develop countermeasures necessary to ensure safety of astronauts. Agencies like The National Aeronautics and Space Administration (NASA) can benefit from tissue engineering models to gain an understanding of the effects of space radiation, microgravity, and oxidative stress on human health and use this information to develop countermeasures to eliminate harmful effects.

Artificial tissue can be used to develop high throughput screening assays for pharmacological agents (28–31) or environmental toxins (32–35). Many of these agents are tested in 2D monolayer cultures followed by *in vivo* assessment with no intermediate steps in between. Artificial tissue can serve as an intermediate step between 2D monolayer and *in vivo* assessment. In the case of new drug development, candidate compounds are screened using *in vitro* monolayer cell culture models, and potential candidates are tested using small animal models. At each step of the process, the number of compounds is reduced from a few thousand to a few hundred. The size and scale of these studies is very large and can add significantly to the total cost of the development of a new drug. If tissue engineering models are used prior to *in vivo* assessment, the total number of compounds that need to be tested using animal models can be reduced; this can lead to a reduction in development time and associated costs.

Mechanical devices are routinely used during surgery and, while they have proven to be effective in several conditions, the attachment of the device to mammalian tissue often proves to be challenging. As an example, left ventricular assist devices (LVADs), which are used in cases of chronic heart failure, serve to pump blood directly from the left ventricle to the aorta (36–38). The LVAD is attached to the apex of the heart using a very invasive procedure, which requires the use of numerous sutures; the interface between the LVAD and mammalian

tissue is nonfunctional. This can be significantly enhanced by the use of artificial tissue at the interface between LVAD and mammalian tissue; which will lead to an increase in adhesion strength and will provide functional coupling between the mechanical device and mammalian tissue. In the future, bioengineered artificial tissue can be developed to replicate many functions of mechanical devices like LVADs; however, one short-term objective would be to serve as anchoring points between mechanical devices and mammalian tissue.

The long-term application of 3D artificial tissue is clinical, designed to replace and restore tissue function for damaged or diseased tissue. However, before achieving the clinical objective, there are numerous applications for artificial tissue, some of which have been discussed here and many of which can be developed in the near future.

1.12 TWO-DIMENSIONAL VERSUS THREE-DIMENSIONAL CULTURE

Cell culture techniques have been developed and optimized to maintain and expand isolated cells on the surface of 2D tissue culture plates (23,39–41) These techniques have become standard across academic research laboratories. We will provide a detailed discussion of the topic in Chapter 2. The culture of isolated cells is critical for tissue engineering studies as well, due to the large number of cells required to support tissue fabrication. During the last several decades, monolayer culturing of cells has added tremendously to our understanding of concepts related to cell biology and pharmacology. The importance of monolayer cell culture techniques cannot be overstated, and these techniques continue to be a mainstay for many areas of investigation. However, there are some limitations associated with monolayer cell culture. During normal mammalian function, cells are not maintained under 2D conditions, but rather under 3D conditions; the cells are in constant communication with other cells and with components of the extracellular matrix. Cell-cell interactions and cell-matrix interactions are important in maintaining cell phenotype and tissue function. These physiologically important cues are not fully reproduced during 2D monolayer culture; this limitation can be overcome by tissue engineering models. Rather than maintaining isolated cells in 2D culture, researchers now have the ability of culturing 3D artificial tissue and utilizing these models to answer many questions in cell biology and physiology that cannot be addressed by 2D culture systems. Tissue engineering models offer several advantages over 2D culture systems, the most important of which is the ability of these models to replicate complex 3D architecture of mammalian tissue; this in turn supports cell-cell and cell-matrix interactions and can increase our understanding of cell biology and cell physiology.

1.13 INTEGRATION OF CORE TECHNOLOGIES

Development of tissue engineering technologies requires collaborative efforts from diverse scientific disciplines. This model of scientific collaboration is fairly

well-established in many scientific endeavors; however, the novelty of tissue engineering places additional challenges in implementing successful collaborations. Development of core technologies for tissue engineering requires expertise from engineering, medical sciences, and life sciences disciplines. At every stage of technology development, experts from engineering, medical sciences, and life sciences participate in different aspects of the process. We will study the technology development process as it applies to the fabrication of 3D artificial heart muscle and assess the relative contributions of experts from different disciplines.

The technology development process is divided into three phases: fabrication of first-generation heart muscle, development of mature heart muscle, and, finally, fabrication of 3D artificial heart muscle similar in form and function to mammalian heart muscle. This can be viewed as early-stage, mid-level, and later stages of technology development. During each of these three stages, researchers from different disciplines (engineering, medical sciences, life sciences) have a very specific role to play during the technology development process.

Let us begin this discussion by assessing the fabrication of early-stage heart muscle tissue. The first step in the technology development process is identification of the need—*why do we need to bioengineer artificial heart muscle?* The need for artificial heart muscle will generally begin in the surgical suite during the treatment of patients with myocardial infarction. From the point of view of the surgeon, while there are several therapeutic options available to treat acute myocardial infarction, each one has severe limitations. Therefore, there is an urgent need to develop novel treatment modalities for patients with myocardial infarction; the ability to fabricate 3D artificial heart muscle is one such treatment modality. The two most essential components of 3D artificial heart muscle are cells and biomaterials; these are necessary pre-requisites to initiate the technology development process. Researchers in life sciences, particularly cell biology, are best trained to assume the responsibilities of cell sourcing while researchers in engineering, particularly material science, are best trained to assume responsibilities for biomaterial fabrication.

Cell sourcing requires identification, isolation, purification, and characterization of a suitable cell source, typically carried out by cell biologists. Development of biomaterials, particularly biomimetic biomaterials, can be spearheaded by the engineering team and would require expertise in biomaterial synthesis, characterization, and induction of bioactivity thereby allowing functional interaction with cells. The ability of cells to functionally interact with biomaterials and promote the formation of 3D heart muscle depends on many factors like attachment of cells to fibers of the biomaterials via integrin mediated binding and ability of cells to maintain differentiated phenotype during scaffold colonization. Understanding and manipulating cell-material interactions necessitates scientific input from engineering as well as life sciences experts.

During the next stage of technology development, the objective is to progress from early stage heart muscle to mature heart muscle. During this stage, there are three important areas of research. First, there needs to be an effort directed toward the development of small animal models to test the effectiveness and safety of

bioengineered heart muscle. This work is best spearheaded by surgeons, who have the necessary skill set to undertake studies of this nature. Surgeons are also best capable of assessing success of the artificial heart muscle as it relates to recovery of myocardial function. Second, the development of bioreactor technology is essential and required to simulate physiological conditions and modulate fluid stress environments. Bioreactors are required to deliver controlled mechanical stretch, electrical stimulation, and controlled fluid flow to guide development and maturation of artificial heart muscle; engineers are best trained to undertake these studies. Third, an accurate assessment of tissue function is required, which involves gene and protein expression along with ultra-structural analysis. These performance metrics are essential to guide the success artificial heart muscle, and this work should be spearheaded by experts in the life sciences.

Let us move on to the final stage of the technology development process, which is focused on the development of 3D artificial heart muscle similar in form and function to mammalian heart muscle. Again, each of the three groups of experts has a specific role in the technology development progress. Large animal models are required to test the effectiveness and safety of tissue grafts; advanced bioreactors are needed with real-time functional assessment of processing variables and noninvasive methods to measure tissue function. As we have seen before, researchers in medical sciences, life sciences, and engineering have specific roles to play in this late stage of technology development; this again demonstrates the need for true collaboration by scientists from each discipline.

A true collaborative effort between various disciplines is imperative to the success of each phase, and it is crucial to promote the exchange of technology between each phase, revisiting the problem definition during every stage of the process. This simple example serves to demonstrate the degree of complex interactions and exchange of information required at the very early stages of scientific development between scientists from medical sciences, engineering, and life sciences. Development of a successful model to accomplish this degree of scientific and technological collaboration will be a significant challenge for the field of tissue engineering, yet is an essential ingredient for success.

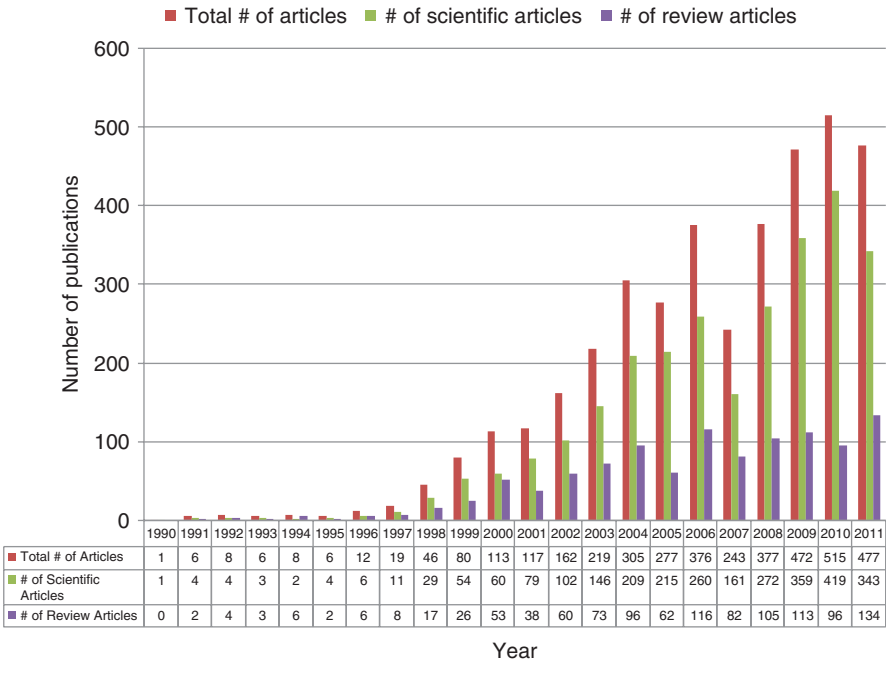
1.14 GROWTH OF TISSUE ENGINEERING

The field of tissue engineering has seen significant growth during the last decade (Figure 1.8).

As has been the case with the development of any new technology, either within academia or industry, initial work starts due to the vision of a single person and is localized to the surrounding environment of this visionary person. During the early stages of technology development, preliminary work is always focused on establishing the feasibility of the work. Expansion of the technology is associated with successful development and evolution beyond initial feasibility studies. A very famous example is the story of Apple, which is now one of the most valuable companies on earth and has touched the lives of hundreds of millions of people around

the globe. With such phenomenal success, it is hard to imagine the modest beginning of Apple, founded as Apple Computer Inc., in a small suburban garage by Steve Jobs, widely regarded as one of the greatest innovators of our time. As we will see in the current discussion, the field of tissue engineering has followed a similar trajectory, starting in the laboratory of a single investigator, Dr. Robert Langer, and expanding to its current status with national and international acceptance and recognition.

Early work in the field began in the Boston area, particularly in the laboratory of Dr. R. Langer. His early work focused on controlled drug delivery, with his first publication in this area appearing in 1976. However, the first publication describing tissue engineering appeared much later in 1988. It showed the survival of primary hepatocytes within 3D scaffolds. During these early years, there were few publications about either controlled drug delivery or tissue engineering. As with most new technologies, the initial work was restricted to a few research centers, which were knowledgeable in the scientific field. However, over time, there was an expansion in the field, as can be seen by the increase in the number of publications from 1990–2011 (Figure 1.8).



	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Total # of Articles	1	6	8	6	8	6	12	19	46	80	113	117	162	219	305	277	376	243	377	472	515	477
# of Scientific Articles	1	4	4	3	2	4	6	11	29	54	60	79	102	146	209	215	260	161	272	359	419	343
# of Review Articles	0	2	4	3	6	2	6	8	17	26	53	38	60	73	96	62	116	82	105	113	96	134

Figure 1.8 Growth of Tissue Engineering (1990–2011)—the growth of tissue engineering as a scientific discipline has seen significant growth over the period spanning from 1990 to 2011. The growth in tissue engineering is evident in terms of the number of scientific articles and review articles that have been published during this time period.

We evaluated the growth in tissue engineering based on the number of scientific publications. We conducted a title search, using tissue engineering as the keyword in Medline. Our search was limited to articles published in English. We distinguished between the total number of articles published and the number of review articles; the difference between these two was considered to be the total number of scientific articles. The results of this search are presented in Figure 1.8. As can be seen from the figure, there was a significant growth in the number of publications in the field of tissue engineering during the time period from 1990–2011. In 1990, there was one scientific publication with the word tissue engineering in the title; this number increased to 113 by the year 2000 and 515 by the year 2010. The growth of tissue engineering is not fully reflected in our data, as we limited our search to articles published with the phrase "tissue engineering" in the title. There are numerous articles which were published in the field and do not have the phrase tissue engineering in the title but focus on diverse research areas like bioreactors, biomaterials, and vascularization.

The growth of tissue engineering is attributed a review article published by Dr. R. Langer and Dr. V.C. Vacanti in the journal Science in 1993. This article was simple and did not provide a great deal of scientific and/or technological insight into the field. However, this article did provide a framework for tissue engineering, defining the scope, challenges, and future potential of success in the field. Publication of this article in a broad based journal like *Science* created a certain awareness of the field. The concept was particularly well received and endorsed by the scientific community. It encouraged researchers to participate in a shared vision, perhaps motivated by the potential impact of the development of tissue engineering technologies. Another driving factor leading to growth in tissue engineering was the availability of existing technologies that could translate into this field. The two major components defined in the paper were cell biology and material science; expertise in these areas was readily available in many research centers across the globe.

The field of tissue engineering has expanded significantly over the last decade, and there are several large research laboratories and tissue engineering centers across the country and across the globe. While the field was initially limited to certain research laboratories in the Northeast region of theUnited States, it has grown nationally to many regions in the United States and globally, particularly in Europe and Asia. This growth is seen in the number of publications in the field, in the number of new research laboratories being established and in the increase in annual research expenditure associated with the field. While the initial recognition of the field took time, tissue engineering as a scientific discipline has been well grounded for future expansion.

1.15 DISCIPLINES IN TISSUE ENGINEERING

The field of tissue engineering has traditionally been dominated by engineers, particularly chemical engineers. This is due to the fact that Dr. Langer, the founding

father of the field, is a chemical engineer. Traditionally, this research has been well supported by Biomedical Engineering Departments, with almost all BME Departments having faculty members whose primary research area is in the tissue engineering or regenerative medicine space. In recent years, there has been a significant expansion in the number of BME Departments in the United States with significant number of faculty hires, many of whom list tissue engineering as an area of interest. This is also reflected in the data, as engineering disciplines accounted for up to two-thirds of publications in the field in 1995.

During later years of tissue engineering, researchers from Medical Schools, particularly from surgical disciplines, entered into the field. This was due to the nature of tissue engineering research, which is focused on the development of artificial tissue/organs for repair and replacement; surgeons are the end users of this technology. There was and continues to be a mutual interest in the development of this technology, and engineers and surgeons have become partners in this work (Figure 1.9). This is reflected in the data, where the participation rate of surgeons in scientific publications has gradually increased over the years, from about 25% in 1995 to about 35–40% in recent years. This trend has been fairly consistent over the years and continues to move forward in a positive manner, with a good partnership between both parties.

Biologists are the third major contributing partner in the field of tissue engineering and represent about 10–15% of scientific papers in the field. The contribution of cell biologists in the development of tissue engineering technology cannot be

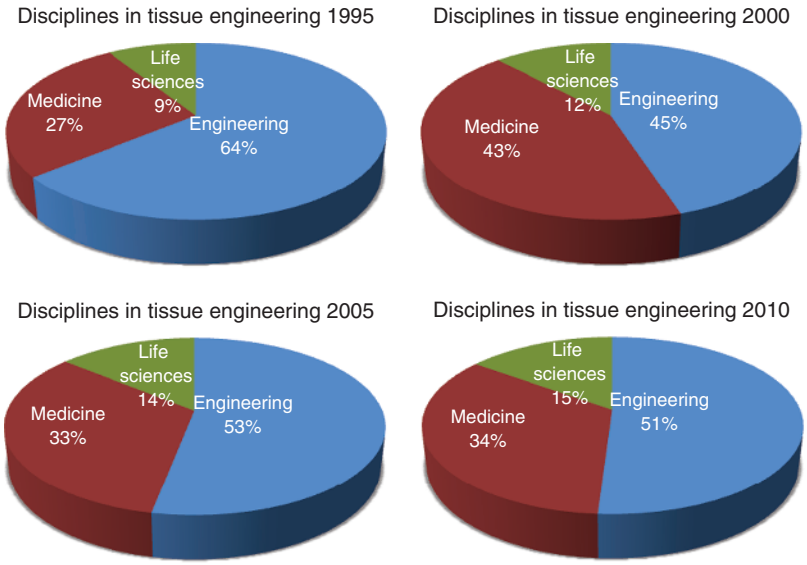


Figure 1.9 Scientific Disciplines in Tissue Engineering—An approximation of the participation rate of researchers from different backgrounds is shown for different time points during the period from 1995 to 2010.

overstated due to the seminal nature of cells in the tissue fabrication process. While the actual percentage may seem lower for researchers from life sciences, this is due to the fact that the research conducted in these fields is not directly geared toward tissue engineering, but rather to gain an understanding into molecular mechanisms of cell behavior; this work then indirectly feeds into the tissue fabrication process. For example, there are numerous publications in the field of stem cell engineering that are focused on developing novel technologies to drive the differentiation fate of stem cells toward a specific lineage. This work is not conducted in the context of tissue engineering, but is important for tissue/organ fabrication. As such, the numbers for life sciences are artificially skewed.

Tissue engineering continues to enjoy strong participation from researchers in engineering, medical sciences, and life sciences, and continued participation of all will remain a central theme for the field, which is important for successful tissue fabrication.

1.16 TISSUE ENGINEERING AND RELATED FIELDS

There are a few fields that are closely related to tissue engineering and will be discussed in this section. There has often been some confusion, disagreement, and debate over the exact definitions for each of these scientific disciplines. The field of tissue engineering is still very fluid and is being defined and redefined on a regular basis. This problem is compounded by the fact that researchers use different terminology to refer to different things; this is expected due to the novelty of the field and due to personal preferences and unique interpretations of the field of tissue engineering. In this section, we present the current understanding of tissue engineering and several related fields, which include gene/protein therapy, controlled release, cell transplantation, cell encapsulation, tissue/organ engineering, and regenerative/reparative medicine. We discuss relative advantages and disadvantages of these fields and scientific and technological challenges for the respective areas of research. We also discuss interrelationships between these scientific disciplines.

The field of tissue engineering is closely related to the field of controlled release and can be viewed as an extension of gene/protein/cell therapy. While it is often difficult to draw comparisons between scientific disciplines, identifying common trends may provide a broader perspective. Lessons learned from the more mature fields like gene and cell therapy will invariably be valuable to fields like tissue engineering, which are still in their infancy. Although research in these fields continues in parallel, the ability to umbrella this body of work under one scientific discipline may be desirable.

Gene Therapy—Gene therapy is defined as the process by which genes, small DNA, or RNA molecules are delivered to human cells, tissues, or organs to correct a genetic defect, or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases[42]. The primary aim of gene therapy is to either increase or decrease the level of a specific protein within target tissue in order to

modify cellular function of the targeted cell or to effect changes to surrounding tissues by altering secreted proteins (42).

The candidate genes for cardiovascular disorders can easily be categorized based on target tissue, which includes the myocardium, vasculature, and cardiac conducting system (43). As one example, the calcium handling proteins play a critical role in maintaining cardiac contractility via excitation contraction coupling. Calcium release from the sarcoplasmic reticulum, in response to depolarization, is regulated by the ryanodine receptors while calcium uptake is controlled by sarcoplasmic endoreticulum Ca-ATPase (SERCA): SERCA activity in turn is regulated by phospholamban. Studies have shown that congestive heart failure in humans is associated with a decrease in SERCA2 (44), measured in terms of gene expression (45), protein level, and activity (46). Several studies have shown that adenovirus-mediated gene transfer of SERCA2 has resulted in improvement of calcium transients in cardiac myocytes after heart failure (47–49).

There are several critical challenges in the field of cardiac gene therapy which need to be addressed (42): 1) mode of delivery, 2) viral myocardial tropism 3) timing of expression, 4) insertional mutagenesis. The mode of delivery of genes has been via transduction, which involves the utilization of viral vectors, or via transfection, which involves non-viral vectors. Transduction is utilized more frequently due to increased efficiency in gene transfer while non-viral vectors are considered safer. The challenge remains to increase the safety of transduction and the efficiency of transfection. Viral myocardial tropism refers to the ability of the virus to specifically target the myocardium, as transfection of non-target tissue can have detrimental effects due to nonfunctional expression of proteins and/or immunogenic effects (42). The timing of expression is important, and it may be desirable to shut off the expression after therapeutic benefit has been observed, thereby limiting the risk of tumorigenesis (42). Insertional mutagenesis refers to the integration of DNA within coding regions of genes and/or regulatory elements; this must be avoided because it can disrupt normal function of the gene[4] (42).

Protein Therapy—Protein therapy is defined as the delivery of proteins to cells, tissues or organs to provide a therapeutic function. Therapeutic angiogenesis, a specialized case of protein therapy, involves delivering angiogenic growth factors to support the formation of new blood vessels, thereby increasing perfusion to infarct tissue (50). In this section, we will discuss therapeutic angiogenesis as it relates to protein therapy in order to gain an understanding of the field and some of its associated challenges. Angiogenesis is defined as the formation of new blood vessels from preexisting vasculature via activation of endothelial cells that proliferate and migrate to construct new capillaries (51).

Vascular endothelial growth factor-A (VEGF-A) has been evaluated extensively to support angiogenesis, and several mechanisms, perhaps acting concurrently, have been proposed for potency of VEGF-A (50). VEGF-A binding to VEGFR-2 promotes survival, proliferation, and migration of endothelial cells, while binding to VEGFR-1 results in vascular permeability and has been shown to promote migration of circulating monocytes and recruitment of hematopoietic progenitor cells (HPCs) from bone marrow to ischemic sites (52–54).

The major challenges that need to be addressed in the field of therapeutic angiogenesis are the mode of delivery of growth factors, concentration of growth factors, and time of delivery. The options for delivery of proteins are the same as for delivery of cells and include intravenous, intracoronary, or intramyocardial delivery (50). The concentration and timing of delivery is also important, as evidence from clinical studies have shown that administration of a single high dose of angiogenic growth factors leads to unstable vessels, while exposure at lower concentrations for extended time periods promotes stable vessel formation (50).

Cell Therapy—Cell therapy involves use of isolated cells that are delivered to damaged or diseased tissue for therapeutic purposes. When applied to the heart, cell therapy is referred to as cardiac cell therapy (55–57); we will discuss cardiac cell therapy to illustrate the challenges associated with cell therapy as a scientific discipline. The field of cardiac cell therapy is centered on the premise that localized delivery of cells to the site of an infarct will result in an increased contractile performance. Initial work involved use of satellite cells and progenitor cells for skeletal myoblasts. The motivating factors to utilize these cells were the availability of an autologous source and contractile activity of mature myoblasts.

Since the initial work involving satellite cells, several cell types have been evaluated as potential candidates for cardiac cell therapy: 1) mesenchymal cells derived from bone marrow and/or adipose tissue, 2) circulating progenitor cells, 3) endothelial progenitor cells (EPCs), 4) resident myocardial progenitor cells, and 5) human embryonic stem cells (55).

The exact mode of action has not been fully elucidated for every cell type, with multiple pathways being postulated (55): 1) differentiation of uncommitted stems to cardiomyocytes resulting in direct increase of contractility, 2) differentiation of EPCs to endothelial cells thereby promoting vascularization, 3) differentiation of stem cells to smooth muscle cells thereby increasing neovascularization, 4) eliciting a paracrine effect thereby activating endogenous stem cells, and/or 5) stimulating survival of border zone cells.

While the field of cardiac cell therapy has tremendous opportunities, several challenges need to be overcome prior to realizing the full potential of the field. These include: 1) mode of delivery for the cells (intravenous, intracoronary or intramyocardial), 2) cell retention at the infarct site, and 3) long term engraftment and survival.

Comparison Between Gene Therapy, Protein Therapy, Cell Therapy and Tissue Engineering—The field of tissue engineering can be viewed as an extension to work in protein, gene and cell therapy. While the goal of these latter fields is to deliver specifically targeted cellular components at the site of infarct tissue, the goal of tissue engineering is to deliver 3D tissue at the site of injury. In the case of gene and protein therapy, the therapeutic agent is the gene or protein of interest, respectively. These strategies are focused at the molecular level and are selective in terms of targeting a specific function. Insertion of a gene or delivery of a protein will replace a very specific function of the cell that has been lost due to injury (as an example, VEGF can be used to increase neovascularization). While gene/protein therapy is designed to mediate changes at the molecular level, cell therapy works

at a larger scale and is designed to mediate changes at the cellular level. While gene/protein therapy aims to selectively replace one function of the cell, the goal of cell therapy is to completely replace the cellular component of damaged or diseased tissue. The major limitation of cell therapy is low retention at the site of delivery, and this problem is addressed by the field of tissue engineering. In the case of cell therapy, isolated cells are transplanted at the site of injury, and a small percentage of these cells are retained at the site of delivery. Tissue engineering strategies are focused on bioengineering 3D artificial tissue using isolated cells and then transplanting bioengineered tissue to the site of injury; the therapeutic agent is 3D tissue, and the problem of low retention has been solved. Cells within 3D artificial tissue are retained at the site of delivery as they are integrated as part of a system.

Controlled Release—The field of controlled release is very closely related to the field of tissue engineering, partly due to research in both fields being started by the same person, Dr. Robert Langer. Controlled release strategies involve the release of a specific drug over time. Rather than providing a single dose at the time of administration, the goal is to provide sustained release over time, preferably with zero order kinetics, which means that the release rate is consistent with time (21,58,59). The main advantage of controlled release strategies involves the ability to maintain therapeutic plasma drug levels without reaching extremely high or dangerously low concentrations. Polymeric scaffolds have been utilized extensively to bind therapeutic drugs; controlled degradation of the polymeric results in the release of drugs. While the field of controlled release is promising, polymer design with controllable degradation kinetics and safe degradation by-products is important. In addition, surgical implantation of the polymer may be required, thereby necessitating biocompatible biomaterials.

Controlled Release and Tissue Engineering—There are clear differences and similarities between the two fields. The most important difference between the two fields is that controlled release is focused on delivery of a therapeutic agent to the injury site and does not involve cells. Tissue engineering, as we have seen, revolves around cells and the development of strategies to support tissue fabrication. This is a very important distinction between the two, as controlled release technologies are focused on delivery of drugs while tissue engineering strategies are focused on development of cell based technologies. The commonality between the two fields is the use of scaffolds. In the case of controlled release technology, properties of the scaffold regulate release kinetics of drugs, which in turn dictate effectiveness of the therapy. Controlled release of the therapeutic agent depends on degradation kinetics of the material—as the material degrades, the therapeutic agent is released within the tissue, and the rate of drug release correlates with the rate of material degradation. In the case of tissue engineering, properties of the biomaterial are important to support tissue fabrication. In one strategy, scaffold degradation is replaced by extracellular matrix (ECM) produced by cells, which in turn support formation of 3D artificial tissue. In the case of tissue engineering, the rate of material degradation correlates with the rate of ECM production by cells, which in turn supports artificial tissue fabrication.

Cell Encapsulation—Cell encapsulation is focused on the culturing of cells within a scaffold, which regulates the release of a therapeutic agent produced by cells into the culture environment[7]. Cells are encapsulated within a 3D scaffold to protect from the host immune system; immune cells like neutrophils and macrophages cannot penetrate the barrier created by the scaffold. The scaffold acts as a semi-permeable membrane and blocks host immune cells; however, nutrients like oxygen and glucose can pass through the scaffold and reach cells. In addition, therapeutic agents, like insulin, produced by the cells can leave the scaffold. The properties of the scaffold are designed to protect cells from the host immune cells, while at the same time supporting diffusion of nutrients to support cell viability and supporting release of therapeutic agents (7). As can be appreciated from the forgoing discussion, cell encapsulation is closely related to controlled release, as both cases require the use of a scaffold for encapsulation of drugs or cells. In the case of controlled release, polymer degradation is used to regulate the release kinetics of the drug while in the case of cell encapsulation, material degradation is not a prerequisite for success of the therapy; rather, the scaffold acts as a semipermeable membrane to support the release of therapeutic agents by encapsulated cells.

Cell Encapsulation and Tissue Engineering—The relationship between the two fields is the utilization of cells for therapeutic purposes. In the case of cell encapsulation, cells function to release specific proteins in the host environment, which serves a therapeutic purpose. In the case of tissue engineering, cells are used to support artificial tissue fabrication, which then acts to replace or restore function in damaged or diseased tissue.

Organ Engineering and Tissue Engineering—The term organ engineering refers to the design and fabrication of entire bioartificial organs and can be considered an extension of the field of tissue engineering (60–62). The holy grail of tissue engineering is indeed the fabrication of bioartificial organs. There needs to be one distinction—the success of tissue engineering technologies should not be judged by the ability to bioengineer bioartificial organs. Artificial tissue itself is a successful endpoint, and in many clinical applications, bioengineered artificial tissue can provide lifesaving options for patients. There are also cases when entire organ transplantation will be necessary and artificial organs will be needed for treatment. As an example, in the case of cardiovascular tissue engineering, technology is being developed to bioengineer artificial heart muscle, tri-leaflet heart valves, blood vessels, heart pumps, ventricles and bioartificial hearts. Depending on the clinical condition, individual tissue constructs may be required and can prove to be beneficial in restoring lost functionality. However, in the case of end-stage heart failure, heart transplantation may be the only viable treatment option, and a complete bioartificial heart will be required. Therefore, both tissue engineering and organ engineering are important and need to be pursued.

Regenerative Medicine and Reparative Medicine—The terms regenerative medicine (63–66) and reparative medicine (67–70) are broad terms used to define therapeutic strategies aimed at regenerating or repairing damaged or

diseased tissue, irrespective of the mechanism or therapeutic agent involved. The therapeutic agent could be a drug, protein, gene, cell (encapsulated or not), or artificial tissue. The fields that we have discussed thus far—gene/protein therapy, cell transplantation, controlled release, cell encapsulation and tissue engineering—are all subcategories of regenerative or reparative medicine. Regenerative and reparative medicine can be viewed as broad overarching themes that refer to any strategy aimed to regenerate or repair damaged or diseased tissue. The specific fields that we have discussed should be viewed as specific therapeutic strategies to achieve this end objective. The term regenerative medicine has been used extensively in the literature while reparative medicine has not been very dominant. We do not distinguish between the two and consider both to be the same. However, due to the dominance of the term regenerative medicine relative to reparative medicine, we will use the term regenerative medicine to refer to any therapeutic strategy with the potential to regenerate mammalian tissue. The term reparative medicine is not used in the remainder of this book and has been included in our discussion for the sake of completion.

Regenerative Medicine and Tissue Engineering—We end this section with a brief discussion distinguishing the fields of regenerative medicine and tissue engineering. Based on our prior discussion, the reader will already have an understanding about the differences between the two fields. However, due to the importance of these two fields in the presentation of the material in this book, we include this discussion. We consider tissue engineering to be a specific therapeutic strategy aimed and repairing, replacing, and/or restoring lost tissue function, which is a subcategory of the broader field of regenerative medicine.

SUMMARY

In this chapter, we have provided a framework for the field of tissue engineering. We started the chapter explaining the chronic shortage of donor organs around the globe and the ability of tissue engineering to provide a viable clinical strategy for artificial tissue and organ development. We provided a formal definition of tissue engineering and outlined an eight-step process to fabricate 3D artificial tissue and organs. We discussed the building blocks of tissue engineering (cell, biomaterials, and bioreactors) and looked at some of the scientific and technological challenges in the field of tissue engineering. We discussed seminal work by Dr. Robert Langer and his contribution to the development of tissue engineering; we also described seminal publications in the field by Dr. Robert Langer. We looked at several applications of the 3D artificial tissue and compared the relative advantages and disadvantages of 2D versus 3D culture. We discussed an integrative model for tissue engineering including participation from different disciplines and the relative contribution of researchers from different disciplines toward development of tissue engineering models. We also presented some data demonstrating the significant growth in the field of tissue engineering and looked at drivers of growth in the field. We concluded this chapter by presenting a comparison of tissue

engineering with related fields including cell transplantation, controlled research, regenerative medicine, gene/protein transplantation, and encapsulation technology. Many of the concepts that were introduced in this chapter will be important in later chapters. In addition, many of the concepts that were introduced in this chapter will be expanded upon in subsequent chapters including cells, biomaterials, bioreactors and vascularization. In conclusion, during the course of this chapter, we have looked at an eight-step process to bioengineer artificial tissue and have identified cells, biomaterials, and bioreactors as the building blocks for tissue engineering.

PRACTICE QUESTIONS

1. Provide a general description of tissue engineering. Without using information from the chapter and using any technical terms, based on your understanding of the field, talk about the field of tissue engineering. Based on your current understanding, what exactly is tissue engineering? Why is it important? What are some potential outcomes of successful tissue engineering technologies?
2. Describe how the development of artificial organs using tissue engineering strategies could alleviate problems associated with the shortage of donor organs.
3. Define tissue engineering.
4. In this chapter, we provided an eight-step process flow sheet for the fabrication of artificial tissue and/or organs. Describe the process of bioengineering artificial tissue and/or organs.
5. In the previous question, you were asked to describe an eight-step process of fabricating artificial tissue and/or organs. If you were to add two additional steps to the process, what would those be and why?
6. During our discussion of the tissue fabrication process, we introduced sensor technology to monitor 3D artificial tissue fabrication. Describe why sensors are needed. What are some important variables that should be monitored? Explain what is meant by real-time noninvasive monitoring of tissue function and explain why this is important.
7. What are the building blocks of tissue engineering? Explain your answer.
8. Describe and discuss five scientific challenges associated with artificial tissue fabrication.
9. What are some potential applications of artificial tissue and organs? Provide four examples, only two of which can be from the chapter.
10. How can 3D artificial tissue be used to support the drug development process?

11. Tissue engineering is a multidisciplinary research field. Describe some of the participating disciplines and explain the relative contribution of each to the process of tissue fabrication.

12. The functional performance of 3D artificial tissue can be assessed by measuring functional, biological, and histological metrics. What do each of these three terms mean?

13. How would you measure the functional performance of 3D artificial heart muscle? Explain in terms of functional, biological, and histological metrics.

14. In this chapter, we discussed three seminal publications in the field of tissue engineering. Explain why these papers are considered to be seminal. What was the contribution of each of these to the field of tissue engineering?

15. What are some of the differences between 2D and 3D culture? Discuss the relative advantages and disadvantages of 2D culture and 3D culture.

16. Cell culture techniques using 2D monolayer systems have been used for decades. The technology for 2D cell culture is well-established. Tissue engineering offers the potential to develop 3D culture systems for isolated cells. The technology for 3D cell culture is not well-established, as the field is very young. What needs to be done to standardize 3D culture techniques?

17. Compare the fields of cell transplantation and tissue engineering. Start by describing these fields. Compare the relative advantages and disadvantages of each of the two fields.

18. What does the term regenerative medicine refer to? What is the relationship between regenerative medicine and tissue engineering?

19. Describe the terms tissue engineering and organ engineering. Select any organ system, excluding the cardiovascular system, and explain how tissue engineering and organ engineering can be used to develop therapeutic strategies.

20. If you had an opportunity to bioengineer any artificial tissue or organ, which one would it be and why?

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