Chapter One

The History and Scope of Tissue Engineering

Joseph Vacanti and Charles A. Vacanti

I. INTRODUCTION

The dream is as old as humankind. Injury, disease, and congenital malformation have always been part of the human experience. If only damaged bodies could be restored, life could go on for loved ones as though tragedy had not intervened. In recorded history, this possibility first was manifested through myth and magic, as in the Greek legend of Prometheus and eternal liver regeneration. Then legend produced miracles, as in the creation of Eve in Genesis or the miraculous transplantation of a limb by the saints Cosmos and Damien. With the introduction of the scientific method came new understanding of the natural world. The methodical unraveling of the secrets of biology was coupled with the scientific understanding of disease and trauma. Artificial or prosthetic materials for replacing limbs, teeth, and other tissues resulted in the partial restoration of lost function. Also, the concept of using one tissue as a replacement for another was developed. In the 16th century, Tagliacozzi of Bologna, Italy, reported in his work *Decusorum Chirurgia per Insitionem* a description of a nose replacement that he constructed from a forearm flap. With the 19th-century scientific understanding of the germ theory of disease and the introduction of sterile technique, modern surgery emerged. The advent of anesthesia by the mid-19th century enabled the rapid evolution of many surgical techniques. With patients anesthetized, innovative and courageous surgeons could save lives by examining and treating internal areas of the body: the thorax, the abdomen, the brain, and the heart. Initially the surgical techniques were primarily extirpative, for example, removal of tumors, bypass of the bowel in the case of intestinal obstruction, and repair of life-threatening injuries. Maintenance of life without regard to the crippling effects of tissue loss or the psychosocial impact of disfigurement, however, was not an acceptable end goal. Techniques that resulted in the restoration of function through structural replacement became integral to the advancement of human therapy.

Now whole fields of reconstructive surgery have emerged to improve the quality of life by replacing missing function through rebuilding body structures. In our current era, modern techniques of transplanting tissue and organs from one individual into another have been revolutionary and lifesaving. The molecular and cellular events of the immune response have been elucidated sufficiently to suppress the response in the clinical setting of transplantation and to produce prolonged graft survival and function in patients. In a sense, transplantation can be viewed as the

Principles of Tissue Engineering, 3rd Edition
ed. by Lanza, Langer, and Vacanti

most extreme form of reconstructive surgery, transferring tissue from one individual into another.

As with any successful undertaking, new problems have emerged. Techniques using implantable foreign body materials have produced dislodgment, infection at the foreign body/tissue interface, fracture, and migration over time. Techniques moving tissue from one position to another have produced biologic changes because of the abnormal interaction of the tissue at its new location. For example, diverting urine into the colon can produce fatal colon cancers 20–30 years later. Making esophageal tubes from the skin can result in skin tumors 30 years later. Using intestine for urinary tract replacement can result in severe scarring and obstruction over time.

Transplantation from one individual into another, although very successful, has severe constraints. The major problem is accessing enough tissue and organs for all of the patients who need them. Currently, 92,587 people are on transplant waiting lists in the United States, and many will die waiting for available organs. Also, problems with the immune system produce chronic rejection and destruction over time. Creating an imbalance of immune surveillance from immunosuppression can cause new tumor formation. The constraints have produced a need for new solutions to provide needed tissue.

It is within this context that the field of tissue engineering has emerged. In essence, new and functional living tissue is fabricated using living cells, which are usually associated, in one way or another, with a matrix or scaffolding to guide tissue development. New sources of cells, including many types of stem cells, have been identified in the past several years, igniting new interest in the field. In fact, the emergence of stem cell biology has led to a new term, *regenerative medicine*. Scaffolds can be natural, man-made, or a composite of both. Living cells can migrate into the implant after implantation or can be associated with the matrix in cell culture before implantation. Such cells can be isolated as fully differentiated cells of the tissue they are hoped to recreate, or they can be manipulated to produce the desired function when isolated from other tissues or stem cell sources. Conceptually, the application of this new discipline to human health care can be thought of as a refinement of previously defined principles of medicine. The physician has historically treated certain disease processes by supporting nutrition, minimizing hostile factors, and optimizing the environment so that the body can heal itself. In the field of tissue engineering, the same thing is accomplished on a cellular level. The harmful tissue is eliminated; the cells necessary for repair are then introduced in a configuration optimizing survival of the cells in an environment that will permit the body to heal itself. Tissue engineering offers an advantage over cell transplantation alone in that organized three-dimensional functional tissue is designed and developed. This chapter summarizes some of the challenges that must be resolved before tissue engineering can become part of the therapeutic armamentarium of physicians and surgeons. Broadly speaking, the challenges are scientific and social.

II. SCIENTIFIC CHALLENGES

As a field, tissue engineering has been defined only since the mid-1980s. As in any new undertaking, its roots are firmly implanted in what went before. Any discussion of when the field began is inherently fuzzy. Much still needs to be learned and developed to provide a firm scientific basis for therapeutic application. To date, much of the progress in this field has been related to the development of model systems, which have suggested a variety of approaches. Also, certain principles of cell biology and tissue development have been delineated. The field can draw heavily on the explosion of new knowledge from several interrelated, well-established disciplines and in turn may promote the coalescence of relatively new, related fields to achieve their potential. The rate of new understanding of complex living systems has been explosive since the 1970s. Tissue engineering can draw on the knowledge gained in the fields of cell and stem cell biology, biochemistry, and molecular biology and apply it to the engineering of new tissues. Likewise, advances in materials science, chemical engineering, and bioengineering allow the rational application of engineering principles to living systems. Yet another branch of related knowledge is the area of human therapy as applied by surgeons and physicians. In addition, the fields of genetic engineering, cloning, and stem cell biology may ultimately develop hand in hand with the field of tissue engineering in the treatment of human disease, each discipline depending on developments in the others.

We are in the midst of a biologic renaissance. Interactions of the various scientific disciplines can elucidate not only the potential direction of each field of study, but also the right questions to address. The scientific challenge in tissue engineering lies both in understanding cells and their mass transfer requirements and the fabrication of materials to provide scaffolding and templates.

III. CELLS

If we postulate that living cells are required to fabricate new tissue substitutes, much needs to be learned with regard to their behavior in two normal circumstances: normal development in morphogenesis and normal wound healing. In both of these circumstances, cells create or recreate functional structures using preprogrammed information and signaling. Some approaches to tissue engineering rely on guided regeneration of tissue using materials that serve as templates for ingrowth of host cells and tissue. Other approaches rely on cells that have been implanted as part of an engineered device. As we gain understanding of normal developmental and wound-healing gene programs and cell behavior, we can use them to our advantage in the rational design of living tissues.

Acquiring cells for creation of body structures is a major challenge, the solution of which continues to evolve. The ultimate goal in this regard — the large-scale fabrication of structures — may be to create large cell banks composed of universal cells that would be immunologically transparent to an individual. These universal cells could be differentiated cell types that could be accepted by an individual or could be stem cell reservoirs, which could respond to signals to differentiate into differing lineages for specific structural applications. Much is already known about stem cells and cell lineages in the bone marrow and blood. Studies suggest that progenitor cells for many differentiated tissues exist within the marrow and blood and may very well be ubiquitous. Our knowledge of the existence and behavior of such cells in various mesenchymal tissues (muscle, bone, and cartilage), endodermally derived tissues (intestine and liver), or ectodermally derived tissues (nerves, pancreas, and skin) expands on a daily basis. A new area of stem cell biology involving embryonic stem cells holds promise for tissue engineering. The calling to the scientific community is to understand the principles of stem and progenitor cell biology and then to apply that understanding to tissue engineering. The development of immunologically inert universal cells may come from advances in genetic manipulation as well as stem cell biology.

As intermediate steps, tissue can be harvested as allograft, autograft, or xenograft. The tissues can then be dissociated and placed into cell culture, where proliferation of cells can be initiated. After expansion to the appropriate cell number, the cells can then be transferred to templates, where further remodeling can occur. Which of these strategies are practical and possibly applicable in humans remains to be explored.

Large masses of cells for tissue engineering need to be kept alive, not only *in vitro* but also *in vivo*. The design of systems to accomplish this, including *in vitro* flow bioreactors and *in vivo* strategies for maintenance of cell mass, presents an enormous challenge, in which significant advances have been made. The fundamental biophysical constraint of mass transfer of living tissue needs to be understood and dealt with on an individual basis as we move toward human application.

IV. MATERIALS

There are so many potential applications to tissue engineering that the overall scale of the undertaking is enormous. The field is ripe for expansion and requires training of a generation of materials scientists and chemical engineers.

The optimal chemical and physical configurations of new biomaterials as they interact with living cells to produce tissue-engineered constructs are under study by many research groups. These biomaterials can be permanent or biodegradable. They can be naturally occurring materials, synthetic materials, or hybrid materials. They need to be developed to be compatible with living systems or with living cells *in vitro* and *in vivo*. Their interface with the cells and the implant site must be clearly understood so that the interface can be optimized. Their design characteristics are major challenges for the field and should be considered at a molecular chemical level. Systems can be closed, semipermeable, or open. Each design should factor into the specific replacement therapy considered. Design of biomaterials can also incorporate the biologic signaling that the materials may offer. Examples include release of growth and differentiation factors, design of specific receptors and anchorage sites, and three-dimensional site specificity using computer-assisted design and manufacture techniques. New nanotechnologies have been incorporated to design systems of extreme precision. Combining computational models with nanofabrication can produce microfluidic circulations to nourish and oxygenate new tissues.

V. GENERAL SCIENTIFIC ISSUES

As new scientific knowledge is gained, many conceptual issues need to be addressed. Related to mass transfer is the fundamental problem associated with nourishing tissue of large mass as opposed to tissue with a relatively high ratio of surface area to mass. Also, functional tissue equivalents necessitate the creation of composites containing different cell types. For example, all tubes in the body are laminated tubes composed of a vascularized mesodermal element, such as smooth muscle, cartilage, or fibrous tissue. The inner lining of the tube, however, is specific to the organ system. Urinary tubes have a stratified transitional epithelium. The trachea has a pseudostratified columnar epithelium. The esophagus has an epithelium that changes along the gradient from mouth to stomach. The intestine has an enormous, convoluted surface area of columnar epithelial cells that migrate from a crypt to the tip of the villus. The colonic epithelium is, again, different for the purposes of water absorption and storage.

Even the well-developed manufacture of tissue-engineered skin used only the cellular elements of the dermis for a long period of time. Attention is now focusing on creating new skin consisting of both the dermis and its associated fibroblasts as well as the epithelial layer, consisting of keratinocytes. Obviously, this is a significant advance. But for truly “normal” skin to be engineered, all of the cellular elements should be contained so that the specialized appendages can be generated as well. These “simple” composites will indeed prove to be quite complex and require intricate designs. Thicker structures with relatively high ratios of surface area to mass, such as liver, kidney, heart, breast, and the central nervous system, will offer engineering challenges.

Currently, studies for developing and designing materials in three-dimensional space are being developed utilizing both naturally occurring and synthetic molecules. The applications of computer-assisted design and manufacture

techniques to the design of these matrices are critically important. Transformation of digital information obtained from magnetic resonance scanning or computerized tomography scanning can then be developed to provide appropriate templates. Some tissues can be designed as universal tissues that will be suitable for any individual, or they may be custom-developed tissues specific to one patient. An important area for future study is the entire field of neural regeneration, neural ingrowth, and neural function toward end organ tissues such as skeletal or smooth muscle. Putting aside the complex architectural structure of these tissues, the cells contained in them have a very high metabolic requirement. As such, it is exceedingly difficult to isolate a large number of viable cells. An alternate approach may be the use of less mature progenitor cells, or stem cells, which not only would have a higher rate of survival as a result of their lower metabolic demand but also would be more able to survive the insult and hypoxic environment of transplantation. As stem cells develop and require more oxygen, their differentiation may stimulate the development of a vascular complex to nourish them. The understanding of and solutions to these problems are fundamentally important to the success of any replacement tissue that needs ongoing neural interaction for maintenance and function.

It has been shown that some tissues can be driven to completion *in vitro* in bioreactors. However, optimal incubation times will vary from tissue to tissue. Even so, the new tissue will require an intact blood supply at the time of implantation for successful engraftment and function.

Finally, all of these characteristics need to be understood in the fourth dimension, time. If tissues are implanted in a growing individual, will the tissues grow at the same rate? Will cells taken from an older individual perform as young cells in their new "optimal" environment? How will the biochemical characteristics change over time after implantation? Can the strength of structural support tissues such as bone, cartilage, and ligaments be improved in a bioreactor in which force vectors can be applied? When is the optimal timing of this transformation? When does tissue strength take over the biochemical characteristics as the material degrades?

VI. SOCIAL CHALLENGES

If tissue engineering is to play an important role in human therapy, in addition to scientific issues, fundamental issues that are economic, social, and ethical in nature will arise. Something as simple as a new vocabulary will need to be developed and uniformly applied. A universal problem is funding. Can philanthropic dollars be accessed for the purpose of potential new human therapies? Will industry recognize the potential for commercialization and invest heavily? If this occurs, will the focus be changed from that of a purely academic endeavor? What role will governmental agencies play as the field develops? How will the field be regulated to ensure its safety and efficacy prior to human application? Is the new tissue to be considered transplanted tissue and, therefore, not be subject to regulation, or is it a pharmaceutical that must be subjected to the closest scrutiny by regulatory agencies? If lifesaving, should the track be accelerated toward human trials?

There are legal ramifications of this emerging technology as new knowledge is gained. What becomes proprietary through patents? Who owns the cells that will be sourced to provide the living part of the tissue fabrication?

In summary, one can see from this brief overview that the challenges in the field of tissue engineering remain significant. All can be encouraged by the progress that has been made in the past few years, but much discovery lies ahead. Ultimate success will rely on the dedication, creativity, and enthusiasm of those who have chosen to work in this exciting but still unproved field. Quoting from the epilogue of the previous edition: "At any given instant in time, humanity has never known so much about the physical world and will never again know so little."

VII. REFERENCES

Langer, R., and Vacanti, J. P. (1993). Tissue engineering. *Science* **260**, 920–926.

Lanza, R. P., Langer, R., and Vacanti, J. P. (2000). "Principles of Tissue Engineering," 2nd ed., p. 929.

Nerem, R. M. (2006). Tissue engineering: the hope, the hype and the future. *Tissue Eng.* **12**, 1143–1150.

Vacanti, C. A. (2006). History of tissue engineering and a glimpse into its future. *Tissue Eng.* **12**, 1137–1142.

Vacanti, J. P., and Langer, R. (1999). Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet* **354**, SI32–34.