ABSTRACT: Reparative medicine is a critical frontier in biomedical and clinical research. The National Institutes of Health Bioengineering Consortium (BECON) convened a symposium titled “Reparative Medicine: Growing Tissues and Organs,” which was held on June 25 and 26, 2001 in Bethesda, Maryland. The relevant realms of cells, molecular signaling, extracellular matrix, engineering design principles, vascular assembly, bioreactors, storage and translation, and host remodeling and the immune response that are essential to tissue engineering were discussed. This overview of the scientific program summarizes the plenary talks, extended poster presentations and breakout session reports with an emphasis on scientific and technical hurdles that must be overcome to achieve the promise of restoring, replacing, or enhancing tissue and organ function that tissue engineering offers.

KEYWORDS: bioreactors; biomaterials, bioscaffolds; cryopreservation; host remodeling

INTRODUCTION

The term reparative medicine is often used to denote the replacement, repair, or functional enhancement of tissues and organs. Reparative medicine has traditionally used materials at hand and the technology of the day to restore or improve function of organs and tissues afflicted with birth defects or the ravages of injury, disease, and age. The driving force for reparative medicine has been clinical need; clinicians, particularly surgeons, have recruited practitioners from multiple disciplines to address complex biological problems that are well beyond a single scientific or engineering discipline. Depending upon the particular organ or tissue at stake, a number of reparative medicine strategies have been employed: (1) substitution of one body part for another (Godbey and Atala report that substitution of one body part for another has been practiced since at least the time of Socrates); (2) repair of the body with non-vital, often synthetic, materials and devices; (3) transplantation of an organ from another individual or from a nonhuman species; (4) use of an external device to augment or substitute for a non-functioning organ; and, (5) use of living cells to restore, maintain, or enhance the function of tissues and organs, that is, what is now known as tissue engineering.
Tissue engineering is the three-dimensional assembly over time of vital tissues/organisms by a process involving cells, signals, and extracellular matrix.\textsuperscript{1,2}

The field of tissue engineering shows enormous potential for reparative medicine, if it can be developed and advanced to the point that living tissues and organs can be routinely assembled and reliably integrated into the body to restore, replace, or enhance tissue and organ functions. Thus, the application of tissue engineering to reparative medicine shows great promise for the treatment of a large number of conditions including birth defects, musculoskeletal disorders, Alzheimer’s and Parkinson’s diseases, diabetes, heart disease, liver and kidney failure, and spinal cord injuries. In addition to its use in reparative medicine, tissue engineering could provide surrogate tissues that could be useful for drug discovery and development and toxicological assessment.\textsuperscript{3}

The engineering challenges posed by generation, insertion, and maintenance of functional tissue are enormous. Complex, multidifferentiated, vascular tissues must be generated either in the body or in bioreactors within the “classical engineering constraints of reliability, cost, government regulation, societal acceptance…” (see Griffith,\textsuperscript{3} this volume). The dynamics of tissue engineering vary from tissue to tissue according to the hierarchy of tissue or organ function, macroscopic structure, and composition. Initial success with the use of tissue engineering in reparative medicine has been with rather delimited applications, such as skin substitutes that are propagated \textit{in vitro} and which, upon implantation, integrate with host skin or introduction of acellular scaffolding—cornea, bone, heart valve, or cell transplantation into damaged organs, such as heart.\textsuperscript{4}

In order to hasten realization of the promise of tissue engineering for reparative medicine, the National Institutes of Health sponsored the two-day symposium “Reparative Medicine: Growing Tissues and Organs.” on June 25–26, 2001, in Bethesda, MD. Scientists, engineers, clinicians, administrators, and other interested parties convened to review and integrate existing knowledge and to identify hurdles to be overcome. The essential elements of tissue engineering, (1) cells, (2) signaling, (3) extracellular matrix, (4) design principles, (5) vascular assembly, (6) bioreactors, (7) storage and translation, and (8) host remodeling and immune response, were reviewed in a series of plenary lectures, breakout sessions, and poster presentations. The current status and some of the future issues associated with each of the elements are considered here.

CELLS

The human body comprises on the order of 100 trillion cells, with about 260 different phenotypes, that divide, differentiate and self-assemble over time and space into an integrated system of tissues and organs.\textsuperscript{5} Although the source and availability of cells for tissue engineering is critical, up until now, the field has been strongly driven from the perspective of biomaterials, based on the recognition that biomaterials can influence cell function and response.\textsuperscript{3–6} Because early attempts with cellular grafts generated outside the body proved problematic, much of the successful work to date, with respect to clinical application, has been acellular.\textsuperscript{4,7} With some exceptions, such as the skin substitute Apligraf, which is able to respond to its wound environment and is not rejected, tissue-engineered constructs containing
cells have exhibited problems with physical properties, maintenance of cell phenotypes, and the host immune response.

The use of cells in tissue-engineered constructs is hampered not only by the lack of information about how to retain and regulate cellular function in the construct and in the host, but also by problems and limitations with both cell expansion and differentiation. It is a challenge to generate sufficient numbers of a single cell type, to orchestrate the assembly of the needed mixture of multiple cell phenotypes, and to maintain stable phenotypes as needed. To alleviate cell shortages, a number of cell sources are being investigated, including adult stem cells, adult differentiated cells, embryonic and fetal stem cells, cells generated by nuclear transplantation, and ex vivo manipulated cells. A major technical hurdle associated with the use of pluripotent progenitor cells is the lack of specific markers for cells in the pluripotent state.

Cells from the above variety of tissue sources can be (1) autologous (self), which offers the advantage of manipulation with minimal risk of adverse host response and disease transmission; (2) allogeneic (nonself, same species), which offers the advantage of banking prior to need, but is more likely to be complicated by the presence of disease-transmitting viruses; or (3) xenogeneic (animal, other species). Both allogeneic and xenogeneic cells would be more likely to generate an adverse response from the host.

The ability to obtain physiologic information on an individual cell basis rather than on the basis of an average response of a population of cells will help to provide needed parameters to design and develop tissue engineering constructs. However, computer software to manage cell images and metabolic data will be required. Cells can also be commandeered within tissue-engineered constructs to serve as cellular bioreactors for the generation of proteins. The generation of proteins at sites where needed offers an advantage over direct protein delivery because the chemical synthesis of proteins greater than 100 amino acids in length can be technically challenging and it can be difficult to deliver and maintain needed protein concentrations at appropriate times.

Cell expansion and differentiation in vivo has been effective in some cases, such as regeneration of cardiac tissue. However generation of tissues and organs in vitro using bioreactors is currently difficult to orchestrate due to problems with supplying oxygen to three-dimensional constructs and the integration of cell expansion, differentiation, and assembly within the extracellular matrix.

**SIGNALING**

Cells respond to the extracellular environment by sensing a chemical signal or physical stimulus that is transmitted to the nucleus to trigger the expression or repression of genes, the products of which regulate cell division, migration, differentiation, and apoptosis. Much of the signaling information that is currently utilized by tissue engineers has been derived primarily from studies with single cell populations cultured in two dimensions and treated selectively with soluble factors. Recently, the importance of spatial signals during three-dimensional culture has been recognized, and time and force are considered the fourth and fifth dimensions that play crucial roles in tissue engineering. Bottaro et al. emphasize the need for a
better understanding of environmental clues given to cells and of how the signals are integrated and assembled into a hierarchy of interactions with cell receptor systems. Also, information is lacking on how pathways specific to cell phenotype are integrated with multimolecular complexes and cellular organelles. Tissue engineering faces the need to switch from two-dimensional to three-dimensional (flexible) cultures and the need to employ design principles in order to become less trial-and-error in approach.\textsuperscript{3,15–20} Investigators also recognize that there are important lessons to be learned from embryonic development and the analysis of postnatal master gene expression.\textsuperscript{16}

It is recognized that cell division, differentiation, and maintenance of phenotype are influenced by the synergy and interplay between soluble factors, insoluble adhesion molecules within the extracellular matrix, and mechanical forces.\textsuperscript{2,18,19} Cellular mechanoregulation refers to the processes by which mechanical forces influence gene expression, metabolic pathways, and tissue patterning and architecture.\textsuperscript{19} Cells in tissues are under constant stimulation by mechanical forces, the very minimum of which is gravity. Mechanical forces influence cell shape, which, in turn, affects how a cell responds to the summation of its signals with either a growth response or apoptosis. Thus, study of three-dimensional multicellular model systems is at a crucial forefront of tissue engineering for reparative medicine. Time can be viewed as the fourth dimension of the cellular environment and gravity or forces as the fifth dimension.

**EXTRACELLULAR MATRIX (BIOSCAFFOLDS)**

It is the insoluble extracellular matrix (ECM) that confers physical, mechanical, and functional properties on tissues and organs, that is, strength of bone, elasticity of skin, et cetera.\textsuperscript{2,3} Throughout the body, ECM is made up of proteoglycans, elastin, fibrillin, and 19 different types of collagen. During development and wound repair, cells synthesize and remodel ECM, and thus all cells spend at least part of their time interacting with the extracellular matrix.\textsuperscript{3,6,21} Insoluble signals/factors provided by the ECM interact with soluble signals and mechanical forces to promote adherence, migration, division, and differentiation of cells. There is an intimate link between cell adhesion and cell signaling. Natural polymers, synthetic polymers, and inorganic composites, collectively known as biomaterials, have been used for tissue engineering usually as temporary, surrogate ECM. Natural polymers used for tissue engineering comprise proteoglycans and collagen; collagen is highly prevalent in the extracellular matrix throughout the body and has been shown experimentally to influence cell phenotype, that is, chondrocyte phenotype is maintained in presence of type II, but not type I collagen.\textsuperscript{3,6,21}

Griffith\textsuperscript{3} reviewed the hierarchy of design scale considerations that apply to scaffolds for tissue engineering: the macroscopic level (on a scale of millimeters to centimeters); an intermediate level (hundreds of microns), involving the topography of pores and channels; and the molecular level, involving surface texture and chemistry (tens of microns). Growing tissues and organs requires that varying numbers of differentiated cells be assembled into a specific architecture in a series of specific events occurring at time intervals ranging from seconds to weeks and months, at di-
mensions ranging from 0.0001 to 10 cm and involving a range of forces from 3 to 15 orders of magnitude difference.

Initially, scaffolds used for tissue engineering were derived from surgical materials; the tendency to adapt materials in current or prior use for other applications offers advantages from the perspective of regulatory agencies such as the Food and Drug Administration, but does not necessarily promote development of optimal materials with regard to performance characteristics needed for different tissues.\(^3,17,21\)

It is desirable that the scaffolding biomaterial can be degraded as cells go through the process of forming their own supportive ECM; the permanent presence of implants almost always can be expected to elicit a foreign-body response. Degradation is influenced by material composition, surface chemistry, and topology.

Bioactive scaffolding materials can be engineered to deliver growth factors/signals, to deliver cells, or to direct the three-dimensional orientation of cells. For example, certain biomaterials can aid hepatocytes to retain epithelial polarization. The number and spatial orientation of cell adhesion ligand moieties is becoming recognized as crucial to cell migration and mechanical signaling and, thus, subsequent differentiation. Scaffolds may also be designed to deliver DNA locally to transduce cells to become bioreactors for production of proteins in situations that require that sufficient local concentrations be produced and that the protein is biologically active.\(^2,3,6,11,21\)

**DESIGN PRINCIPLES**

When faced with the challenge of replacement of whole organs and tissues, there is the need to understand tissue and organ properties that result from scaled hierarchies, from molecular to cellular to macroscopic organ length, that are currently best understood at the cellular and molecular level.\(^3,17,20,22\) It is considered to be unlikely that the properties of final tissue-engineered constructs will be completely understandable by investigation of the respective components, cell types, and matrix, and vasculature in isolation because of the need to consider properties resulting from issues of hierarchy.\(^3,22,14\)

Tissue engineering, if it is to fulfill its promise for reparative medicine, will require standards and fundamental principles that cross organ-based disciplines and will require a judicious mix of design and iteration.\(^17,20\) It is crucial that rational design be added to studies that have been up until now somewhat trial-and-error in nature. Goldstein\(^17\) and Guilak and copanelists\(^20\) presented design principles for functional tissue engineering that start and end with patients’ needs, such as the biomechanics for bone strength and electrical conduction needed for heart contraction. It was noted that the repair of load-bearing structures (tissues that serve a biomechanical function) is problematic and that there is a need to define biomechanical properties of native tissue. The need for early consideration of complexities of design and running clinical trials and for the selection of clinically relevant end points was emphasized. In view of the long lag period from project initiation to clinical application,\(^1\) it is advisable to choose carefully and perhaps limit the numbers of scaffolds and cell sources to be developed.

The fundamental elements of embryonic development may be expected to have direct applicability to functional tissue engineering. As work progresses from animal
models to clinical trials, it will be highly desirable to have surrogate markers of function, as the methodology to assess function in the patient must be much less invasive. The continued development of imaging approaches is expected to help greatly in assessment of function.21,23,24

VASCULAR ASSEMBLY

A major roadblock to the achievement of three-dimensional tissue-engineered constructs is ability to vascularize the tissues. The need for a vasculature is pervasive throughout the body, as all cells need adequate oxygen levels. Hirschi and coauthors14 stressed the need to apply an integrative approach to determine the principles that guide tissue and organ formation together with a continued reductionist approach to develop a more sophisticated understanding of vessel formation at the molecular and cellular levels. It appears unlikely that the final vascular structure can be pre-determined in the micro fabrication stage of tissues and that the natural adaptive powers of resident cells will be needed to achieve a vasculature that is sufficient for large, complex tissues. Lessons from embryonic development may be helpful here. Key issues in need of investigation include identification of the various differentiation stages of cells with respect to vasculogenesis, and the mechanism by which differentiation is regulated and cells are supported and directed to sites of injury or tissue repair. Also, there is the need for molecular modeling of the vascular network and study of blood vessel pattern formation using time-lapse microscopy, computational analyses, and computer modeling.14, 25

BIOREACTORS

Tissue-engineering efforts must include an early decision as to whether tissues should be generated inside or outside the body. Success has recently been achieved in the area of implantation of cells into diseased tissue (bone marrow stem cells and skeletal muscle to heart).1,2,13,14,21 In other cases, the approach has been to implant tissues assembled in vitro as in the case of bioengineered bladder.1 The challenges associated with design and implementation of bioreactors, in which functional tissues and cells are produced at the laboratory bench, are enormous, in view of the need for multiple cell phenotypes and an adequate vasculature.20 A number of non-technical regulatory issues are associated with bioreactors and bioprocessing, and there is a dilemma with how to establish and evaluate research milestones. In some situations, the bioreactor approach to tissue engineering can be utilized in the form of extracorporeal assist devices such as the bioartificial liver and kidney.1

STORAGE AND TRANSLATION

There are a number of translational issues associated with taking a tissue-engineered construct from the laboratory to the clinic. These pertain to cell isolation, cell and tissue culture and differentiation, scale-up of bioreactors, biomaterials, and scaffolds, long-term storage strategies, and safety and regulatory policies.
In order to provide for storage of living cells or tissues, freeze-thawing and vitrification approaches are being considered.\textsuperscript{27,28} A great deal of basic information is needed about physicochemical changes associated with intracellular ice formation, how ice is propagated, and about how cryoprotectants protect and interact with cells.

In the vitrification process, cells don’t freeze but, instead, become “glassy” in the presence of high concentrations of cryoprotectants. Vitrification is considered to be suitable for preservation of complex three-dimensional structures; a challenge is how to load and remove high concentrations of cryoprotectants. Nontoxic sugars have been used at lower concentrations, but cells need to be permeabilized. Gain of an understanding of wound healing at the level of the cell membrane may facilitate cryoprotection by stabilization of cell membranes.\textsuperscript{29} One desirable goal for reparative medicine would be the direct administration of freeze-thawed cells without the removal of cryoprotectants.

Cell recovery is a major technical hurdle for translation from the laboratory bench to the clinic. It is currently being investigated in this regard whether maintenance of tissues in the dried state may be preferable to freeze-thawing. Desiccation based on principles of anhydrobiosis, which is used in nature by some microorganisms, and which involves a glassy state in which sugars protect membranes, proteins, and supramolecular structures from effects of drying, is being investigated. Here, research is needed on the stability of the dry state as light, oxygen, and other features of the storage environment affect it.\textsuperscript{27}

### HOST REMODELING AND IMMUNE RESPONSE

Host remodeling is viewed as an inevitable and often beneficial stage of the tissue-engineering process.\textsuperscript{21} Remodeling is an essential part of development throughout life, and varies with age, disease state, and species. When organ regeneration is to be achieved in the body, scar-tissue formation is a major barrier. While host remodeling is closely related to the immune response, integration of the tissue engineering and immunology disciplines has been extremely limited.\textsuperscript{30,31} Badylak and copanelists\textsuperscript{21} commented that “it is likely that the traditional dividing lines that tend to exist between our understanding of the processes of inflammation, immunity, scar tissue formation, developmental biology and wound healing will require rethinking and/or elimination.” The need for better methods to track tissue-engineered implants was recognized as was the great need for a registry of human patients in order to establish predictors for success and failure of tissue-engineered products.

Previous work in reparative medicine involving allograft and xenograft transplantation has shown that both innate and adaptive immunity represent formidable barriers. Even autologous materials will undergo remodeling and induce immune reactions.\textsuperscript{30} The host immune system may be expected to influence tissue-engineered constructs whether generated in the body or in a bioreactor. In many situations, remodeling may facilitate or be necessary for successful integration of the tissue-engineered construct. One approach that has been used by tissue engineers is encapsulation to isolate the construct from the body’s immune response by biomaterial or by a fibrous capsule generated by the body itself. Immunologists\textsuperscript{30} consider the body’s immune system to comprise the innate immune system, an ancient, “hard wired” system in which macrophages play a prominent role that recognizes molec-
ular patterns found on pathogens, but not higher eukaryotic organisms. In addition to innate immunity, higher organisms possess an adaptive immune system that utilizes T and B lymphocytes to recognize specific antigens that have been processed and presented by the host. Currently, the immunology community is considering a greater role for innate immunity in the immune response, in that the role of the immune system may be to recognize danger to the body, rather than self or non-self, as has been thought for a number of years. A greater understanding of the interplay between remodeling and innate and adaptive immunity is needed to realize the potential of reparative medicine.

CONCLUSION

This volume is a compilation of plenary manuscripts, panelist overviews, breakout session summary reports, and extended poster abstracts covering the range of elements into which tissue engineering can be reduced: cells, signaling, extracellular matrix, design principles, vascular assembly, bioreactors, storage and translation, and host remodeling and immune response. The poster paper manuscripts present current research activities of some of the symposium participants. There was consensus that multidisciplinary efforts involving surgeons, physicians, engineers, physicists, mathematicians, chemists, cell biologists and allied specialties will be needed to achieve the promise of tissue engineering for reparative medicine.  

REFERENCES