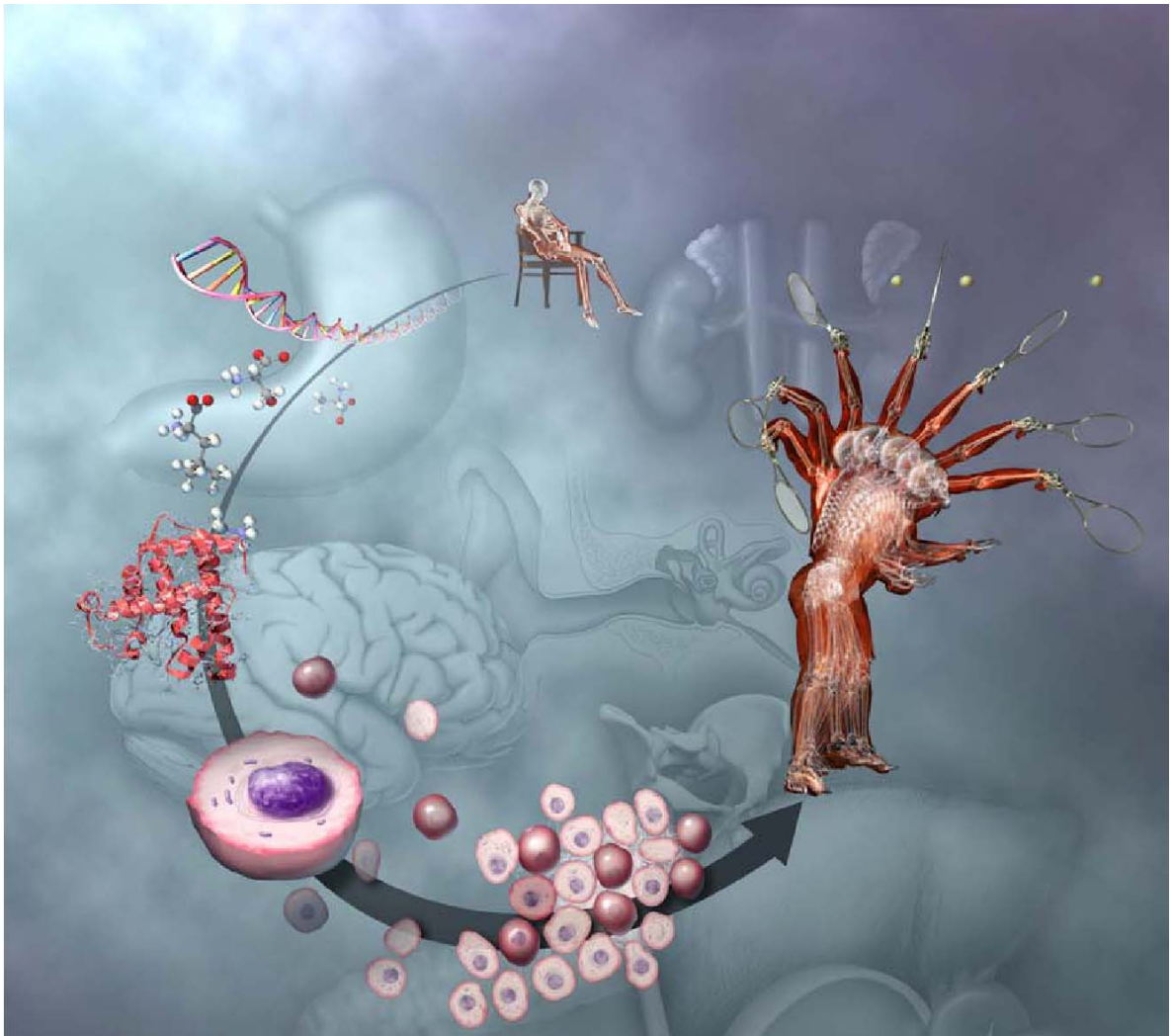

# Reparative Medicine: Growing Tissues and Organs

## June 2001

## Symposium Report

**National Institutes of Health Bioengineering Consortium**

NATIONAL INSTITUTES OF HEALTH

BECON

**Reparative Medicine: Growing Tissue and Organs**
National Institutes of Health Bioengineering Consortium (BECON)
Natcher Conference Center
June 25 - 26, 2001

## Foreword

The full promise of reparative medicine has only begun to be realized. Sometimes referred to as regenerative medicine or tissue engineering, reparative medicine 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. In an effort to continue to move this field rapidly forward and to seek new ways in which these advances can provide better health and quality of life to patients, the fourth annual NIH Bioengineering Consortium (BECON) Symposium on *Reparative Medicine: Growing Tissues and Organs* was held at the National Institutes of Health, Bethesda, MD, June 25-26, 2001.

The symposium was structured to provide a forum where clinicians, engineers and scientists from a variety of disciplines would be able to exchange knowledge, identify obstacles to be overcome and identify collaborators for future research. The keynote address, plenary talks, poster and exhibit presentations and breakout sessions were designed to assist the Institutes and Centers of the National Institutes of Health in the formulation of research and training funding programs to hasten the development of reparative medicine.

The proceedings of the symposium have been published as Volume 961 of the Annals of the New York Academy of Sciences. The volume is available online at www.annalsnyas.org. Access is free of charge to institutional subscribers and to Academy members.

We thank the NIH Program Planning Committee, listed on page 49, for their excellent ideas and hard work in developing this program and organizing and executing this event. BECON also owes a special thanks to Dr. Wendy Baldwin, Deputy Director of Extramural Research, for her efforts in expanding appreciation and support of bioengineering at NIH. As the administration of BECON moves into the new National Institute of Biomedical Imaging and Bioengineering (NIBIB) it will continue to ensure that multidisciplinary support for biomedical and biological engineering enjoys strong support across the NIH.

*External Co-Chairs*
Robert Nerem, Ph.D.
Georgia Institute of Technology

E. Helene Sage, Ph.D.
Hope Heart Institute

*NIH Co-Chairs*
Christine A. Kelley, Ph.D
National Heart, Lung and Blood Institute

Lore Anne McNicol, Ph.D.
National Eye Institute

**Above:** NIH Co-chairs Christine Kelley and Lore Ann McNichol. External Co-Chairs Robert Nerem and E. Helene Sage. **Below:** John Watson (NHLBI) and Wendy Baldwin (OER).

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Workshop Participants listen intently

## Executive Summary

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. Reparative Medicine is a critical frontier in biomedical and clinical research. At the same time that researchers are discovering new knowledge, they are developing new opportunities to advance medicine. In an effort to continue to move this field rapidly forward and to seek new ways in which these advances can provide better health and quality of life to patients, the fourth annual NIH Bioengineering Consortium (BECON) Symposium on *Reparative Medicine: Growing Tissues and Organs* was held at the National Institutes of Health, Bethesda, MD, June 25-26, 2001. The goals and objectives of the symposium were to:

- **Develop a vision for reparative medicine;**
- **Identify challenges and opportunities in the field;**
- **Identify short- and long-term research needs and strategic goals; and**
- **Recommend the means to address the research needs and to achieve the goals.**

In addition, the symposium was intended to provide a forum for exchange of knowledge, help investigators identify collaborators for future research, and educate the community in the state of the science.

Approximately 500 participants, including leading figures in the field of tissue engineering and reparative medicine, attended the meeting, which featured a keynote address, five plenary talks, ten breakout sessions, as well as posters and exhibits. The objective of the keynote address and plenary talks was to provide a broad overview of the major research areas that impact reparative medicine. Gail Naughton, Ph.D of Advanced Tissue Sciences, Inc. delivered the keynote address. She discussed the scope of research, ranging from "gene to clinic", that is necessary to accomplish biological repair and replacement of tissues. Examples included human dermal equivalents, heart valves, cartilage, and liver. The five plenary talks covered a range of topics germane to issues that pertain to tissue engineering. Linda Griffith, Ph.D. from the Massachusetts Institute of Technology presented fascinating data on molecular design based on established and novel principles of cell adhesion. Nancy Parenteau, Ph.D. of Organogenesis, Inc. focused on an approach to organ design/repair based on the use of stem cells, and other cell types. A new field of tissue informatics, as well as the need for tissue engineering standards, was presented by Peter Johnson, M.D. of Tissue Informatics, Inc. Strong attention to clinical outcomes and experimental modeling of a tissue-engineered system (bladder replacement) was provided on the second day by Anthony Atala, M.D. of Harvard Medical School and Steven Goldstein, Ph.D. from the University of Michigan.

Discussion of the symposium goals, including recommendations to the NIH on programs in reparative medicine, were stimulated by the plenary presentations and further developed in

the breakout sessions. The ten breakout sessions addressed topics ranging in scope from basic science to therapeutic development and application and were designed to provide a diverse blend of scientific and clinical perspectives of investigators, clinicians and advocates.

The specific topics were:

- **Vascular assembly in engineered and natural tissues**
- **Biomaterials and scaffolds in reparative medicine**
- **Bioreactors and bioprocessing**
- **Cells for repair**
- **Functional assessment of engineered tissues and elements of tissue design**
- **Genetic approaches to tissue engineering**
- **Immune responses to engineered tissues and cells**
- **In vivo remodeling**
- **Molecular signaling**
- **Storage and translational issues in reparative medicine**

The major conclusion from the breakout group discussions was that despite enormous progress, there are many opportunities and challenges for the future. The recommendations that were formulated fall into two categories: 1) cross-cutting intellectual themes; and 2) programmatic initiatives. For each of these two categories, the key points are summarized as follows:

**1. Cross-Cutting Intellectual Themes**

These cross-cutting themes represent intellectual targets of opportunity and are logically grouped into the following four major areas.

- **Cell Technology.** Critical issues for reparative medicine are: a) cell source; b) characterization of cells (biosynthetic profile, cell cycle/proliferation); c) "management" of differentiation such that cells can be directed into the appropriate phenotype and/or response; d) intercellular interaction; and e) cell-ECM communication and signaling. Another important topic was centered on stem cell technology, the plasticity of stem cells, and the appropriate understanding of developmental biology.
- **Rational Design.** This topic includes: a) the development, fabrication, and analysis of novel biomaterials and scaffolds; b) the assembly of cells into three-dimensional structures that mimic the architecture and function of native tissue; c) the integration of molecular signals (including responses to signals) in these assembled cellular systems; d) the influence of biochemical and biophysical factors; and e) determination of the requisite functionality. Of critical importance is the development of strategies for innervation and for angio/vasculo/lympho genesis.
- **Integration into the Living System.** This theme includes: a) remodeling of tissue-engineered implants; b) other biological responses such as inflammation and thrombosis; and c) development of strategies for the engineering of immune acceptance. The development of methods for tracking

the fate of implanted tissue-engineered devices through non-invasive imaging is an additional mandate**.**

- **Technologies for Going from Benchtop to Clinic**. If patients are to realize benefits from our research, reparative medicine must deliver products that can be commercialized. Therefore, enabling technologies, such as new generations of bioreactors, separation and purification techniques for use in growing tissues/organs on a commercially relevant scale, and strategies for preserving living-cell products with off-the-shelf availability will be required.

**2. Programmatic Initiatives**

To foster the advancements required if reparative medicine is to achieve its potential as a technology, participants in the Symposium proposed a variety of program initiatives including training, cross-training, and retraining programs, funding initiatives that foster cross disciplinary research in order to enhance the pool of talent, and establishment of Centers in Reparative Medicine that are both research centers and resource centers. It was considered that high priority should be given to funding of projects directed toward development of the core, enabling technologies, which will emerge largely from the intellectual themes discussed. A topic considered to require thoughtful consideration is that involving the type of tissues/organs that should be grown. To date, many of the achievements have been in the area of relatively simple tissues, with the initial products entering commercialization being primarily skin substitutes. The real potential for tissue engineering, however, lies in addressing those vital organs for which transplantation is not meeting the patient need, i.e., where there is a tremendous disparity between patient need and the availability of donor organs. These vital organs include the heart, kidney, liver, and pancreas. If tissue engineering can address these organs and thus provide an alternative supply, the crisis in transplantation can be confronted successfully. It will therefore be important to support research on the development of models, simulations, and statistical theories to describe biochemical, biomechanical, and biomolecular behavior.

As tissue engineering moves into its next generation of development as a field, it must build on a strong foundation of science. This means that it must move from an alliance of engineers and clinicians to one that also involves basic biologists. Clearly, an integrated team approach, involving in some instances academic and industrial partnerships, would be advantageous. Only in broadening itself to include a more diverse array of disciplines will the field of reparative medicine be able to achieve its true potential.

## Symposium Report

This report of the fourth NIH/BECON symposium, *Reparative Medicine: Growing Tissues and Organs,* held on June 25 - 26, 2001, summarizes the keynote and plenary lectures delivered by six distinguished speakers. Also summarized are the ten panel discussions led by 61 eminent clinicians, engineers and scientists. Approximately 500 participants from academic institutions, research foundations, government and the private sector joined in the discussions to contribute their views. The perspectives captured in these overviews and summaries are invaluable not only to the NIH as it formulates programs to facilitate fruitful and effective research in reparative medicine, but to all stakeholders in restoring or replacing functional organs and tissues to those afflicted with organ malfunctioning tissues. NIH welcomes additional suggestions for improving its extramural research funding mechanisms to facilitate research in tissue engineering and reparative medicine. Parts of this report have been published in Volume 961 of the Annals of the New York Academy of Sciences, Jean D. Sipe, Christine A. Kelley and Lore Anne McNicol, editors, and are reprinted here with permission.

## Overview of Presentations

### Repair And Replacement: From Lab Bench To Market

*Gail K. Naughton, Ph.D., Advanced Tissue Sciences*

Tissue engineering integrates science and engineering, using the fields of biomaterials, cell biology, biochemistry, biomedical engineering and clinical medicine. It creates tissues and organ substitutes that function to repair or replace damaged or diseases tissues and organs. Unlike traditional devices, tissue constructs are engineered to remain biointeractive after implantation, thereby offering structure as well as the physiologic functions of the replaced tissue. The most usual technology used in tissue engineering is the cell-scaffold approach, and is one that Advanced Tissue Sciences has combined with the use of completely closed bioreactor systems. This maintains sterility while mimicking a physiological environment in which cells, seeded onto biocompatible three-dimensional scaffolds, divide and secrete all human growth factors and matrix proteins to form a functional tissue. In-process testing is performed to measure sterility and cell activity and tissue formation while closely monitoring oxygen concentrations, pH, temperature and nutrient uptake. The same bioreactor is utilized to seed the cells, grow, freeze, ship and store the tissue. This system allows for lot-to-lot reproducibility and maintenance of sterility.

Advanced Tissue Sciences is currently focusing on the development of human-based tissue engineered products for wound care (burns and diabetic foot ulcers), orthopedics (articular and meniscal replacements), craniofacial (periodontal repair and cartilage) and cardiovascular surgery (small diameter blood vessels and cardiac angiogenesis patches). The wound care products are the most advanced, with TransCyte TM having been already approved by the FDA as a temporary human skin substitute for full-thickness and partial-thickness burns, and Dermagraft$^{TM}$ under expedited PMA review as a human dermal replacement for the treatment of hard-to-heal full-thickness diabetic foot ulcers. Both

products are approved in several global markets and commercialized by our joint venture partner, Smith & Nephew plc. Dermagraft™ products are manufactured using neonatal dermal fibroblasts from routine circumcisions. Such cells offer multiple advantages including greater reproducibility (cells are always same age, sex and anatomical location) and expansion potential (one sample yield cells sufficient for manufacture of 250,000 $ft^2$ of Dermagraft). Cells are extensively tested for known viruses and passages at several stages including Master Cell Bank, Manufacturer's Working Cell Bank and end-of-production testing. Final product testing includes tests for sterility, matrix uniformity and content, and metabolic activity after cryopreservation.

The cell-scaffold approach has been used successfully in cartilage tissue engineering. Cartilage constructs were formed using PGA scaffolds in specially designed bioreactors, and implanted into osteochondral defects in the knees of rabbits. These repaired the defects up to 24 months after surgery. In additional studies a new scaffold was designed to overcome challenges of fixation encountered when using larger animals. Feasibility studies showed this approach to be successful. Modulation of the mechanical environment during culture has been shown to increase mechanical properties of the constructs. The manufacturing and testing methods utilized for Dermagraft™ have now been used to form human cartilage constructs. There is now substantial evidence that the cell-scaffold technology can be used for repair of major articular defects.

Cardiovascular diseases represent a major challenge in medicine, and one that tissue engineered products have the potential to address. Small diameter tissue engineered vascular grafts have been developed using bioreactors that impose fluid flow representative of native blood flow. Using allogeneic smooth muscle cells seeded onto a tubular scaffold, and with an endothelial cell lining, constructs have been formed that have the cells and matrix aligned similarly to native blood vessels. These grafts have been found to remain patent in animal studies for over 1 month. The cell-scaffold tissue engineering approach is now being investigated (in major collaborative program lead by University of Washington) to produce cardiac tissue, with the intent that new devices, including ventricular assist devices, can be engineered. The Dermagraft™ product has been shown to induce angiogenesis in vitro and in vivo. In feasibility small animal studies, ischemia or infarct was induced in heart muscle of animals, and Dermagraft was shown to induce angiogenesis at these sites, and to induce retention of heart function.

Our platform technology has shown proven feasibility in a number of additional clinical applications including periodontal repair, acute wound repair, tendon/ligament replacement, bone repair, reconstructive surgery and replacement of functional liver and pancreatic implants. There is the potential for tissue engineering to impact almost every tissue of the body.

For tissue engineering to meet the true clinical demands, the products must be manufactured reproducibly, in large numbers, and be available on demand. The use of an allogeneic cell source permits an "off-the-shelf" product to be made. Scaleable manufacturing processes permit many units to be made in a uniform manner, and allow

diseases that affect large patient populations to be addressed in an efficient and cost-effective way.

Tissue engineering has the potential to redefine tissue and organ repair and replacement. Uniform criteria for cell sourcing and testing, formulation of enhanced biomaterials, process developments to insure reproducible manufacturing on a large-scale basis, advancements in cryopreservation, an enhanced understanding of cell-cell and cell-matrix interactions and bioreactor design will all advance the field. Critical to the future of tissue engineering is the establishment of clear regulatory pathways and requirements. Combined, these will result in the successful growth of this exciting new industry.


Gail Naughton, Keynote Speaker

**Biomaterials/Scaffolds for Reparative Medicine**

*Linda G. Griffith, Ph.D., Massachusetts Institute of Technology*

Tissue engineering inherently involves recreation of a 3-D tissue structure from a source of cells that may be derived from an endogenous source in the patient (e.g., bone wound healing) or from a donor (e.g., skin). In some sense, the field of tissue engineering dates back to the classic experiments on tissue morphogenesis in vertebrates conducted by Townes and Holtfreter in the mid-50's. It then follows a path through the pioneering work of Steinberg, who developed a biophysical framework for self-organization of mixed cell populations as a contributing factor in tissue morphogenesis in the 60's and 70's. The experiments and analysis conducted in this early work demonstrated that dispersed cells had some intrinsic ability to organize into tissue structures; that the organization of cells was at least partly predictable, based on measurable cellular properties; and that self-organization into tissue would occur only over some finite length scale-different for each tissue-on its own. Beyond a size a few tens or hundreds of microns, however, cells need assistance to form into useful tissue structures.

Creation of large-scale tissue structures requires biomaterials in the form of scaffolds to guide the organization, growth, and differentiation of cells in the process of forming functional tissue. The molecular structure of the material provides both physical and chemical cues to direct cell behavior, while the microscopic and macroscopic architecture of the scaffold foster the organization of cells into 3-D structures.

A particular challenge in addressing materials issues for tissue engineering is that the biological processes are not yet understood well enough to allow a clear set of design parameters to be specified a priori. Indeed, evolution of materials/devices and knowledge of biological processes occur simultaneously. New materials/devices illuminate the enormous complexity of biological responses - which then inform new, better, designs of materials and scaffolds. The process is autocatalytic. As information emerges about one system, the same principles can often be applied to others, pushing the field forward at a faster and faster rate.

One of the first tenets defined in the now-established field of tissue engineering is that materials and scaffolds should be degradable, leaving a natural tissue replacement. The first scaffold materials to be widely used in tissue engineering-degradable polyesters (polylactides, polyglycolides and their copolymers) and collagen-were adapted from other surgical applications. The scaffold structures were either directly adapted (e.g., woven, knitted, or entangled surgical fabrics made from degradable polyesters) or designed (e.g., collagen scaffolds for skin). The relative success of these original materials and scaffolds, particularly for connective tissue applications, has made them enduring tools in tissue engineering. Their limitations in effecting repair of complex tissues such as nerve and liver have also become apparent.

These limitations are pushing design of new materials and scaffolds that interact with cells via tailored control of growth factor and adhesion receptor ligation, and that actively respond

to the repair environment by degrading on cue. The rational design of such materials requires a quantitative understanding of how cells respond to molecular signals and integrate multiple inputs to generate a given response - a significant challenge considering that the number of cellular regulatory molecules identified so far represents only a fraction of the total which exists in the normal tissue environment. Model polymeric and oligomeric systems, synthesized without constraints of in vivo biocompatibility or cost, and thus with potential for very precise control of molecular and supramolecular structure, are emerging as tools to study these issues. Model systems allow these quantitative, physical issues to be understood and thus provide the design basis for clinical implant materials, where design constraints include composition, mechanical properties, stability, processibility, and cost.

At the meso- and macroscopic levels of scaffold architecture, performance needs are also increasing. New approaches to materials processing are being developed to create scaffolds with complex architectures and macroscopic shapes, and which allow composition variation to accommodate variations in evolving tissue structure. In summary, tremendous advances are occurring in integrating molecular and macroscopic design of materials with the needs of growing tissues.

**The Use of Cells in Regenerative Medicine**

*Nancy L. Parenteau, Ph.D. Organogenesis, Inc.*

**Abstract**

Cells are the functional elements of regenerative medicine and tissue engineering. The use of living cells as a therapy presents several challenges. These include identification of a suitable source, development of adequate methods, and proof of safety and efficacy. We are now well aware that stem or pluripotent cells offer an exciting potential source for a host of functional cell types. Their true potential will only be realized through a continued effort to increase basic scientific understanding at all levels, the development of adequate methods to achieve a functional phenotype and attention to safety issues associated with adequate control of cell localization, proliferation and differentiation. There is also new understanding regarding the immunology of parenchymal cells and new, promising approaches to immune modulation, which will open the door to broader therapies using allogeneic cell sources without prohibitive immune suppression. Control of cell growth and phenotypic expression does not end in the culture vessel but goes beyond to the patient. A living therapy is not static but dynamic, as is the host response. The cells or tissue construct in most cases will not behave as a whole organ transplant. It is, therefore, important that we understand a cell or tissue therapy's ability to react and interact within the host since clinical effectiveness has proven to be one of the most difficult milestones to achieve. A living cell therapy offers great potential to alter the human condition encompassing alteration of the current biological state of a targeted tissue or organ, augmentation of depleted or lost function, or absolute functional tissue replacement. The extent to which we are able to achieve effective cell therapies will depend on assimilating a rapidly developing base of scientific knowledge with the practical considerations of design, delivery and host response.

**Who Will Lead the Way?**

Fundamental knowledge is integral to effective design in tissue engineering; whether the goal is the development of a novel scaffold material to promote tissue regeneration, or a living cellular implant. Without it, technology is developed in the dark, using an iterative approach often lacking the dimension and understanding to produce a successful, predictable outcome in a timely manner. Unless the appropriate knowledge and experience are put to the problem, the potential of living therapy will not be realized.

Academic research must lead the way in establishing the knowledge base that will lead to product opportunities. In the biological disciplines at least, we cannot ask or expect industry to create, research, and develop, for there are too many inputs needed for any one group to take on the entire job. In addition, we cannot expect industry support to correctly target and fund academic research for the broad advancement of the field. Academic research must be where things begin, helping to create the opportunities and the paths to those opportunities through basic experimentation and the acquisition of fundamental understanding of biological processes and cell behavior. Industry is expected to take the discovery, knowledge, and opportunity and bring it to meaningful use. This will require innovation, technology development, and ongoing discovery. Universities and industry must work in partnership to bring the technology forward, because without industry participation, products of scientific discovery and knowledge are unlikely to get to the patient in an effective way.

Biotechnology will be the proving ground for many of these opportunities, but basic research must be there to continually provide support of theories, scientific validation, and additional knowledge.

**Cells – There's No Escaping Them**

Repair of the body implies a re-growth, a re-formation of tissue, and a re-establishment of function. Cells are the functional elements of reparative medicine, no matter whether we are concerned with creating a noncellular implant that will guide tissue formation or are concerned with the growth and manipulation of cells outside the body to form living tissues for transplantation. For example, much of tissue engineering over the last ten years has been focused in the area of biomaterials to control cell response.[4] To use an example from my company's own work, we have been concerned with the development of a small-caliber vascular graft for some time. The graft has been further refined and key chemical and physical parameters are being defined as part of the development process; in preparation are large animal studies in both the carotid and coronary positions as the team proceeds to the next step, preclinical efficacy. As the graft gets closer to human use, the team of engineers, biologists, chemists, and clinicians now must not only investigate gross measures of success such as graft patency, but also analyze more dynamic biological parameters such as rate of cell infiltration, cell phenotype, behavior, physiological function, tissue development, and physical properties over time. Knowledge of vascular cell biology, vascular physiology, cell-matrix interaction, and mechanisms of inflammation and vascular disease progression are all key elements that must be brought to the analysis of graft performance and likely outcome. Only with such an analysis, with each discipline involved, will we be able to recognize issues that might mean the success or failure of the construct in the clinic. A sophisticated supporting technology such as bioimaging could greatly facilitate assessment of progress and outcome, preclinically and clinically.

**Cells for Therapy and Functional Implants**

Effective use of cells for therapeutic purposes hinges on our ability to:

1. accurately predict cell response;
2. acquire the appropriate cells, either through recruitment *in situ*, expansion *ex vivo* from self or non-self, or manipulation of novel sources such as from different species or pluripotent embryonic or adult cells; and
3. direct and control cell response toward the desired phenotype to achieve the desired outcome, level of function, and assurance of safety.

We are only now beginning to understand cell response *in vitro*, a highly artificial environment, with limited dimension. We can only begin to appreciate that cells implanted in a living human being, often under varying conditions, are an even bigger challenge, not "automatic" in outcome, but not insurmountable either. There will be different immunological considerations, differing donor-host responses and a dynamic interaction, as the living tissues react, respond, and repair. One of the greatest challenges is delivering cells, tissues, or organ "equivalents" in a form that will be biologically meaningful. Our experience with the living skin substitute, Apligraf®,a illustrates this point.

Apligraf is a bilayered skin construct of human skin reconstructed in vitro through the use of organotypic culture techniques.[20] Apligraf is able to respond to its environment, go through the biological and structural aspects of wound healing *in vitro*, and take routinely as a skin graft lasting at least one year on immune-compromised mice. There is no evidence of rejection in humans.[25] It became the first manufactured living product to be approved for two indications through the traditional U.S. Food and Drug Administration Pre-Market Approval route, having demonstrated clinical efficacy in the treatment of both venous leg ulcers and later, diabetic foot ulcers.

Away from the controlled environment of the athymic or SCID mouse, Apligraf appears to respond to the patient's wound in multiple ways to provide its benefit.[26] We are allowed at least ready gross observation of this response since Apligraf is applied to the body surface. Even with that, it is sometimes difficult to ascertain the exact nature of the response. As understanding grows, through observation and clinical experience, clinical outcomes become increasingly predictable even though the mechanisms may not yet be well understood. Continued scientific study will provide this understanding. Further analysis is also important in setting a course of future development as we must consider ways to improve the technology.

**Scientific and Technological Advances in other areas of Science will Play Key Enabling Roles**

There are a number of ways in which basic scientific understanding of cell genetics and cell processes will enable the successful use of cells for repair. Accurate prediction of cell response is important, not only in enabling us to control proliferation and differentiation pathways *in vitro*, but also in understanding how the responses and factors controlling them will influence their action, reaction, persistence, and safety *in vivo*. This fundamental knowledge is integral to the direct manipulation of cells, and also to the design and development of the enabling technologies surrounding them; such as the development of novel scaffolds, new biomaterials, immunotherapy, microinvasive surgery, and bioimaging.

**Immunology**

Clinical and basic research on the immunology of the allogeneic fibroblasts and keratinocytes in Apligraf has demonstrated that at least certain allogeneic cells of a nonprofessional nature may be transplanted without immune recognition and rejection.[31,32] Basic research indicates that this is in part due to lack of an operable costimulatory pathway,[32] necessary for T-cell activation. Further work continues to help us to understand other biological factors affecting the ability of the graft to persist in different types of wounds. This information not only helps us to understand the skin construct, but also provides important understanding that we can apply to other cell therapies as well.

One important, but neglected aspect governing cell and/or functional persistence is the cell or tissue response to inflammation. This will be a critical area of research going forward, as will immunological research in the areas of immune tolerance, which is expected to play a key role in allowing the transplantation of many cell types and tissues.[33-35] We must

appreciate that lack of persistence is due to multiple factors, only one of which may be the recognition of non-self, and that allogenicity does not always equate with rejection.

**Genomics and Proteomics**

Both genomics and proteomics have the potential to fuel advances in the use of cells for reparative medicine. Methods of cultivation and organotypic culture technologies, i.e., methods that foster and support maximum phenotypic expression, will advance using this knowledge. These technologies will help not only the development of technology, but our ability to predict outcome. Both genomics and proteomics will help foster advances in cell culture methodology as well. This, combined with innovative bioreactor designs that foster cell-cell interaction and allow dimension for tissue growth, such as the microgravity rotary wall vessel originally developed by NASA (Synthecon Inc., Houston, TX), will play important roles in achieving the cell development and control needed for the generation of functional tissue implants. We are rapidly gaining insights into transcription factors that are important in certain aspects of tissue development.[44] Innovative developmental biological research is illustrating the plasticity of cell phenotype and the potential of the cell to exhibit pluripotency.[45, 46] But for the correct enabling in vitro environment, for example, a skin construct might be made containing not only epidermis and dermis, but also appendages, such as glands and hair, derived from a single epithelial cell source. Technology must advance to support this scientific potential.

**Issues of Cell Sourcing**

The choice of cell source has an impact on a technology beyond immunology and safety. A more in-depth discussion of cell sourcing issues can be found elsewhere,[47] but a few points deserve mention. The use of autologous cells often implies an assumption of minimal manipulation and an inherent safety in the use of one's own cells. This is not entirely correct, as culture processes and reagents can alter cells, regardless of the origin. The use of allogeneic (non-self) and xenogeneic (animal) sources presents additional, but different, immunological and safety considerations.

The choice of cell source can have an impact upon the research strategy, the design requirements, the clinical strategy, and the potential target market. For example, an extracorporeal liver-assist device containing porcine cells will have certain research and development issues, such as a controlled source, reproducible methods of isolation, assurances of batch-to-batch quality and physiological function, immune isolation, etc.

**Conclusion**

We have used a few examples from our own experience to illustrate the need for multidisciplinary approaches, open minds, and new tools. We have also attempted to highlight some of the important complexities cells bring to the task. The outlines presented in Figure 3 summarize the contributions both basic research and industry can make to cellular reparative medicine. Awareness, focus, and support must be built into many areas to adequately address the complex tasks ahead. The successful delivery of a living cell or tissue therapy to the patient will require the participation of all of us.

(Text excerpted from Parenteau, N., and J.H. Young, 2002. The Use of Cells in Reparative Medicine. Ann NY Acad Sci 961:27-39)

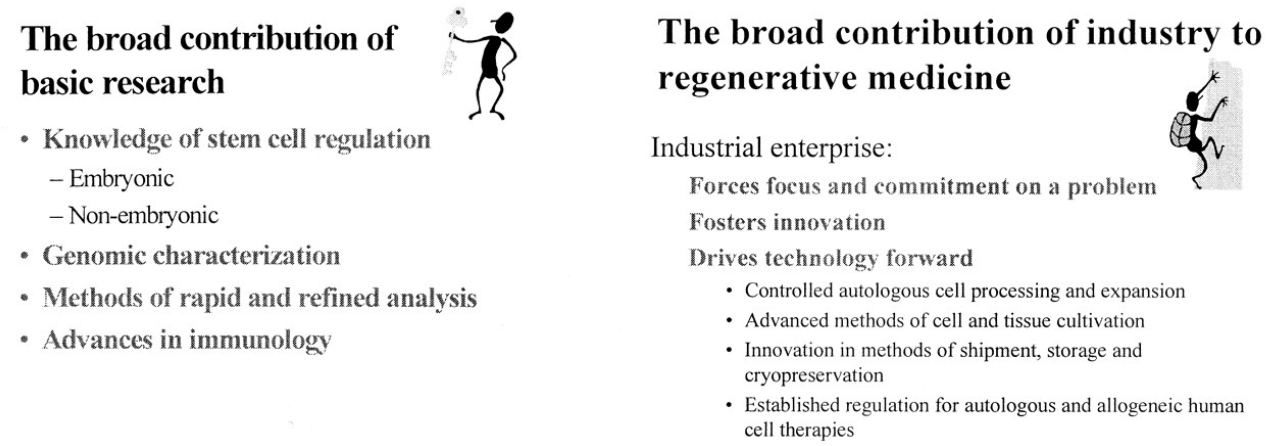


Figure 1. Development of a successful therapy requires strong research, technology, and management (Parenteau, N., and J.H. Young, 2002. The Use of Cells in Reparative Medicine. Ann NY Acad Sci 961:27-39, reprinted here with permission.)

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## Emerging Technologies and Tissue Design

*Peter C. Johnson, M.D., TissueInformatics, Inc.*

Tissue engineering began with the realization that the regenerative potential of tissues could be harnessed through the joint application of tissue culture structural support technologies. In most cases, the development of present engineered tissues followed a course of empirical design in which tissues were grown to compare in appearance to normal tissues or to achieve functional parameters, or both. This approach has provided a great deal of information on the patterns of tissue growth in vitro and has been sufficient for the generation of diffusion-limited, planar tissues. As we enter an era of three-dimensional tissue engineering, the concurrent availability of new analysis technologies will enable us to approach the design of such tissues in a more planned fashion. These technologies include the analysis of gene expression using microarrays, the assessment of protein structure and function using proteomics technologies, cellular response archiving using massively parallel cell response assays, digital tissue analysis methodologies and information systems that track clinical outcomes when engineered tissues are used. Data from such analyses will eventually power in silico systems that will enable customized engineered tissues to be modeled before manufacture. The need for design, the components of design and the effect of these new technologies on tissue design, quality assurance and regulation will be discussed.

### The Tissue Design Process

Tissue engineering includes a number of stakeholders, all of whom are important in bringing a product from initial research stages into the market. These stakeholders include scientists, engineers, clinicians, funding agencies, businesses, regulators, buyers, patients, and the society at large. Currently, a prevalent design method in tissue engineering begins with work done by independent researchers and over time moves progressively through the various groups of stakeholders. This process may have limited success, but is particularly problematic if the design initiated by researchers does not adequately address the problems of other groups, such as clinicians or businesses, or does not meet regulatory requirements. In such a case, the initial investment in the technology may be wasted.

To most effectively and efficiently deal with the entire design process, the needs of all stakeholders must be addressed throughout the design process. Driving forward progress of tissue engineering requires all stakeholders to take an equal role in the development process. Since tissue engineering is so complex, communication and collaboration between disparate groups is crucial.

**Informatics as an Aggregating Mechanism**
Communication between different stakeholders may take place in scientific meetings or formalized group events, but the importance of informatics in facilitating concurrent design cannot be underestimated. Effective collaboration requires a common language and data reference system. A tissue informatics system that includes information on tissue structure, function, and performance, and that can be accessed by people of different languages and educational levels, would be incredibly effective in facilitating aggregation of data. Continuous access to such a system allows cross-correlation and exchange of information in a common data bank. Additionally, informatics systems allow the development of normative standards for individual tissues. The presence of this information provides a context for stakeholders to realize the volume and kind of information available. Early on, this system should use all stakeholders to eliminate surprises and to benefit everyone.

**Elements Needed for a Successful Tissue Engineering System**
Currently, the ability of researchers to engineer functional tissues is limited by a lack of information about normal tissue. As clinicians wish to produce more artificial tissues, the design process is likely to enhance demand for basic research data. Normative tissue parameters can come from a number of different technologies, and include data from DNA microarrays, mass spectrometry, cellular arrays, structural and functional imaging, mechanical testing, and performance metrics in both the clinical and industrial setting. Currently, hyperquantitative data acquisition is needed. Data stored in a digital, cartesian format would allow questions about location and distance to be answered. Integration of this data into a common format would facilitate data processing.

This information can be put into data banks and made available for other researchers. Such a data bank would include not only direct tissue characterization and spatial information, but also information about the clinical outcomes of specific tissue pathologies. It would have a composite, open dataset. An informatic system would also need data mining systems that allow one to parse through the available data.

Additionally, stakeholders should have access to non-proprietary information held by private companies. Sharing this data would allow a more rapid advancement and increase the success of the field as a whole. Regulatory and funding agencies can play a role in this by supporting automated systems for tissue analysis, assisting the development of digital tissue banks, assisting in the creation of mathematical analyses for tissue characterization, and providing general support for informatics infrastructure.
Lastly, issues of public reception are essential for continued progress. Thus far, society has reacted positively to emerging tissue engineering technologies. With ethical issues concerning stem cells and cloning reaching the public, this field must be aware of its association with these areas and be cautious with its public image.

**Standards for Tissue Engineering**
Assessment of tissue morphology has historically been dependent upon the subjective judgment of individual pathologists and clinicians. Development of genetic and genomic analyses has made concrete markers of tissue genotype available. These markers can be used to construct normative standards for the structure, function, and performance of

tissues. These standards include genomic information and are objective, quantitative, and reproducible.

In addition to standards for normal tissues, there should be a greater investigation into the acceptable range of characteristics for artificial tissues. There should also be information about acceptable tolerances in tissues, including oncogene activity, developmental stage, and relative stages of different cells. This information would be extremely useful for tissue engineering since the success of engineered constructs is not necessarily predicted by their appearance. Collection of this data will allow the creation of *in silico* modeling systems that allow researchers to examine cells and tissues prior to manufacture of constructs.

**Conclusion and Future Directions**

In summary, successful development of engineered tissues is enhanced by communication with all groups of stakeholders throughout the production process. Informatics is a critical tool for tissue engineering, and stakeholders should utilize data banks that contain quantitative, reproducible data to construct normative tissue standards and standards for engineered tissues. There should be a greater capture of knowledge based on informatics, a stress on normative standards, and definition of normal ranges of tissue characteristics that allow successful tissue design. Tissue engineering will only fulfill its full potential within health care systems by addressing these issues and embracing tissue informatics.

## Functional Assessment and Clinical Outcome

*Steven A. Goldstein, Ph.D., University of Michigan, Ann Arbor*

This paper provides a general framework for the process of bringing tissue-engineering constructs from the laboratory bench to the patient's bedside, rather than presenting a detailed review of the engineering or biologic principles or mechanisms that are necessary for successful tissue engineering. Many of the principles are animated by using examples from current studies being developed in my laboratory or those of my collaborators. In all likelihood, multiple solutions or approaches will be found that lead to successful tissue-engineering constructs. The focus here is on the identification of critical parameters to be considered rather than specific design solutions. The review is therefore organized to reflect feasible sequences of activities formulated to take tissue engineering from concept to clinical reality.

### Construct Conceptualization

Successful tissue engineering begins with a clear and precise definition of the clinical demand or problem being addressed. Regardless of whether the application is focused on replacing or augmenting failing heart tissue or on creating bone graft substitute materials, the design objective should begin with the delineation of the multiple specific attributes of the identified clinical problem. It is important to begin by identifying these targeted functional properties in a quantitative manner, since they will become the reference of comparison for outcome measures that will be used to qualify the tissue-engineering construct as being successful or unsuccessful.

### Design and Function Hierarchy

Following a paradigm similar to that used to solve an engineering design problem, the next phase associated with successful tissue engineering is focused on characterizing the specific design attributes and technology that will serve as the backbone of the construct under consideration. I have found it particularly valuable to consider the design attributes and targeted functions within a hierarchical framework. This concept can be demonstrated in Figure 1 by reviewing the images used in designing a bone tissue-engineering construct.

In practice, the ability to perform this segmentation can facilitate a design optimization process. Since all tissue engineering constructs involve the use of cells, matrices, and biofactors, this hierarchical paradigm can be used to assess how the source or manipulation of cells (*in vivo* or *ex vivo,* for example), the design of the matrices, and the choice of biofactors can influence the behavior of the construct. At the conclusion of this design phase, the hierarchical analyses, both within scale and across scale, provide the proof of concept for the technology. Most often, this proof of concept involves cellular, molecular, or physiologic analyses *in vitro* and, importantly, *in vivo*.

### Design of Pivotal Preclinical Studies

Typically, the development of a tissue engineering construct requires the performance of several preclinical studies prior to evaluation in human subjects. As discussed earlier, some preclinical studies are a part of the evaluation of proof of concept and are followed by

expanded studies that are designed to evaluate efficacy in a relevant or well-accepted model. Usually, parallel studies are also performed to evaluate safety.

For many tissue-engineering constructs, the pivotal preclinical studies designed to demonstrate efficacy involve a transition from the use of small animals to large-animal models. The safety studies, in contrast, are focused on evaluating toxicity, either locally or systemically, bioavailability, and the relationship between bioavailability, toxicity, and dose. Often, the safety studies can also provide assessment of the effects of storage or sterilization on the properties of the construct.

The designs, as well as the success of preclinical studies, are dependent on a number of issues. These include:

1. the experimental model;
2. functional assays;
3. dose response;
4. correspondence of preclinical and clinical variables; and
5. safety considerations and assessments.

**Experimental Model**
The appropriate choice of an experimental model is critical to the success of the preclinical studies. Part of the criteria associated with choice or design of the experimental model is related to the targeted properties and, often, the expected commercial market of the tissue-engineered construct. For example, if the tissue-engineered construct is designed for a very specific clinical application, and a market strategy includes a relatively narrowly defined use, then the experimental model should be designed to simulate the clinical condition that is targeted. On the other hand, if the goal is to seek approval of a construct to be used in a broad range of clinical applications, then the experimental model may not precisely resemble any one specific clinical condition, but instead be general enough such that the results can be translated to this broad set of clinical applications.

**Quantitative Functional Assays**
Quantitative and reproducible functional assays must be developed and utilized in the pivotal preclinical study. The quantitative aspects of these functional assays cannot be underestimated, since without highly quantitative assays, the ability to statistically evaluate the efficacy of the tissue-engineering construct may be compromised. In fact, the overall statistical robustness of both the experimental model and the experimental design of the study that utilizes the model must be considered carefully. It is recommended that investigators seek consultation with biostatistical experts.

**Preclinical versus Clinical Studies**
Considering that many of these tissue-engineering constructs are based on cellular or molecular mechanisms for incorporation and regeneration, the ability to have quantitative functional assays is limited to existing imaging modalities or minimally invasive or non-invasive measures. This is in stark contrast to preclinical studies in which the animals can be sacrificed and assays ranging from macroscopic to molecular in scale can be performed in

highly quantitative ways. This limitation in translating studies into the clinical arena provides an incentive for additional considerations in the design of the preclinical studies. Investigators should consider developing surrogate assays as part of the preclinical studies. In other words, the preclinical studies should include outcome measures that are similar to those that are anticipated to be utilized in the clinical studies.

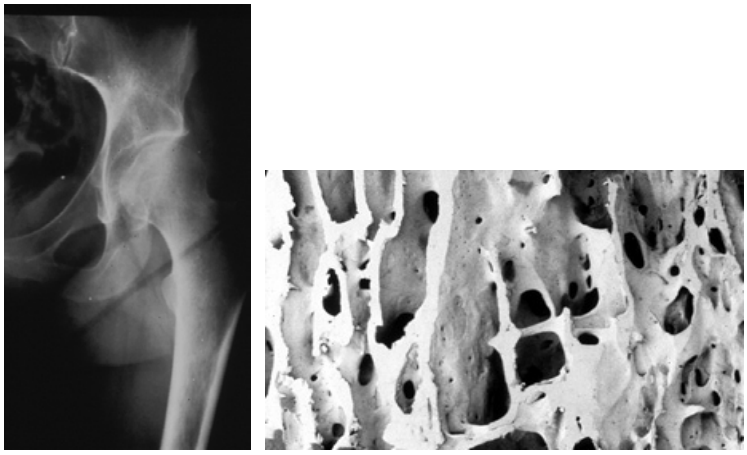
There are very few, if any, assays available to non-invasively characterize mechanical properties of tissue-engineering constructs. Therefore, a successful outcome depends on the development of surrogate assays for these mechanical properties during the preclinical studies.

**Summary**

The principles that must be considered for successful tissue engineering include the following:

1. comprehensive description and characterization of the properties of the native tissue;
2. thorough design optimization based on clinical need and targeted functional properties;
3. conceptualization of the tissue-engineering construct within a hierarchical paradigm that includes functional assessment across multiple scales from the cellular and molecular level, through the organ level;
4. assessment of both biomechanical and biologic function and adaptation; and
5. the development of preclinical studies that include considerations of characterizing surrogate variables in the preclinical studies that may aid in functional assessment during the clinical phase.

(Excerpted from Goldstein, S.A. 2002 Tissue Engineering: Functional Assessment and Clinical Outcome. Ann NY Acad Sci 961: 183-192).




a. b.

c.

Figure 2. A simplified hierarchical pattern for bone is illustrated. Clinical function is defined at the organ level (a) as depicted by the finciton of the whole femur. The function of the femur, however, is dependent on the properties/function of the bone tissue architecture and properties (b), which is dependent on the cell/matrix interactions. (Goldstein, S.A. 2002 Tissue Engineering: Functional Assessment and Clinical Outcome. Ann NY Acad Sci 961:183 – 192, reprinted with permission)

## *In vitro* Systems for Tissue Engineering

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### Introduction

### Strategies for *in Vitro* Tissue Engineering

Tissue engineering follows the principles of cell transplantation, materials science, and bioengineering towards the development of biological substitutes that would restore and maintain normal function. *In vivo* tissue engineering relies on the body's natural ability to regenerate over non-cell-seeded biomaterials. This approach may require proteins, DNA, or mRNA additives for enhanced regeneration. This technology has been applied to patients for decades. In contrast, *in vitro* tissue engineering involves the manipulation of cells *in vitro*, prior to implantation into the *in vivo* environment. Although this technique also relies on the body's ability to regenerate, additional cues are provided with the presence of cells or tissue at the time of implantation. When cells are used for tissue engineering, donor tissue is dissociated into individual cells that are either implanted directly into the host or expanded in culture, attached to a support matrix, and re-implanted after expansion.

### Biomaterials for Cell-Based Tissue Engineering

Biomaterials function as an extracellular matrix (ECM) and elicit biological and mechanical functions of native ECMs found in tissues in the body. Native ECMs brings cells together into tissue, control the tissue structure, and regulate the cell phenotype.[8] Biomaterials facilitate the localization and delivery of cells and/or bioactive factors (e.g., cell adhesion peptides and growth factors) to desired sites in the body, define a three-dimensional space for the formation of new tissues with appropriate structure, and guide the development of new tissues with appropriate function.[9] Direct injection of cell suspensions without biomaterial matrices has been utilized,[10,11] but it is sometimes difficult to control the localization of transplanted cells. In addition, some mammalian cell types are anchorage-dependent and will die if not provided with a cell-adhesion substrate. Biomaterials provide a cell-adhesion substrate and can be used to achieve cell delivery with high loading efficiency to specific sites in the body. The configuration of the biomaterials can guide the structure of an engineered tissue. The biomaterials provide mechanical support against *in vivo* forces, thus maintaining a predefined structure during the process of tissue development. The biomaterials can be loaded with bioactive signals, such as cell-adhesion peptides and growth factors that can regulate cellular function.

### Design and Selection of Biomaterials

Generally, the ideal biomaterial should be biocompatible, promote cellular interaction and tissue development, and possess proper mechanical and physical properties. If the selected biomaterial is biodegradable, the degradation products should not provoke inflammation or toxicity and must be removed from the body via metabolic pathways. The degradation rate and the concentration of degradation products in the tissues surrounding the implant must be at a tolerable level.[12]

The biomaterials should provide an appropriate regulation of cell behavior, such as adhesion, proliferation, migration, and differentiation in order to promote the development of

functional new tissue. Cell behavior in engineered tissues is regulated by multiple interactions with the microenvironment, including interactions with cell adhesion ligands[13] and with soluble growth factors.[14] Cell-adhesion-promoting factors (e.g., Arg-Gly-Asp [RGD]) can be presented by the biomaterial itself or be incorporated into the biomaterial in order to control cell behavior through ligand-induced cell receptor signaling processes.[15,16] The biomaterial can also serve as a depot for the local release of growth factors and other bioactive agents that induce tissue-specific gene expression of the cells. The biomaterials should possess appropriate mechanical properties to regenerate tissues with predefined sizes and shapes. The biomaterials should provide temporary mechanical support sufficient to withstand *in vivo* forces exerted by the surrounding tissue and to maintain a potential space for tissue development. The mechanical support of the biomaterials should be maintained until the engineered tissue has sufficient mechanical integrity to support itself.[17] This can be potentially achieved by an appropriate choice of mechanical and degradative properties of the biomaterials.

**Types of Biomaterials**

Many classes of biomaterials have been used for cell-based tissue engineering. These can be categorized as naturally derived materials (e.g., collagen and alginate), acellular tissue matrices (small intestinal submucosa), and synthetic polymers (e.g., polyglycolic acid [PGA], polylactic acid [PLA], and poly(lactic-co-glycolic acid) [PLGA]). Naturally derived materials and acellular tissue matrices have the potential advantage of biological recognition. An advantage of synthetic polymers is reproducible large-scale production with controlled properties of strength, degradation rate, and microstructure.

**Cell Sources and Bioreactors for In Vitro Tissue Engineering**

The fields of cell transplantation and tissue engineering have been actively studied for several decades, yet there have been relatively few clinical advances. This is mostly related to the inability to grow and expand numerous cell types in large quantities. It is known that 70% of a liver can be surgically resected, and 6 months later it can regrow to its initial volume. Yet, even today, if a biopsy of liver tissue were to be obtained, it would not be possible to grow and expand all the cell types in any appreciable manner. However, scientists have made major advances in the areas of cell growth over the last decade.[39,40] Many different cell lineages have been tried for *in vitro* tissue engineering. These include cells from either autologous or heterologous sources and from allogeneic or xenogeneic lineages. More recently, stem cells have also been applied for tissue engineering, either from embryonic or adult sources.[42,43]

*In vitro* systems used in tissue engineering span a wide area of subject matter, from pumps and bioreactors to constructs intended for tissue or organ replacement. *Ex vivo* approaches to alter or otherwise control cell behavior are included in this range, and are important for the possible attainment of novel treatments for many human diseases such as diabetes or muscular dystrophy. Bioengineered organs are another goal of tissue engineering, with current approaches aimed at partially constructing and growing the organ in an *in vitro* setting.

As sophisticated as tissue construction *in vitro* can be today, the *in vivo* environment is crucial for the final development of the tissue or organ. Thus, the human body often serves as the "terminal incubator." It has often been stated that cells possess all the genetic information required to reconstruct tissues, whole organs, and even an entire human being. This concept has become even more important today as scientists, governments, and ethicists grapple with the controversies surrounding stem cells and cloning. One concept is certain: that if specific cells are placed in the right environment within a living organism and are given the right cues, then appropriate tissue and organ development may ensue.

The term "bioreactor" refers to a system where conditions are closely controlled to permit or induce a certain behavior in living cells or tissues. The behavior could simply be cell proliferation, or could be as complex as having several sets of cells that sense one or more variable parameters and produce specific chemicals accordingly. The former example could be applied to the common laboratory incubator, while the latter could serve as a guide for a theoretical glucose sensor for the control of insulin and glucagon levels.

**Biomaterial-Cell Interactions**

Virtually every tissue in the body has been considered for some form of tissue engineering application. Reactions between the matrix itself and the transplanted cells are important for development of successful tissues. For instance, in studies involving the engineering of liver tissues, chitosan matrices have been modified with collagen, gelatin, or albumin in an effort to enhance hepatocyte attachment.[65] Here it was found that albumin modification of chitosan provided a good surface for cell attachment. Another example is the use of poly(l-lactic acid) sponges as matrices for hepatocyte culture, where it was found that the use of collagen coatings on the sponges or collagen-embedded cells could aid in cell attachment and culture.[66] Others have foregone the use of artificial matrices by using the native architecture of existing livers.[67] Similar findings regarding matrix modification have been noted with various other cell systems.

**Cell Encapsulation into Microspheres**

The principle behind cell encapsulation is to surround cells with a coating that prevents contact between the cells and their surface antigens with host immune components. The coating must be porous to allow for the exchange of nutrients and cellular products between the interior and exterior of the microcapsules. While the encapsulated cells are used in an *in vivo* setting, their creation is by necessity an *in vitro* process. The level of protein production and the duration of the secretion period can be regulated by modulating the number of engineered cells that are encapsulated per microsphere, as well as the number of microspheres injected.

**Gene Delivery**

A powerful tool that is becoming increasingly useful to the tissue engineer is that of gene delivery. Gene delivery itself is a very large field that is itself going through rapid changes and expansion. There are many carriers available for gene delivery, and each comes with its own set of advantages and disadvantages.[87] The most common use of gene delivery for tissue engineering is *ex vivo* transfection. The goal of such work is typically either to engineer cells with particular characteristics to repair a defect or replace somatic cells that

are lacking in the specific behavior, or to manipulate existing cells to produce a product that will induce the body to respond in a specific way after implantation.

**Clinical Applications**

*In vitro* tissue engineering techniques have been used clinically for a variety of organ systems. Like most areas involving biologicals, the translational lag from experimental work to clinical application has varied depending on the complexity involved. In the field of cell transplantation and tissue engineering, there has usually been a 10- to 20-year lag for most tissue types applied clinically to date: skin (7 years),[101,102] pancreas (19 years),[103,104] cartilage (17 years),[11,105] liver (12 years),[106,107] cornea (15 years),[3,108] and bladder (9 years).[109,110] In addition to these specific examples, several other tissue technologies are in the process of being applied clinically. Tissue-engineering techniques using cell-based approaches are already expanding the options for treatment in patients with deficient tissues and organs.

(Excerpted from Godbey W.D. and A. Atala. 2002. In vitro tissue engineering. Ann NY Acad 961: 10 – 26).

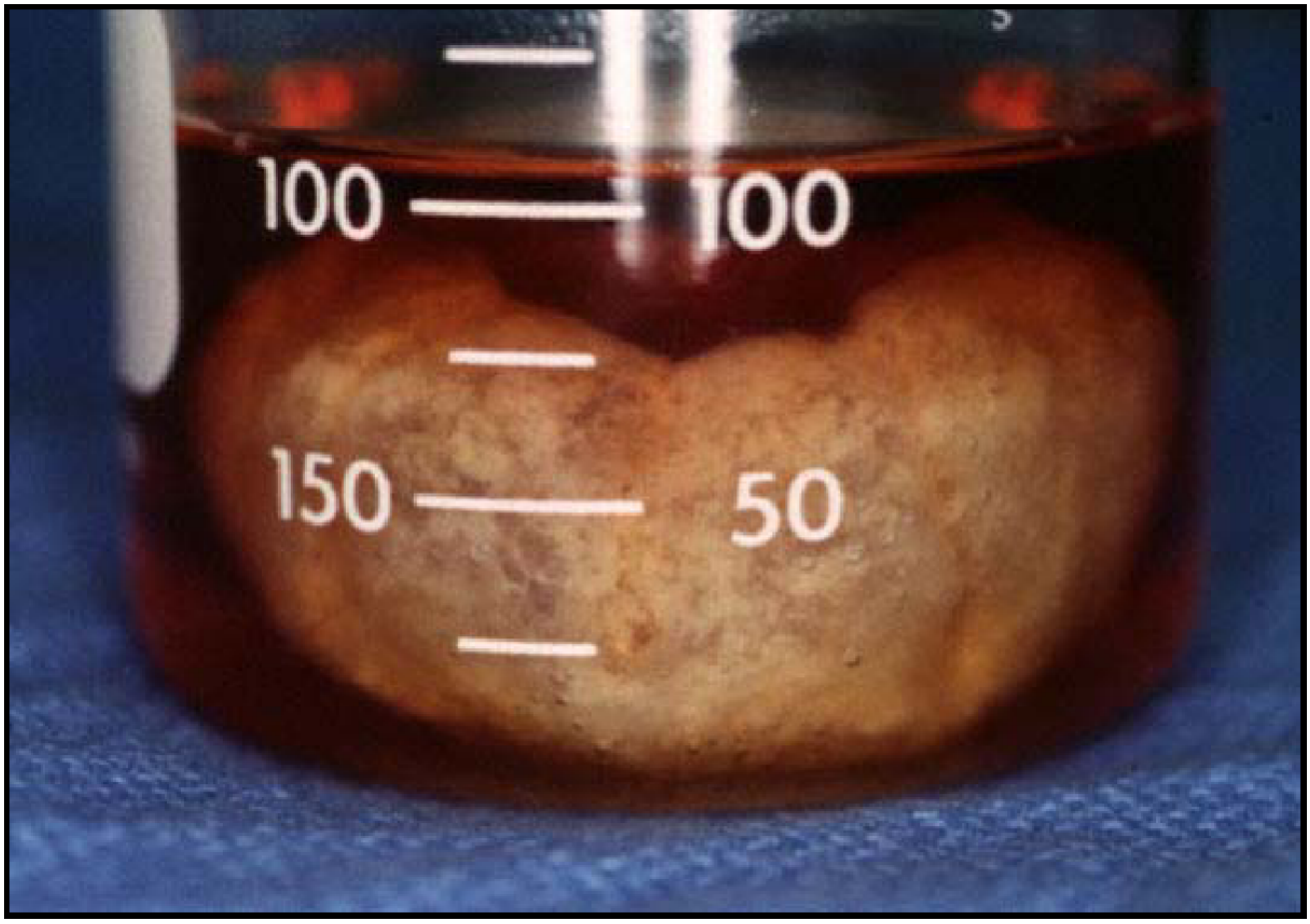


Figure 3. A tissue engineered bladder. An acellular matrix consisting of PLGA-coated PGA was seeded with bladder smooth muscle cells to form the structure pictured. [Photograph Anthony Atala. Godbey W.D. and A. Atala. 2002. In vitro tissue engineering. Ann NY Acad 961: 10 – 26 reprinted here with permission.

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## Reports of Panel Sessions

## BIOMATERIALS/SCAFFOLDS FOR TISSUE REPAIR

**Moderators**
*Elliot Chaikof, M.D., Ph.D., Emory University*
*Howard Matthew, Ph.D., Wayne State University*

**Panelists**
*Joachim Kohn, Ph.D., Rutgers University*
*Glenn Prestwich, Ph.D., University of Utah*
*Antonios Mikos, Ph.D., Rice University*
*Christopher Yip, Ph.D., University of Toronto*

## BREAKOUT SESSION SUMMARY

**Broad Statement.** The versatility of many of the approaches currently pursued or contemplated within the framework of *Reparative Medicine* is dependent on our ability to: 1) synthesize or otherwise generate novel materials, 2) fabricate or assemble materials into appropriate two dimensional and three dimensional forms, and 3) precisely tailor material-related physical and biological properties so as to achieve a desired clinical response.

**Vision.** The development of *innovative materials and scaffolds* that are capable of modulating those cellular responses required for tissue repair and regeneration will be achieved through multidisciplinary, collaborative interactions between investigators working in the physical and biological sciences.

**Objectives.** To facilitate this vision, future research should address four different areas. The first is methodologies for macromolecular design and synthesis. There is the need to 1) design recombinant matrix proteins with properties that exceed those associated with native proteins, 2) develop strategies for the synthesis of polymers with controlled molecular weight, architecture and conformation and 3) optimize solution and solid-phase methods to generate complex natural products, as well as nonnatural biomacromolecule mimics.

The second critical area is the development of hybrid, composite and complex biomaterial/scaffolds. There is the need to 1) extend techniques for the utilization of naturally occurring biopolymers either alone or in combination with other natural or synthetic materials and 2) to characterize the effect of material degradation and related byproducts on reparative processes.

The third critical area is the fabrication and processing from the nanoscale to the macroscale. There is the need to 1) optimize fabrication methods that operate on nano-, micro- and macroscale levels via two and three-dimensional assembly processes and 2) to elucidate the effects of processing conditions on material properties and function.

The fourth critical area is biorelevant structure-property analysis. There is the need 1) to develop appropriate instrumentation and techniques that can measure physical or biological properties or other material attributes over a wide range of dimensional length scales and 2)

to design integrated strategies that evaluate material properties in real-time, in istue, and under physiologically relevant conditions.

**Challenges.** Rational approaches for material design and fabrication will require collaborative interactions between biologists, engineers and physical scientists. Elucidation of structure/function relationships of tissues and natural matrices is required to serve as a starting point and reference base for material design. Novel synthetic approaches will be needed for enhancement of the materials design process. Identification of promising biomaterial candidates will require the development of analytical tools and physiologically relevant testing methods.

**Recommendations.** NIH should facilitate the cultural union of the intellectual frameworks of biologists and engineers through new funding initiatives for projects involving both disciplines. Support of centralized core facilities such as for microarray analysis or combinatorial synthesis for readily available use by any NIH funded investigator would accelerate progress in biomaterials/scaffold development.

## BIOREACTORS AND BIOPROCESSING

**Moderators**
*Laura E. Niklason, M.D., Ph.D., Duke University Medical Center*
*Anthony Ratcliffe, Ph.D., Advanced Tissue Sciences, Inc.*

**Panelists**
*Kelvin Brockbank, Ph.D., Organ Recovery Systems, Inc.*
*Duane F. Bruley, Ph.D., P.E., University of Maryland Baltimore County*
*Kyung A. Kang, Ph.D., University of Louisville*

**Vision.** Biomedical engineers are rapidly developing procedures and techniques to produce functional cells and tissues at the laboratory bench. *The next challenge is to translate this research into large-scale production of safe, reproducible tissues that are clinically appropriate for reparative medicine.* This will require research to develop technologies to initiate cell proliferation under conditions that maintain functional phenotypes; to design bioprocessing systems that promote large-scale growth and histogenesis; and to develop efficient separation and purification systems. Progress in these areas is essential to ensure the availability of reparative cells, three-dimensional tissues, and tissue composites for clinical repair and regeneration treatments.

**Successes, Challenges, and Opportunities.** Bioprocessing involves both upstream (bioreactors) and downstream (separation and purification) elements to produce bioproducts. Bioprocessing is fundamental to the pharmaceutical and biotechnology fields for producing large quantities of products for clinical and research use at a reasonable cost. The healthcare industry will become increasingly dependent on products of bioprocessing that improve quality of life and economic competitiveness.

Currently, the expansion of cell lines and the growth of three-dimensional tissues are major areas of focus in developing new bioprocessing techniques. Solving these important technical issues requires a better understanding of processes such as interactions between cells and biodegradable scaffolds, angiogenesis in large three-dimensional tissues, transport of materials within living tissues, maintenance of sterility from cell seeding to implantation, growth of cells and tissues under varying conditions of biomechanical loading, identification of defined culture media (lacking animal serum), and signaling between cells.

Much of the recent bioprocessing research has been done using bioreactor systems adapted for the production of reparative tissues for transplantation, such as vascular substitutes, skin, or cartilage. However, bioreactors are also being investigated for use as short-term extracorporeal cell-based therapies such as the bioartificial liver or kidney. Miniaturized bioreactors, or microencapsulation devices, have been adapted for either extracorporeal or implanted cell-based drug delivery, for hormones such as insulin or erythropoetin. These devices present other research challenges. One is the development of high flux immunoprotective membranes that do not inhibit the essential mass transport

functions. Another is engineering processes to attach functional cells to durable biomaterials, rather than having cells seeded onto biodegradable materials.

Bioreactors are also used in the pharmaceutical industry, both for drug production and testing. This application would also benefit from three-dimensional human tissues that more accurately mimic *in vivo* function.

Further advances in separation and purification will be essential to the quality, reproducibility and throughput of bioprocessing. Challenges include extending cell viability, preventing biological fouling, developing control biosensors, and implementing continuous processing and quality control procedures. In addition, improved *in silico* computational modeling of flow distribution and mass transport within the porous media of bioreactor membranes would lead to more successful design for *ex vivo* and *in vivo* bioprocessing systems.

Production and distribution of reparative cells and tissues will require advances in several areas. These include storage technology, where engineering of improved cryopreservation procedures is of utmost importance. Improvements are also necessary in the isolation of pure cell populations, the guarantee of disease-free cell sources, and identification and isolation of stem/progenitor cells. Techniques for efficient gene transfection of large numbers of cells would also be of benefit.

Treatment of certain clinical conditions may require the patient to bioprocess the engineered cells and tissues *in situ* in order to provide healing and repair. Challenges include understanding how natural and synthetic pathways can be utilized to induce cell and biomechanical signaling, how matrices are formed, and how nano-machines can be adapted to build reparative tissues. Implanted biodegradable scaffolds might be engineered to guide self-processing or reparative tissues. Alternately, engineered products might induce existing tissues to resume their natural function.

Several non-technical areas present challenges to bioprocessing. These include the development of standard reference materials, guidelines, and educational strategies. Achievement of all the identified goals will require strategies to stimulate and strengthen multi-disciplinary research collaborations. The success of reparative medicine relates directly to rate of advances in bioprocessing capabilities to produce adequate quantities of engineered material.

**Recommendations.**

- A new generation of bioreactors, separation approaches and purification methods are needed to fulfill the promise of reparative medicine. System design should include *in vitro*, paracorporeal, *in vivo* and in situ approaches
- It will be necessary to promote administrative strategies that emphasize multi-disciplinary research projects, education, and collaborative review and funding.
- Bioprocessing research rarely relates to any specific clinical application. Thus such research often does not find a home within a categorical NIH Institute. A

multidisciplinary NIH group such as BECON is essential for promoting funding opportunities for bioprocessing and reparative medicine.

**Implementation.** Few of the barriers and opportunities identified above will lead to research initiatives having significant commercial potential by themselves. Thus, one funding strategy to spur innovation to meet these technical challenges would be two-stage, SBIR-like grants for the development of innovative enabling technologies. A small, first-year award would be of low risk while permitting a proof of the feasibility of the technology development. Rapid follow-on funding would turn the most promising ideas into optimal techniques or technologies.

## CELLS FOR REPAIR

**Moderators**
*Denise L. Faustman, MD, Ph.D., Harvard Medical School*
*Roger A Pedersen, Ph.D., University of California, San Francisco*

**Panelists**
*Seung K. Kim, MD, Ph.D., Stanford University School of Medicine*
*Ihor R. Lemischka, Ph.D., Princeton University*
*Ronald D. McKay, Ph.D., National Institute of Neurological Disorders and Stroke, NIH*

**Vision.** Recent advances in stem cell biology hold great promise for regenerative medicine in humans. The identification of stem/multipotent progenitor cells in fetal and adult tissues and organs has many scientific and clinical consequences. It is hoped that stem/progenitor cells could be manipulated, both *in vitro* and *in vivo*, to differentiate into many different types of cells therefore overcoming supply problems of traditional organ transplantation**.**

**Successes, Challenges and Opportunities.** Cell sourcing is a major issue in the development of all *in vitro* tissue engineered products and *in vivo* cell therapies to repair or replace the loss of tissue function due to damage, disease, age or other complications. The cell sources currently available are autologous, allogeneic or xenogeneic and include adult differentiated cells, adult stem cells, embryonic and fetal stem cells, ex-vivo manipulated cells and cells generated by nuclear transfer. The generation of any optimal cell source for a particular application will depend on a rigorous characterization of these various cell sources in regards to their plasticity, propagation, and control of differentiation both *in vitro* and *in vivo*. Basic research that will elucidate the properties of stem cells must then be integrated with the multidisciplinary aspects of tissue engineering to successfully deliver the appropriate number and types of cells and promote a positive outcome: formation of new, functional tissue. The strategy of cell sourcing and use of cells for tissue engineering and cellular therapy is a central and important topic of basic and applied sciences.

There is an accumulating body of evidence that self-renewal of tissues and organs is possible from adult bone marrow as well as stem cells residing within a regenerating organ. There has been a recent explosion of literature demonstrating that adult stem cells from one tissue or organ can be made to differentiate into cells of other organs, both *in vivo* and *in vitro*. Demonstration of these concepts includes bone marrow cells into muscle and brain and liver; adult neural cells into blood and heart and perhaps skins cells to brain cells. Although many of these studies are in preliminary stages of investigation, these cells could serve as a potential source of cells for replacement therapy, and therefore warrant further study.

Although the therapeutic potential of stem cells shows promise, more basic research is needed to overcome the obstacles that exist in translating these findings into clinical treatments. For instance, many adult origin stem cells that are cultured *in vitro* followed by *in vivo* reintroduction, fail to differentiate into the correct cell type and indeed in may cases

demonstrate metastatic potential. The prevalence and location of multipotent stem/progenitor cells in various adult tissues is not clear. The expansion of purified clonal populations of cells in conjunction with the characterization of these cells using appropriate clonogenic and/or functional assays has not been achieved. In most cases, the signals/signaling pathways needed to promote a particular cell lineage are not well understood. The degree of host engraftment may not be robust or properly sustained for functional phenotypes to persist and confer phenotypes that permanently reverse disease.

Pluripotent embryonic stem (ES) cells derived from mouse or human can give rise to many different cell types in culture, and are capable of self-renewal. If ES cells are to be used therapeutically to generate tissue for transplantation, then information on how to manipulate undifferentiated cells efficiently down different developmental pathways is critical. The derivation of additional human pluripotent embryonic stem cell lines and their characterization and direct comparison to stem/progenitor cells derived from human adult tissues is needed.

The known challenges to this field include:

- Defining the molecular and cellular features, location and lineage-specific gene programs of stem cells in developing and adult organs and tissues.

- Understanding the molecular signals/signaling pathways that regulate self-renewal, cell lineage, proliferation, and differentiation of stem/progenitor cells.

- Understanding the complex interplay between “environmental” factors and “intrinsic” factors that regulate self-renewal and differentiation.

- Understanding the mechanism of how stem/progenitor cells continue to maintain residency, respond to insult, and regenerate during adult life and in different disease states.

- Understanding cell migration and homing of stem cells/progenitors to their environment.

- Understanding the plasticity of hematopoietic, neural, mesenchymal, and other tissue-specific stem cells.

- Designing strategies for the propagation and differentiation of embryonic stem cells or multipotential progenitor cells to specific cell types *in vitro*.

- Developing quantitative clonogenic assays that are reliable and convenient for characterizing stem/progenitor cells and determining if the cells are capable of giving rise to functional progeny.

- Studying stem cell tumorogenecity after *in vitro* culture and expansion.

- Determining the function, reliability, long-term survival, and safety of isolated cells upon reintroduction into the host.

The severity of the limitations of conventional organ and cell transplantation cannot be overstated, i.e. supply and demand, recurrent disease, rejection, long-term immunosuppression, etc. The recent flurry of research events in stem cell biology is exciting and could have a significant impact on future disease treatments. Diseases such as diabetes, cardiac failure, strokes, CNS degeneration and hematopoietic diseases could be amenable to restorative therapies that validate the therapeutic effectiveness of these novel modalities.

Goal. To produce *in vitro* or enable *in vivo* immune disguised cells that can differentiate into diverse targets, maintain a stable phenotype, and restore *in vivo* function.

**Recommendations.**

- Support is needed for a broad range of studies in basic cell and developmental biology of stem cells. This is particularly needed to rigorously characterize the properties of all types of candidate stem cells with respect to the following criteria:

    1. Estimated *in vitro* and *in vivo* proliferative capacity;
    2. Determination of precise molecular pathways that control *in vitro* and/or *in vivo* differentiation and
    3. Determination of capacity for clonal growth (to distinguish between *bona fide* pluripotent stem cells and mixtures of specialized progenitors of terminally-differentiated cells).

- Identify and support enabling technologies from diverse scientific disciplines that promote and advance the goal of regenerative medicine.

- Identify and support innovative and enabling technologies that investigate and promote immune compatibility of reparative cells and the host.

- Identify and support research aimed at deriving diverse sources of donor cells that can ultimately yield a desired phenotype and provide a functional cellular replacement in diverse targets.

- Support research that determines the parameters that allow an indefinite supply of cells but also result in desired vivo phenotypes and function.

- Support research that utilizes molecular and biochemical approaches to understand the host environmental factors that:

    1. Aid exogenous supplied cells or endogenous cells to home to the correct site and
    2. Aid long term survival and function of exogenous supplied or introduced cells.

- Recommend the rigorous testing of novel cells for repair in spontaneous small and large animal models of disease to understand the host disease (diabetes, neurological, orthopedic, etc) as well as to understand *in vivo* regeneration.

**Implementation.** Animal models will be essential in understanding and optimizing the host environment in order to promote the regeneration of internal organs, and promote survival and correct targeting of introduced cells. Increased funding for these animal models is needed (including non-human primates, domestic species, laboratory animals) so that the function, safety and efficacy of cell culture-derived grafts can be tested *in vivo*.

Increased funding is needed for equipment for investigators to support non-destructive imaging (e.g., 2-photon confocal microscopy), real-time PCR, array facilities for the characterization of source and end products of differentiation pathways.

Funding is needed for development of public (community) databases, reagents such as cDNAs, antibodies, and cell lines, and other resource repositories (e.g., ongoing or increased funding for facilities such as ATCC, Developmental Biology monoclonal resource).

Support is needed to increase interdisciplinary interactions through on-site NIH conferences, mini-sabbatical visits (including short time research support) to enable investigators to visit other laboratories to expand their research expertise in the area of stem cells and regenerative medicine.

## MOLECULAR SIGNALING

**Moderators**
*Caroline Damsky, Ph.D., University of California – San Francisco*
*Mohammad Heidaran, Ph.D., BD Technologies, Research Triangle Park, NC*

**Panelists**
*Donald Bottaro, Ph.D., EntreMed, Inc.*
*Kathryn Crossin, Ph.D., Scripps Research Institute*
*Patricia Ducy, Ph.D., Baylor College of Medicine*
*Donald Ingber, M.D.,Ph.D., Harvard Medical School/Children's Hospital*

**Broad Statement.** Tissues and organs consist of specialized living cells arrayed within a complex structural and functional framework generally known as the extra-cellular matrix (ECM). ECM composition is an important factor that contributes to the function and characteristics of each organ and tissue such as the rigidity and tensile strength of bone, the resilience of cartilage, the flexibility and hydrostatic strength of blood vessels, and the elasticity of skin. Also important is the role of the ECM during growth, development, and wound repair where it provides a reservoir for soluble signaling molecules and, through its own dynamic composition, a source of additional signals to migrating, proliferating, and differentiating cells.

Artificial substitutes for the ECM, called scaffolds, consisting of natural and/or synthetic polymers have been used successfully alone or in combination with cells and soluble factors to induce tissue formation and promote tissue repair. Cells are also central to many tissue engineering strategies, and significant efforts have been made to identify and propagate pluripotent stem cells, to identify signaling events important for proper differentiation, and to identify ideal micro-environments for maximum cellular function. These efforts have led to a convergence of research in bioengineering, biomaterials, ECM, cell growth and differentiation, and soluble factors that control cell fate.

Recent developments in the multi-disciplinary field of tissue engineering have provided a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered ECM (scaffolds), cells, and biologically-active molecules.

**Vision.** The goals of reparative medicine are to enhance normal tissue regeneration and to engineer artificial tissues for use as replacements for damaged body parts. While significant advances have been made in the development of prosthetic devices that can repair structural defects (e.g., vascular grafts) and even replace complex mechanical behaviors (e.g., artificial joints), the challenge for the future is to develop therapies and devices that restore the normal biochemical functions of living tissues in addition to their structural features. To accomplish this objective, precise design criteria must be established to guide developmental efforts. These criteria must be based on a thorough understanding of the molecular signaling networks, cellular interactions, and biophysical aspects of tissue

formation. Such understanding will accelerate our abilities to promote optimal tissue reconstruction and repair.

**Objectives.** To facilitate this vision, near-future NIH investment in molecular signaling should support research in areas associated with growth factors (i.e., hormones, cytokines, and other factors that regulate cellular transduction and control cell behaviors), mechanical and stress-induced signaling, ECM and scaffolds (including cell-to-matrix interactions), cell adhesion receptors and molecules, and temporal (aging) effects. NIH investment should also encourage and support multi-disciplinary and multi-organizational approaches to addressing these research needs as well as the need for student education and training programs.

**Challenges.** Among the major challenges for tissue engineering are the needs for complex functionality and biochemical stability in laboratory-grown tissues destined for transplantation. Realization of the potential benefits offered by tissue engineering in the development of true human replacement parts will require convergence of molecular signaling principles with research advances in tissue, matrix, growth factor, stem cell, and developmental biology. Specific challenges associated with developing a thorough understanding of molecular signaling and tissue regeneration include:

- **Understanding how to manipulate signaling through cell adhesion receptors and molecules to promote the desired endpoints for specific tissue engineering problems.** Cell-cell and cell-ECM adhesion receptors and molecules play critical roles in both anchorage and signal transduction. A key feature of receptor function is their ability to organize signaling complexes at sites of contact with their extra-cellular environment. Depending on other aspects of the environment including the nature and organization of the ECM, the presence of growth and differentiation factors, and the presence of mechanical stimuli, these signals can promote or restrain cell proliferation, promote differentiation, trigger matrix remodeling, or promote enhanced tissue organization. The ability to manipulate these variables to control the balance between proliferation and maintenance of the differentiated state for particular cell types is essential for designing effective cellular replacement therapies.

- **Establishing continuous molecular bridges between cell physiology, signal transduction, and gene expression.** For a cell to respond to its environment, divide, migrate, or differentiate, signals from the extra-cellular compartment need to be sensed, reach the nucleus, and then trigger expression or repression of specific factors. Understanding of this information transmission process is currently incomplete. For example, while it is common to know which ligand/receptor interaction will activate a specific kinase pathway, specific insights into the transcriptional events and target genes that will eventually be involved are not known. Also, although numerous nuclear factors are known to control expression of specific genes (e.g., cell differentiation programs), very few signaling cascades have been defined as controlling their activity. Filling these gaps by establishing

continuous molecular bridges is key to understanding cell transduction and differentiation.

- **Understanding the impact and mechanisms of growth factor signaling in complex systems where multiple biochemical and physical stimuli modify intracellular signaling and biological responses.** Although much insight has been gained from studies of growth factor signaling using simple, well-characterized cultured cell models, studies involving more complex systems are necessary. The acquisition of high-resolution, three-dimensional extra-cellular matrix component/protein complex structures and the discovery of specificity among ECM components that impart high-affinity protein binding are currently at the forefront of research in this area. Information resulting from related research will facilitate the development of complex artificial scaffolds for tissue regeneration, repair , and replacement.

- **Translating knowledge of molecular events and molecules known to be important during embryonic development to the understanding of adult physiology and physiopathology.** It is unlikely that these genetic programs involve totally different sets of regulatory genes. In fact, a small but growing number of genes have been shown to fulfill roles after as well as before birth. A systematic analysis of expression patterns and functions of developmental "master genes" in post-natal and aging models could provide useful information to define novel pathways and strategies for therapeutic intervention and regenerative repair.

- **Understanding how cells sense mechanical forces and integrate them with signals from other tissue control elements (e.g., growth factors and ECM).** Cells in tissues constantly experience mechanical stimuli. Even cells in static culture experience the effects of gravity. Stimuli such as shear-stress, fluid-flow, compression, stretch, etc. not only alter the organization and distribution of structural elements and organelles within cells, but also become transduced into biochemical input that modulates intra- and inter-cellular signaling networks and in turn, gene expression. Understanding the importance of mechanical stresses and micro-architecture in cell signaling is important for the design of medical devices (i.e., for promoting wound healing) as well as the engineering and manufacture of artificial replacement tissue.

- **Fundamental research on cell response to 3-dimensional, non-rigid extra-cellular matrices.** Most work with cells *in vitro* has involved culture on 2-dimensional rigid substrates. For many cell types, this is not a biologically-relevant environment. Studies with 3-dimensional matrices have shown that cells behave very differently under such conditions. Mechanisms by which signals from the ECM, neighboring cells, and growth/differentiation factors synergize to regulate cell growth and survival, affect commitment to tissue-specific differentiation programs, and regulate tissue remodeling need to be evaluated at the molecular level with regard to the more relevant 3-dimensional microenvironments. Increased knowledge of the

impact of 3-dimensional microenvironments at the molecular level will expedite and improve tissue engineering strategies and biomaterials design.

**Recommendations.** Recommendations for near-future NIH investment and programs to meet the objectives and challenges outlined in the preceding text include:

- Actively encourage and support multi-disciplinary and multi-organizational approaches to molecular signaling research that require such collaborations for the proposed effort.

- Include the following specific research areas in program announcements relating to bioengineering or biomaterials:

  - Cell adhesion receptors and molecules.
  - Continuous molecular bridges between cell physiology, signal transduction, and gene expression.
  - Growth factor signaling in complex systems.
  - Translation of molecular events from embryonic to adult physiology.
  - Effects of mechanical forces on cell response.
  - Cell response in 3-dimensional, non-rigid extra-cellular matrix environments.

- Support research on the development of modeling, simulation, and statistical theories to describe chemical and molecular behavior to parallel empirical observations. Modeling efforts should focus on the complex, dynamic network behaviors in signal transduction and biochemical/genetic regulatory pathways.

- Encourage and support the development and application of enabling technologies for tissue engineering research.

- Encourage and support education and training programs that incorporate the molecular signaling “challenges” described in the preceding text into bio- and tissue engineering research endeavors. These programs should include support for graduate-level training and the advanced education of research professionals through organized meetings, conferences, and symposia.

## VASCULAR ASSEMBLY IN ENGINEERED AND NATURAL TISSUES

**Moderators**
*Thomas C. Skalak, Ph.D., University of Virginia,*
*Charles D. Little, Ph.D., University of Kansas*

**Panelists**
*Larry V. McIntire, Ph.D., Rice University*
*Karen K. Hirschi, Ph.D., Baylor College of Medicine*
*Robert T. Tranquillo, Ph.D., University of Minnesota*
*Mark Post, M.D., Ph.D., Dartmouth Medical School*
*John Ranieri, Ph.D., Sulzer Biologics*

**Broad Statement.** A convergence of problems in reparative medicine today requires renewed attention to improving our basic understanding, developing predictive modeling techniques, and creating rational therapeutic methods of guiding vascular assembly in diseased natural tissues as well as in engineered tissues. Pioneers and leaders in this field now recognize that a complex spatial and temporal interplay of multiple molecular and environmental signals determines vascular assembly, and that many hurdles may be overcome though integrative, quantitative studies of molecular genetic determinants, cell behaviors *in vivo*, and functional cell aggregates. Several key processes and objectives drive both current investigation and future goals:

- Analysis of natural vascular assembly processes, including gene expression, spatial and temporal growth factor actions, stem cell lineages, and extracellular matrix-cell interactions in development and in adults.
- Analysis and harnessing of adaptive/remodeling processes in vascular systems.
- Quantitative experimental analysis and predictive computer modeling of vascular system assembly and maintenance.
- Design of synthetic constructs that guide/optimize vascular assembly.
- Physical linkage of micro- and macro-scale vascular systems.

**Vision.** Rational design of vascular systems assembly will take a prominent place in the practice of both preventative and restorative medicine. It is already clear that a complex spatial and temporal interplay of signals, including both genetic and environmental cues, are needed for normal small vessel growth, remodeling, and maintenance. Thus, the next phase of research and development in this field will almost certainly require innovative new ways to study and design multicomponent, multisignal vascular systems, both in natural and engineered tissues. An emerging theme is that pre-determination of final vascular or tissue structure in the microfabrication stage of engineered tissues is unlikely to succeed alone, but rather the harnessing of the natural, adaptive powers of the resident cells to guide functional vascular assembly and maintenance will provide the ability to supply large, complex tissues.

Biomedical engineers, vascular biologists, and developmental biologists are ideally suited to form teams to help address the behavior of these complex systems. The long list of

components typically addressed today via the reductionist approach to studying therapeutic vascular assembly may be expressed either as a part of normal vascular development (vasculogenesis), later during vessel remodeling to a differentiated functional network with arterioles, capillaries, and venules, or during adult maintenance of a functional network. The implication of these observations is that several different design paths for engineering of de novo vascular networks in engineered constructs or remodeled networks *in vivo* can be envisioned.

**Objectives.**

- Advance fundamental understanding of vascular assembly: this includes stem cell biology, cell behaviors in development of functional networks, network growth and maintenance by environmental cues, artery wall remodeling, and venous/lymphatic assembly**.**

- Construct quantitative, predictive models of vascular assembly.

- Fabricate engineered constructs (large and small blood vessels and networks) as well as *in vivo* remodeling processes.

**Obstacles.**

- Network development involves a complex temporal and spatial interplay of cellular, chemical, and mechanical stimuli. The tissue microenvironment affects local remodeling events.

- Imaging and measurement tools for monitoring of vessel development, including gene expression, protein localization, chronic functional and cell phenotypic study, and mechanical forces *in vivo* are poorly developed or lack the needed spatial resolution, with the exception of optical methods used in the embryo or thin chambers.

- Persistence of functional networks is little studied at present.

- Control of stem cell differentiation is not quantitatively understood.

- Self-assembly of tissue-engineered vessels must be scaled and modified to offer reproducible, economical production/storage/distribution capabilities.

- Identification of embryonic processes that can be recapitulated in the adult or in synthetic vascular constructs.

- Diffusive constraints limit cell seeding densities in large constructs, necessitating novel approaches to vascularization.

**Challenges.**

- Understand the genetic circuits (i.e. genes and interplay of gene products) controlling vascular assembly and incorporate them into optimized designs and models.

- Move from a “single important molecule” approach to a multisignal, multicomponent approach.

- Develop modeling and simulation methods to predict cell motions, associations, and differentiation state based on the secretion, diffusion, binding, and functional effects of a complex interplay of molecular signals in a field of interacting discrete cells that include endothelial, smooth muscle, and fibroblast precursors.

- Develop methods for delivery of cell-based therapies and multiple growth factors via microcarriers in a prescribed temporal and spatial sequence to perform guided arterialization in areas demanding blood supply.

- Assembly of arteries, veins, and lymphatics by tissue engineering processes.

- Design methods for guiding the inosculation process (connection of engineered constructs to host vessels) - a micro to macro interfacing problem.

- Understand genetic regulation of on/off switches for various steps in vascular assembly and maintenance.

- Identify gene families that control vascular assembly in development and adult.

- Understand the biology of mesodermal precursor cells.

- Develop site- and cell-specific sampling methods and DNA microarray analysis to identify new candidates and gene circuitry of existing candidates.

- Develop micro- and nano- fabrication methods allowing for creation of spatial gradients in ECM, signal molecule, and cell structures, and for high-spatial resolution actuation and sensing of mechanical stimuli.

- Increase the respect in academia for integrative, interdisciplinary, collaborative scholarship that seeks to build new functional understanding from existing knowledge bases.

**Recommendations.**
Broad recommendations:

- Fund larger number of research grants addressing the behavior of cellular assemblies (vascular and pan-NIH).

- Create a NIH study section focusing on fundamental understanding and application of non-disease-based vascular biology and system function.

- Promote the parallel development of and linkage between reductionist, discovery-oriented work with integrative systems modeling, *in vivo* measurements of vascular assembly, and function and design-oriented studies (perhaps joint between NHLBI and NIBIB).

- Fund increased numbers of individual and institutional training grants at all levels, which focus on vascular assembly/remodeling *in vitro* and *in vivo.*

- Establish national resource programs to screen vascular mutants resulting from large scale mutagenesis projects to identify new genes involved in vascular development.

- Train young researchers/educators in the culture of interdisciplinary studies of complex living systems (pan-NIH).

Detailed recommendations:

- Support development of new computer modeling methods capable of multicomponent, multisignal simulation of vascular pattern formation.

- Support development of site and cell-specific sampling methods, DNA microanalysis, and proteomics characterization of new candidates and to define functional circuitry of existing candidates.

- Support development of experimental tools for spatial and temporal control of growth factor delivery/stimulation and cell-based therapies *in vivo.*

- Support development of fabrication methods for vascular tissue engineering (creation of spatial gradients in extracellular matrix, signals, cell structures).

- Support long-term monitoring methods for small vessel assembly in intact tissues and engineered constructs.

- Support integration of discovery work with *in vivo* vascular systems analysis.

## Functional Assessment of Engineered Tissues and Elements of Tissue Design

*Moderators:* *Farshid Guilak, Ph.D., Duke University Medical Center*
*Ravi Kapur, Ph.D., Cellomics, Inc.*

*Panelists:* *Michael V. Sefton, Ph.D., University of Toronto*
*Herman H. Vandenburgh, Ph.D., Brown University School of Medicine*
*Alan P. Koretsky, Ph.D., National Institutes of Health*
*Andres Kriete, Ph.D., Tissue Informatics, Inc.*
*Regis O'Keefe, M.D., University of Rochester*

**Broad Statement.** Tissue engineering is a rapidly growing field that seeks to repair or replace tissues and organs by delivering combinations of cells, biomaterials, and/or biologically active molecules. Tissue engineering merges several aspects of engineering, biology, and medicine, and many rapid achievements in this field have arisen from significant advances in the integration of these fields. Despite early successes, however, few functional tissue engineered products are currently available for clinical use. The development and application of rational design criteria and technologies for the assessment of tissue function would be expected to improve the success of engineered products.

**Vision.** Tissue engineering has the potential to dramatically alter the treatment of numerous diseases by enabling the repair of injured or diseased tissue with living replacements. The overriding vision of this field is to improve the speed, extent, and duration of tissue repair over currently available methods. Most tissue-engineered products must serve multiple, complex functions, including inter-related metabolic and structural (i.e., biomechanical) demands. Many challenges still remain in the ability to restore the native function of various organs and tissues. In the next 5-10 years, advances in two important and related areas are presented as mechanisms to improve the outcome of engineered tissue replacements: 1) the assessment of function in engineered tissues, and 2) the application of rational design principles. Incorporation of these approaches must span multiple hierarchical scales, from the macroscopic level, directed at satisfying the clinical requirements of the product, to the microscopic level, directed at satisfying the cell and molecular requirements for long-term functional success.

**Challenges and Objectives.** The ultimate goal of tissue engineering is to restore the function of injured or diseased tissues. However, many challenges remain, particularly with respect to functional assessment and the design of tissue replacements. Many of the complex structural and metabolic functions of tissues and organs are not fully understood, even for native tissues. In this respect, what constitutes "success" needs to be defined *a priori* and will be expected to differ among tissues. For example, tissues or systems that are designed to prolong life may tolerate a lower margin for error than those that are designed to improve the quality of life. The difficulty in performing a surgical procedure, and the duration that a specific treatment lasts will influence the cost-effectiveness of a procedure and therefore may also factor into its perceived success. For example, therapies of replacement or regeneration of blood vessels or cardiac muscle might be expected to last

the lifetime of the individual, while replacement of cartilage may be considered successful if it delays total joint replacement for five to ten years. The following objectives establish an initial set of directives that are hoped to significantly influence the quality of the implants that tissue engineers design and the success of repair procedures after surgery.

- **Defining standards of success for tissue repair**. A critical step in achieving success in tissue-engineering and reparative medicine will be the development of appropriate standards of clinical success and the completion of prospective outcomes studies to compare the safety, efficacy, and cost-effectiveness of different procedures.

- **Understanding the function and properties of native tissues**. A thorough understanding of the interactions between the metabolic and physical (e.g., biomechanical, physicochemical, electrophysiological) properties of native tissues will facilitate the design and engineering of repair tissues that provide the appropriate functional properties.

- **Prioritization of specific functional properties as design parameters**. The relative importance of the many different properties and functions of native tissues in the design of an engineered repair tissue is not known. Given the difficulties in providing all of these functions in an engineered construct, a key issue in the success of engineered repairs will be the prioritization of different properties as design parameters. In tissues that serve a predominantly biomechanical role, emphasis should be placed on the material properties that are necessary for appropriate function. Where appropriate, these parameters must be considered in light of other critical issues such as the interaction of multiple cell types, vascularization and nutrient supply, thrombogenicity, immune and inflammatory responses, and manufacturing, storage and quality control.

- **Quantitative measurement of the *in vivo* environment in native and repair tissues**. In attempting to define design parameters for the restoration of function of repair tissues, knowledge of the biochemical and physical history that normal and repair tissues may encounter for different *in vivo* activities will provide important insight by establishing the limits of expected usage. Inherent in this process will be the further development of minimally-invasive methods for the long-term assessment of cell and tissue function *in vitro* and *in vivo*. In particular, radiological imaging tools such as MRI, ultrasound, CT, PET, and SPECT should be developed to asses engineered tissues. In addition, optical techniques, surrogate biological markers, biosensors, novel biomechanical testing devices) and other techniques such as genomics, proteomics, and cell-based techniques will provide means of profiling the activity of cells, tissue, and organs prior to and following implantation.

- **Development of computer-based tools that support functional tissue engineering and tissue design.** The modeling and functional simulation of organs has to respect the inherent hierarchies of biological structures, their properties and interdependencies from cellular organelles to macroscopical, anatomical structures. With recent progress in 3-D image acquisition, processing, visualization, and computer modeling a complete representation of cells, tissues and organs is now an attainable goal. In addition to

"structural" models of cells and tissues, a number of different approaches have been developed to model cell physiology, such as the "Virtual Cell" or the "E-cell" program. The ability to predict and validate functional models that span the hierarchy of the molecule to the organ will greatly improve the process of designing engineered tissues.

- **Investigation of the effects of mechanical and other biophysical factors on tissue repair *in vivo***. Once implanted, tissue-engineered constructs will be subjected to significant loads and deformations *in vivo*. Both the mechanical and biological consequences of *in vivo* loading must be understood to improve the success of engineered repairs and to develop appropriate rehabilitation protocols for regenerative medicine.

- **The use of biophysical factors to enhance tissue regeneration *in vitro***. Biophysical factors play an important role in the modulation of cell physiology, and there is significant evidence that physical factors may be used to improve or accelerate tissue regeneration and repair *in vitro*. A more thorough understanding of cell and tissue response to mechanical stress and other physical factors within native and artificial extracellular matrices would improve the ability form functional tissue replacements *in vitro*.

Realization of this vision for tissue engineering will hopefully lead to the establishment of both functional criteria as well as design parameters for tissue regeneration. Other rapidly evolving new technologies may have a significant impact on functional tissue engineering, and it is important to consider these principles of functional tissue engineering in light of the role of novel growth factors, new biomaterials, gene therapy, and other changing technologies. Incorporating such principles in the design of functional tissue replacements, from the macroscopic to the molecular scale, will hopefully result in safer and more efficacious tissue repairs and replacements.

**Scientific Recommendations.**

- A primary recommendation in this field is the development of fundamental principles and standards that cross multiple disciplines. In this respect, researchers with different expertise and backgrounds will be more likely to seek common goals in the development of engineered tissues.

- As it is unlikely for an engineered replacement to meet all of the functional demands of native tissue or organ, it is critical to prioritize the needs and to determine the relationship between specific functional properties and the overall clinical success of product. The definitions of "function" should be broadened to include biomechanical, electrophysiological, and metabolic properties, where appropriate.

- Trial-and-error approaches have led to rapid advances in the field of tissue engineering, but are inherently limited in many cases. A balance of "rational" design approaches with trial and error methods is recommended. Such approaches will require further basic science studies in tissue engineering systems in addition to translational research.

- Interdisciplinary collaborations are the foundation of tissue engineering and will be necessary for significant advances in the field. Such approaches must be fostered from the student level to that of research partnerships through increased interaction and communication among disciplines.

**Recommendations to the NIH.** Numerous mechanisms currently exist for the funding of research and training in the general areas of functional assessment and tissue design in tissue engineering. Several specific areas are suggested as potential topics for funding initiatives in the general area of tissue engineering:

- Minimally-invasive imaging in tissue engineering.

- High-throughput cellular activity profiling and molecular characterization.

- Modeling of biological systems in reparative medicine.

- The influence of biophysical factors on engineered tissues.

- Controlled, prospective, and randomized outcomes studies in reparative medicine

## Genetic Approaches to Tissue Engineering

**Moderators**
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*Savio L.C. Woo, Ph.D., Mount Sinai School of Medicine*

**Panelists**
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*Steven C. Ghivizzani, Ph.D., Harvard Medical School*
*David Schaffer, Ph.D., University of California, Berkeley*

**Broad Statement.** Genetic approaches for tissue repair and regeneration lie at the interface of two exciting fields, gene therapy and tissue engineering. Typically, the therapeutic goal is to induce new tissue formation in an injured site or to inhibit an exaggerated tissue-repair response. Researchers involved in tissue engineering strive to repair lost tissue function through transplantation of living cells grown on bioresorbable scaffolds. Exciting advances have been made in the regeneration of bone, skin, and blood vessel, yet significant challenges remain. With the advent of modern gene transfer technologies, damaged cells and tissues can be repaired through somatic gene delivery and expression. Gene therapy is the use of genes as medicines for the purpose of preventing, ameliorating, or curing disease.

**Vision.** Genes may be transferred to cells cultured on bioresorbable scaffolds in order to:

- Improve the manufacture of tissue-engineered constructs.
- Promote the biocompatibility of these constructs once implanted into patients.
- Deliver therapeutic genes to surrounding tissues.

It is anticipated that gene transfer will augment current tissue engineering technology, resulting in improved treatment outcomes for patients with diseases of the cardiovascular, renal, endocrine, musculoskeletal, nervous and other organ systems.

**Objectives.** The modern medical marketplace will favor the development of cost-effective gene delivery technology that will be:

- Easily managed by physician and patient.
- Simple, safe, stable, and relatively inexpensive to manufacture.
- Capable of producing therapeutic protein in a controllable manner.

**Obstacles and Challenges.** The field must have safe, effective, and acceptable gene delivery technology at its disposal. Toward this end, the scientific community needs to:

- Develop efficient and safe gene transfer vectors, and rigorously investigate the basic mechanisms of gene transfer.

- Establish and quantify the control parameters (e.g. biodistribution, pharmacokinetics and pharmacodynamics) of gene delivery.

- Develop targeting vectors capable of tissues-specific gene transfer and expression.

- Develop regulated gene expression as a way to achieve desired therapeutic outcome while minimizing or avoiding potential toxicities. A system conferring regulated gene expression should feature low baseline transgene expression, a high induction ratio, and tight control by a small molecule drug.

- Develop vector systems for genetic modification of stem/progenitor cells, and rigorously investigate the functional consequences of vector transduction into these cells.

- Develop vectors that do not induce innate/adaptive immune responses and those that induce local immune tolerance to the grafts.

- Establish multi-disciplinary approaches toward clinical translational research, so that novel therapeutics in tissue engineering can be validated in the clinical trials while maximally protecting patient safety.

- Carefully and meticulously establish database and tissue repository in support of future clinical translational studies.

- Engage the public in earnest about the real promises and challenges in tissue engineering and regenerative medicine. Integrity and open disclosure of scientific and clinical translational activities will be required to gain the public trust in gene-delivery technology.

**Recommendations.**

- Most scientific discoveries have been, and will continue to be, made by investigators conducting independent research funded through the mechanisms such as the R01. However, due to the complexity and diversity of disciplines that are needed to expedite progress in this high impact area, the traditional R01 mechanism will need to be complimented by programmatic strategies such as program project and center of excellence grants, as well as those in establishing databases/repositories.

- Due to the fact that tissue engineering is a new field in biomedicine, it will need to be nurtured by encouraging exploratory project grant applications through the R21 mechanism. These are applications for projects that explore and develop scientifically sound ideas without substantial preliminary results.

- Support for scientific conferences and workshops, particularly those of multi-disciplinary nature, and comprehensive clinical gene-transfer training courses.

- Support of cross-training/retraining of investigators in order to enhance the talent pool. Attention to education of teachers, students, patients and lay public including the press about possibilities afforded by, and limitations of, gene transfer/tissue engineering strategies.

- Last and not the least, there should be a multi-disciplinary Study Section on Tissue Repair and Regenerative Medicine to facilitate the evaluation of grant applications in this novel area of medicine. In addition to the traditionally hypothesis-driven grants, the NIH needs to also support technology-driven and engineering/design-driven as well as application-oriented grants.

## IMMUNE RESPONSE TO ENGINEERED TISSUES AND CELLS

**Moderators**
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*David H. Munn, M.D., Medical College of Georgia*
*Richard M. Ransohoff, M.D., Cleveland Clinic*

**Broad Statement.** Remarkable progress has been in the generation of cells and tissues for organ repair and replacement. However, further advances in the engineering of biological materials will face the difficulty of immune acceptance of the altered materials. In the case of allografts and xenografts, rejection through several mechanisms including both innate and adaptive immunity, represents a formidable barrier. Inflammation is likewise important in preventing successful engraftment, but may also be pivotal for proper tissue remodeling. In light of the critical need to understand the role of immunity in successful tissue implantation, the goal of this panel was to identify how immune responses are initiated, to review newer approaches to prevent rejection, to identify the role of inflammation in tissue remodeling, and to compare the results obtained in different model systems. In addition, the panel considered how the NIH could foster increased interdisciplinary research in the vital area of immunity to tissue engineered tissues and organs.

**Vision.** Understanding the interplay between implanted tissues/materials and the host response to these implants represents a growing concern in the field of biomedical engineering. Further advances will only occur with increased focus on this important issue.

**Objectives.** In considering advancing the design, delivery, and function of bioengineered tissues, the host response to these tissues needs to be fully considered: Specifically, we need to understand:

- Inflammatory cell signaling and trafficking.
- The pivotal role of antigen presenting cell subsets.
- Whether Th1 versus Th2 activation is important for implantation success.
- Specialized immune mechanisms at various anatomical sites, *e.g.*, nervous system, mucosal sites.
- The potential need for inflammatory cytokines for successful transplantation.
- The role of the galactose epitope in xenotransplantation.
- Tolerance and initiation of the immune response to danger signals.

**Obstacles and Challenges**. As bioengineers increase their efforts to fabricate artificial tissues and organs in attempts to improve natural polymers, the response of the immune system will almost certainly thwart progress. Unfortunately, there often appears to be lack of understanding of the likely immune consequences of tissue/organ manipulations and of

possible approaches that might be exploited for overcoming these consequences. Conversely, there seems to be little appreciation among the immunological community for the challenges facing the tissue engineering field. The field of tissue engineering is characterized by a lack of communication between tissue engineering and immunology scientific communities and by a lLack of a multidisciplinary approach.

**Recommendations.** Increased efforts must be made to integrate the fields of immunology and tissue engineering. There is currently a gaping hole in the opportunities available for examining immunological responses related to bioengineered materials. To overcome this:

- NIH should develop funding initiatives (RFAs) to form partnerships between tissue engineering community and immunologists to apply innovative immunological concepts and approaches.
- NIH should develop RFAs to support multidisciplinary approaches to the field of tissue engineering.
- NIH should promote education/training through conferences to bring together scientists in tisue engineering and immunology to address issues of common interest.
- Through collaborative efforts, scientists should develop strategies for *in situ* tissue regeneration
- Through collaborative efforts, scientists should incorporate strategies to allow acceptance by the immune system and, when appropriate, rejection by the immune system.
- NIH should promote an enhanced awareness among bioengineers of immunological issues that face attempts to optimize tissue function.
- Investigators should emphasize use of natural components rather than artificially created or altered biomaterials.

## *In vivo* Remodeling

**Moderators**
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*Markus Grompe, M.D., Oregon Health and Science University*

**Panelists**
*Arnold Caplan, Ph.D., Case Western Reserve University*
*Howard Greisler,M.D., Loyola University Medical Center*
*Robert Guldberg,Ph.D., Georgia Institute of Technology*
*Doris Taylor, Ph.D., Duke University Medical Center*

**Vision.** The implantation of tissue engineered devices or cells into living hosts is accompanied by unavoidable remodeling of both the implant and the host tissue. Tissue remodeling is a dynamic process that is related to normal developmental biology and is present from the earliest moments of fetal development. In the adult, this process exists as part of the tissue repair response and can result in several possible outcomes including tissue regeneration, scar tissue formation, or complete tissue loss. An in-depth understanding of the signals and mechanisms that control this process at the molecular level is necessary to maximize the constructive outcomes and therapeutic applications that can potentially result from *in vivo* remodeling.

The patterns of *in vivo* remodeling differ among species, within species and even as a function of age or body location within the same individual. The remodeling events are fundamentally protective responses against tissue injury or tissue exposure to harmful substances; thus are attempts to assure survival of both the individual and the species. Efforts to understand the molecular signals that control the remodeling process will have multiple positive payoffs including an improved understanding of developmental biology, inflammation and immunity, scar tissue formation, tissue and organ regeneration, and wound healing.

***Goals of In vivo Remodeling Research and Application.*** *In vivo* remodeling is the inevitable fate of all efforts at tissue engineering and reparative medicine. Regardless of whether the therapeutic approach is gene or cell based, scaffold based, or based upon the use of selected growth factors or a combination approach, it will be subjected to the biologic variability that is part of *in vivo* remodeling. All events that precede the *in vivo* step including the selection of specific cell types, the in-vitro cell or tissue bioreactor conditions, and the utilization of desired scaffold materials can generally be tightly controlled. However, the *in vivo* remodeling that occurs after application to the mammalian system has much less predictable results based upon our current knowledge base and current methodologies. Goals of research in this area should include:

1. **To characterize and understand at the molecular level the differences between wound healing events that occur at different ages; especially the differences between fetal vs. adult wound healing.**

Rationale: The genetic makeup of an individual is constant throughout life, yet tissue response to injury can differ markedly between the fetus and the adult. Fetal wounds can heal with complete regeneration of tissues and organs whereas adults tend to heal by scar tissue formation or, in selected instances, wound healing can fail completely. What are the specific differences in this wound healing response? What molecular signals control these differences based upon age of the individual? Can these signals be modified by therapeutic intervention? Without a thorough understanding of these issues, it is likely that only incremental advances will be made in this field.

2. **To understand at the molecular level the differences between wound healing in normal and diseased individuals.**

   Rationale: Reparative medicine and tissue engineering efforts are targeted at the repair and restoration of damaged or missing tissues and organs. By definition, therefore, these efforts will be targeted at otherwise healthy individuals with congenital deformities, healthy individuals with traumatic injuries, and diseased individuals with degenerative, inflammatory, or neoplastic conditions. It is important to understand the differences that may exist in the *in vivo* remodeling events for these different conditions. Even if the therapeutic methods that are considered optimal are utilized for all of these different states, it is unlikely that the short and long term expectations for success will be the same.

3. **To understand at the molecular level the events that control and regulate the formation of scar tissue; especially in the nervous system.**

   Rationale: Scar tissue formation is a common result of *in vivo* remodeling following tissue injury in the adult. Scar tissue is the bane of surgeons and is associated with loss of tissue or organ function, undesirable adhesions, and less than optimal cosmetic results. Scar tissue is considered to be one of the major barriers to regeneration of central and peripheral nerve tissue. The formation of scar tissue has significantly more detrimental sequelae in the central nervous system than in other body systems such as the integumentary system or musculoskeletal system. An understanding of the controlling signals for scar tissue formation is essential before therapeutic methods can be developed to minimize or eliminate it.

**Obstacles to Understanding *in vivo* Remodeling.** Several significant obstacles exist to a more thorough understanding of the events that regulate *in vivo* remodeling in mammalian systems. Identification and enumeration of these obstacles will permit the development of a rationale approach to overcoming these limitations and provide for advancement in the field. These obstacles provide targets for future resource deployment . Although the following list is by no means comprehensive, it represents the panel's collective opinion as to most dominant current rate limiting issues:

1. **Methods to quantitatively and qualitatively study gene expression in host and donor cells at the single cell level are poorly developed.**
   Until such methods can be developed, it will be very difficult to make significant progress in our understanding of normal developmental biology events, the mammalian response to tissue injury, transplanted cells or foreign materials (e.g., scaffolds), or the regulators of cellular proliferation, hypertrophy, differentiation or spatial three dimensional organization.

2. **Inter-individual biologic variability in higher mammals makes controlled study of the events that regulate *in vivo* remodeling very difficult.**

   The reasons for the differences in *in vivo* remodeling events following identical types of tissue injury in separate individuals is very poorly understood. Are these differences in the rate of remodeling or the extent of remodeling or the effects of nutrition or age inevitable? Can these differences be controlled? The presence of this biologic variability makes controlled study design difficult and expensive. This variability also raises significant questions with regard to choice of animal models for particular types of tissue injury.

3. **There is a paucity of methods for tracking the fate of implanted tissue engineered genes, cells, scaffolds and composite devices in real time.**
   The ability to monitor in real time the fate of cells or scaffolds, gene expression patterns, and the degradation of implanted tissue engineered devices is essential to the iterative research process and thus, progress in the field.

**Recommendations of Panel for Deployment of NIH Resources in the Area of *In vivo* Remodeling**

1. **A concerted multidisciplinary effort to identify and understand the molecular events that control wound healing, especially the differences in wound healing between the fetus and the adult, is imperative.**

   Such an effort will likely require the combined and coordinated efforts of molecular biologists, developmental biologists, and tissue engineers. Such an effort should be inclusive of individuals not only with specialized expertise in selected basic science fields, but also those individuals with knowledge of the translational efforts necessary to quickly mobilize this knowledge to the tissue engineering laboratory and the bedside.

2. **Support of studies to understand, at the molecular level, those controlling events that result in constructive remodeling of tissues vs. those that result in destructive remodeling or lack of remodeling.**

   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.

*In vivo* remodeling incorporates all of these events simultaneously. Understanding the similarities and differences in these events will allow the design of rational cell, gene, scaffold or combination approaches as therapeutic methods.

3. **Support for the development of improved methods to track the fate of implanted tissue engineered devices. These methods should include strategies for monitoring the ultimate fate of implanted cells, degradation of implanted scaffolds, and quantitative expression of specific genes**.

   It is likely that autologous, allogeneic, and xenogeneic genetic material, cells and scaffold materials will have potential benefit in future strategies for reparative medicine. Furthermore, both synthetic and naturally occurring materials are likely to have roles in optimal therapeutic modalities. The biologic fate of these products is of critical importance in the development of rational treatment methods. Without the tools to track the *in vivo* fate of these products and the function of the target tissue or organ, progress will be severely limited.

   And finally,

4. **It is recommended that a registry of human patients that receive tissue-engineered products be developed. This database should include such information as gender, ethnic background, selected genotypic information, age and state of health/disease.**

   This information can be used to later establish predictors of success and failure for tissue engineered products.

In summary, *in vivo* remodeling is an unavoidable and inevitable event in all tissue engineering and reparative medicine efforts. There is strong and unanimous consensus that resources must be deployed to first understand the molecular events that control *in vivo* remodeling. Once normal events are understood, it will then be possible to rapidly and rationally design therapeutic methods for the optimal repair and restoration of injured or missing body parts. Traditional NIH funding mechanisms including investigator initiated RO1 applications, RFAs and multidisciplinary research partnerships are all appropriate and would significantly enhance progress in this important field.

## STORAGE AND TRANSLATIONAL ISSUES IN REPARATIVE MEDICINE

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**Broad Statement.** Several issues must be addressed in translating any basic science research result into a clinical treatment. When the therapeutic agent is living tissue or cells, the translational issues become more complex. There are research questions relating to efficient and high -volume cell production, tissue typing or characterization, the health and consistency of cells, the long-term storage of living cells and tissues using cryopreservation and drying approaches, and the distribution of therapeutic products. There are also basic studies required to answer questions about efficacy and safety, regulation, and potential ethical issues related to the distribution of scarce resources. A third area of research is in clinical studies, starting with pilot studies and carrying through to phase three clinical trials.

**Vision.** Develop key technologies and approaches in order to seamlessly translate basic science ideas to clinical reality in reparative medicine. Especially focus on long-term storage issues to provide readily available supply of cells and engineered tissues to end-users including medical centers, hospitals, clinics, and doctors' offices.

**Objectives.**
- Provide off-the-shelf readily available supply of cells and tissues for clinical applications.
- Preserve cells and tissues with minimal structural and functional compromise from storage and processing, especially for applications where immediate functional capacity is necessary such as the use of hepatocytes for the treatment of acute fulminant liver failure.
- Reconstitute full biological potential with minimal post-storage manipulation, and develop novel storage technologies using nontoxic cryoprotectants to directly infuse cells into patients immediately upon thawing.
- Investigate new strategies for stabilization of cells and engineered tissues in storage including molecular ice blockers, cell death modifiers (e.g., apoptosis inhibitors, membrane stabilizers), and others.
- Understand basic cell and molecular biology of cell injury associated with various cryopreservation strategies including conventional slow freezing in the presence of 1-2 molar cryoprotectants and vitrification approaches utilizing 6-8 molar cocktail of cryoprotectants.

- Investigate mechanisms by which various organisms and animals survive extreme dehydration and cold temperatures. Engineer key aspects of anhydrobiotic organisms into mammalian systems using genetic and metabolic engineering approaches.
- Overcome the existing technological barriers including reliable large-scale controlled rate freezers, and smart packages with built-in sensors to non-invasively monitor the stability of storage conditions.
- Bring together engineers, physicists, biologists, and clinicians to create multidisciplinary groups to address complex biological and physicochemical processes associated with biopreservation.

**Obstacles and Challenges.**

- Fundamental knowledge about the mechanisms of damage inflicted on cells by cryopreservation-induced stresses need to be obtained, and strategies to minimize cellular damage using genetic and molecular approaches need to be developed.
- Alternative approaches to cryogenic storage need to be developed such as dry storage at ambient temperature.

**Recommendations.**

- Basic cell and tissue preservation technologies require multidisciplinary approach involving physical sciences, cell and molecular biology, clinical medicine, and engineering.
- Preservation technologies lag behind other cell processing technologies such cell culture, isolation, purification, genetic engineering, and bioreactor design. There needs to be more emphasis put into biopreservation principles and technologies by funding agencies.
- Encourage and develop collaborative funding opportunities among multiple agencies with complementary missions. Especially emphasize multidisciplinary partnerships to tackle the current lack of fundamental understanding of cellular damage under extreme dehydration and thermal conditions encountered during cryopreservation.

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