Transforming Regenerative Medicine: An Interdisciplinary Approach

Final Report

May 19–20, 2008

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Executive Summary

A Workshop on "Transforming Regenerative Medicine: An Interdisciplinary Approach" was held on May 19–20, 2008, in Bethesda, Maryland, by the National Institutes of Health (see Appendix A for list of Workshop Co-Chairs and Planning Committee Members). The primary objective of this critical workshop was to bring together leaders in the multiple fields that constitute regenerative medicine to explore strategies for better coordinating biological knowledge, engineering technologies, and clinical needs, with resources, to promote transformation of regenerative medicine. The Workshop consisted of an opening session, four scientific sessions, a plenary talk, and a concluding session (the full meeting agenda can be found in Appendix B).

The four scientific sessions included:

Session 1: Generation and Regeneration: Learning from Nature
The goal was to discuss optimal strategies for translating advances of developmental and post-developmental biology into practical strategies for tissue and organ regeneration.

Session 2: Cell-Instructive Technologies for in vivo Regeneration
The goal was to discuss reprogramming or instructing cells for *in vivo* regeneration within the context of cutting edge biological paradigms for dedifferentiation or regeneration of adult cells and technological advances for local or remote delivery of remodeling/regenerative instructions *in vivo*.

Session 3:3-Dimensional Engineered Tissue in vitro and in vivo
The goal was to illustrate how the study of cells and tissues in complex environments that closely resemble the true physiologic state can lead to the design of successful engineered tissue implants.

Session 4: Functional Integration of Engineered Tissues: More Than the Sum of Its Pieces
The goal was to identify a common set of considerations and outcome measures to gauge progress and define the success of functional integration, and compare common issues across different tissue systems to achieve better coordination to transform regenerative medicine.

The overarching questions for the workshop and the responses are summarized below:

1. How do you build an effective regenerative medicine interdisciplinary team?
Interdisciplinary teams in Regenerative Medicine should include experts in a number of areas (biology, engineering, clinical needs, product development, business strategies, and regulatory policies). Engineers and biologists need to work together to a greater extent. Incentives need to be in place for clinicians to participate. Relationship-building and cross-disciplinary training should be aimed at the post-doctoral level.

2. How do you coordinate biological knowledge, engineering technologies, and clinical needs?
Look at a few of the successful regenerative medicine therapy approaches and learn from these what worked to successfully coordinate biological knowledge, engineering technologies and clinical needs. Several elements are key to the coordination process, including the exchange of results/experience between labs and standardization of cell sources, culture protocols, and assessment methods.

3. What information and expertise is needed to effectively translate regenerative medicine research to patients/products?
If a product is to be successfully used in the clinic, it must address a real clinical need and have a long enough product life to be practical to invest in its development. Expertise in putting together a product development plan and business plan are essential.

4. *What can the NIH do to better coordinate regenerative medicine research to achieve transformation of this field?*
The NIH can consider new funding models that promote goal-oriented research; invest in training courses to teach investigators the skills they need for true interdisciplinary research; consider granting mechanisms that specifically support interdisciplinary research teams; and meet the need for better communication across the regenerative research community.

This report is intended to provide an overview of the presentations and discussions that took place during the workshop. It describes those subjects discussed at the workshop and is not intended to be a comprehensive review of the field. A list of invited speakers can be found in Appendix C and their slide presentations are available on the website of the National Institute of Biomedical Imaging and Bioengineering at www.nibib.nih.gov.

Opening Session

Dr. Roderic Pettigrew, Director of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) and Dr. Christine Kelley, Director of the Division of Discovery Science and Technology in the NIBIB, opened the workshop with welcoming remarks and background information on the NIH Roadmap 1.5 Regenerative Medicine Coordinating Committee and the origin of the idea for the Workshop. Dr. Richard Maas, one of the Workshop Co-Chairs, followed with an overview of the workshop including goals, overarching questions, and the charge to the group. Next, Dr. William Heetderks, Associate Director of Extramural Science Programs in the NIBIB, provided an overview of Regenerative Medicine at the NIH and Dr. Alan Krensky, Director of the new NIH Office of Portfolio Analysis and Strategic Initiatives (OPASI), gave an overview of OPASI and the role of trans-NIH collaboration and coordination in regenerative medicine.

Session 1
Generation and Regeneration: Learning from Nature

Session 1 began with introductory remarks by the session chair, Dr. Frederick Schoen, who articulated that the session would focus on discussion of optimal strategies for translating advances of developmental and post-developmental biology into practical strategies for tissue and organ regeneration. His introductory remarks were followed by a series of individual presentations and concluded with a panel discussion between the session speakers and the audience.

1. Michael Levin
Morphogenetic Information and Biophysical Signals as Key Areas for Progress in Regenerative Medicine

Mechanistic connections between tissue morphogenesis, regeneration and cancer.
Dr. Levin proposed that tissue regeneration represents a special case of morphogenesis. Since organisms make tissues and organs during development, it should be possible to reactivate this program in the adult life for the purpose of tissue regeneration. Dr. Levin also pointed out that there exist fundamental connections between cancer, embryonic development, and tissue regeneration because each reveals how 3-dimensional shape can be stored, re-created, or impaired in a biological organism. The key challenge for regenerative medicine is to learn practical lessons from these related disciplines for orchestrating productive regenerative responses in the setting of the adult organism. Since projects dealing with cancer, development, and regeneration are usually reviewed by different NIH study sections, and the work in these fields is usually performed in different university departments, the crucial mechanistic connections between development, regeneration, and cancer are often missed.

Regeneration as a high-order global process.
All productive morphogenetic systems possess multi-scale interactive control programs that guide the final shape of developing structures and detect deviations from the final shape. These systems also sense their current morphogenetic state and “know” the steps that must be taken to restore the desired final pattern. This ability underlies both normal morphostasis (adult growth and remodeling) and the cessation of growth when damaged structures have finished regenerating. Failure to regenerate can result from defects in any of the multiple steps in the program, and understanding these steps on a systems level is central to the success of regenerative medicine. It has proven difficult to obtain funding for proposals that address these questions on a global level focusing on morphogenetic information flow and storage. The current funding paradigms tend to support research addressing a single model system and a single protein or pathway. This situation must be changed in order to revolutionize regenerative medicine.

Dr. Levin pointed out that while it is often presumed that the capacity to regenerate tissues and organs is limited to simple organisms, and that higher animals do not regenerate, in reality the regenerative capacity is sprinkled throughout phyla, and is not tied to specific regenerative species or particular gene products or cell-level mechanisms.

Regeneration constitutes a fundamentally higher-order global process and it must be understood from the perspective of the dynamics of the multi-scale morphogenetic information. The current fragmented view of regeneration and its mechanisms in the various model systems is an artifact of focusing at the wrong level (individual molecules and pathways instead of the larger questions of how cells and tissues store complex 3-D patterns).

Bioelectric signals: master regulators of tissue morphogenesis and regeneration.
Dr. Levin spoke briefly about his work on the role of bioelectric signals in regulating tissue regeneration and morphogenesis. He proposed that these signals (long-term ion fluxes, electric fields, voltage and pH gradients), that are present in most cells, act as additional and very powerful "control knobs" that can be used to control morphogenesis and regeneration. In support of this hypothesis, his recent work revealed that modulation of bioelectrical signaling can provide organ-level control of morphogenesis and regeneration in vertebrates. For example, they found that mis-expression of a single hydrogen transporter protein is sufficient to initiate and complete the cascade of tail regeneration in *Xenopus laevis*. It thus appears that ion fluxes may represent upstream master regulators in *Xenopus* and, according to data from other laboratories, many other systems. It is likely feasible to activate regenerative programs via high-level control of these master regulators without the need for "micromanaging" the processes on the lower level of individual genes and pathways. The next challenge will be to quantitatively describe the sets of bioelectric parameters and their downstream signaling pathways that define regenerative vs. non-regenerative states, and to develop methods for rational modulation of these parameters for productive tissue regeneration.

2. Christopher Chen
Extra- and Intracellular Signaling, the Engineering of Cell Function, and Potential Utility to Regenerative Medicine

Sub-systems and global biological processes.
Dr. Chen argued that while many genes, cellular pathways, and bioactive molecules have been identified during the last 30 years of research providing information about the individual sub-systems of biological processes, we presently have only modest information as to the importance of these sub-systems in the global processes occurring *in vivo*. For example, during scarring and regeneration many sub-systems of inflammation, angiogenesis, and tissue remodeling become activated or repressed. However, because these processes are composed of multiple stages, occur in parallel, and influence each other; we still do not have a complete picture of the role of individual sub-systems in the choice between scarring and regeneration. It is thus difficult to design adequate interventional tools to guide tissue regeneration away from scarring. Thus a major enabling capability for regenerative medicine research would be to develop approaches to be able to control, mimic, or otherwise perturb the behavior of these subsystems (within the context of the whole system), and then to be able to do so with multiple subsystems simultaneously. Currently, nearly all tools to intervene *in vivo* are genetic tools targeted at individual cell types, as opposed to tools targeted at subsystems or processes. This will require some shift in our perspective.

Limitations of extrapolation of *in vitro* results to *in vivo* systems.
The advantage of working *in vitro* is in the opportunity to break down problems into individual sub-problems for derivation of simpler working models of the *in vivo* processes. Another advantage is the availability of interventional tools that are often lacking *in vivo*. We only need to look at the impact of standard cell culture on biomedical research to realize how important model systems can be to the research establishment. One needs to be cautious, however, when reconnecting *in vitro* insights into the *in vivo* models, because this process is prone to errors. An important source of such errors results from the inadequacy of a biological context, such as local and global tissue architecture, biophysical forces, ligand presentation to cells, extracellular matrix composition, and cell-cell interactions, in *in vitro* experiments. More complex *in vitro* culture systems are just now being explored by a few laboratories, and a great deal of potential remains to be tapped from developing such model culture systems that might better recapitulate *in vivo* cellular phenotypes.

Integrative frameworks to build system models of biological processes.
It will be important to develop frameworks to help the integration of disparate *in vitro* and *in vivo* experiments into system models of biological processes. These frameworks should be built through coordination of work from different laboratories to standardize cells, reagents, data, and systems employed by various researchers. It will also be necessary to develop better experimental systems that could capture essential features of the *in vivo* informational context. Further, a new generation of robust and quantitative *in vivo* interventional tools will be needed to enable continuous, real time temporal and spatial control and readout from *in vitro* and *in vivo* systems. If we compare our efforts in biomedicine to those in electronics, we begin to realize how standardization of protocols and processes can truly catapult a field into a regime where every researcher's efforts are additive at minimum, and synergistic at maximum, with others in the field. Much of this is based on better cooperative frameworks.

3. Frederick Schoen
Harnessing Insights from Developmental and Post-Developmental Biology

Plasticity and regenerative potential of adult tissues.
Dr. Schoen proposed that adult tissues may posses more plasticity and regenerative potential than was previously thought. He used the aortic valve as an example and spoke about the role of valvular interstitial cells (VICs) in regulating aortic valve health and pathobiology. VICs, which are the most common cells in the valve, can exist in five distinct phenotypes serving different functions: embryonic progenitors (developmental source of VICs), quiescent VICs (resting state of VICs), activated VICs (mediate remodeling, adaptation and disease), circulating endothelial cell precursors (post-developmental source of VICs; maintain tissue homeostasis), and osteoblastic VICs (mediate calcification and disease). Intriguingly, new results suggest that the phenotypes of VICs are plastic and that interconversion among different VICs is regulated by environmental stimulation, such as mechanical stress and presence of specific growth factors, and that some phenotypic modulation of VICs may be reversible. It is thus possible to envision that the regenerative potential of a heart valve could be controlled via

external modulation of the VIC phenotypes. Results implying aortic valve plasticity are emerging from studies of pulmonary-to-aortic valve transplantation. For example, during the early period following transplantation (3-6 months), pulmonary valves transplanted into the systemic circulation undergo extracellular matrix remodeling mediated by activated VICs in order to maintain their functional properties. However, with time (more than 3 years), they not only acquire the structure and function of normal aortic valves, but also the VICs return to quiescence.

Adaptation of developmental and post-developmental principles to regenerative medicine.

Dr. Schoen showed that when *in vitro* grown heart valves are subjected to mechanical and flow stimulation approximating that of the normal *in vivo* heart valve, this drastically improves function of the engineered valve following its transplantation *in vivo*. In particular, after 5 months *in vivo* these engineered valves resembled normal heart valves in microstructure, mechanical properties, and extracellular matrix architecture. Further, multiple lines of evidence suggest that the endogenous environment and normal host pathophysiological processes significantly contribute to remodeling and functional maturation of transplanted grafts *in vivo*. Circulating Endothelial Progenitor Cells (EPCs) may participate in this process on multiple levels since EPCs can be mobilized from the bone marrow and homed to specific sites in the body through mediators and cell-cell and cell-matrix interactions similar to those involved in physiological inflammation. It might be possible to develop practical strategies for exploiting the regenerative capacities of these cells. In fact, one could envision that by targeted manipulation of EPCs and other components of the inflammatory response, it might be possible to significantly enhance intrinsic tissue regeneration.

Role of genetic variation and in tissue regeneration.

Dr. Schoen proposed that genetic variation among otherwise normal individuals may exert significant effects on the tissue regenerative capacity of an individual. From studies of pharmacogenetics it is known that in the human populations there exist clinically important inherited individual variations in drug metabolism, drug transport, drug tissue distribution, and interaction of drugs with their therapeutic targets. Extensive research is now in progress to identify predictive markers defining this variability in drug response. The tissue regenerative response has not yet been extensively studied from this perspective. However, interesting data is already available to show, for instance, significant genetic heterogeneity in the rate of wound healing between different inbred strains of mice. Genetic variability in the inflammatory response has also been documented. It will be important to develop easily measurable predictive biomarkers that characterize various aspects of tissue remodeling and regenerative response. Availability of such biomarkers could greatly enhance prediction, monitoring, and directed intervention, tailored to an individual patient's needs, that would enhance the outcome of regenerative medicine therapeutics in the same manner as pharmacogenetics will identify the optimal drugs and doses for each patient.

4. Gordana Vunjak-Novakovic
Creating Tissues for Therapeutic Use: Biological Principles and Engineering Designs

Engineering the environments, so the cells can engineer tissues.
Dr. Vunjak-Novakovic spoke about enabling components of tissue engineering and regenerative medicine. She emphasized the critical role of engineering suitable cellular environments providing the extracellular matrix, nutrients, oxygen, cell-cell interactions, molecular factors, and physical forces for building functional tissues. Dr. Vunjak-Novakovic argued that proper environments can enable the cells to become competent "tissue engineers" for generation of functional tissues. She also asserted that assaying function of engineered tissues on multiple levels should be one of the central priorities of the field. It is imperative to develop robust high fidelity assays to properly measure cell and tissue function. To this end, it will be important to design advanced high fidelity *in vitro* culture systems that recapitulate the *in vivo* environment, can be easily controlled, and can be effectively correlated with the corresponding *in vivo* systems. Building such *in vitro* systems will have to rely on a cross-disciplinary approach of biologists, engineers and clinicians.

Biomimetic approach to tissue building.
Dr. Vunjak-Novakovic argued that nature provides us with the blueprints for constructing tissues. Heart, for example, which is an excellent illustration of "engineering by nature", lends us design principles for generating functional cardiac muscle. Dr. Vunjak-Novakovic's laboratory is using tissue bioreactors to incorporate the biomimetic design principles into their cardiac tissue constructs. As more details about normal heart development and function become available, this information will be translated into perfecting the scaffold design, optimizing mechanical and electrical stimulation parameters, and improving the cellular environment of the engineered constructs. She has already found that cells in the bioreactors readily respond to the appropriate environmental cues; a combination of mechanical and electrical cell stimulation for example, improves *in vivo*-like structural and functional properties of the engineered cardiac muscle. Transplantation of *in vitro* generated tissues into appropriate animal models is essential for completion of tissue development initiated *in vitro*. Analysis of tissue function *in vivo* is also essential. In this regard, development of appropriate *in vivo* real time functional imaging methodologies and assays to measure biomechanical properties, integration, and function of engineered constructs in the host environment are of primary importance.

In *vitro vs. in vivo*: a two way road.
Dr. Vunjak-Novakovic proposed that developing technologies to build *in vitro* micro-tissues that recapitulate normal *in vivo* tissue structure and function would revolutionize both basic science and medicine. Not only would such *in vitro* organoids serve as invaluable tools for developing therapeutic tissue replacements, they would also be used by developmental and cancer biologists to study mechanisms of normal and abnormal tissue morphogenesis and regeneration, wound healing, and cancer progression. This work will facilitate the generation of new hypotheses to be refined and tested *in vivo* and

in vitro, and will guide transformation of regenerative medicine for the benefit of human health.

Panel Discussion

During the panel discussion several questions were raised concerning strategies for extrapolation of knowledge obtained in simpler cell and animal model systems, and from mathematical modeling of biological processes, to more complex vertebrate systems.

In response to these questions, a general consensus was reached regarding the importance of understanding the underlying basic phenomena in simpler organisms to glean key features and mechanisms of development and regeneration in more complex organisms. It was emphasized that it will be necessary to develop non-invasive micro-level and high-throughput molecular imaging methodologies for *in vitro* culture systems to monitor cell and tissue behavior and to screen and compare results obtained in different systems. These technologies will help us to select specific combinations of physical and chemical parameters to be used for the development of regenerative strategies in complex systems. One should be aware, however, that a mathematical apparatus to reliably describe complex biological systems has not yet been fully developed.

There was also a discussion about the amount of pre-determined information that we will need to provide to regenerating tissues. Since regeneration is a complex and multistage process, how much instruction should we give? How do we know when it is enough? During this discussion, several comments were made on the importance of taking full advantage of the tools that nature uses to regenerate tissues. In other words, we should aim at capitalizing on the intrinsic tissue regenerative capacity, and at minimizing the exogenous intervention. It is likely that the tissues will not need to be built from scratch. Mechanistic insights into tissue regeneration will teach us how to tune the key parameters of the intrinsic tissue regenerative machinery, and then to get out of the way to let the tissues heal themselves.

Session 2
Cell-Instructive Technologies for *in vivo* Regeneration

The chair, Dr. Richard Maas, provided an introduction to this session. He explained that the focus of the session was on the reprogramming or instructing of cells for *in vivo* regeneration within the context of cutting edge biological paradigms. Dr. Maas considered the following questions: Do we need to have a comprehensive understanding of developmental regulatory networks in order to regenerate or form desired tissues (a complete "molecular blueprint"), or will more empirical approaches, not based on systematic information, suffice for achieving this goal? Both strategies have value. Two considerations were discussed: 1) the sufficiency of specific genes and signaling pathways – e.g., *Pax6;* an important gene or node within a larger gene regulatory network. While genes do not operate in isolation, multiple entry points can yield the same regulatory result due to inherent cross-regulatory features of network architecture; and, 2) the principle of autonomy – i.e., once initiated and provided with the proper microenvironment, organogenic regulatory circuitry can precede largely to completion; for example tooth formation in ovarian teratomas. Dr. Maas' opening comments were followed by a series of presentations as summarized below.

1. Richard Maas
Organ Induction *in vivo*

Dr. Maas stated that there are two potential strategies for tissue formation: 1) *In vivo* regeneration, which takes advantage of endogenous stem cell niches, and is less dependent on knowledge of the molecular blueprint of development (since unknown factors may be provided by the in vivo context); and, 2) *In vitro* regeneration, followed by transplantation or engraftment, which takes advantage of abundant cell sources (ES, iPS) and controlled fabrication strategies, and is more dependent on knowledge of the molecular blueprint of development.

Tooth-germ model

Epithelial-mesenchymal interactions represent a conserved developmental mechanism from which fundamental principles can be deduced. Indeed, stimulation of Wnt signaling via activation of beta-catenin or via inactivation of APC can cause induction of supernumerary teeth in vivo, from either embryonic or adult oral ectoderm. Ectopic tooth buds develop from multiple regions of oral and dental epithelia. They are robust, the enamel is normal, and there is innervation. One of the advantages of in vivo regeneration is that vascularization and innervation, two recurrent problems in tissue engineering, are properly realized. What happens if this pathway is turned on the adult animal? Even in adult mice, ectopic teeth form with APC targeted inactivation. Latent tooth-forming potential likely exists within a dormant stem cell niche in the adult jaw. In addition, there are cell-nonautonomous effects in this system since only some cells activate Wnt signaling but they are apparently able to recruit their neighbors into tooth formation. Dr. Maas concluded that it appears possible that only a few components need to be manipulated in vivo in order to induce an entire developmental pathway that leads to

odonotogenesis and possibly, by extension, organogenesis. That is, prior knowledge of the entire molecular blueprint of organogenesis is not required in such in vivo systems.

An important question to answer is if activation of the Wnt pathway can induce human dental tissues. If so, then this process might possibly be effectively regulated in situ. Alternatively, another route might involve tissue manipulation *ex vivo*, followed by reimplantation. It is advantageous to use reprogramming methods because these are autologous cells. Sequential reprogramming needs to be experimentally applied as opposed to a master switch. Future work must determine if key features of major gene regulators is sufficient. Once determined, applications can be developed for tooth formation *in vitro*, which may be used for tooth replacement.

It was commented that Wnt pathway activation can lead to many outcomes; hence, it is unclear why other tissues are not made. One possibility is that environmental factors play a role. However, experiments have only been done in embryonic stem cells. It was noted that the tooth is a great paradigm, but the idea is also to move toward heart valves and pancreatic islet cells. There is a need to understand one model well, however, and to adapt that knowledge to inform different tissue models.

2. Kathrin Plath
Generating Human Induced Pluripotent (iPS) Stem Cells

The two major known reprogramming methods of nuclear transfer and cell fusion have many ethical and technical limitations. Another way, that is ethically less challenging, is the generation of induced pluripotent (iPS) cells by retroviral overexpression of four transcription factors; Oct4, Sox2, cMyc, and Klf4 in somatic cells. These four factors are required for reprogramming and can induce pluripotency in human and murine fibroblasts. It was commented that reprogramming occurs when the respective endogenous promoters are turned on and retroviral promoters are turned off. Dr. Plath discussed the therapeutic promise of induced pluripotent stem cells and the need for a detailed understanding of the mechanisms underlying reprogramming to be able to achieve reprogramming more efficiently and safely.

Human iPS cells are easy to derive from fibroblasts, don’t require sophisticated technologies, and have been reproducibly generated by several laboratories. An important question is; are human iPS cells similar to existing embryonic stem (ES) cell lines? Dr. Plath suggested that this is the case, based on expression profiling results demonstrating similar gene expression patterns and teratoma formation. However, further analysis is required to understand the relationship of iPS cells to ES cells. Currently, reprogramming of iPS cells takes about 4 weeks and occurs with low efficiency. Additional limitations of this method include retroviral integrations of the four transcription factor and the fact that tumors can develop due to reactivation of the reprogramming factors or retroviral insertional mutagenesis. Concerns about tumorigenesis can be addressed to some extent by omitting cMyc, but this approach leads to even slower and less efficient generation of iPS cells. Clearly, new methods need to be developed to generate safer iPS cells.

In addition to their use in cell replacement studies and basic research on pluripotency, research using iPS can enable the development of experimental systems to evaluate cellular defects of complex disease, and can be used potentially to screen drugs.

Dr. Plath stated that it was important to answer several questions in order to move the field forward:

- Can we achieve this without retroviral integration?
- Can iPS cells be differentiated into functional cell types that could be transplanted
- Are human iPS cells indeed ES like (and not EC like)?
- Can the master regulators be replaced by small molecules
- Can any cell be reprogrammed into any other cell type via direct transdifferentiation?

Dr. Plath fielded many questions from the workshop participants. For example, it was asked if cellular age affects reprogramming efficiency and if iPS cells can be generated from patients of any age. Dr. Plath responded that these experiments have not been done yet. Others wondered if telomere length could be reset, which it can. Another participant noted that one might not want complete pluripotency to get clinically useful cells without the tumorigenic problem. Similarly, using different starting cells might reduce the requirement for ectopic factors. It is becoming clear in the field that if one uses multipotent cells then one can use fewer factors. In addition, Dr. Plath pointed out that everyone in the field is trying to establish methods for iPS generation that do not depend on the use of retroviruses.

3. Quiao (Joe) Zhou
Reprogramming Pancreatic Cell Fates i*n vivo*

Dr. Zhou spoke about new methods to reprogram cells in situ. He described his work on conversion of non-islet pancreatic cells into insulin secreting beta-cells by direct reprogramming. The results suggest that appropriate combinations of embryonic genes can reprogram adult cells. Dr. Zhou explained that an initial list of 1100 transcription factors as potential candidates for reprogramming was reduced to 28 based on gene expression patterns, of which 9 were considered candidates for inducing a beta cell phenotype based on three expression categories: panendoderm, islet progenitors, and mature beta-cells. The group made high titer adenoviruses of each of 9 candidate transcription factors along with a GFP reporter and introduced these into the adult mouse. They observed that non-islet (exocrine) cells turned into GFP-positive, insulin-positive beta-islet cells, and then looked for factors which were dispensable. Ultimately, only 3 factors were needed to convert exocrine pancreatic cells into beta cells in vivo: Ngn3, Pdx1, MafA. The effect was rapid; the first transdifferentiated cells were seen within 3 days post-infection with increasing numbers evident still after 10 days. Dr. Zhou noted that reprogrammed beta cells are identified on the basis of EM appearance, expression of known beta cell markers, and lack of expression of other endocrine or exocrine genes, such as glucagon, etc. Additionally, transgene expression is not required to maintain a stable beta cell state. Insulin is produced and secreted by the reprogrammed beta cells.

These cells also produce VEGF which enables them to attract endothelial cells, as do endogenous beta cells, and induce angiogenesis.

In summary it was noted that indirect reprogramming with iPS methods (i.e., *in vitro*) causes most epigenetic marks to be removed and there is extensive proliferation of the cells. However, with direct reprogramming methods (i.e., in situ repair), epigenetics plays some role and there is minimal proliferation. Workshop participants wanted to know the efficiency and speed of reprogramming, and questioned if there is a tug of war going on with the cell's identity and a need to knock down other programs, perhaps with siRNA. Another comment was that the epigenome has not been studied in pancreatic tissue cells but one hypothesis is that the observation that addition of just 3 transcription factors allows specific trans-differentiation of these cell types suggests that exocrine and endocrine pancreatic cells may have sufficiently similar epigenomes that only minor changes are necessary.

4. David Mooney
Polymers for *In Situ* Cell Programming

Dr. Mooney's presentation focused on the design of polymer systems that are capable of programming cells in the body as a strategy to bypass the need for cell isolation, *ex vivo* manipulation, and subsequent transplantation. These polymer systems may be designed to regulate the adhesive cues (e.g., integrin binding, mechanical signaling) and morphogen/cytokine signaling that can be used to: 1) recruit host cells; 2) instruct those cells in terms of proliferation and differentiation; and, (3 disperse the cells to the desired tissue or organ where they may participate in regenerative processes. Recent work on presentation of adhesive peptides focuses on using a multi-scale modeling approach to design the appropriate nanoscale organization and spacing of the ligands within the material; materials are also being developed to enable sequential factor delivery to drive processes such as angiogenesis. Recombinant proteins are embedded in a biodegradable polymer to enable sequential delivery. Specific peptide and island-spacing effects are used to alter integrin binding that, in turn, regulates cellular response. These material systems have shown utility in enhancing the regenerative ability of transplanted skeletal muscle and endothelial progenitor cells. Proof of principle that host cells can be recruited, manipulated by a material, and subsequently dispersed to distant sites was provided with dendritic cells.

Dr. Mooney concluded his talk noting that it was important for the NIH to build more effective teams at the pre- and post-doc fellowship levels, and suggested that offering individual pre and post-doc fellowships in regenerative medicine, with a requirement to attend a workshop each year would facilitate team-building.

5. Samuel Stupp
Self-Assembly and Supramolecular Structure for Cell Signaling

Dr. Stupp began his talk describing supramolecular elements that are biomimetic (i.e., ordered, hierarchical, self-assembling) and abiotic (i.e., technology to trigger events that

are not spontaneous) and how these components are needed for instructive matrices. Self-assembly approaches are unique because therapies can reach deep into tissues as liquids and assemble in situ into solid structures. Self-assembly also offers a way to multiplex biological signals by simple mixing of components rather than requiring chemical synthesis of a specific structure. An example of this is the use of nanofibers which mimic collagen fibrils in an infarcted mouse model. Formation of the nanofibers is triggered by ions in the physiological environment. Recovery of function was observed 30 days post-infarct.

Another example described used aggregates of neural stem cells mixed in suspension with solutions of self-assembling molecules. Ions in culture media triggered self-assembly of bioactive nanofibers around the cells and induced their rapid and selective differentiation into neurons within 24 hours of exposure. The neural stem cells did not become astrocytes in the presence of the bioactive nanofibers.

Dr. Stupp showed recent work on self-assembling macroscopic sacs that can encapsulate cells and function as mini-cell biology labs to carry out experiments or vehicles to deliver cell therapies (Science 319: 1821, 2008). Human stem cell differentiation and survival within the sac was observed over the period of one month. The self-assembling sacs exhibit unusual hierarchical structure which allows diffusion of large proteins such as growth factors through their walls. The hierarchal structure of the sac can accommodate 3 different bioactive compartments; 1) surfaces; 2) walls; and, 3) interior structure. The sac has potential for use in cell delivery because the sacs are stable in media over weeks but are biodegradable in vivo. Additionally, cells can be injected into the sac and it reseals itself by self-assembly. Multiple sacs can also be fused together temporarily to study paracrine interactions. It was noted that the macroscopic sacs can be enzymatically biodegraded in vivo, however, Dr. Stupp noted that for certain applications the chemistry can be especially designed to maintain the sac structure for long periods of time.

It was noted that advances in biological signaling are critical for the design of sophisticated instructive supramolecular matrices that can include both structural and kinetic features for optimal performance. Matrices could be designed that have the capability of repelling or attracting different cell types.

Session 3
Engineered Tissue *in vitro* and *in vivo*

The overall goal of this session was to illustrate how the study of cells and tissues in complex environments that more closely resemble the true physiologic state can lead to the design of more successful engineered tissue implants. Tissues and organs are three-dimensional (3-D), but the tools used to study them have been largely two-dimensional (2-D), even though cells in such systems differ considerably in their morphology, cell-cell and cell-matrix interactions, and differentiation from their *in vivo* counterparts. This core conundrum was presented by Dr. Gordana Vunjak-Novakovic (Columbia University), the Session Chair, who gave a general introduction. She emphasized the need for realistic mimics of cells in their native environments with on-board monitoring and controllable features to probe complex questions about physiologic responses. Many of the 3-D systems currently under development are also being used for near-term ends: to produce circulating blood cells from precursors in a closed, controllable system, or provide robust, reliable, reproducible mini-organoids for high throughput screening applications.

1. Larry Lasky
Cell-Instructive Technologies for *in vivo* Regenerative Medicine

Dr. Lasky outlined his concept of how to design and build an *in vitro* system to produce red blood cells on demand. The "RBC Machine" will integrate classical *in vitro* methods with key *in vivo* concepts and some *de novo* approaches to deliver an engineering "tour-de-force"— a device to produce red cells in clinically useable numbers. He presented a schematic view of a multistage modular bioreactor system in which starting cells (expanded and then induced to hematopoietic differentiation) migrate to sections of the device with controlled levels of cytokines for further development, and finally liquid cultures for terminal differentiation to fully functional erythrocytes. The process is tightly monitored, automatable, minimally complex, and highly scalable. Dr. Lasky identified some of the potential challenges, including the variety of candidate cell sources—cord blood, embryonic stem cells, cell lines, marrow/peripheral blood stem cells; massive requirements for media and growth factors; diverse and well-defined scaffolds that recapitulate the physical structure and surface characteristics of the marrow hematopoietic niche; scale up protocols for sustained production; and managing/funding such a multi-disciplinary project.

2. Dan Kaufman
Progress and Challenges for Large-Scale Erythroid Development from Human Embryonic Stem Cells

Dr. Kaufman delivered a complementary presentation to Dr. Lasky's focusing on the use of embryonic stem cells to produce precursors for the hematopoietic lineage. Limiting oxygen to its *in vivo* concentrations aids cell maturation probably by inducing some of the cytokines necessary for development of specific cell types. It is clear however that

even the most rigorous differentiation protocol does not produce pure populations, and that in addition to scalability, predictability and control are critical considerations.

3. Nancy Parenteau
Translating What We Know About Liver Biology to Achieve Functional Authenticity in Vitro—What Does Hepatocyte Function Really Represent

Dr. Parenteau discussed the development of liver organoids and covered the basic biology considerations in working towards an authentic *in vitro* model of human liver. She initially highlighted lessons learned from other organ systems (skin, pancreas, and cornea), particularly emphasizing that 1) parenchymal development has a large autonomous component; 2) the most conducive environments can be permissive and enabling but not necessarily instructive, and 3) accessory cells are powerful influential factors. Fully functional engineered liver tissues will need to recapitulate the polarity, positional modulation, differentiation/regenerative history, cellular interactions, responsiveness, and metabolic robustness of the intact organ. It will be critical to look at how nature puts things together and regulates cellular behavior over time and in different circumstances. Strategic decisions will have biological implications with issues like choice of starting cell population, complexity of the matrix, flexibility of bioreactor conditions, etc. determining outcomes.

4. Sangeeta Bhatia
Translating What We Know About Liver Biology to Achieve Functional Authenticity in Vitro—What Does Hepatocyte Function Really Represent (Part 2)

Dr. Bhatia addressed the specific technical challenges in building the authentic *in vitro* model of human liver described by Dr. Parenteau. She described the importance of phenotypic stability, cell source, and preservation for *in vitro* tissue generation. Cues derived from stromal cells are important for hepatocyte proliferation *in vitro*. Translating information about embryonic liver development into *in vitro* models is still difficult because it is not clear how much of the environmental context is required to achieve the target performance. She cautioned that for some high throughput screening applications, limited complexity may be sufficient. For example, benchmark assays for liver function can be performed in parallel in micropatterned wells, by establishing different compartments for zonal hepatotoxicity studies. Such tissues may not be suitable for transplantation where the needs will be different (e.g. tissue persistence, effective host-integration, genetic stability, and resistance to a possible underlying disease state at the transplant site). However, *in vitro* systems provide important steps toward building complex tissues for implantation. Audience members were curious about the use of alternative sources of stem cells (e.g. induced pluripotent cells, amniotic fluid stem cells). Both Drs. Parenteau and Bhatia commented that deriving functional hepatocytes for *in vitro* culture was more efficient and effective using committed progenitor cells, rather than more primitive cell types. Dr. Bhatia further stated that cell sourcing was a major hurdle in more aggressively moving toward clinical applications.

5. Samuel Stupp
Self-Assembling Nanofibers for Regeneration in the Central Nervous System

Dr. Stupp described a novel system using nanofibers for regeneration of the central nervous system. He focused on use of bioactive peptides (specifically IKVAV, a pentapeptide derived from laminin) injected as a liquid for *in-situ* self-assembly into nanofibers customized for regeneration post spinal cord injury. Following implantation of nanofiber matrix at the injury site, motor and sensory axons regenerate, with enhanced functional recovery. Scale up and preclinical studies in large animals are planned. There are also promising results using encapsulation of mouse embryonic neural stem cells in nanofiber matrix which became neurons within a day.

Dr. Stupp emphasized the importance for involving clinicians earlier in such experiments in regenerative medicine. This set the tone for the general discussion on how to best build multi-disciplinary teams to address key questions in designing and using complex systems *in vitro* and *in vivo*. NIH has a broad array of mechanisms that can be used to support the groups and/or efforts needed, but the research community needs to effectively frame the challenges and subsequent path forward. Key considerations might be deciding the point at which the engineering is sufficient to put the major focus on translation. At what point, does the work move beyond “research” (which is primarily supported by public monies) to “development” (which is where industry takes precedence)? Dr. Bhatia added that a major part of “translation” is the need to transition methods as well as results. Many laboratories that want to model her platforms do not want to adapt the technology in house but rather desire a ready made system. This offers a commercialization opportunity. Standardization of data acquisition and interpretation is also ripe for commoditization.

Along the road toward translation, a crucial element will be the integration of interdisciplinary teams to the extent that data can be acquired *and interpreted* by all members. Key experimental milestones might be the establishment of phenotypic stability, and the identification of master regulators for organ development. The importance of understanding check points in normal development (even if the underlying mechanisms are not readily apparent) was emphasized. On the practical side, it was noted that good tracking systems for human cells are not yet in hand. Anther critical need for larger tissues is vascularization/angiogenesis, which was not addressed at the session. Ultimately, participants reinforced a common theme of the workshop—the most transformative event will be to “provide a permissive environment, and then get out of the way”.

Session 4
Functional Integration: More than the Sum of Its Pieces

The chair for this session, Dr. Rocky Tuan, began with a working definition of functional integration. He stated that for the purpose of this session, functional integration was the *integration of regenerated/engineered tissues within a host organism, addressing the complex physiological interactions across multiple tissue types, while maintaining long-term viability, survival, and safety*. Functional integration encompasses systemic interactions, including, but not limited to, biological signaling, vascularization, innervation with respect to neural integration and physiology, structural and mechanical function, interaction of regenerated tissues with innate and adaptive immune systems, survival (host and graft), and safety. The session used four case studies to explore these systems.

The goal of the session was to identify a common set of considerations and outcome measures to gauge progress and define the success of functional integration, and compare common issues across different tissue systems to achieve better coordination to transform regenerative medicine. To develop standardized metrics for assessing functional integration across fields, the panel proposed a workshop to write a consensus white paper on standardized phenotypic endpoints.

1. Rocky Tuan
Case Study #1: Sensory System with a Focus on Innervation: Axon Regeneration in the Optic Nerve

Grafts from the peripheral nervous system, which does regenerate, have been used to study what blocks functional regeneration in the mammalian central nervous system (CNS). Mature CNS neurons can be stimulated to regenerate their axons *in vivo*. Inflammation and growth factor secretion from macrophages caused extensive regeneration in the optic nerve. Axon regeneration in the optic nerve can be induced by counteracting the cell-extrinsic signals that normally suppress axon growth, but only if the neuron's intrinsic growth state has been activated. *Thus, the regenerative process requires both stimulation and overcoming inhibition.*

Considerations for the NIH:

- Outcome parameters for sensory system regeneration: axon growth to central targets; topographically organized projections; myelination; nerve conduction; functional responses.
- Basic principles from case study of the body's innate regenerative potential
    - Innate regeneration requires overcoming inhibitory factors in the environment as well as activating regenerative capacity of the cells.
    - Factors involved in initial development need to be explored for roles in regeneration.
    - Inflammation's effect on CNS regeneration is context-dependent. More needs to be learned on how to modulate the inflammatory response to tilt the balance towards the production of pro-survival and pro-regenerative factors and away from cytotoxic factors.

- One can distinguish neurite promotion from survival neurotrophic factors.

2. Doris Taylor
Case Study # 2: Cardiac Regeneration and Vascularization: Vascular Functional Integration: Take Heart

Challenges in tissue engineering include: 1) cell sources; 2) scaffolding/matrix; and, 3) functional perfusion. Stem cells (from neonatal or adult hearts) can be expanded and differentiated into endothelial cells, smooth muscle and cardiac-like cells. Autologous cells from bone marrow and blood can give rise to subsets of these. Although engineers can design 3-D matrices or create self-assembling scaffolds, artificial matrices do not yet support creation of viable thick 3-D cardiac tissue. An alternative is to borrow an existing natural scaffold by chemically decellularizing any organ to create an extracellular-matrix cast. Adult heart cells were injected or infused into scaffolds prepared from adult hearts. Vascular conduits were preserved after the decellularization process. After recellularization with cells, the constructed hearts exhibited developmentally appropriate biomechanical and perfusion properties and contained integrated blood vessels and muscle. Perfusion reendothelialized heart scaffolds yielded constructs that exhibit much less thrombosis than scaffolds without reendothelialization. In animal models, the decellularization/recellularization approach taken with hearts has been extended to every perfusable organ or tissue. *Give nature the tools to regenerate and get out of the way.*

Considerations for the NIH:

- With endogenous repair, stem cells exist or can be recruited to any adult tissues.
    - Depletion of stem cell pools with aging may explain reduced regenerative capacity: Creating appropriate chronic animal models is an unmet need.
    - Inflammation is the body's message to recruit cells—and (chronic) inflammation persists until proper cells are recruited. Chronic inflammation recruits deleterious cells.
    - Therefore, regenerative medicine could involve exogenous therapy to increase appropriate stem cell delivery to site of damage.

- Extracellular-matrix casts may be sufficient 3-D matrices for complex organs.

3. Farshid Guilak
Case Study # 3: Functional Tissue Engineering of Cartilage: A Joint Venture

Applying engineering principles will improve success of tissue engineering (TE): 1) define standards of success from outset (ex: "successful" cartilage construct could postpone the need for joint replacement for five years); 2) define requirements and functional properties of native tissues; 3) prioritize subset of properties as design parameters; 4) use reductionist design of constructs with controlled properties; 5) study response to biomechanical factors (wear testing analogous to plastics industry testing). This case study approached the unique challenge of TE mechanical stability with scaffolds of 3-D fiber weaves. Mechanical properties can be engineered by fiber type and weave density specifications. Growth factors can be embedded into the fibers and used to seed adipose-derived adult stromal cells as a source of chondrocyte progenitors. Use of

this approach has resulted in a product that approximates the mechanical properties of native cartilage.

Considerations for the NIH:

- Treat the patient, not the scientist/clinician: project should address dominant clinical problem. (Osteoarthritis is significantly more prevalent than focal cartilage defects).

The disease process must be taken into account in construct design.

4. Jeffrey Platt
Case Study #4: Immunological Barriers to Transplantation and Regeneration of Tissues

Survival and functional integration of foreign tissues is limited by sequential immunologic barriers including 1) ischemia; 2) sensitization; 3) the impact of immunity on the tissue. Ischemia generates oxidants, activates complement, coagulation, platelets, and toll-like receptor 4, and recruits antibodies and T cells. Foreign antigens from engineered tissues, the medium, or infectious agents can lead to sensitization. Unusual/large amounts of peptides can stimulate "autoimmune" sensitization. *The impact of sensitization depends on the tissue's vascular supply.* Engrafted organs, carrying foreign blood vessels are subject to injury by both cellular and humoral immune responses. By contrast, cell and tissue grafts fed by blood vessels of the host are subject mainly to cellular immune responses. Accommodation refers to an acquired resistance of tissues to injury by inflammation and immunity. In physiology, accommodation allows the immune system to attack foreign organisms without causing inadvertent injury to nearby cells.

Key immunological considerations for functional integration of regenerative medicine:

- All implanted tissues suffer ischemic injury. Minimizing/reversing it may help integration.
- Propensity of immune responses to cause injury depends on vasculature and immunogenicity. Blood vessels are the most vulnerable to immune attack: antibodies cross blood vessels poorly. Are there parts of the vasculature with higher rejection potential?
- High degree of immunogenicity in skin and transplanted bone marrow.
- What is the functional difference between chronic versus acute rejection of organs?
- Fetal tissues may exhibit a non-immunogenic "window" in gestation.

Overarching recommendations to the NIH to better coordinate Regenerative Medicine

Interdisciplinary teams and training

1. Encourage and support interdisciplinary, translational research. For functional integration, interdisciplinary teams should involve molecular and developmental biologists interested in applied/translational research, virologists, neuroscientists and

tissue engineers. The NIAMS BIRT award is a good model to build interdisciplinary teams in regenerative medicine (http://grants2.nih.gov/grants/guide/rfa-files/RFA-AR-08-001.html).

2. In addition to CTSA, SBIR/STTR, interdisciplinary conferences, and core centers, prioritization of multi-disciplinary training and translational research is critical. Training awards can encourage co-mentoring or pairing a PI and trainee with different backgrounds. Also multi-disciplinary environments can be fostered in large laboratories containing a wide range of scientific backgrounds, traveling fellowships and an annual workshop for RM fellows; (e.g. NIBIB Trainees workshop).

Coordination of research

3. Create web-based tools for academicians to educate themselves about team based approaches required for regenerative medicine.
4. Help investigators find ways to publish lessons learned and consider those as positive findings for the field and for granting purposes.

Improving the review process to increase innovation

5. Consider anonymous peer review to enhance funding of “outside-the-box” research projects.
6. Study sections should match better to programmatic goals.
7. Better educate reviewers about RFAs and the different review processes, especially for high risk and multidisciplinary research.
8. Fund enabling technology-based, non-hypothesis driven research programs.

Concluding Session
How Can We Better Coordinate and Transform Regenerative Medicine?

The concluding session of the Workshop was moderated by Dr. Jeanne Loring and consisted of a panel discussion with invited comments from Drs. Michael Lysaght, Tony Ratcliffe and Don Fink, as well as audience input, to address the following four overarching questions:

1. How do you build an effective Regenerative Medicine interdisciplinary team?
2. How do you coordinate biological knowledge, engineering technologies, and clinical needs?
3. What information and expertise are needed to effectively translate regenerative medicine research to patients/products?
4. What can the NIH do to Transform Regenerative Medicine?

The panel made specific recommendations that point the way toward transforming regenerative medicine that will require coordination, strategy and communication. When we move from where we are now, with a few regenerative medicine products on the market, to having significantly more regenerative medicine products available to patients, we will know we have achieved true transformation of the field.

Panel discussion and recommendations:
Dr. Loring introduced the session by reading a quote from a patient advocate to remind the group that our mission to transform regenerative medicine for the sake of the patients who suffer from debilitating disease.

The major discussion points and recommendations are provided below by addressing each of the overarching questions.

1. How do you build an effective Regenerative Medicine interdisciplinary team?

The panel members proposed that an interdisciplinary team should include individuals who provide essential scientific expertise, but to be effective the team also must have expert project management. Ideally, a team would all be focused on a single project, would share the same goals, and would be composed of experts in: biology; engineering; medicine/clinical needs; preclinical *in vitro* and animal model studies; analysis and interpretation; leadership and management; regulatory policies and procedures; product development; and business strategies and practices.

These interdisciplinary teams of experts need to meet at the interface and communicate effectively. Interdisciplinary teams and teams of people from different disciplines are two very different things and it is the interdisciplinary teams that need to be emphasized to make transformative changes to the field of Regenerative Medicine.

It was agreed that, in general, engineers and biologists are not currently working together to the extent necessary and that this is a serious obstacle to a field that needs input from both. One key solution might be to provide more opportunities for truly interdisciplinary interactions. The group agreed that a major goal should be to support communication and cross-training of young investigators at the postdoctoral level. One suggestion was the creation of a funding program to support exchange of junior investigators between laboratories with diverse expertise. In addition to an exchange program, the group recommended a one-week course for pre and post docs to learn cross-disciplinary techniques, build professional and personal relationships, and obtain a real experience in multiple fields.

The importance of encouraging cross-training at the pre and post doctoral level was heavily emphasized because it was thought to be the real pivotal point in the career spectrum for developing interdisciplinary teams of the future. Participants also pointed out that NIH could provide seed money to develop a "farm" system for recruiting and training students in interdisciplinary fields. Research institutes that create truly interdisciplinary departments would be the beneficiaries of such funding. It was also suggested that a training mechanism be created that builds on the Howard Hughes Medical Institute model that combines training in science and medicine (http://www.hhmi.org/news/20060215.html) and also permits scientists/engineers (as Ph.D. students and potentially beyond) to have some experiences in the hospital seeing problems first hand as well as brainstorming with clinicians about clinical needs and potential solutions.

Several participants also noted that fitting the clinical translational piece into the team is difficult because it is a challenge to involve clinicians in meaningful laboratory research. There is currently a chasm between basic and clinical research that needs to be crossed. A major issue is not only the "two cultures" but a fundamental contemporary problem in health care reimbursement that requires academic clinician-scientists to "differentiate" into all clinical or all scientist. There is a need to create incentive for clinicians to participate.

Another point that was raised in the discussion was that the community of researchers in regenerative medicine does not communicate well with each other. Though their long term goals are similar, they do not comprise a coherent group and have few opportunities to interact. To emphasize this, it was pointed out the there are currently multiple annual meetings focused on Regenerative Medicine (ex. TERMIS and ISSCR), and different disciplines often populate one meeting and are unaware of the others. It was suggested that there should be one "go to" regenerative medicine international meeting a year and that would bring more of the disciplines together. Another suggestion was that the NIH holds more cross-disciplinary workshops, like this one, where a cross-section of individuals is invited to attend. It was felt that the networking benefits of these types of workshops may even outweigh their formal purpose. The key is in selecting the right cross-disciplinary-minded attendees.

2. How do you coordinate biological knowledge, engineering technologies and clinical needs?

In response to this second question, one strong recommendation was to start by looking at a few of the successful regenerative medicine therapy approaches, some of the in vivo applications that are currently on the path to translation, as well as examples of *in vitro* 3D culture model systems, and learn from these what worked or didn't work and what is needed to successfully coordinate biological knowledge, engineering technologies and clinical needs. However, a word of caution was placed on looking at things that are "currently on the path to translation" because "it ain't over until it's over". In the future, the NIH might also think about performing regular economic surveys of the tissue engineering/regenerative medicine industry.

Exchange of results/experiences between labs and standardization of cell sources, culture protocols, and assessment methods were also considered to be a key to the coordination process. It was agreed that coordination also requires pre-established goals and pathways, teams with strong leadership, management and authority, experts in all disciplines, established project management processes, standards, and the ability to recognize and solve major technical hurdles.

3. What information and expertise are needed to effectively translate regenerative medicine research to patients/products?

In response to this third question, the Workshop participants recognized that there are many practical issues that need to be addressed in order to effectively translate regenerative medicine research concepts into products. Regenerative Medicine researchers, especially academic scientists, must recognize that if a product is to be successfully used in the clinic, it must address a real clinical need and have a long enough product life to be practical to invest in its development. For a product to be transferred to the clinic it needs to be attractive to commercial entities, and have the potential be profitable. With this in mind, expertise in putting together a product development plan (preclinical, regulatory pathway, clinical, process development, manufacturing, IP, marketing) as well as a business plan (realistic, robust) are essential. The NIH should create programs for regenerative medicine that are specifically focused on translation.

Several participants also pointed out that enabling tools and resources would need to be developed to translate research to products. These include methods for cell expansion/characterization, animal models, and functional imaging. National core laboratories could be established for providing quality-controlled cells and biomaterials, relieving individual researchers from the burden of being a supplier.

4. What can the NIH do to transform Regenerative Medicine?

The panel had several important recommendations for the NIH.

First, transformation was largely viewed as moving from where we are now, with a few regenerative medicine products on the market, to having significantly more regenerative medicine products available to patients. To achieve that goal, the panel recommended that NIH consider new funding models that promote more goal-oriented research (ex: Genome Project) with a full team-based approach (expertise in leadership and management, regulatory policies and procedures, clinical needs, product development, business strategies and practices, engineering and biology), and measurable and achievable goals.

Second, the panel specifically recommended that the NIH invest in training courses to teach investigators the skills they need for true interdisciplinary research with emphasis on productive interactions between engineering and the life sciences as well as translation of the research for medical applications.

Third, there was agreement that the NIH consider granting mechanisms that specifically support interdisciplinary research teams. These teams could be two PIs with complementary expertise (e.g. a biologist and an engineer) who are eager to work together but have no practical means to do so. An example would be a short-term grant for pilot studies that specifically require two diverse areas of expertise. Also, since the most innovative approaches may result from collaborations between geographically separate PIs, it would be a valuable feature for such grants to allow or even require exchange of researchers between laboratories and face-to-face meetings between PIs.

Fourth, there was discussion about how the NIH can meet the need for better communication across the regenerative research community. A specific suggestion was that the NIH establish a "virtual network" of investigators in regenerative medicine. The goal of the virtual network of investigators would be to facilitate exchange of published work, information about funding opportunities, and materials. This could be a website or wiki. It was also suggested that this might be done through the Multi-agency Tissue Engineering Science (MATES; www.tissueengineering.gov) Working Group.

In addition to the major recommendations made in response to question 4 summarized above, the following recommendations were emphasized:

- There should be more representation in the NIH intramural program for regenerative medicine and more collaborations with Universities/Institutions

- NIH should promote multi-PI grants (biologist, engineer, and clinician) to a greater extent and applications focused on the development of enabling technologies, new tools, and translation should be emphasized.

- Regular interdisciplinary Regenerative Medicine Workshops, like this one, should be held.

Appendix A: Workshop Planning Committee

Workshop Co-Chairs

Jeanne Loring, Ph.D., Director of the Center for Regenerative Medicine, The Scripps Research Institute

Richard Maas, M.D., Ph.D., Professor of Medicine, Harvard Medical School; Division of Genetics, Brigham and Women's Hospital

Planning Committee Members

Tony Beck, Ph.D., Division for Clinical Research Resources, National Center for Research Resources, National Institutes of Health

Olivier Blondel, Ph.D., Director, Endocrine Systems Biology Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Deborah Carper, Ph.D., Special Assistant to the Director, National Eye Institute, National Institutes of Health

Sherry L. Dupere, Ph.D., Chief, Biology of Development and Aging IRG, Center for Scientific Review, National Institutes of Health

Nancy Freeman, Ph.D., Program Director, Hearing and Balance/Vestibular Sciences, National Institute on Deafness and Other Communication Disorders, National Institutes of Health

Shefa Gordon, Ph.D., Program Analyst, Office of Program Planning and Analysis, National Eye Institute, National Institutes of Health

Susan R. Haynes, Ph.D., Program Director, Division of Genetics and Developmental Biology, National Institute of General Medical Sciences, National Institutes of Health

Tanya Hoodbhoy, Ph.D., Program Director, Division of Strategic Coordination, Office of Portfolio Analysis and Strategic Initiatives, Office of the Director, National Institutes of Health

Rosemarie Hunziker, Ph.D., Program Director, Tissue Engineering and Regenerative Medicine, Division of Discovery Science and Technology, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health

Christine A. Kelley, Ph.D., Director, Division of Discovery Science and Technology, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health

Ronald Kohanski, Ph.D., Program Director, Cardiovascular Biology Program, Aging Physiology Branch, Biology of Aging Program, National Institute on Aging, National Institutes of Health

Nadya Lumelsky, Ph.D., Director, Tissue Engineering and Dental and Craniofacial Regenerative Medicine Program, Integrative Biology and Infectious Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health

Martha Lundberg, Ph.D., Program Director, Tissue Engineering, Advanced Technologies and Surgery Branch, Division of Cardiovascular Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health

Alan Michelson, M.D., Ph.D., Associate Director for Basic Research, Office of the Director; Senior Investigator, Division of Intramural Research; Chief, Laboratory of Developmental Systems Biology, National Heart, Lung, and Blood Institute, National Institutes of Health

David Owens, Ph.D., Program Director, National Institute of Neurological Disorders and Stroke, National Institutes of Health

Neelakanta Ravindranath (Ravi), Ph.D., Scientific Review Officer (DEV2 Study Section), Biology of Development and Aging IRG, Center for Scientific Review, National Institutes of Health

Leslie Reinlib, Ph.D., Health Scientist Administrator, Division of Extramural Research & Training, National Institute of Environmental Health Sciences, National Institutes of Health

Katherine Serrano, **B.S.E.**, Biomedical Engineer, Division of Discovery Science and Technology, National Institutes of Biomedical Imaging and Bioengineering, National Institutes of Health

Jean D. Sipe, Ph.D., Scientific Review Officer, Musculoskeletal Tissue Engineering Study Section, Center for Scientific Review, National Institutes of Health

Sonia I. Skarlatos, Ph.D., Acting Director, Division of Cardiovascular Diseases, National Heart, Lung and Blood Institute, National Institutes of Health

Jonathan Vogel, M.D., Senior Investigator, Dermatology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health

Fei Wang, Ph.D., Director, Musculoskeletal Development, Tissue Engineering and Regenerative Medicine Program, Division of Musculoskeletal Diseases, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health

Daniel Wright, M.D., Senior Scientific Advisor and Program Director, Hematology Research, Division of Kidney, Urologic, and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Da-Yu Wu, Ph.D., Program Director, Genetics and Molecular Neurobiology Research Branch, Division of Basic Neuroscience and Behavioral Research, National Institute on Drug Abuse, National Institutes of Health

Appendix B: Meeting Agenda

Transforming Regenerative Medicine:
An Interdisciplinary Approach

May 19–20, 2008
Bethesda Marriott, Pooks Hill

AGENDA

Day 1: Monday May 19, 2008

7:30 a.m. Continental Breakfast

8:00 a.m. **Welcome and Introductions**

Roderic I. Pettigrew, Ph.D., M.D.
Director, National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health

Christine A. Kelley, Ph.D.
National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health

8:15 a.m. **Symposium Overview, Overarching Questions, and Charge to the Group**
Co-chairs:
Jeanne Loring, Ph.D.
The Scripps Research Institute
Richard Maas, M.D., Ph.D.
Brigham and Women's Hospital, Harvard Medical School

8:45 a.m. **Regenerative Medicine at the NIH**
William J. Heetderks, M.D., Ph.D.
National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health

9:00 a.m. **OPASI and the Role of Trans-NIH Collaboration and Coordination in Regenerative Medicine**
Alan Krensky, M.D.
Office of Portfolio Analysis and Strategic Initiatives
National Institutes of Health

Session 1: Generation and Regeneration: Learning from Nature

9:15 a.m. **Opening Remarks from Session Chair**
Frederick Schoen, M.D., Ph.D.
Brigham and Women's Hospital, Harvard Medical School

9:20 a.m. **Endogenous Bioelectrical Signals as Control Points for Modulating Cell Behavior and Regeneration**
Michael Levin, Ph.D.
The Forsyth Institute for Regenerative and Developmental Biology
Harvard Medical School

9:45 a.m. **Extra- and Intracellular Signaling, and the Regulation of Cell Function**
Christopher Chen, M.D., Ph.D.
University of Pennsylvania

10:10 a.m. Working Break

10:40 a.m. **Harnessing Insights from Developmental and Post-Developmental Human Biology to Transform Regenerative Medicine**
Frederick Schoen, M.D., Ph.D.
Brigham and Women's Hospital, Harvard Medical School

11:05 a.m. **Creating Tissues for Therapeutic Use: Biological Principles and Engineering Designs**
Gordana Vunjak-Novakovic, Ph.D.
Columbia University

11:30 a.m. **Session Concluding Remarks and Panel Discussion**

11:45 a.m. Lunch

Session 2: Cell-Instructive Technologies for i*n vivo* Regeneration

1:00 p.m. **Organ Induction i*n vivo***
Richard Maas, M.D., Ph.D., Session Chair
Brigham and Women's Hospital, Harvard Medical School

1:30 p.m. **Generating Human Induced Pluripotent Stem (iPS) Cells**
Kathrin Plath, Ph.D.
UCLA, California Institute for Regenerative Medicine

2:00 p.m. **Reprogramming Pancreatic Cell Fate i*n vivo***
Quiao (Joe) Zhou, Ph.D.
Harvard University

2:30 p.m. Working Break

3:00 p.m. **Polymers for In Situ Cell Programming**
David Mooney, Ph.D.
Harvard University

3:30 p.m. **Self-Assembly and Supramolecular Structure for Cell Signaling**
Samuel Stupp, Ph.D.
Northwestern University

4:00 p.m. **Day 1 Wrap Up**
Co-chairs

4:30 p.m. Break
5:00 p.m. **Keynote Address:**
Fetal Tissue Engineering for the Treatment of Structural Congenital Anomalies
Dario Fauza, M.D.
Children's Hospital Boston

6:00 p.m. Adjourn for the day

7:00 p.m. Group dinner at local restaurant

Day 2: Tuesday May 20, 2008

8:00 a.m. **Charge for Day 2**
Co-chairs

Session 3: 3-D Engineered Tissue *in vitro* and i*n vivo*

8:15 a.m. **Overview: 3D Tissues for Transforming Regenerative Medicine**
Gordana Vunjak-Novakovic, Ph.D., Session Chair
Columbia University

8:20 a.m. **Cell-Instructive Technologies for i*n vivo* Regeneration**
Larry Lasky, M.D.
The Ohio State University

Progress and Challenges for Large-Scale Erythroid Development from Human Embryonic Stem Cells
Dan S. Kaufman, M.D., Ph.D.
University of Minnesota

9:00 a.m. **Translating What We Know About Liver Biology to Achieve Functional Authenticity *in vitro* – What Does Hepatocyte Function Really Represent?**
Nancy Parenteau, Ph.D.
Parenteau Bioconsultants, LLC

Sangeeta Bhatia, M.D., Ph.D.
Massachusetts Institute of Technology

9:40 a.m. **Self-Assembling Nanofibers for Regeneration in the Central Nervous System**
Sam Stupp, Ph.D.
Northwestern University

10:00 a.m. **Panel Discussion**

10:20 a.m. Break

Session 4: **Functional Integration of Engineered Tissues: More Than the Sum of Its Pieces**

10:45 a.m. **Functional Integration of Engineered Tissues: An Overview**
Rocky Tuan, Ph.D., Session Chair
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health

10:55 a.m. **Axon Regeneration in the Optic Nerve**
Rocky Tuan, Ph.D.
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health

11:15 a.m. **Vascular Functional Integration: Take Heart**
Doris Taylor, Ph.D.
University of Minnesota

11:35 a.m. **Functional Tissue Engineering of Articular Cartilage**
Farshid Guilak, Ph.D.
Duke University

11:55 a.m. **Immunological Barriers to Transplantation and Regeneration of Tissues**
Jeffrey Platt, M.D.
University of Michigan

12:15 a.m. **Panel Discussion**

12:45 p.m. Lunch

2:00 p.m. **Concluding Session**
Michael Lysaght, Ph.D.
Brown University

Donald W. Fink Jr., Ph.D.
Center for Biologics Evaluation and Research
Food and Drug Administration

Anthony Ratcliffe, Ph.D.
Synthasome, Inc.

3:30 p.m. Adjournment

Appendix C: Invited Speaker Listing

Larry Benowitz, Ph.D.
Children's Hospital Boston
Harvard Medical School

Sangeeta Bhatia, M.D., Ph.D.
Massachusetts Institute of Technology

Christopher Chen, M.D., Ph.D.
University of Pennsylvania

Dario Fauza, M.D.
Children's Hospital Boston

Donald W. Fink Jr., Ph.D.
Center for Biologics Evaluation and Research
Food and Drug Administration

Farshid Guilak, Ph.D
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National Institute of Biomedical Imaging and Bioengineering
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