Cardiovascular Tissue Engineering Research Support at the National Heart, Lung, and Blood Institute

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Abstract: Tissue engineering aims at building 3-dimensional living substitutes that are equal to or better than the damaged tissue to be replaced. The development of such a tissue replacement requires a multidisciplinary approach and careful attention to the optimal cell source, the interactions of growth factors and extracellular milieu, and the scaffolding design. This article is a review of the tissue engineering programs of the National Heart, Lung, and Blood Institute, which support research efforts to translate novel approaches for the treatment of cardiovascular disease. Recent progress is discussed, which highlights some major questions relevant to cardiovascular tissue engineering. The National Heart, Lung, and Blood Institute has a strong interest in tissue engineering and will continue to foster the practical, clinical, and commercial development of research discoveries in this emerging field. (Circ Res. 2013;112:1097-1103.)

Key Words: biomaterials • cardiovascular • regenerative medicine • stem cells • tissue engineering

Over the last 40 years, death rates from cardiovascular diseases (CVD) have declined significantly because of advances in medical treatment options and improved healthcare. Americans are living longer, healthier lives with CVD but along with this reality comes additional consequences. For example, the total economic cost of CVD in the United States rose to ~$298 billion in 2008\(^1,2\); CVD is becoming a chronic and expensive ailment, which is compounded by an aging population. Our ability to achieve alternative and more cost-effective therapies is an urgent need, especially for the treatment of atherosclerosis, peripheral arterial disease, and congestive heart failure. Recently, improved understanding of cells, tissue properties, and specialized biomaterials has enabled teams of scientists and engineers to create tissue substitutes destined for heart valve repair, reduction of scarring after myocardial infarction, blood vessel replacement, and correction of pediatric heart malformations. Development of novel tissue engineering strategies for heart failure and vascular disease is a research priority for the National Heart, Lung, and Blood Institute ([NHLBI], http://www.nhlbi.nih.gov/about/strategicplan/documents/StrategicPlan_Plain.pdf).

The Multi-Agency for Tissue Engineering Science (MATES) Interagency Working Group defines tissue engineering and science as the use of physical, chemical, biological, and engineering processes to control and direct the aggregate behavior of cells (http://www.tissueengineering.gov/advancing_tissue_science_&_engineering.pdf). At the core of tissue engineering is the construction of 3-dimensional (3D) tissues using biomaterials. These materials provide overall mechanical support and cell-instructive cues to guide cell growth and tissue construct stability. Tissue engineering, often used synonymously with regenerative medicine, endeavors to combine the use of cells, engineering, and biomaterials to empower the body’s own repair mechanisms to heal damaged tissues or organs.

Since Weinberg and Bell’s first described the idea of growing a living blood vessel in 1986, the field of tissue engineering has grown tremendously. For example, healthcare is already being changed by tissue engineering approaches; a search on tissue engineering in ClinicalTrials.gov brings up almost 50 trials, primarily in the eye, bone, cartilage, and dental fields (http://clinicaltrials.gov/). Clinical studies are also beginning to emerge in the cardiovascular, liver, kidney, and endocrine areas.

At the National Institutes of Health, a survey of NHLBI-specific projects in tissue engineering was conducted using the electronic research administration system. Overall, this survey showed that NHLBI supports >200 projects in tissue engineering and regenerative medicine, which represents ~$96.9 million in funding for fiscal year 2012. The NHLBI portfolio is spread over a number of mechanisms, including Small Business Innovation Research (R42, R44) but >50% fall in the R01 category. There is a sizeable training component in the F, K, and T mechanisms. Comparable data can also be obtained using the...
**Nonstandard Abbreviations and Acronyms**

CVD  cardiovascular disease  
ECM  extracellular matrix  
hESCs  human embryonic stem cells  
MATES  Multi-Agency for Tissue Engineering Science  
NHLBI  National Heart, Lung, and Blood Institute  
3D  3-dimensional

public domain National Institutes of Health Research Portfolio Reporting Tools system (http://www.projectreporter.nih.gov/reporter.cfm). The purpose of this article is to describe NHLBI-supported cardiovascular tissue engineering research programs, discuss recent advances made possible by federal-wide collaboration through the MATES interagency working group (http://www.tissueengineering.gov/), and summarize some of the major questions that may impact the future directions of this field.

**Innovative Technologies for Engineering Small Blood Vessels**

Demand for alternative vascular conduits has been driven by the poor clinical efficacy of existing synthetic grafts for small diameter artery applications in patients who lack adequate saphenous veins. To address this demand, NHLBI released a targeted initiative, Innovative Technologies for Engineering Small Blood Vessels. Four R01 grants were funded for a total of $13.7 million for 5 years in 2006 (http://grants.nih.gov/grants/ guide/rfa-files/rfa-hl-05-013.html). The major objective of this program was to apply a broad range of multidisciplinary approaches to conduct research, and to design, fabricate, and engineer small (<5.0 mm) blood vessel substitutes with improved biocompatibility and durability. The anticipated outcome was to create an environment for establishing optimal conditions (eg, cell types, protocols, animal models, and assessment tools) for vessel replacement therapy in humans. Investigators who were funded under this program have accomplished significant preclinical proof of principle studies in animals, and these novel technologies are being further explored for translation into human use by industry. As of October 2012, this program has stimulated the tissue engineering area by producing 72 publications in peer-reviewed journals, which have been cited >860 times by others (excluding review articles).

**Enabling Technologies for Tissue Engineering and Regenerative Medicine**

One ultimate application of tissue engineering is to develop strategies for in vivo regeneration to permanently restore function to compromised tissues in humans but a more near-term application is to develop in vitro disease models for drug testing. However, a critical roadblock is the difficulty in unifying a broad array of disciplines and applying focused tools from developmental and cell biologists, geneticists, clinicians, engineers, materials scientists, and mathematicians to better understand tissue formation. In particular, there is a critical need for multidisciplinary teams to work together to identify the mechanisms of cellular behavior that affect tissue dynamics.

In collaboration with the MATES working group, NHLBI cosponsored another program, Enabling Technologies for Tissue Engineering and Regenerative Medicine, and solicited for grant applications (http://grants.nih.gov/grants/guide/pa-files/par-06-504.html) in an effort to cross-fertilize the field with other scientific disciplines. In 2007, funding support for 7 R01 multidisciplinary research projects began, and our commitment totals $14.1 million. Active support continues through 2014. The overall outcome for this transagency effort was to promote collaborative development of new technologies, tools, methods, and devices that enable the engineering of functional tissues. As of October 2012, the NHLBI-funded investigators supported by this program have published 122 articles since 2007 on their findings, often in high-impact journals.

**New Strategies for Growing 3D Tissues**

Although the long-term goal of tissue engineering is to enhance in vivo regeneration, a short-term opportunity is to improve our understanding of how cells respond structurally to the features of their environment, and to develop accurate approaches that may guide the creation of 3D engineered cellular aggregates. Consistent with this short-term goal, the NHLBI initiated a new program in 2011 entitled, New Strategies for Growing 3D Tissues (http://grants.nih.gov/grants/grants/guide/rfa-files/RFA-HL-11–025.html, and http://grants.nih.gov/grants/grants/guide/rfa-files/RFA-HL-11–026.html). The anticipated outcome for this program is to demonstrate reproducible recapitulation in the laboratory for events, such as differentiation, proliferation, migration, and maturation. This program is jointly funded with the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the National Institute of Biomedical Imaging and Bioengineering. Total support for 12 projects shared between the 3 institutes is $18.4 million. Of the 12 projects funded, key multidisciplinary research questions being addressed include how cells form a 3D structural response to the dynamic elements of humanized heart (7), lung (3), blood (1), and musculo-skeletal (1) biomatrices, as well as the design of bioreactors that will sustain the growth, development, and vascularization of functional human tissues. Funding support for the R01 projects continues through 2015 and is expected to make significant advancements in this area.

**NHLBI Support for Federal-wide Efforts in Tissue Engineering Research**

Parallel to efforts at the NHLBI, a federal-wide strategic plan was developed by the MATES Interagency Working Group regarding tissue science and engineering. Since the term tissue engineering was initially defined in 1988,4 several fundamental questions about how cells work within engineered matrices still remain unsolved. The MATES plan laid out the priorities and implementation steps for Federal Government agencies to focus their activities to have the greatest impact in advancing tissue science and engineering. New innovations that address understanding the cellular response, formulating biomaterial scaffolds and the tissue matrix environment, and developing enabling tools are recognized needs to launch tissue engineering applications into an increasingly sophisticated medical marketplace.

An explosion of interest and research in tissue engineering has occurred during the last 10 years, and the NHLBI has
played a critical role in accelerating our understanding of the field. First generation tissue-engineered products have been designed as simplified model systems to answer fundamental cell biology and engineering questions needed for moving to the next level of functional organ complexity. Additionally, stem cell research has contributed much toward the scientific underpinnings of cell biology and engineering, as well as providing reliable, robust sources of characterized cells. Many near-term challenges and opportunities for integrating current knowledge and best practices are now being sought, so that gap areas can be rapidly identified and addressed. Recent examples of where the NHLBI has funded the growth of federal-wide strategic research areas, as described in the MATES plan, are provided in 3 broadly defined categories below.

Understanding the Cellular Response
An important challenge for the field of tissue engineering and science is to model the normal process by which cells self-assemble into tissues and then organs; in general, the cell is considered the central functional unit within a given organ system. Cells receive messages from physical, chemical, and electric sources, each of which begins a cascade of internal cellular events that ultimately determines phenotypic change. Cells also share information by releasing molecular signals that regulate their own behavior and that of nearby or even distant cells.

In the cardiac regeneration field, the biological complexity of the tissue has driven researchers to focus efforts toward that of in vivo repair as opposed to replacement. Animal studies are in progress to understand the ability of human embryonic stem cells (hESCs)—derived cardiomyocytes to repair injured hearts. Shiba et al6 used a guinea-pig model to show that transplanted heart cells, grown from human stem cells and delivered with a prosurvival cocktail of Matrigel, insulin-like growth factor-1, and multiple cell death pathway inhibitors, electrically coupl and beat in sync with the own muscle of the heart. More surprising is that with transplantation of the cells, the overall incidence of arrhythmia was lower, an effect that may be clinically useful if shown successful in larger animals (Figure 1).

Furthermore, matrices that promote differentiation and proliferation of stem cells for use in tissue engineering are of great interest. For example, hESCs have been used to promote neovascularization and myogenesis in the areas damaged by a heart attack; however, because of minimal cell-based retention, more suitable biomatrices are needed to improve survival and integration of cells transplanted into the host tissue. Duan et al7, funded under PAR-06-504, aim to further our understanding of hESCs in a 3D environment to determine whether native cardiac extracellular matrix (ECM) hydrogels can drive differentiation of hESCs into cardiac lineages, and whether the use of cardiac ECM hydrogels can alleviate the need for supplemental growth factors. The authors show that hydrogel with a high ECM content (75% EM, 25% collagen) increased the fraction of hESCs expressing cardiac marker troponin T, improved mature striation patterns, and improved overall contractile function. These results serve as the basis for future studies of the mechanisms by which the native ECM hydrogel regulates cardiomyocyte phenotype and the use of the native ECM hydrogel as a cell delivery vehicle for heart repair.

Formulating Biomaterial Scaffolds and the Tissue Matrix Environment
Because of advancements in materials science, there are a wide variety of synthetic and natural polymers that may be used for tissue scaffolds. Increasingly, materials are being selected based on the properties for a particular application. Scaffold porosity and matrix stiffness are both important considerations for structural integrity, cellular infiltration, and final maturation of the construct. Following up on studies that demonstrated the clinical feasibility of engineered vascular grafts in humans, Hibino et al8 developed a tissue-engineered vascular graft composed of a biodegradable, polyglycolic acid scaffold with autologous bone marrow–derived mononuclear cells. The authors demonstrated in mice that bone marrow is not a significant source of endothelial or smooth muscle cells in the formation of neovessels, and further, that the adjacent vessel wall is the principal source of cells, making up 93% of the proximal neotissue. The authors conclude that in this setting, the tissue-engineered construct functions by mobilizing the innate healing capabilities of the body to regenerate neotissue from preexisting committed tissue cells. This technology is currently in clinical trial to evaluate the safety and growth potential in children undergoing surgery for congenital heart disease.

A major limitation for use of tissue-engineered vascular grafts is that it often requires months-long processing to culture a functional and mechanically robust arterial prosthesis that are suitable for repair. Recently although, Quint et al9 funded under RFA-HL-05-013 showed that 1 mm decellularized human tissue–engineered vessels can be used as a biological graft that resists both clotting and intimal hyperplasia. Their results demonstrated that engineered vessels can be grown from banked cells, rendered acellular, and then be used for tissue regeneration in vivo. Even more remarkable was that the decellularized human tissue–engineered vessels were found to have recruited endogenous cells that included a confluent endothelium and coaxial subendothelial smooth muscle cells (Figure 2). Their results offer an inventive solution to the widespread scarcity of vessels available for revascularization procedures. Moreover, they show that engineered vessels can feasibly be produced offline, not requiring cells from the actual recipient, and thereby reducing the waiting time for the production of the vascular substitute.

There are ≈80 000 heart valve replacements or repairs performed in the United States each year, however, mechanical or bioprosthetic devices for heart valve disease are associated with significant drawbacks, such as the fact that they cannot grow, remodel, or repair in vivo. The field of tissue engineering has emerged as an exciting alternative in the search for improved heart valve replacement structures, especially for those individuals who cannot receive conventional therapy. For example, studies by Tseng et al10 are exploring the fabrication of trilayer hydrogel quasilaminates. This novel approach looks at the challenge of layer-specific valve mechanical properties for the purpose of tailoring matrix-specific formulas for cell encapsulation. In another study, Tedder et al10 describe the development of 2 novel types of acellular collagen scaffolds, one scaffold was designed to mimic natural valve fibrous layers, and the other scaffold was developed to mimic the delicate and
highly hydrated spongiosa layer. Human bone marrow stem cells were seeded onto both scaffolds. Trilayered constructs were created using a cell-seeded spongiosa scaffold sandwiched between 2 fibrous scaffolds using a protein-based glue and then placed into anatomically analogous 3D heart valve shapes. The valves were conditioned in bioreactors to induce cellular differentiation, and cell viability was assessed after 8 days. To evaluate biocompatibility, the structures were implanted subdermally in juvenile rats and showed good integration after 5 weeks. These examples are foundational toward the development of new approaches to reduce the burdens associated with conventional, mechanical, and bioprosthetic (animal or human) heart valve replacement therapies.

**Developing Enabling Tools**

Tissue development is the result of molecular and supramolecular interactions, and great progress has been made toward that understanding; yet additional research is needed to improve our appreciation between living and nonliving components of engineered tissues. Reliable protocols and tests, as well as improved real-time imaging, are required for collecting data that are comparable, and to inform predictive models for assessing the state of engineered tissues.

Currently, studies of engineered tissues often are conducted by implantation of the construct into an animal; however, this approach can be inefficient because of inadequate vascularization. Vascularization is a limiting factor because of...
the role of oxygen and nutrient diffusion limitations within bulk engineered tissues, which underlies the importance of preformed vasculature. In engineered tissues with preformed vascular networks, this is attributable to the inability of preassembled vascular networks to connect with the host’s microcirculation. To overcome this limitation, Kang et al.\textsuperscript{11} has developed a technology for generating vascularized tissue–engineered constructs. In this study, the authors designed a transplantation model in immunodeficient mice, which consisted of human endothelial colony forming cells and mesenchymal progenitor cells in Matrigel. The cell-containing Matrigel implants were harvested for microvessel density analysis or transplanted into secondary mice at day 7. In vivo labeling of human-specific cells with fluorescently conjugated lectins allowed for the visualization of functional connections between bioengineered and host vessels. Importantly, their results show that prebuilt perfused human vessels are transplantable from one in vivo site to another (Figure 3).\textsuperscript{11} Kang’s work extends the range of applications of cell-based technology for transplantable tissue–engineered constructs. NHLBI looks forward to the expansion of this field using new approaches for scaffold design.

One advantage of studying cell behavior at the microscale level is to identify mechanisms of in vivo multicellular migration.\textsuperscript{12,13} Hydrogels represent a mostly biocompatible class of materials that are capable of mimicking the basic, 3D properties of native tissues. Their properties can be tailored to enable precise control of surface features to study cell behavior mechanisms in vitro. For example, Du et al.\textsuperscript{14} developed a clever method using hydrophilic microgels (20% [wt/wt] poly(ethylene glycol)-diacrylate) mixed with a photo-initiator and applied to a glass slide as a way to study cells discretely. Specifically, a drop of photocrosslinkable polyethylene glycol diacrylate prepolymer was pipetted onto a glass slide. A cover glass slide was applied on top of the solution drop, which formed an evenly distributed film of prepolymer solution. A photomask was put on top of the cover slide, and microgels were formed by exposing the prepolymer solution to ultraviolet light. The resulting hydrogel provided a platform for the study of cell behavior with high spatial resolution.

**Table. Some Major Questions in Cardiovascular Tissue Engineering**

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<thead>
<tr>
<th>Domain</th>
<th>Question</th>
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<tbody>
<tr>
<td>Soft tissues</td>
<td>What is the best way to establish a blood supply to a tissue-engineered construct, such as a cardiac patch?</td>
</tr>
<tr>
<td>Cells</td>
<td>How do very few hESC–CMs surviving in the heart affect ventricular arrhythmia? Are patient-specific (autologous) approaches best, or can we address issues with allogenic donors?</td>
</tr>
<tr>
<td>Imaging</td>
<td>What approaches and methods are best to assess viability and function of an engineered tissue in vivo?</td>
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hESC–CM indicates human embryonic stem cells–cardiomyocytes.
light through the photomask. The glass slide of prefabricated array of microgels was immersed in mineral oil, and the microgels were assembled into tubular structures by swiping a needle underneath them. The assembly was subsequently stabilized by a secondary crosslinking step. By varying the internal designs of each individual microgel, the sequential assembly allowed for the creation of vascular-like microchannels with a circular lumen and was interconnected in a network to model the bifurcating structure of native vasculature. This simple yet elegant design may be used as a powerful research tool for building biomimetic tissue models, such as for studying cardiac progenitor cells differentiated to mimic native muscle fibers.

Future Directions
The use of tissue engineering approaches for the treatment of CVD holds significant promise for new therapeutic approaches, especially given the growth of nanoscale approaches for biomaterial design. Further exploration of stem cell technologies, particularly the use of cells differentiated from hESCs and induced pluripotent stem cells, and the creation of a suitable microenvironment for long-term tissue formation are directions likely to advance our understanding of how tissues repair and regrow. These advancements should lead to improved options for arterial revascularization, heart valve repair, arrhythmias, and congenital malformations in pediatric populations.
However, the practicality of implementing clinically viable tissue-engineered constructs on a day-to-day level will require answers to some of the major questions and provocative issues facing this field (Table). Some of these concerns are hampered by research gaps, which include: (1) validated biomarkers to identify the intracellular machinery that guides cells to become functional tissues; they are also required to assess the immunologic requirements and fate of engineered tissues over time; (2) quantitative biological assays to improve the assessment of tissue-engineered construct safety, function, and stability that assist the development pathway and regulatory acceptance of these products; and (3) computationally efficient methods and models, to link gene and protein dynamics to cellular phenotypic outcomes. Additional constraints are universal standards and protocols for long-term storage of cells and tissue constructs, and standardized clinical and preclinical approaches consistent with regulatory requirements. Perhaps the greatest roadblock to therapeutic application is how to combine the vast number of parameters that influence 3D biological responses to produce the necessary outcome.

In terms of NHLBI support for tissue engineering research, the RFA-HL-11–025, New Strategies for Growing 3D Tissues program has 3 more years to run. It is hoped that this program, together with other Agency efforts, will provide a robust scientific underpinning to aid tissue engineering approaches for heart, lung, and blood diseases. Leveraging both nano- and microsystem technologies will lead to innovations for the treatment of disease in vivo and may serve as a conduit to spur corporate growth activity in this area. For example, microsystems could be useful for the identification of regeneration-inducing ligand systems to treat chronic inflammation and ischemia or potentially enable the monitoring of real-time angiogenesis. Strategies that improve nondestructive assessment tools for regenerative medicine remain an important goal (http://www.nist.gov/mml/bbd/biomaterials/functional_imaging_regenerative_medicine_workshop.cfm). It is anticipated that these efforts will be highly interactive with discovery science and clinical research and thereby, provide design blueprints for realistic expectations and marketplace needs for tissue-based tools and approaches.

Summary

Considerable progress has been made in the field of tissue engineering, however the search for the best technologies to create functional cardiovascular tissues are still being investigated. The targeted research programs of the NHLBI are aimed toward improving the understanding of the physical, electric, and chemical factors that affect 3D cellular structures and transferring that knowledge into the testing and translation of novel CVD treatments. As a medical treatment concept, tissue engineering aims to control cell phenotype and direct tissue formation that is functionally equal to or better than the tissue to be replaced. The development of such a tissue replacement will require careful attention to the cell/growth factor/matrix interactions, optimal cell sources, and the design of biomimetic scaffolds. The NHLBI has a strong interest in tissue engineering and will continue to foster the practical, clinical, and commercial development of research discoveries in this emerging field. Future steps will be taken to identify opportunities for leveraging between the research priorities of the NHLBI and other Federal initiatives.

Additional information regarding the research funded at the NHLBI can be found at www.nhlbi.nih.gov and by using the National Institutes of Health Research Portfolio Reporting Tools available at: http://www.projectreporter.nih.gov/reporter.cfm.

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Disclosures

None.

References

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