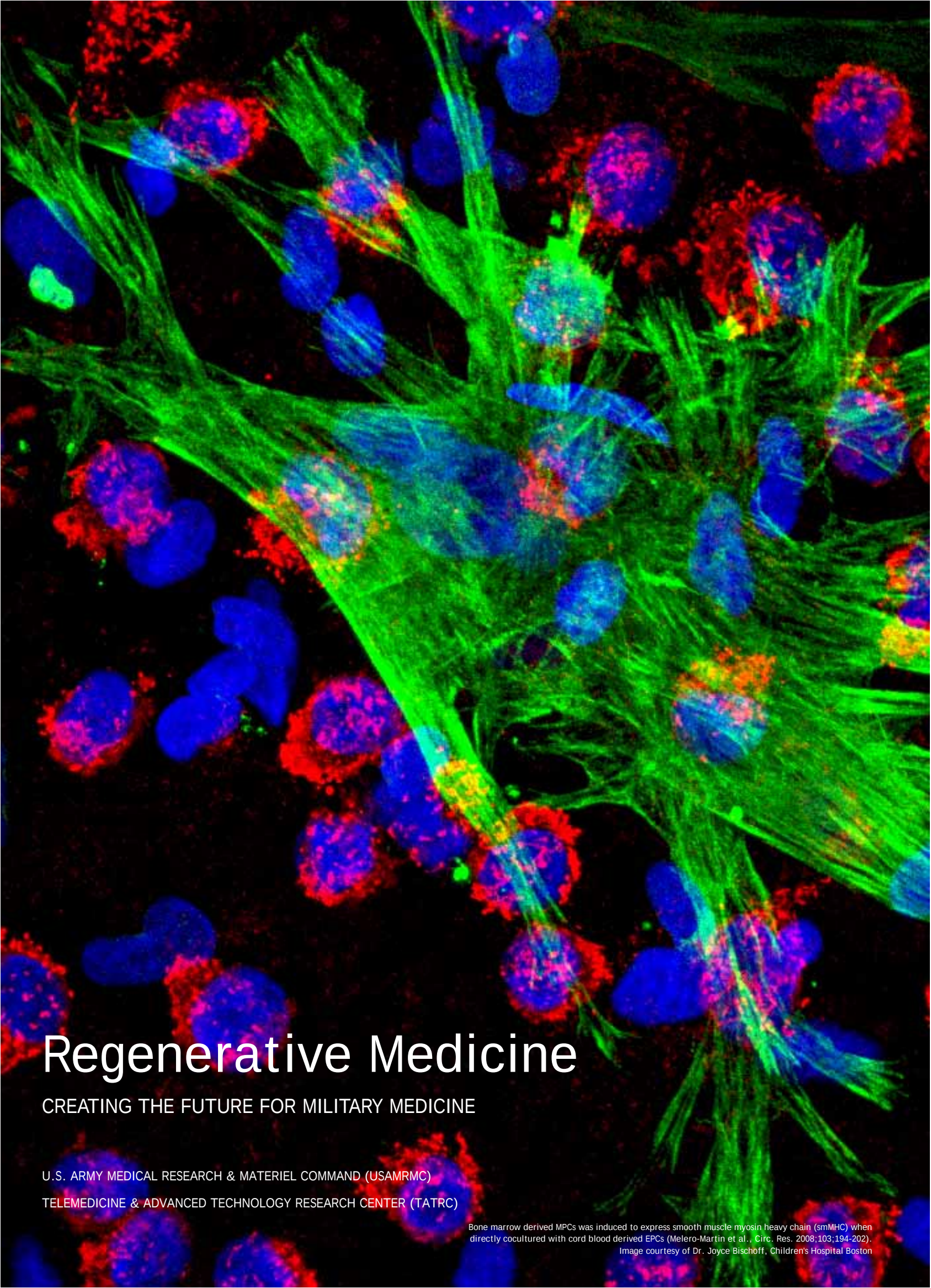




Regenerative Medicine

CREATING THE FUTURE FOR MILITARY MEDICINE

U.S. ARMY MEDICAL RESEARCH & MATERIEL COMMAND (USAMRMC)

TELEMEDICINE & ADVANCED TECHNOLOGY RESEARCH CENTER (TATRC)

Bone marrow derived MPCs was induced to express smooth muscle myosin heavy chain (smMHC) when directly cocultured with cord blood derived EPCs (Melero-Martin et al., Circ. Res. 2008;103:194-202).
Image courtesy of Dr. Joyce Bischoff, Children's Hospital Boston

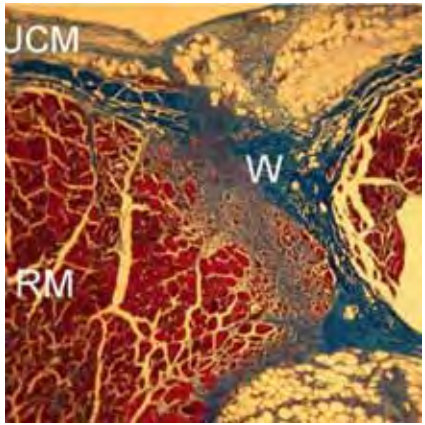
Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE AUG 2009		2. REPORT TYPE		3. DATES COVERED 00-00-2009 to 00-00-2009	
4. TITLE AND SUBTITLE Regenerative Medicine: Creating the Future for Military Medicine				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Army Medical Research and Materiel Command ,Telemedicine & Advanced Technology Research Center (TATRC),Fort Detrick,MD,21702				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 16	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

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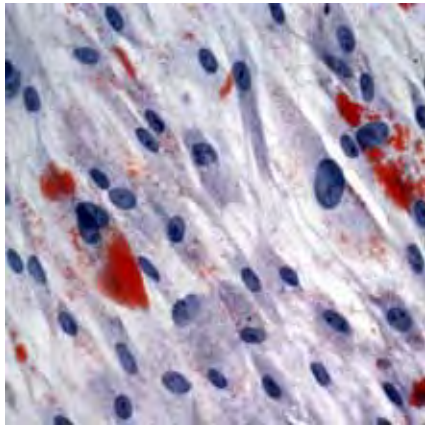
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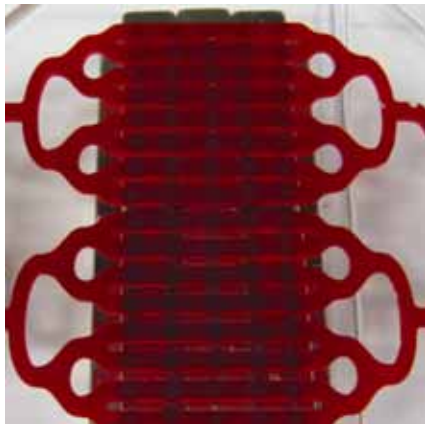
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“The future of regenerative medicine is truly amazing. We’re embarking on a new generation of research that’s going to redefine Army and military medicine as we know it today.”

Lieutenant General Eric B. Schoomaker, The Army Surgeon General, April 2008

A Highlight on the TATRC Regenerative Medicine Portfolio

The Telemedicine & Advanced Technology Research Center (TATRC) has been funding and managing research in the area of regenerative medicine and tissue engineering since 2001. Starting with research in understanding the healing and regeneration of bone in murine models, creating gene- and cell-based therapies to improve healing of soft and hard tissue injuries and developing cell sorter technologies, to name a few. Over the years, the number of projects in this area has grown significantly as the number of injuries and in particular, the increasing severity of injuries resulting from the Global War on Terrorism (GWOT). Thus, military medicine is being challenged from the frontline, looking at how to stabilize and transport the patient quickly without negatively impacting their reconstructive surgeries to rebuilding the injured service member whole again both in appearance and in functional outcome. Due to the complexities of these injuries where large composite tissues are severely damaged and/or missing, development of novel treatment options based on regenerative medicine and tissue engineering concepts is an attractive option compared to the alternatives such as forced amputations due to lack of functional recovery or prosthesis for the resulting amputated limb. In cases of trauma to the face, oftentimes multiple surgeries are required and do not result in pleasing appearance, appropriate plasticity, and/or function.

Currently, the Regenerative Medicine portfolio has over 70 unique active projects totaling more than \$115M in investments to date and includes almost 20 additional projects, totaling at least \$25M in funding that fall under other TATRC portfolios/programs such as Neurotrauma and Nano-Medicine & Biomaterials. Funding and management of these projects have successfully paved the way for many investigators to understand the military challenges in developing innovative medical treatments for traumatic injuries. In 2007 TATRC also supported the U.S. Army Medical Research and Materiel Command (USAMRMC), including the Research Area Directorates (RAD) and the U.S. Army Institute of Surgical Research (USAISR) on its first major initiative in advancing innovative medical treatments for wounded warriors with its successful launch of the Armed Forces Institute of Regenerative Medicine (AFIRM) in 2008.

Purpose

The Regenerative Medicine portfolio aims to invest in and manage research projects and programs that will benefit service members with traumatic injuries by restoring them to the fullest function possible. The current GWOT may have the lowest lethality of war wounds among U.S. soldiers (10%) compared to past wars, due to advances in body armor, medical evacuations, and battlefield surgeries (Gawande, *N Engl J Med* 2004). These advances, however, only protect the torso and its major organs. Hence, a significant number of U.S. service members suffer devastating extremity injuries including the head, face, neck, and limbs. In fact, extremity trauma accounts for 82% of war injuries (Oct 2001 – Jan 2005), often severe and with multiple injuries to the arms and legs that include damage to nerves, tendons, muscles, vessels, bones and soft tissues; head and neck trauma accounts for 21% (Xydakis et al, *Otol Head Neck Surg* 2005; Brennan, *Otol Head Neck Surg* 2006; Wade et al *J Trauma* 2007).

Portfolio Strategy

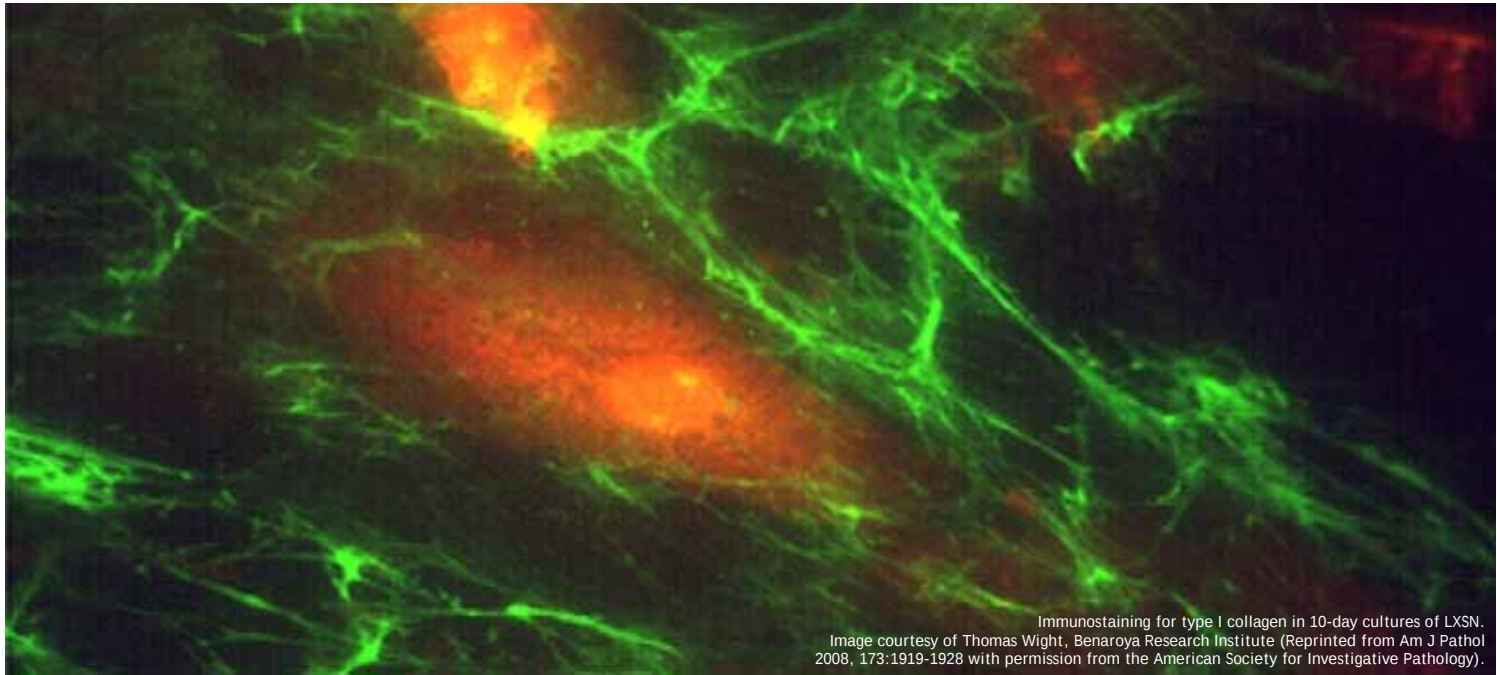
The Regenerative Medicine portfolio strives to support TATRC’s mission through a synergistic research planning and investment strategy. This strategy includes partnerships with relevant government agencies and programs such as the U.S. Army Institute of Surgical Research (USAISR), Clinical and Rehabilitative Medicine Research Program (CRM RP), Combat Casualty Care Research Program (CCCRP), Armed Forces Institute of Regenerative Medicine (AFIRM), Defense Advanced Research Projects Agency (DARPA), NIH National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Department of Veteran Affairs, and many other Federal programs/agencies, resulting in an elaborate Federal and Department of Defense interagency network of communication and coordination. Through these partnerships, TATRC’s Regenerative Medicine portfolio aims to improve and excel the research of its funded partners in academia, nonprofit research institutes, and/or small/large businesses by identifying and facilitating appropriate partnerships as well as recommending more targeted military-relevant problems to address.

Strategic Enabled Objectives

This strategy enables TATRC to actively and strategically direct Congressional Special Interest projects to address the unmet, unsolved, and highly challenging problems of treating traumatic injuries resulting from service to our country – one of the major objectives for this research portfolio. It ensures complementary research initiatives can be leveraged, such as directing some overlapping research areas that tackle similar or the same problems with different approaches – a second objective of this research portfolio. It also enables TATRC to take calculated risks in funding high risk, highly innovative concepts through small pilot projects – a third objective of this research portfolio. Discoveries and innovative developments resulting from TATRC’s Regenerative Medicine portfolio benefit both the service members and the public at large – a fourth objective of this research portfolio. A long term objective for this research portfolio is to invest in and manage research that will achieve the Armed Forces’ goal to return injured service members to full function or to minimize their disabilities. In summary, this portfolio strategy identifies research gaps, minimizes research overlaps, directs research for high value and utility, brings collaborators together, and builds partnerships. One key characteristic of this strategy is fast, effective decisions can be made to advance research including taking calculated risks on highly innovative, out-of-the-box ideas.

Contents & Organization

There are many ways to organize the projects. Here the Regenerative Medicine portfolio is simply divided into 5 major categories including Cell Biology, Therapeutics, Tissues & Organs, Technologies, and Enabling Tools. Most projects, in particular research programs, may encompass more than one of these categories.



Cell Biology

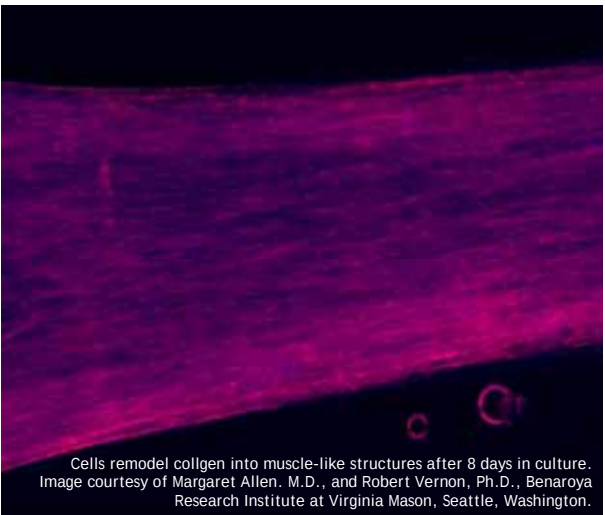
UNDERSTANDING THE FUNDAMENTALS TO
UNCOVER DISCOVERIES AND MAKE ADVANCES

Advances and discoveries in stem cell science and tissue engineering are made through understanding of the biology behind how cells communicate, grow, differentiate, organize, and react/interact with the environment.

Controlling the Body’s Responses toward Functional Healing and Regeneration

Investigators at Benaroya Research Institute in Seattle, Washington have created a research program to evaluate for ways to control inflammation and tissue repair with the goal to develop new therapies to regulate these natural reparative processes, specifically to redirect the body’s responses to injury toward functional healing and regeneration. This program consists of several projects and spans across almost all of the portfolio categories since information gained from the study of inflammation and cell response could be used to design novel therapeutics as well as for controlling tissue regeneration.

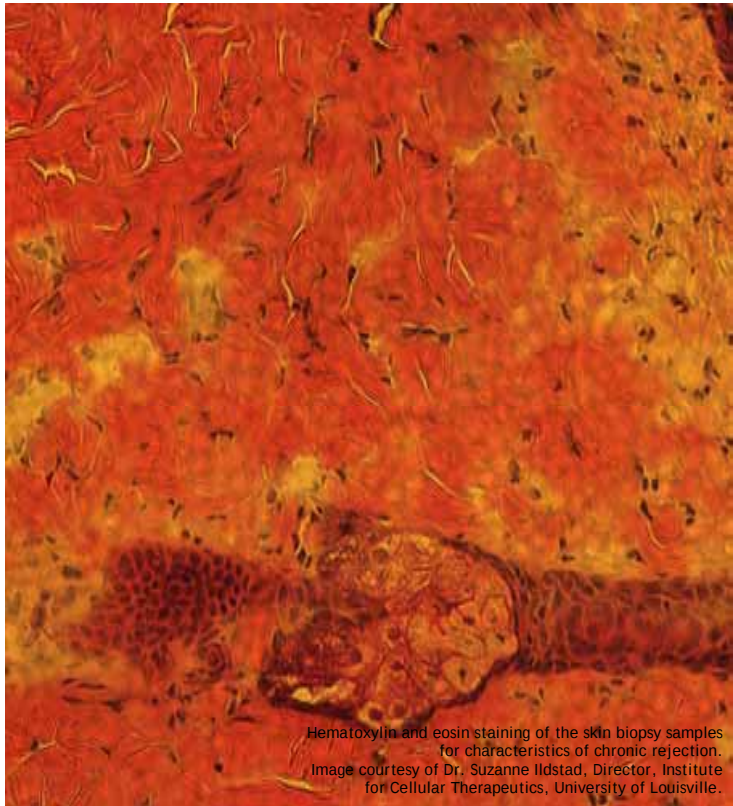
In one thrust area, the investigators are looking at how to utilize signaling mechanisms to regulate inflammatory and fibrotic processes to direct cell behavior and orchestrate tissue repair in acute and chronic disease. For example, studying the role of the matricellular protein HEVIN and its role in inflammation and wound repair could help understand the factors that regulate inflammatory processes and use this information to guide the development of novel therapeutic treatments aimed at manipulating cellular trafficking to direct healing responses toward defined positive outcomes. As a case in point, a unique population of T cells was found that respond to acute inflammation and then elaborate chemokines to attract further inflammation-suppressing cells to migrate to the site, a natural mechanism for the resolution of inflammation that might be exploited for therapeutic use (Koch MA et al. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nature Immunology 10(6): 595-602, June, 2009). Another project looks at how neuronal injury and survival depend on interactions between the motor neuron and the surrounding cellular matrix compartments in transgenic models of amyotrophic lateral sclerosis (ALS), where the information could be used to develop therapeutic targets to improve



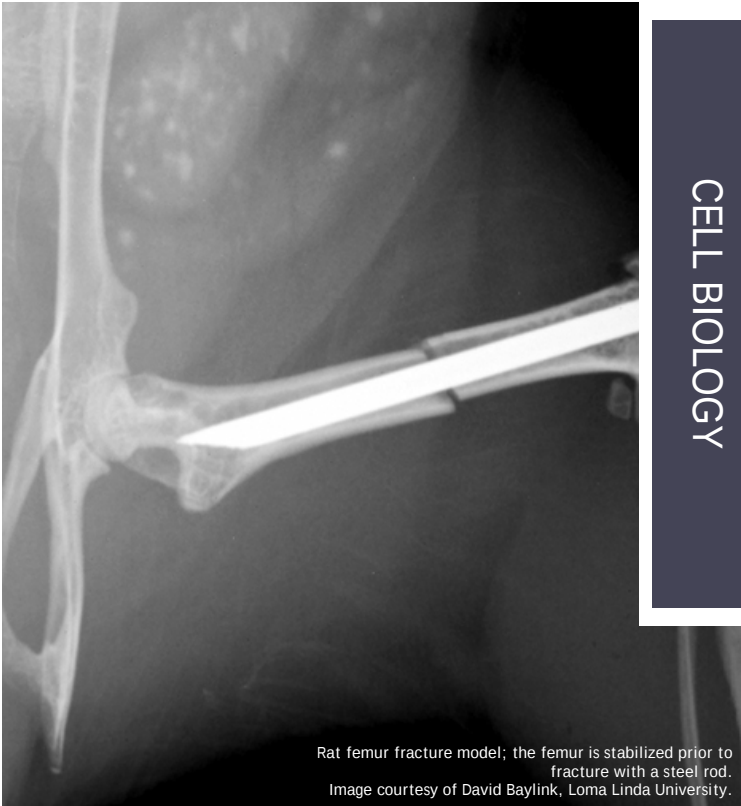
neuronal survival in trauma or disease applications (Ravits J, Traynor BJ: Current and future directions in genomics of amyotrophic lateral sclerosis. Phys Med Rehabil Clin N Am 19: 461-77, 2008). This is relevant to the military since veterans deployed in the 1991 Persian Gulf War have almost twice the risk of ALS compared to non-deployed veterans. Currently, the investigators are studying ways to enhance skeletal muscle survival following ischemic injury since this is a problem encountered in battlefield injuries due to blunt trauma or tourniquet application.

A second thrust area focuses on designing and developing tissue replacements, starting at the fundamental level and a mechanistic view. Investigators are asking two fundamental questions, specifically how to control the cellular alignment in tissues where axial orientation of cells with their extracellular matrix is a prerequisite in tissues such as blood vessels, tendons, and ligaments, and how to regulate elastic fiber formation in tissues where the extracellular matrix component is crucial, such as in blood vessels and skin. A multidisciplinary team of matrix biologists, surgeons, and immunologists are working together to develop examples of prototype replacement tissues such as blood vessels, ligaments, and skeletal muscle, all generated on biocompatible natural matrix scaffolds to facilitate integration with host tissues. The absence of artificial prosthetic material should allow implantation even into contaminated wound beds, a situation commonly encountered in battlefield medicine.

The third thrust area studies the early signaling when there is cellular damage to include developing a model system to characterize radiation damage detection system, identifying small molecular inhibitors to components of the radiation damage detector, and characterizing and manipulating dendritic cells toward an anti-inflammatory phenotype. The overall goal is to exploit inherent signals of cellular damage such as radiation-induced DNA damage and/or immune activation with the intent to develop early intervention strategies.



Hemafoxylin and eosin staining of the skin biopsy samples for characteristics of chronic rejection. Image courtesy of Dr. Suzanne Ildstad, Director, Institute for Cellular Therapeutics, University of Louisville.



Rat femur fracture model; the femur is stabilized prior to fracture with a steel rod. Image courtesy of David Baylink, Loma Linda University.

CELL BIOLOGY

Inducing Immunological Tolerance

Researchers at the University of Louisville are investigating immunological tolerance to composite tissue allotransplantation using several low toxicity reduced-intensity conditioning approaches to develop a treatment protocol for complex tissue allotransplantation. In this research, there are three research objectives including defining the role of a putative tolerogenic facilitating cell in the establishment of hematopoietic chimerism using murine and rat models, employing graft-facilitating cells to enhance engraftment of donor-derived stem cells to avoid graft versus host disease (GVHD), and translating these findings into strategies to promote composite tissue allograft acceptance. Such studies could help advance the field of transplantation immunology toward its goal of immune tolerance and impact on red blood cell disorders and autoimmune disorders, thereby benefitting millions worldwide.

Dr. W.P. Andrew Lee and co-investigators at the University of Pittsburgh are also conducting fundamental research aimed at developing cutting-edge cell-based immunomodulatory therapies that could minimize the risks and complications of maintenance immunosuppression after composite tissue allotransplantation (CTA). Insights into the expression patterns, profiles, kinetics and dynamics of cytokine and chemokine production and interactions during tissue rejection are just emerging. In this regard, a comprehensive analysis of cytokine interactions and networks during the onset, evolution and progression of rejection in the microenvironment of CTA could define strategies that monitor, prevent and treat rejection through a better understanding of its pathogenesis.

Dr. Lee’s research is directed at identifying the nature, pattern, and timeline of cytokine expression and complex network interactions in cellular and humoral components after CTA in experimental models. The overall goal is to use such modeling of early and ongoing cytokine behavior to predict the probability of downstream immune events and to identify the targets for diagnostic, prophylactic and therapeutic management of rejection, thus optimizing immunological and functional outcomes after CTA.

Understanding the Cellular and Molecular Mechanisms of Skeletal Augmentation and Repair

Dr. David Baylink along with co-workers at the Loma Linda University has a three-prong research program studying the cellular and molecular mechanisms involved in skeletal augmentation and repair. The overall focus of this research program is to establish a molecular foundation for the development of future approaches for the application of molecular medicine for the prevention and treatment of battlefield injuries. One of these projects looks at the coupling of bone formation to bone resorption, specifically to determine whether osteocyte-derived IGF-I is a potential coupling factor and plays a role in the mechanical stimulation of bone formation. Since it is known that osteocyte function in the bone is to sense mechanical strain and to be involved in signals during the fracture healing process, the investigators propose to examine fracture healing and bone formation response to mechanical stress in transgenic animals. A second project focuses on performing quantitative analysis of cell lineages during fracture repair as a function of time after injury. The information gained will provide insights into the cellular and corresponding molecular aspects of gene-based therapy and thus could be used to improve its efficacy. The third project involves developing and characterizing human hematopoietic stem cells-based transplantation strategy.

Looking into the Future

An understanding of the fundamental biology and science remains crucial in making progress in this field. Much remains to be determined, including how to control cell responses to an injury site such that it will result in healing versus scarring, which molecules mediate the desired outcomes, how to control and improve cell differentiation, and so forth.

CELL BIOLOGY

Number of Projects: 15*

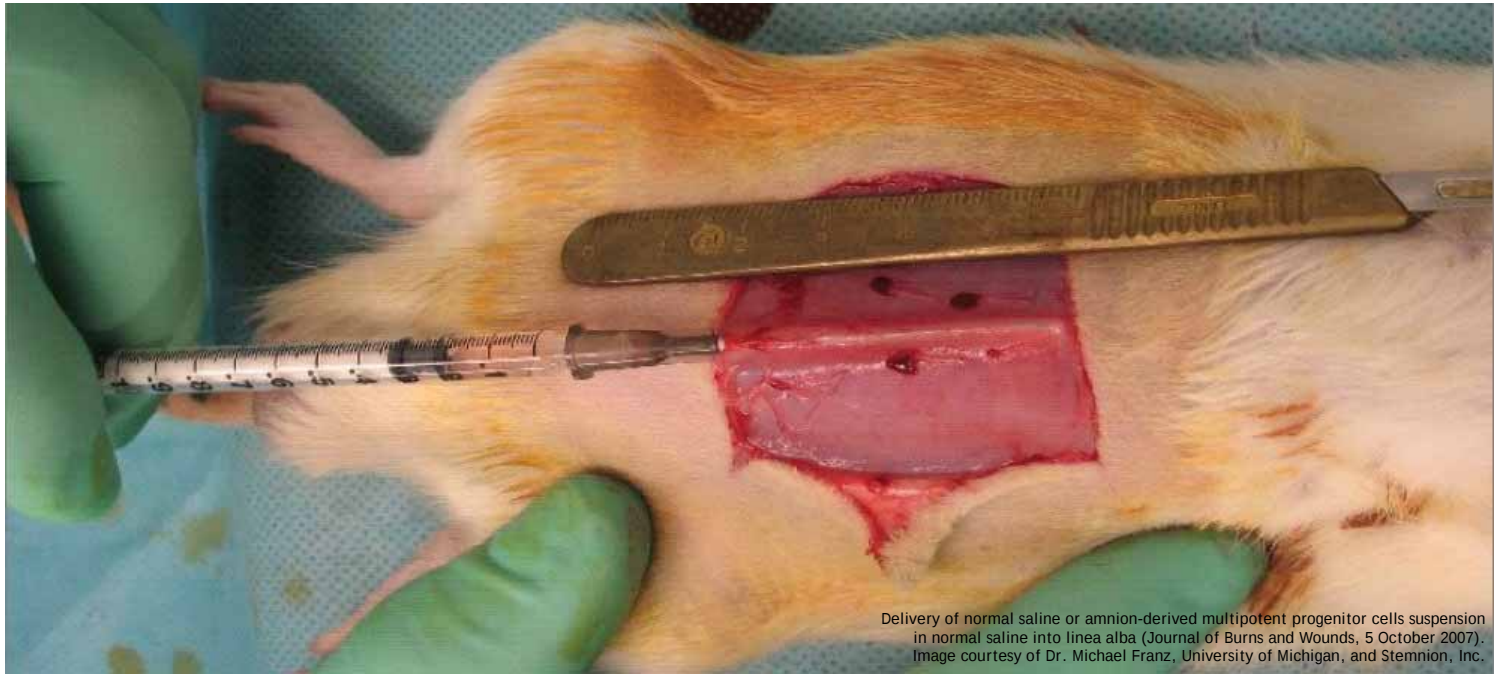
Publications: 52*

Patents: 5 *

Number of PIs: 9*

Funding for FY07 & FY08: \$18,451,384

* Numbers reflect ALL active projects, which include projects started earlier than FY07.



Delivery of normal saline or amnion-derived multipotent progenitor cells suspension in normal saline into linea alba (Journal of Burns and Wounds, 5 October 2007). Image courtesy of Dr. Michael Franz, University of Michigan, and Stemnion, Inc.

Therapeutics

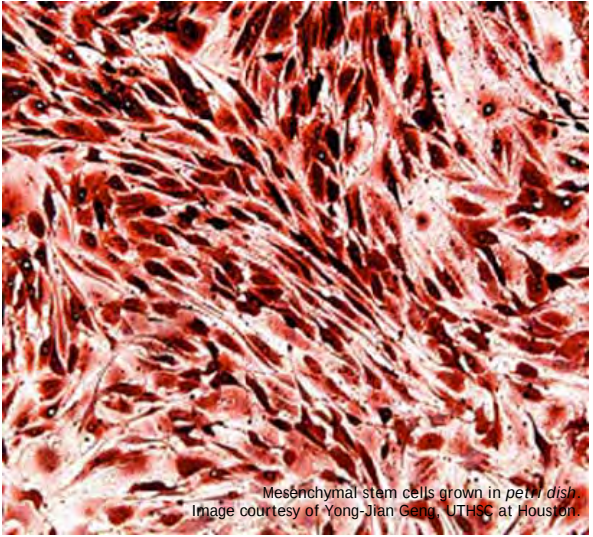
CREATING NOVEL THERAPIES

Therapeutics based on application of cells, genes, and/or growth factors could help improve wound healing of diseased or injured tissues or restore cellular function that has been damaged or missing.

Enhancing Wound Healing in a Variety of Tissue Injuries

Stemnion, Inc., a small privately held company based in Pittsburgh, Pennsylvania is working with leading clinical and research scientists specializing in wound healing to develop therapies that use amnion-derived multipotent progenitor cells and/or their products in the treatment of damaged tissues. These amnion-derived multipotent progenitor cells are formed very early in embryonic development from the same embryonic cell layer that forms the fetus. Thus, these cells have strikingly similar characteristics to embryonic stem cells, but possess important characteristics that distinguish them from embryonic stem cells and adult stem cells. One of these characteristics includes defined cell growth, meaning they do not grow and multiply indefinitely, and thus are not considered immortal. This characteristic substantially reduces the risk of transplanted amnion-derived cells forming spontaneous tumors following transplantation. In addition, these cells are readily available and easily harvested without any ethical issues associated with embryonic stem cells.

The amnion-derived multipotent progenitor cells exhibit a unique combination of properties known to be associated with wound healing including extensive cell proliferation potential, differentiation into multiple cell types, and secretion of cytokines and many growth factors. Currently, the company is taking on the challenge to develop and bring to the market an innovative wound healing agent based on the byproducts of amnion-derived multipotent progenitor cells for treatment of burns. In addition, the company is also working with other TATRC funded partners as well as the Walter Reed Army Institute of Research to evaluate the neuroprotective and neurorestorative effect of amnion-derived multipotent progenitor cells for spinal cord injury



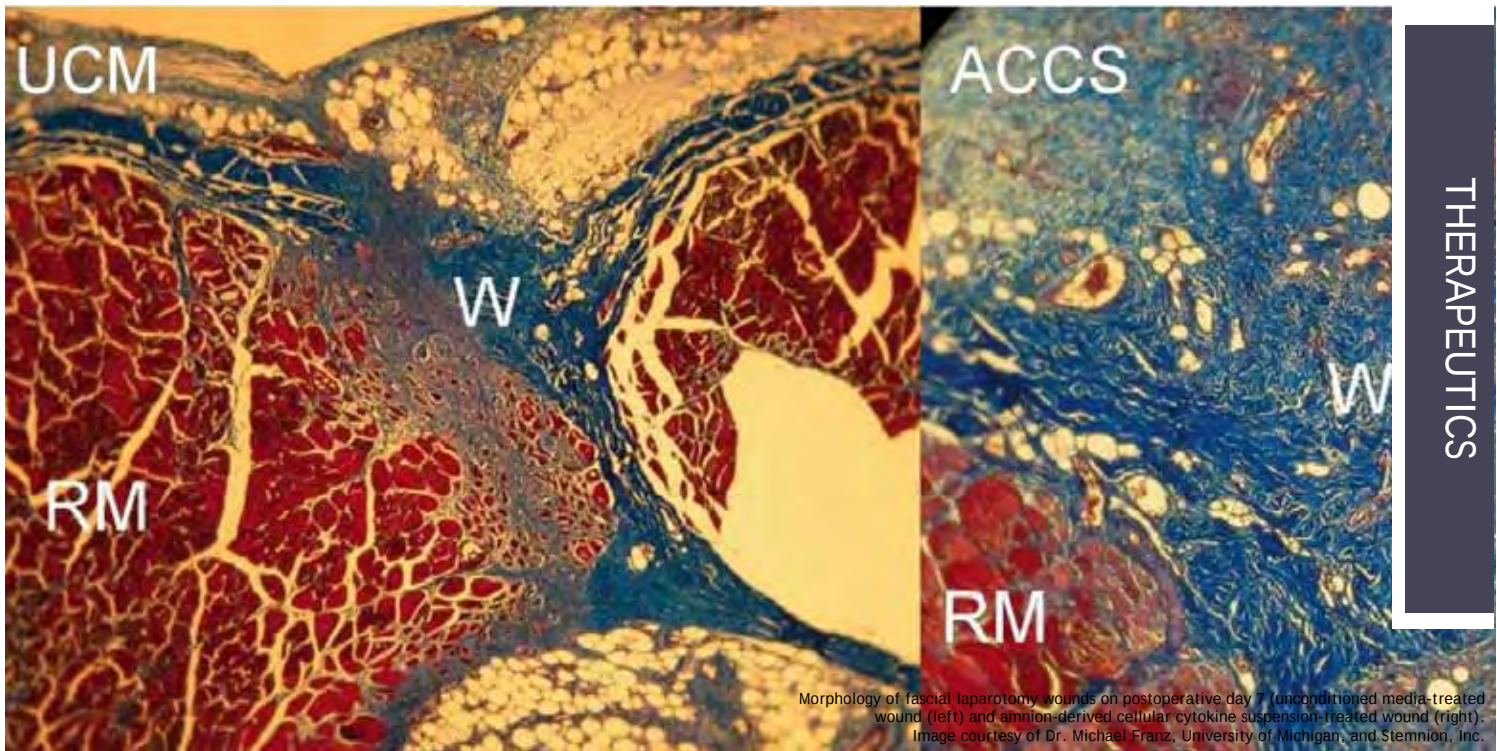
Mesenchymal stem cells grown in petri dish. Image courtesy of Yong-Jian Geng, UTMSI at Houston.

and for traumatic brain injury. It is anticipated that the transplanted cells will produce growth factors and cytokines in the damaged area and thereby will enhance the survival of injured neurons, and potentially replace lost neurons. Thus, if successful, such therapies could substantially change the lives of patients suffering from spinal cord injury or traumatic brain injury.

Repairing Damaged Cardiac Muscle and Spine

Pioneering investigators, including Drs. Eduardo Marbán and Dan Gazit, at Cedars-Sinai Medical Center in Los Angeles, California will study ways to improve stem cell-based therapies for treatment of damaged cardiac muscle and spine. Recently Dr. Marbán led a research team to perform the first procedure that used a patient's own cardiac stem cells to treat that same patient's heart that was previously damaged by a heart attack. This pioneering procedure was performed as part of a Phase I clinical trial. The investigators will soon be conducting additional research under a grant from TATRC to further develop and improve this promising cell-based therapy.

Under the TATRC grant, Dr. Marbán and his team will study retention and early cell migration of labeled cardiac stem cells in animal models to improve engraftment of stem cells to host tissue. To overcome some of the current research limitations that require sacrifice of animals at different time points, the investigators will develop and validate multiple noninvasive imaging tools to track the fate of



Morphology of fascial laparotomy wounds on postoperative day 7 (unconditioned media-treated wound (left) and amnion-derived cellular cytokine suspension-treated wound (right)). Image courtesy of Dr. Michael Franz, University of Michigan, and Stemnion, Inc.

cardiac derived stem cells following intramyocardial transplantation in mouse models and also in preclinical large animal models. Besides obviating the need to use large numbers of animals in the research, the imaging modalities the team will develop will be directly applicable to use in human patients. This will be very important clinically, since there will likely be considerable variability in how individual patients respond to injection of cardiac stem cells after suffering a heart attack.

goal to find the optimal conditions for effective treatment. They plan to compare hematopoietic stem cell populations of varying purity for its potential to restore cardiac function following implantation of these cells into the infarcted myocardium of a mouse model. In addition, the investigators will examine whether exposure of hematopoietic stem cells to specific re-programming factors will promote the ability of these cells to trans-differentiate into cardiomyocytes following transplantation into damaged heart tissue.

In another regenerative medicine research study, also to be funded under the TATRC grant, Dan Gazit, Ph.D., and co-investigators will utilize several molecular and tissue imaging modalities to develop immune-selected stem cell-based therapies for treating traumatic and osteoporotic spinal vertebral injuries. The envisioned stem cell-based therapy will be applied to the patient through a minimally invasive injection into the vertebral body of immune-selected, autologous mesenchymal stem cells with a biocompatible scaffold. Autologous adult mesenchymal stem cells will be exploited, since the group has previously demonstrated that these cells are able to induce rapid bone regeneration and fracture repair in several *in vivo* bone loss models. These same studies have also led the group to develop a unique immune-selection method that enables the immediate implantation of freshly isolated mesenchymal stem cells without the need for prolonged culture periods to expand the cell populations. In addition, the researchers have found a method that will induce the desired differentiation pattern of the cells, which in turn should result in accelerated tissue regeneration.

Restoring Cardiac Function of Damaged or Diseased Heart

Given that cardiovascular diseases remain the leading causes of deaths in the United States regardless of economic and/or age groups, there is significant interest in developing therapies to repair damaged or diseased heart tissue since current therapeutic modalities are insufficient and tissue/organ transplantation could be limited in supply and comes with the risk of rejection and reduction of lifespan as well as complications associated with taking immunosuppressive drugs. Further, development of novel regenerative medicine based treatments for this tissue could be translated for other similar tissue structure that requires repair or regeneration due to other causes of injuries.

At the University of Hawaii, Dr. Lawrence Burgess and colleagues will evaluate the efficacy of cell-based therapy to restore cardiac function using different populations of hematopoietic stem cells with the

Over at the University of Texas Health Science Center at Houston, Dr. Yong-Jian Geng

is also leading a research study focusing on developing a cell-based therapy for treating heart failure and myocardial infarction, specifically using cardiovascular stem cells. Here Dr. Geng and his colleagues are exploring ways to optimize the dosage and timing of stem cell transplantation for heart failure and infarction. To date, they have established stem cell culture methods for cardiac therapy, identified the molecular pathways leading to better survival and differentiation of stem cells into cardiovascular cell types in the heart wound, developed successful delivery of stem cells into the lesions of cardiovascular tissues, and obtained useful data and information on the efficacy and biosafety of cardiac stem cell therapy in experimental infarction and heart failure in small and large animal models.

Looking into the Future

Therapeutics derived from growth factors, genes, cells, or cellular byproducts that will enhance wound healing, minimize or prevent scar tissue formation, or repair and/or regenerate damaged tissues are needed to address wounds and injuries where current medical treatments have not been able to produce the desired results in terms of appearance as well as function.

THERAPEUTICS

Number of Projects: 20*

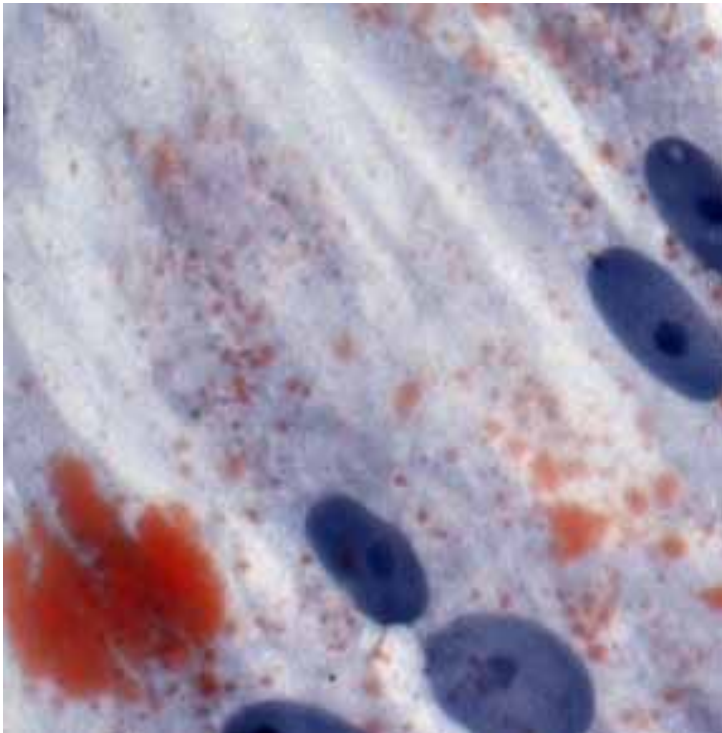
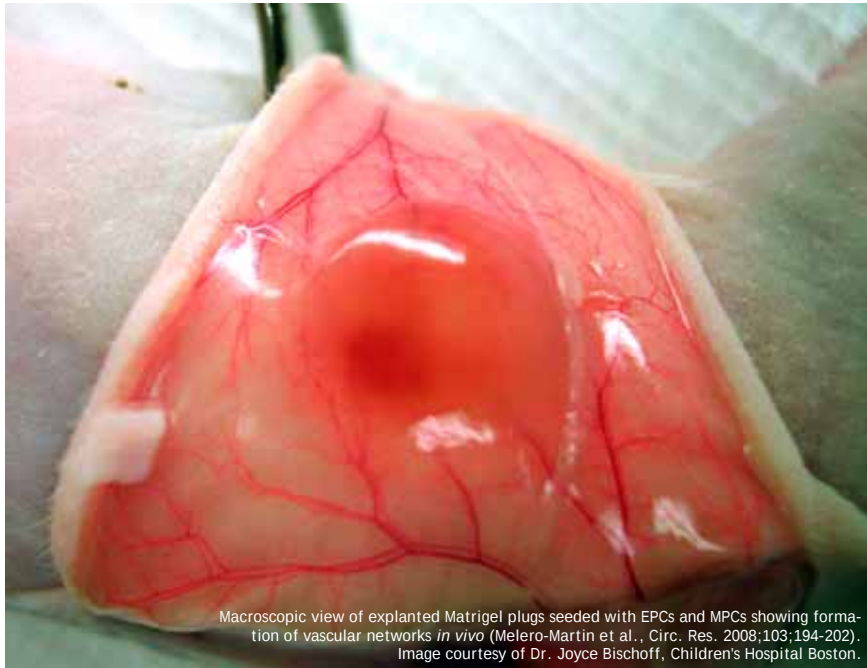
Publications: 42*

Patents: 2*

Number of PIs: 16*

Funding For FY07 & FY08: \$9,422,050

*Numbers reflect ALL active projects, which include projects started earlier than FY07.



Tissues & Organs

REGENERATING FUNCTION AND RESTORING LIVES

Engineering of red blood cells, organs, and complex tissues to include skin, muscle, bone, nerve, tendons, and blood vessels requires a combination of understanding the fundamental cell biology as well as development of technologies and enabling tools. Thus, oftentimes the funded research in this area is a multi-prong program with long term plans that consists of multiple, interdisciplinary projects that span across several of the portfolio's categories from cell biology to therapeutics to technologies to enabling tools. The largest area of focus has been in bone repair and regeneration, followed by musculoskeletal and soft tissue engineering addressing an array of tissue types and injury types.

Generating Microvasculature that will Support New Tissues Formation

Dr. Joyce Bischoff and co-investigators at the Children's Hospital Boston have developed a robust *in vivo* model for blood vessel formation using human endothelial progenitor cells (EPCs), isolated from peripheral or cord blood, and mesenchymal stem/progenitor cells (MPCs), isolated from adult bone marrow or cord blood. The *in vivo* model uses immune-deficient mice in order to avoid immune attack on the human cells. The most recent peer-reviewed research, published in Circulation Research 2008 (Melero-Martin et al., Circ. Res. 2008; 103; 194-202), demonstrates that a perfused vascular network can be established *in vivo* via implantation of these two cell populations. As noted in the editorial of this issue, this research provides "a significant step forward in the vascularization process necessary for tissue engineering and regenerative medicine." Creation of a microvasculature network that is connected with the host is necessary to support new tissue formation and tissue implantation/

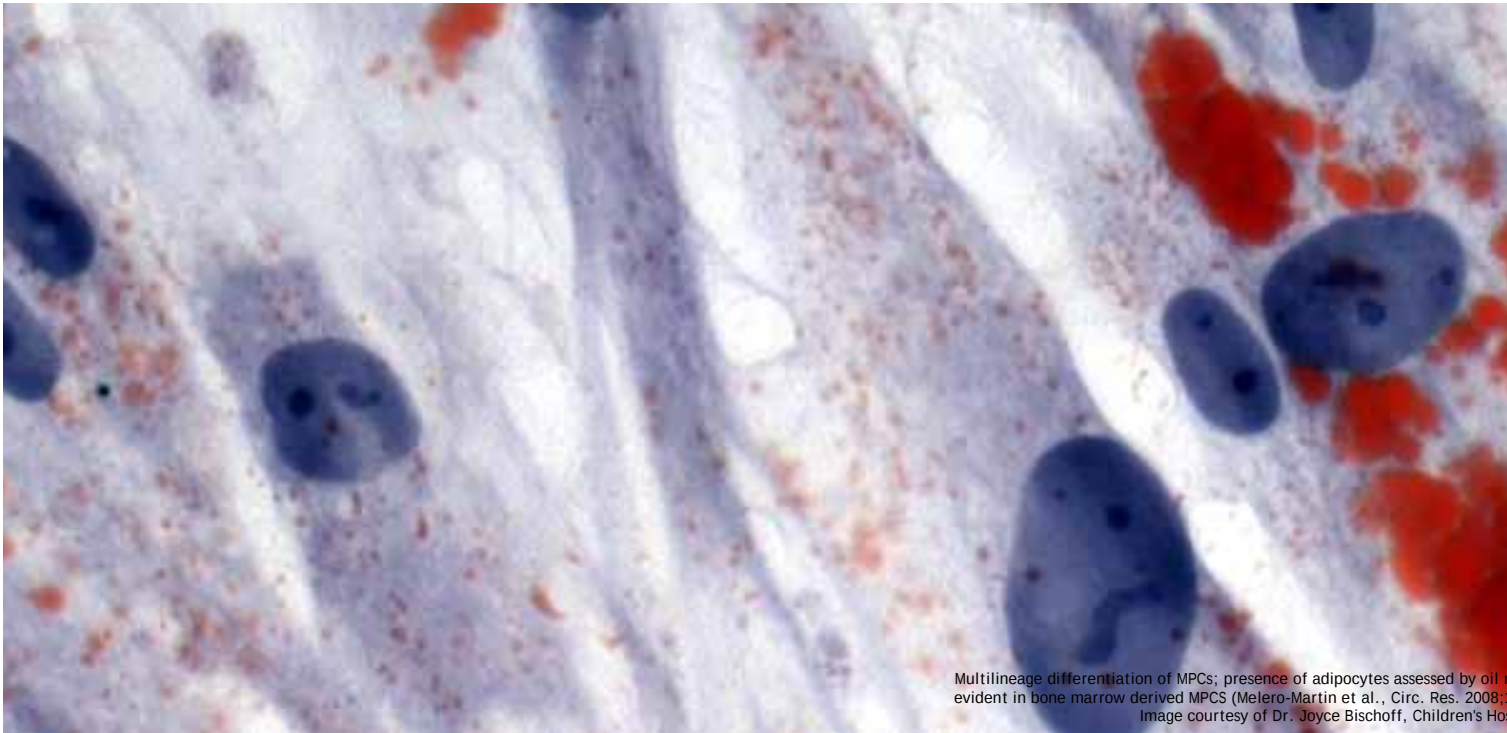
engraftment because the supply of oxygen and nutrients is vital to the cells that make up the tissue. Thus, demonstrating an extensive and functional microvascular network connected with the host blood supply is a significant achievement. Furthermore, that this is achieved through the use of adult autologous human progenitor cells, obtained through a minimally invasive procedure, is another important milestone.

Engineering Tissues for Replacement

The Center for Military Biomaterials Research (CeMBR), located at Rutgers, The State University of New Jersey, and spearheaded by Joachim Kohn, Ph.D., has developed a comprehensive research program that specifically focuses on developing treatments that target the military's most urgent health care needs on and off the battlefield, from treatments for acute wounds to regenerative medicine and tissue engineering to restore form and function to patients suffering from severely damaged and/or lost tissues. Of the several ongoing active projects under this program, there are currently three that fall within regenerative medicine and tissue engineering.

One project, in particular, focuses on the continued development of injectable, resorbable polyurethane/bone particle composites and implantable polycarbonate scaffolds containing bone growth factors and adult stem cells for fracture fixation devices (pins and rods) and for repair of large cranio-orbital defects. The latter is of particularly high military interest since there is a critical need for regeneration of the cranio-orbital region of patients having avulsed soft tissue and bone. Current therapies for massive traumatic cranio-orbital loss utilize non-physiological, non-degradable biomaterials such as poly(methyl methacrylate). While these non-degradable plastic implants temporarily restore form, they do not remodel or integrate with host tissue, leading to other complications such as infection. Thus, reconstruction of the craniofacial region with plastic implants often requires subsequent revision surgeries that come with additional complications, pain, and risks. CeMBR's proposed research is advancing the design of injectable composites and bioactive scaffolds as physiological cranio-orbital implants that will integrate with host tissue, remodel, and restore enduring form and function through bone regeneration.

Another ongoing CeMBR project focuses on the creation of innervated vascularized skeletal muscle. This therapy is an



Multilaneage differentiation of MPCs; presence of adipocytes assessed by oil red O staining evident in bone marrow derived MPCs (Melero-Martin et al., Circ. Res. 2008;103;194-202). Image courtesy of Dr. Joyce Bischoff, Children's Hospital Boston.

TISSUES & ORGANS

important, unmet need for the military. Initial research goals target small muscles in the face or hand, but in the long-term, it will become possible to regenerate larger muscles to restore movement of arms or legs. Muscle tissue has already been grown in several laboratories from isolated muscle cells, but such engineered muscle tissue is not useful, unless it can be connected to the patient's own nerves. Therefore, an objective of CeMBR's research is to develop innovative nerve conduits. This technology can then be leveraged to develop innervated, vascularized skeletal muscle. Preliminary studies show that CeMBR's novel nerve conduits provide the requisite mechanical strength properties and biocompatibility with nerve tissue *in vivo*.

To successfully address complex, multidisciplinary research problems such as the projects above, CeMBR operates as an open network that allows for outside institutions and organizations to join the Center, and thus, able to bring the best scientists, engineers, and clinicians with varying backgrounds and expertise to develop innovative solutions. This model enabled CeMBR, in partnership with 15 leading academic institutions and over 20 industry partners, to be awarded as one of the two consortiums to start the Department of Defense Armed Forces Institute of Regenerative Medicine (AFIRM).

Accelerating Healing and Improving Functional Recovery

Investigators at the Oregon Biomedical Engineering Institute, Inc. are focused on developing a research program addressing tissue replacement and regeneration technologies to save organs, accelerate healing, and improve the functional recovery for the injured soldier. In 2008, the research focus includes three areas that are critical to solving military traumatic injuries. One of these will focus on the need to improve healing, muscle regeneration, and functional recovery following extremity compartment syndrome injury using autologous bone marrow derived progenitor cells. A second research area will focus on creating the first biocompatible, durable small vascular graft that would be equivalent to autologous vein grafts using recombinant elastin and collagen proteins that are woven into nano-fibers to reproduce the normal vascular four-layer architecture. The third research project will aim to induce endogenous dermal fibroblasts to create a flexible skin replacement that also reduces collagen scar disability by applying recombinant dermal matrix proteins to severely burned skin. The Oregon Biomedical Engineering Institute is also one of the research partners for the Department of Defense Armed Forces Institute of Regenerative Medicine, which is funded through the U.S. Army Medical Research and Materiel Command.

Looking into the Future

Given the types and severity of combat injuries, the targeted areas of tissue for improved healing, repair, and regeneration include skin, blood vessels, muscle, bone, tendons, and nerve. While some injuries may just need to focus on orthopedic repair and regeneration or skin replacement, others are even more complicated and require tissue engineering of composite tissues. Challenging hurdles that remain to be overcome in tissue engineering/regeneration include how to keep the tissue mass viable, especially following implantation of *ex vivo* engineered tissue into the host or regenerating the tissue *in vivo* directly within the host. Additional issues of consideration include how to ensure appropriate cell growth and differentiation. More importantly, how to ensure the regenerated tissue will provide the same or similar level of function as the pre-injured state. Current traumatic injuries requiring regenerative medicine and tissue engineering solutions to address include complex orthopedic injuries to the cranio-facial region as well as to the extremity of the limbs. Oftentimes these polytrauma injuries also involve large tissue loss and thus, may require engineering of large section of the limbs that includes bone, muscle, nerve, blood vessels, tendons, and skin. Control of bacteria and pain provides additional challenges to an already very complex tissue engineering and regeneration effort.

TISSUES & ORGANS

Number of Projects: 14*

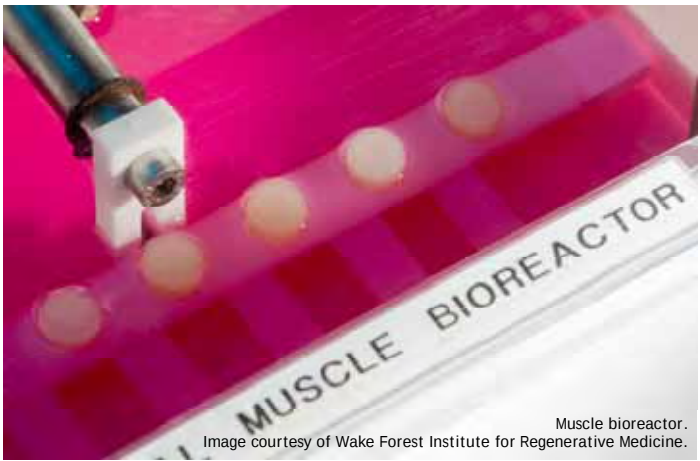
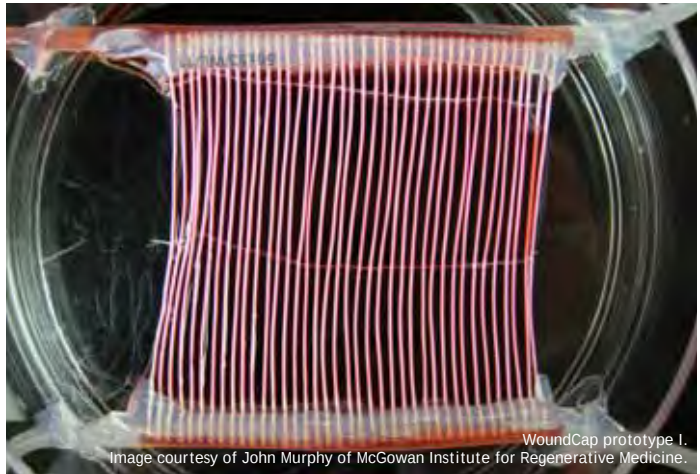
Publications: 31*

Patents: 15 *

Number of PIs: 13*

Funding for FY07 & FY08: \$12,314,709

* Numbers reflect ALL active projects, which include projects started earlier than FY07.



Technologies

ADVANCING RESEARCH AND THERAPEUTIC
POSSIBILITIES

Technologies that enable cell harvesting, sorting and multiplication, cell/tissue preservation and storage, and tissue creation such as bioreactor or cell printing are important pieces to advancing the research and innovative therapeutics possibilities.

Creating Organized Functional Muscle and More

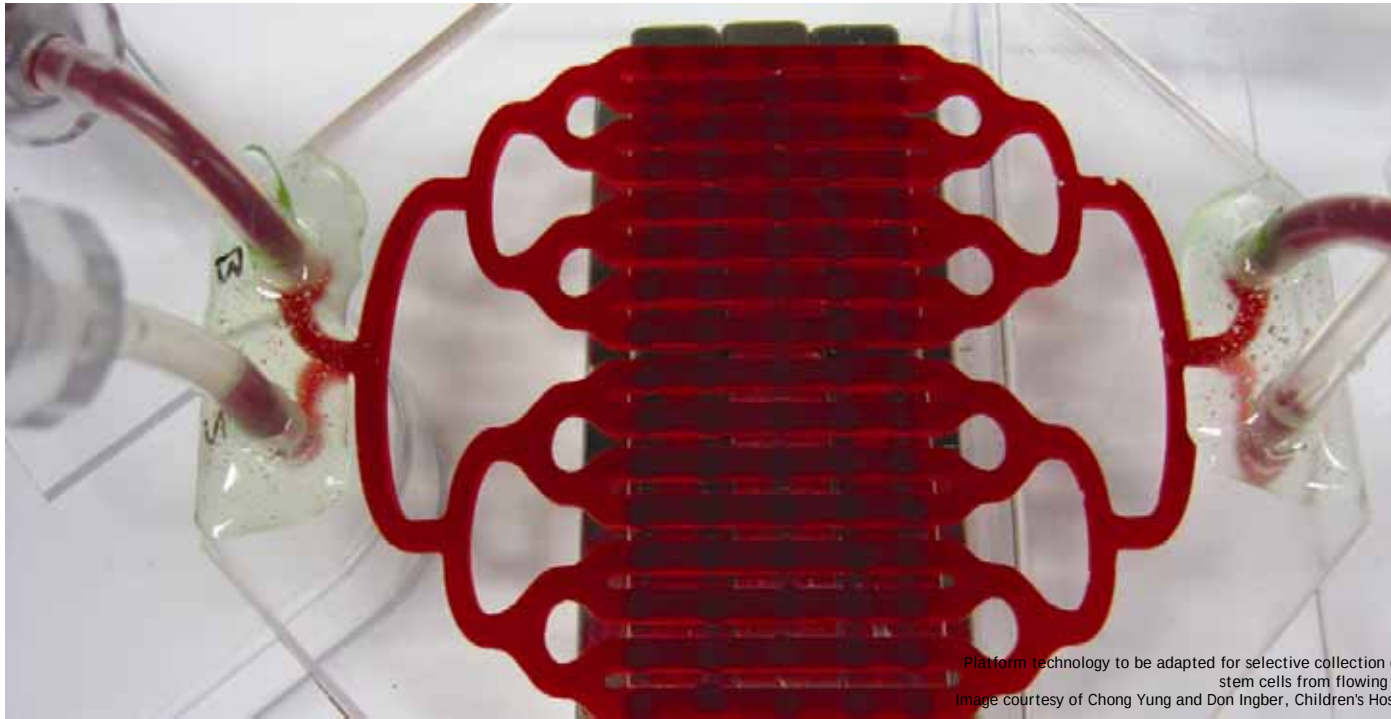
Dr. Anthony Atala and co-investigators at the Wake Forest Institute for Regenerative Medicine (WFIRM) in North Carolina are taking on some of the greatest challenges in tissue engineering and regenerative medicine. The group is currently conducting research to develop tissue engineered implants that can be made from the patient's own cells with the goal to improve functional outcome in patients injured in severe trauma. In order to accomplish this long-term goal, several research areas are being conducted simultaneously to overcome the current limitations of engineering clinically relevant masses of tissue, which include the need to provide vascular support and innervations to regenerated tissues foremost. At the basic level, these research areas include developing technologies to expand progenitor cells in culture, designing novel biomaterials to facilitate cell attachment and tissue growth, constructing a three-dimensional tissue bioreactor system to enhance cellular organization and advanced tissue maturation *in vitro*, and devising growth factor delivery strategies to enhance angiogenesis and neuroinductive processes.

This research program is built on the Institute's many successes, including being the first to demonstrate that complex layered tissue structures can be engineered using cells and creating the world's first laboratory-engineered organ, bladder tissue that has been successfully implanted in children and adults. The group consisting of research scientists, engineers, and clinicians at the Institute is also working on a number of other research projects in addition to the TATRC funded research program to include using amnion stem cells for engineering insulin-producing cells, cell-based therapies, and other tissues and organs for

replacement as a result of diseases or trauma. These demonstrated successes and research foci helped the Institute competitively win an award to be one of the co-leading consortiums to start the Department of Defense Armed Forces Institute of Regenerative Medicine (AFIRM). The primary goal of the AFIRM is to solve some of the most severe and complicated traumatic injuries incurred by soldiers in combat, which current treatments are not able to provide the optimal or ideal results in restoring the soldier's life with near normal appearances and functions.

Making Tissue Revascularization Possible

Donald Ingber, M.D., Ph.D. and co-investigators at the Children's Hospital Boston are currently developing a device based on a novel platform technology to isolate circulating vascular stem cells, also known as "endothelial precursor cells" (EPCs), from a patient's blood that might be used for blood vessel tissue engineering in the injured soldier one day. The potential impact of this technology could be significant in advancing regenerative medicine to address both wound healing and tissue reconstruction of traumatic injuries. Current treatments for some of the traumatic war injuries are inadequate and in some cases, require re-vascularization for optimal wound healing in severe burns or for tissue reconstruction. Ideally, autologous stem cells would be the best source to obtain the needed tissue reconstruction materials to regenerate this vasculature. Hence, this ongoing research explores ways to improve the design and to enable it to continuously harvest large quantities of circulating stem cells directly from the bloodstream of wounded patients in a minimally invasive way or prior to injury and could transform the laborious process of harvesting, isolating, and sorting of needed stem cells for regenerating blood vessels and for other stem cell based-therapeutics possible in a clinical setting.



TECHNOLOGIES

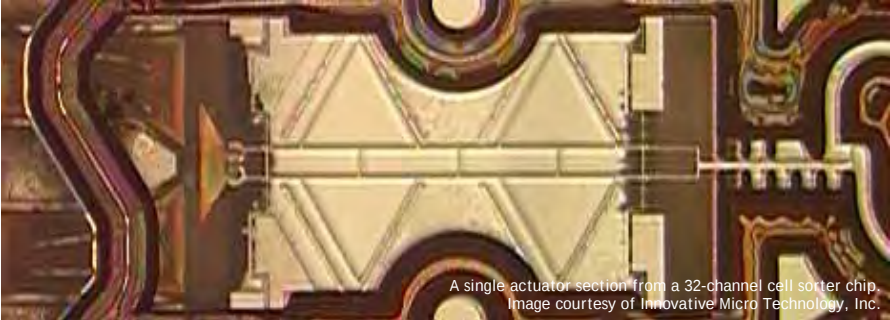
Platform technology to be adapted for selective collection of circulating stem cells from flowing whole blood. Image courtesy of Chong Yung and Don Ingber, Children's Hospital Boston.

Improving Wound Healing of Burned Patients and Other Traumatic Injuries

Researchers and clinicians at the Pittsburgh Tissue Engineering Initiative (PTEI), spearheaded by Dr. Alan Russell, have created the Advanced Regenerative Medicine program composed of an array of projects that provides innovative medical solutions to help save soldiers lives and restore their function so they can continue to lead active lives. These projects span across all categories of the TATRC regenerative medicine portfolio to include developing technologies to control the microenvironmental niche for promoting epimorphic regeneration in amputated digits, designing bioreactor to control inflammation, creating novel active, on-demand biodegradable wound dressing device that provides temporal delivery of growth factors for improved healing, and developing a “SkinGun” delivery system to address burns and other traumatic wound injuries.

The “SkinGun” delivery system combined with an active wound dressing research focuses on the development of technology to distribute, grow, and support skin cells for burned patients using a skin cell deposition system (aka “SkinGun”) and the development of an active wound dressing to support the sprayed skin cells (aka “WoundCap”). The unique aspect of this approach is the application of relevant cells suspended in solution can be evenly distributed via an innovative device for spraying a cell suspension under controlled conditions onto the wound. Such an approach could overcome some of the limitations found in current methods for treating skin wounds from skin grafting (i.e. split-thickness and full-thickness grafts, micro-grafting, and mesh graft) to cultured epithelial autografts, and to other mechanical hand driven spray techniques (Navarro et al 2000 and Wood et al 2003).

The SkinGun research focuses on developing a methodology that allows for high yield autologous skin cell isolation in the intra-operative setting, and to deliver an expanded population of skin cells to wound sites in a controlled spray pattern. The controlled spraying allows for a more even distribution of cells to be deposited in the wound and enables a larger surface to be treated compared to current treatments such as cell sheets for a given therapeutic cell number. This provides an advantage for the spray deposition concept over cell sheets, which is avoidance of blister formation that results in irregular scar formation and insufficient take rates. These sprayed cells could be supported by an active, on-demand biodegradable wound dressing device that also provides temporal delivery of growth factors to enhance healing. The concept of an active, on-demand biodegradable wound dressing device is



A single actuator section from a 32-channel cell sorter chip. Image courtesy of Innovative Micro Technology, Inc.

to not disturb the healed layers of tissue. Currently, both devices are in research development.

PTEI research program in advancing regenerative medicine is built on solid foundation and successes. One of the unique aspects of the Advanced Regenerative Medicine research program includes development of an innovative professional training and education program for students, which allow them to work with scientists and clinicians at the U.S. Army Institute of Surgical Research (USAISR) in San Antonio. This helps ensure a pipeline of trained scientists who will be able to take on the challenges of solving some of the most complex injuries, especially those sustained from combat. The successes of PTEI foundation helped win them an award to be one of the co-leading consortiums to start the Department of Defense Armed Forces Institute of Regenerative Medicine (AFIRM).

Looking into the Future

Technologies that can help advance the regenerative medicine and tissue engineering include ways to obtain stem cells of interest in a minimally invasive procedure and in a sterile environment, create and support complex tissue for implantation/regeneration, and rapid production of stem cells to enable faster tissue creation.

TECHNOLOGIES

Number of Projects: 15*

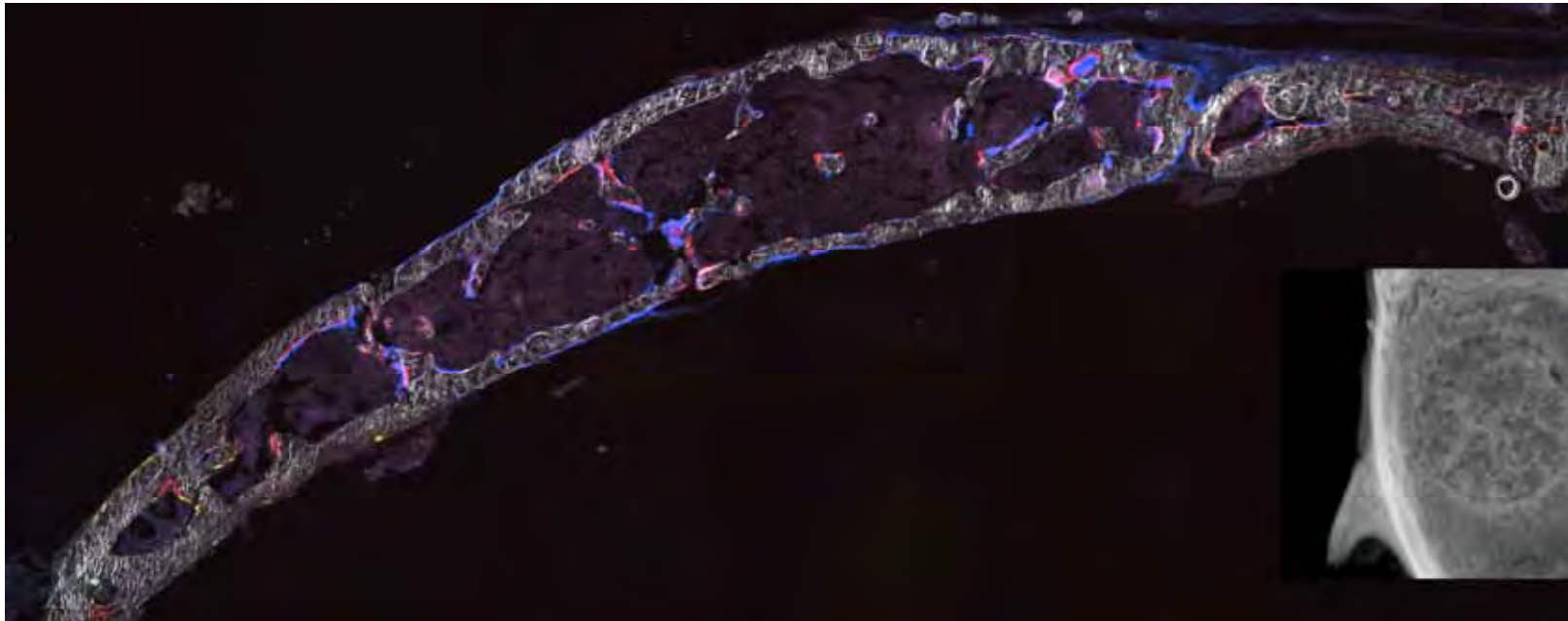
Publications: 27*

Patents: 22*

Number of PIs: 9*

Funding for FY07 & FY08: \$9,082,648

* Numbers reflect ALL active projects, which include projects started earlier than FY07.



Enabling Tools

MAKING TISSUE REGENERATION POSSIBLE

Enabling tools include developing scaffolds to create an environment for cells to attach, differentiate, organize, and grow, using imaging technologies to track movement and effectiveness of cell-based interventions, creating novel media to sustain cell growth or control differentiation, and applying computational modeling to describe and predict complex biological pathways.

Improvised explosive devices (IEDs) create significant traumatic injuries that are previously unseen in prior combats, resulting in difficult to impossible salvageable extremities that require extensive soft and hard tissue regeneration and reconstruction. In order to make complex tissues that are implantable and become grafted to the patient's remaining tissue or wound site, scaffolds may be one solution to help create and support regeneration of soft tissues as well as support of bone tissue.

Making Broken Bones Whole Again

It is well known that bones do self-heal and regenerate to some degree. However, the severity of the injuries sustained from IED necessitates medical intervention. In fact, bone regeneration is one of the largest tissue engineering areas within the TATRC regenerative medicine portfolio ranging from understanding the cell biology to enabling tools, spanning across all categories within the portfolio. Below are some projects that focus on creating scaffold for bone tissue engineering.

Advanced Ceramics Research, Inc., located in Tucson, Arizona, is working on producing tissue engineering scaffold designs in consultation with Dr. Jeffrey Hollinger and partners at the University of Texas, San Antonio (UTSA), the Carnegie Mellon University, and the University of Texas Health Science Center San Antonio (UTHSCSA). Their approach, known as the Plasti-Bone Implant System, involves tissue engineering of healthy bone using fully customized porous bone scaffolds that are produced using computer aided manufacturing techniques. The intent is to produce biocompatible polymer scaffolds with a porous structure similar to that of healthy cancellous

bone and coated with nanocrystalline calcium particles and growth factor protein to enhance osteoinductivity and osteoconductivity. It is envisioned that the resulting resorbable scaffold be applied to the repair of segmental bone defects.

A multidisciplinary team led by principal investigator David Puleo, Ph.D., at the University of Kentucky is investigating multifunctional materials to treat traumatically induced orthopedic and craniofacial injuries. Because such war injuries are often contaminated with debris and subsequently microbes, from which even an indolent infection initiates a chronic inflammatory response that undermines tissue repair, there is a need to develop materials that both eliminate bacteria and regenerate injured tissues. Hence, the team is designing a moldable, biodegradable bone graft substitute that will provide localized, controlled, sequential release of antimicrobial and osteogenic agents for repair of large orthopedic and craniofacial injuries. It is anticipated that delivery of an antimicrobial agent directly from the osteogenic defect filler will result in improved and accelerated bone healing as compared to either individual treatments alone or bone grafting in conjunction with systemic antibiotics.

Dr. Jody Redepenning and co-investigators at the University of Nebraska are planning to conduct research to examine possible candidates for construction of bioceramic bones to treat severe orthopedic trauma. The goal is to construct these biocomposites based on composites of poly-L-lactide (PLLA) and hydroxyapatite (HA) to exhibit macroscopic morphologies and mechanical properties similar to those of mammalian cortical bone. Surface-initiated polymerization of cyclic lactones will be the method used to construct these biomaterials since it is less likely to cause an undesirable immune response when the scaffolds will be used for bone repair. Further, it is anticipated that the new materials will be resorbed without any compromise to the original mechanical properties of the devices during the resorption, new bone formation, and subsequent remodeling processes. Once the process of generating these biocomposites has been defined and optimized, the investigators plan to introduce antibiotics into the biocomposites under conditions that will provide for controlled release of the agent that will remain in active form.

Developing a Tool for High-Throughput Screening

David Rowe, M.D. and colleagues at the University of Connecticut Health Center are developing an animal model for assessing the bone



reparative potential of progenitor cell therapy and in collaboration with material scientists, they are also evaluating different composite materials and scaffolds for bone regeneration. The importance of this work is that it allows for a systematic way of assessing efficacy and determining the optimal scaffold, cells, and/or growth factors best for tissue repair and/or regeneration. Development of such a research tool could help avoid a trial and error approach and drive decision-making to be based on more data that can concretely identified how well a scaffold, cell source, and/or growth media would work in tissue repair and/or regeneration. In addition, development of this research tool could make it more cost effective. Currently, the group is demonstrating how such a tool, based on using a series of GFP-reporter mice and bone repair models, could provide for a fast, informative, quantitative, and biologically relevant process to initially screen for the most promising candidate factors. The level of information that could be gotten will help distinguish the contribution of tissue regeneration due to the tissue engineering and regenerative medicine applications or due to the host native response. Thus, such a tool could help identify what works and determine the optimal candidates for further evaluation. A long term goal for this research group includes adapting the models and reporter systems for evaluation of human derived progenitor cells.



Engineering Autologous Blood Vessels

Drs. William Wagner and David Vorp at the University of Pittsburgh have been working on a project to create autologous blood vessels for use in vascular bypass grafting procedure or for situations where blood vessels replacement be needed due to traumatic injuries. Engineered autologous blood vessels would be a better alternative to the current options that include synthetic grafts or autologous saphenous vein. The latter material is preferred, but may not always be available and often fails within 10 years. Synthetic grafts have only been successful for larger diameter vessels, but smaller diameter applications have not been successful due to thrombotic response, ingrowth of synthetic smooth muscle cells, and calcification. Hence, the researchers have been working to develop a novel, bioresorbable scaffold bulk-seeded with human mesenchymal stem cells and cultured acutely *in vitro* to yield a histologically- and biomechanically-equivalent vascular graft suitable for implantation. The idea is to make these bioresorbable scaffolds readily available as an off-the-shelf product that can be quickly bulk-seeded with the patient's own bone marrow-derived mesenchymal stem cells and be used for replacement of small sized blood vessels.

Looking into the Future

Enabling tools remain an important component for regenerating tissue. Innovations that could improve and sustain cell attachment, growth, and implantation of the resulting tissue for host to take or result in native recruitment of circulating stem cells for repair and regeneration at the damaged site are sought. Tools that could help discriminate, determine, and/or predict what variables or combination of variables to result in the optimal formulation will help reduce overall research and development cost and streamline the research and development timeline from bench to translation are of interest.

ENABLING TOOLS
Number of Projects: 17*
Publications: 12*
Patents: 4 *
Number of PIs: 17*
Funding for FY07 & FY08: \$12,658,813
* Numbers reflect ALL active projects, which include projects started earlier than FY07.

TATRC partnered with Principal Investigators from academia, nonprofits, hospitals, industry, the Research Area Directorates (RAD) at the U.S. Army Medical Research and Materiel Command (USAMRMC) to include the newest RAD, the Clinical and Rehabilitative Medicine Research Program (CRM RP), and the U.S. Army Institute of Surgical Research (USAISR) to work together (1) to advance the science and technology in the regenerative medicine area, (2) to create innovative medical solutions, (3) to solve some of the most complex injuries, and (4) to save and restore Soldiers functions. These life-saving and function-restoring therapies will also benefit the general public. To learn more about the additional research supported through the USAMRMC / TATRC, please peruse through the CD and TATRC website, which contains short descriptions of all actively funded projects highlighting the overall goals, impacts, and accomplishments.

Advances and discoveries are made possible through the contributions and collaborations of our partners, in particular the Principal Investigators. We greatly appreciate their efforts in working hard with us and a special thanks to the Principal Investigators, Contract/Grant Officer Representatives, Project Officers, and the TATRC Graphics/Marketing Team (in particular, Ms. Sarah Perrie, Graphic Artist, and Ms. Lori DeBernardis, Director, Marketing and Public Affairs) in making this report and CD possible.

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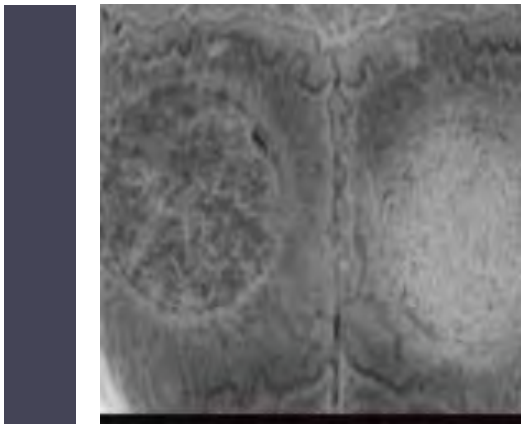
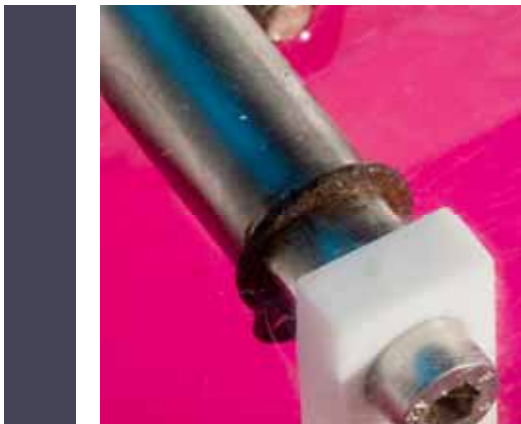
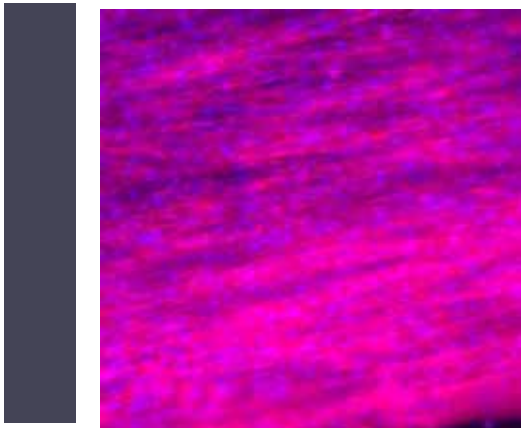
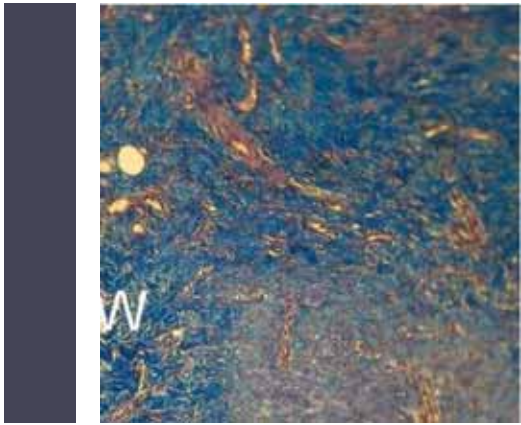
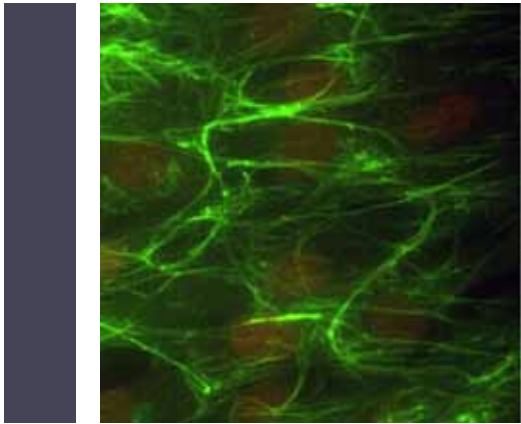
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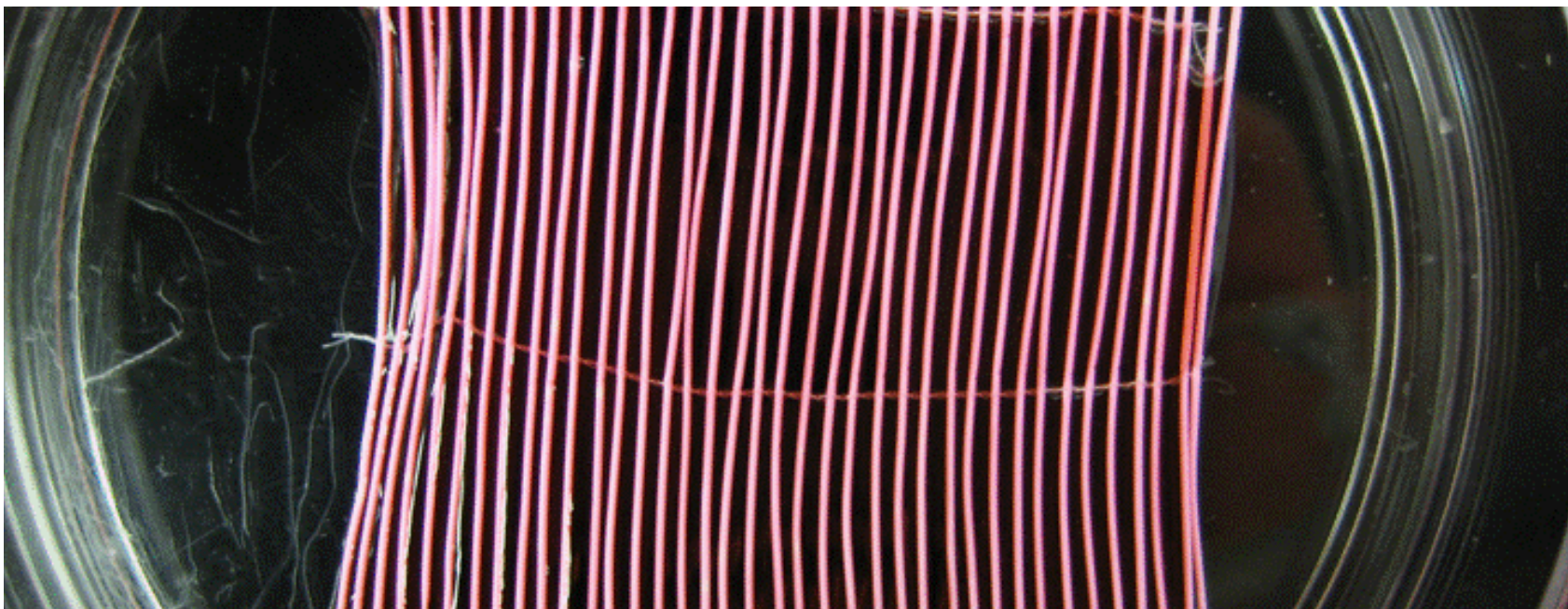
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For more information on these
groundbreaking projects and other exciting
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