Armed Forces Institute of Regenerative Medicine

Annual Report 2013

AFIRM OUR SCIENCE FOR THEIR HEALING

Armed Forces Institute of Regenerative Medicine

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This report contains nontechnical summaries of all currently funded Armed Forces Institute of Regenerative Medicine research projects. The technical progress reports for these projects are contained in the AFIRM Annual Report 2013 – Technical Progress Reports.

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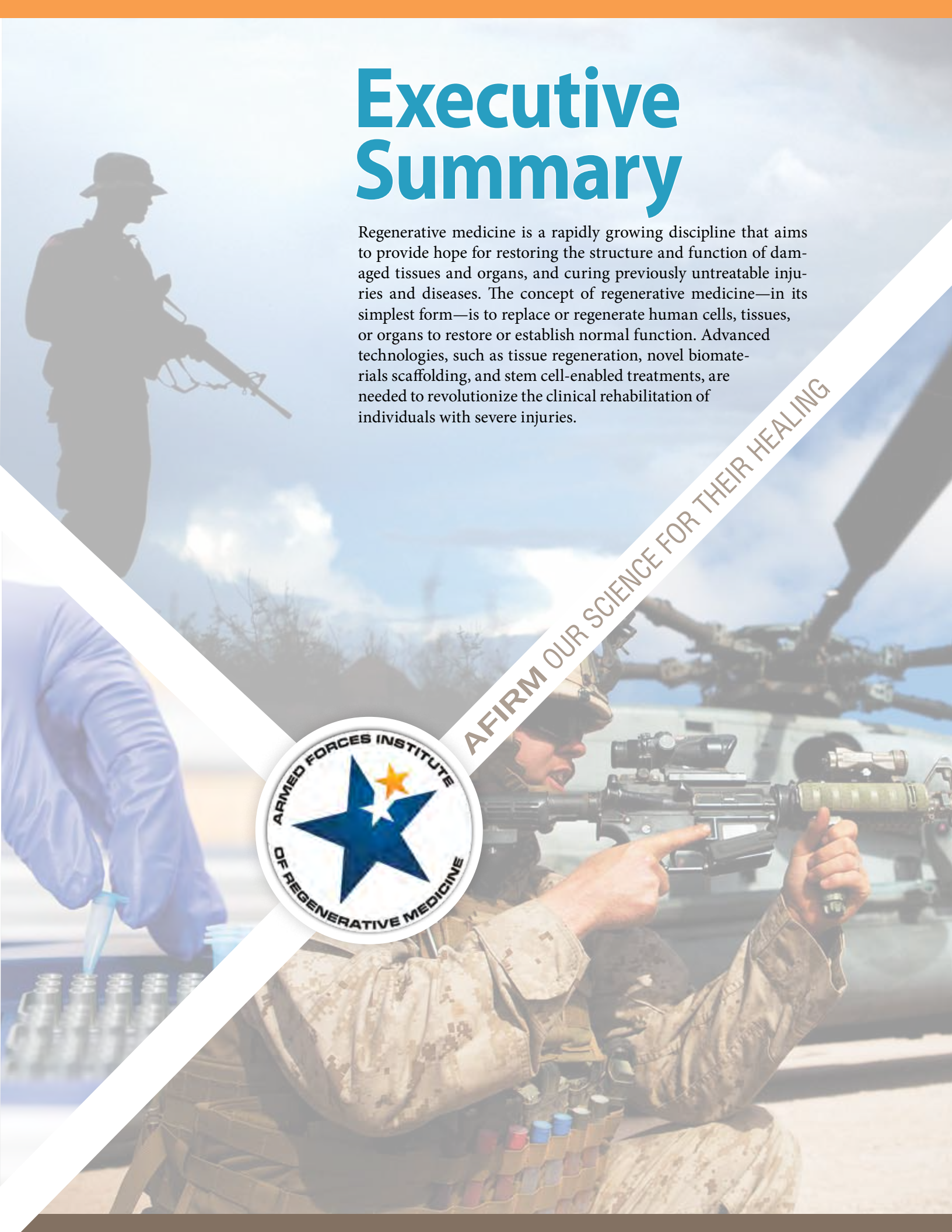
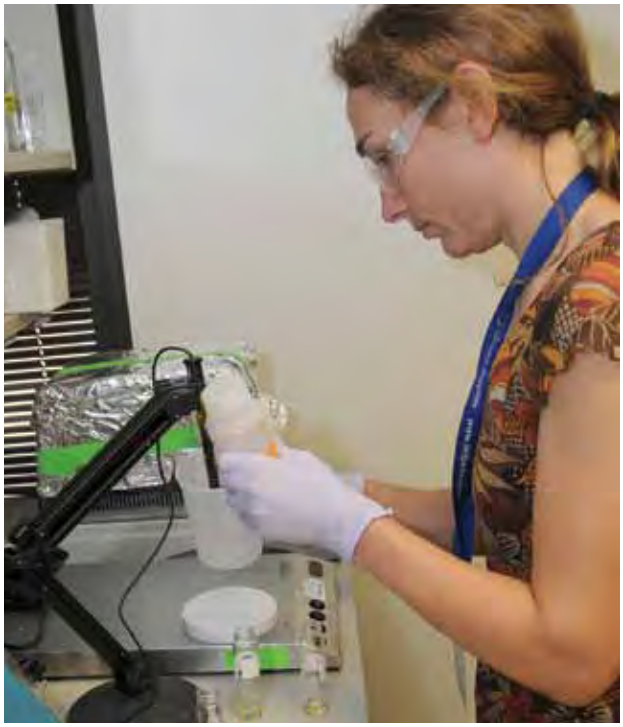
Executive Summary

Regenerative medicine is a rapidly growing discipline that aims to provide hope for restoring the structure and function of damaged tissues and organs, and curing previously untreatable injuries and diseases. The concept of regenerative medicine—in its simplest form—is to replace or regenerate human cells, tissues, or organs to restore or establish normal function. Advanced technologies, such as tissue regeneration, novel biomaterials scaffolding, and stem cell-enabled treatments, are needed to revolutionize the clinical rehabilitation of individuals with severe injuries.





Dr. Krista Niece, a research associate at USAISR, measures fluoride levels in media from cells cultured with the antifungal drug voriconazole. Elevated serum fluoride levels have been reported in patients taking voriconazole.

In 2008, the Department of Defense established the Armed Forces Institute of Regenerative Medicine (AFIRM) to help treat injured warfighters who have survived serious injury (potentially due to advances in body armor, battlefield evacuation, and/or military medical care) only to face the challenge of overcoming severe limb, head, face, and burn wounds caused by the use of improvised explosive devices. As a multi-institutional, interdisciplinary network of scientists, the AFIRM's mission is to accelerate the development of new regenerative medicine-based products and therapies to treat the severe injuries suffered by U.S. Service members. Utilizing a team of well established, proven research investigators, the AFIRM has expanded the regenerative medicine knowledge base, developed models of injury, and tested advanced technology products in an effort to propel the field forward.

The AFIRM has exceeded the U.S. Army's expectations regarding the quickness of translating new therapies from the research laboratory into clinical trials. These advances were made possible by a cultural transformation among the participating laboratories. Usually, academic laboratories receive competitive but rather small, short-term research grants that foster innovation but do not necessarily allow translational research or the formation of collaborative networks. The AFIRM changed this culture by (1) fostering an understanding among its researchers that clinical impact is the ultimate goal, and (2) focusing its mission on synergy through collaboration among the laboratories. This understanding permeates all levels of the AFIRM program including its leadership.

Creating Partnerships and Collaborations

Over the past five years, the AFIRM has achieved success through a six-member partnership that includes the U.S. Army, Navy, and Air Force; the Veterans Health Administration; the Defense Health Program; and the National Institutes of Health. This effective collaboration has yielded more than 85 funded projects to date, with research activities being organized into five primary focus areas that include Limb and Digit Salvage, Craniofacial Reconstruction, Scarless Wound Healing, Burn Repair, and Compartment Syndrome. A Program Synergy Group was established early on to identify collaborative opportunities and to build bridges between the focus areas and projects.

Limb and Digit Salvage

The Limb and Digit Salvage focus area seeks to develop novel solutions using regenerative medicine that will allow victims of severe extremity trauma to recover rapidly, reliably, and completely so they can return to productive lives. In total, 22 projects received funding in Year 5. Projects span the following clinical challenge areas: Bone, Soft Tissue, and Nerve Repair/Regeneration; Composite Tissue Injury Repair; and Epimorphic Regeneration.

Research under the AFIRM is conducted through two independent research consortia working with the U.S. Army Institute of Surgical Research (USAISR). One research consortium is led by Rutgers, the State University of New Jersey, and the Cleveland Clinic (RCCC [Rutgers–Cleveland Clinic Consortium]), while the other is led by Wake Forest University Baptist Medical Center and the McGowan Institute for Regenerative Medicine in Pittsburgh (WFPC [Wake Forest–Pittsburgh Consortium]). Each consortium is a multi-institutional network, together comprising more than 35 members, which are primarily academic institutions. Notably, AFIRM member organizations have established collaborations/partnerships with more than 100 academic or industrial institutions located within and outside of the U.S. (including Australia, China, Finland, France, Germany, Italy, and the Netherlands).

As the AFIRM consortia continue to exhibit a substantial level of collaboration and coordination, a web of new working relationships has evolved between the two academic consortia (RCCC and WFPC) and USAISR, and between academic and military scientists and clinicians. This type of successful collaboration is illustrated by a Craniofacial Reconstruction project that is focused on developing vascular tissue engineering treatments using bioactive scaffolds. Funded in 2010 by an AFIRM supplement, this project links the Longaker group at Stanford University (WFPC member) with the Anderson/Langer team at the Massachusetts Institute of Technology (RCCC member). Another example of successful collaborative activity within the AFIRM involves a number of strong working relationships that have developed over the past two years between RCCC and WFPC investigators and military clinicians at the Walter Reed National Military Medical Center (WRNMMC). For example, a WRNMMC patient who received a double hand transplant by WFPC researchers at Johns Hopkins University was recently transferred back to WRNMMC to continue to receive hand therapy and rehabilitation.

The development of new partnerships with scientists at various academic institutions as well as with industry partners has led to the establishment of 7 new AFIRM projects in the areas of Craniofacial Reconstruction, Burn Repair, and Limb and Digit Salvage since 2012. These projects are being led by the following individuals: Dr. Brian Barnes of Arteriocyte Medical Systems, Inc.; Dr. Stephen Feinberg of the University of Michigan; Dr. Bohdan Pomahac of Brigham and Women's Hospital; Drs. David Sachs and Curtis Cetrulo, Jr., of Massachusetts General Hospital; Drs. Douglas Smith and D. Kacy Cullen of the University of Pennsylvania; Dr. Richard Clark of NeoMatrix Formulations, Inc.; and Dr. Thomas Tulenko of Cooper Medical School of Rowan University. Partnerships such as these will add new technologies to the existing research portfolio, helping to fulfill the mission of the AFIRM.

Craniofacial Reconstruction

The Craniofacial Reconstruction focus area aims to generate both soft and hard tissues through novel regenerative medicine approaches to reduce the impact to wounded warriors of devastating, disfiguring facial injuries. In total, 18 projects received funding in Year 5. Projects span the following clinical challenge areas: Bone Regeneration, Soft Tissue Regeneration, and Cartilage Regeneration (with a focus on the ear).

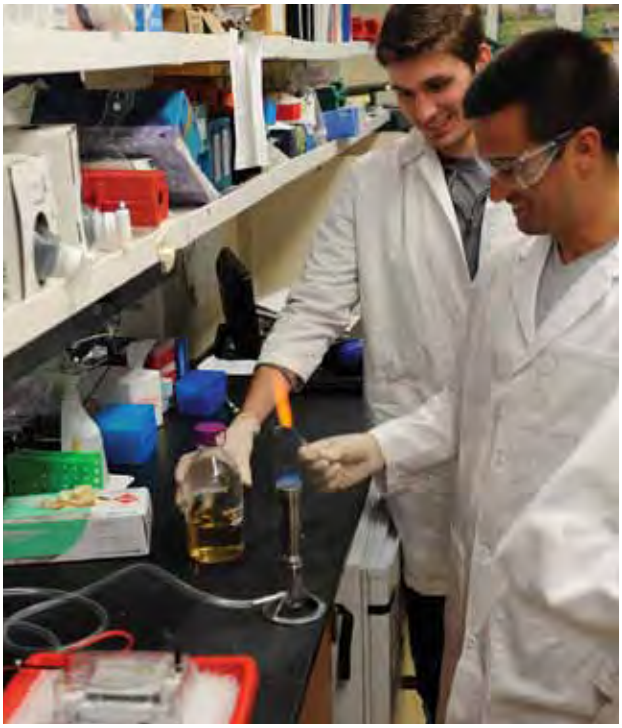
Year 5 Program Highlights

The AFIRM program involves the efforts of nearly 500 individuals, which include faculty members, postdoctoral fellows, graduate students, scientific and technical staff, and undergraduates. AFIRM faculty members are highly accomplished scientists: During the fifth year of the program, AFIRM faculty received 43 honors or awards, including selection to membership or leadership positions in professional societies, invited presentations and lectureships, receipt of honorary degrees, awards from private foundations, recognition of excellence at conferences, and faculty teaching awards.

AFIRM-sponsored researchers are making substantial contributions to the scientific literature: In the fifth year of the program, the group published 85 articles in peer-reviewed journals and produced 70 presentations and non-peer-reviewed publications. AFIRM scientists also have been making novel patentable discoveries in the field of regenerative medicine: During the fifth year of the program, the researchers filed 8 invention disclosures and 8 U.S. Government patent applications.

The Technology Readiness Levels (TRLs) of products generated by AFIRM-funded researchers have been steadily rising. At the start of the program, 58 of 63 research and development products (92%) were nearly evenly distributed across TRLs 1, 2, and 3. By the end of the fifth year of the program, approximately one-third of the products were at TRLs 2 and 3, one-third at TRL 4, and one-third at TRL 5 or greater. The shift to higher TRL numbers demonstrates the increasing translation of products into preclinical studies and human clinical studies.

The AFIRM has recruited a substantial number of talented young scientists into the field of regenerative medicine. During the fifth year of the program, more than 100 graduate and undergraduate students received practical scientific training through AFIRM-sponsored research projects. The number of advanced degrees (master's or doctoral) awarded to students who received training through AFIRM-sponsored projects has risen from 3 in the first year of the program to 24 in the fifth year.



WFPC postdoctoral fellows prepare a glass pipette for sterile technique.

Scarless Wound Healing

The Scarless Wound Healing focus area encompasses technologies aimed at the various stages of wound healing to find new treatment options to prevent and manage scars. In total, 9 projects received funding in Year 5. Projects span the following clinical challenge areas: Control of Wound Environment and Mechanics, Therapeutic Delivery to Wounds, and Attenuation of Wound Inflammatory Response.

Year 5 Research Highlights

Research efforts over the past 5 years have led to dozens of noteworthy accomplishments, including the following:

Dr. Matthew Tirrell and colleagues at the University of California, Berkeley, and the University of Chicago (Limb and Digit Salvage, Project 4.4.5, WFPC), are pursuing an approach to induce peripheral nerve growth following traumatic amputation by modulating components of the naturally occurring extracellular matrix (ECM). The researchers have developed a peptide-based hydrogel system as injectable ECMs with nanofibrous structures. They can incorporate bioactive peptide sequences and/or growth factors into the hydrogels. The concentration of the hydrogels, which is directly linked to stiffness, can be tuned to promote the spreading, proliferation, and migration of a model cell type, Schwann cells. The researchers will choose the hydrogel concentration that performs best in their in vitro

models, and they will use this concentration in animal studies.

Dr. Scott Guelcher and colleagues at Vanderbilt University (Craniofacial Reconstruction, Projects 4.5.1a/4.5.7, RCCC) are developing and evaluating allograft bone/polymer composites for treating trauma-related bone defects. The researchers are pursuing two related projects in collaboration with researchers at the USAISR and Medtronic. In Project 4.5.1a, they are developing injectable, settable LV® bone grafts for the repair of long bone defects. In Project 4.5.7, they are developing injectable, settable LV bone grafts augmented with recombinant human bone morphogenetic protein-2 (rhBMP-2) for the repair of craniofacial and long bone defects. The researchers have demonstrated that their LV grafts support bone remodeling and healing in rabbit and sheep leg bone defect models. They also found that delivery of rhBMP-2 from LV grafts enhanced new bone formation in a rat skull defect model. Pending 510(k) regulatory clearance of the LV bone graft and successful completion of the preclinical studies, preparation for a clinical trial in patients requiring ridge augmentation will begin.

Dr. Sang Jin Lee and colleagues at Wake Forest University (Compartment Syndrome, Project 4.3.5, WFPC) are focused on developing an approach to enhance the recruitment of endogenous stem and progenitor cells to the site of compartment syndrome injury to increase the regenerative response. The researchers are using biomaterials containing myogenic (muscle cell)-inducing factors that can be implanted within the injured muscle compartment. They have demonstrated that host muscle stem cells can be recruited into implanted biomaterials in situ, and that these cells can be transformed into muscle cells using myogenic-inducing factors. They have developed novel injectable and implantable scaffolding systems using heparin-conjugated gelatin microparticles and heparin-conjugated cell-free muscle matrix, respectively. The researchers demonstrated the mobilization of host stem and progenitor cells, as well as new muscle tissue regeneration in vivo in rats, using these scaffolding systems.

Dr. Thomas Mustoe and colleagues at Northwestern University (Scarless Wound Healing, Project 4.6.3, RCCC) are investigating the wound-healing capability of curcumin (a yellow-colored component of the Indian spice turmeric). The researchers found that the intravenous delivery of curcumin accelerated healing and reduced scarring in a rabbit ear ischemia/reperfusion (I/R) model. They also found that they could apply curcumin via tiny nanospheres to the wound site in the rabbit I/R model with no apparent toxicity. They recently confirmed the effectiveness of curcumin in reducing necrosis and supporting the survival of skin flaps in a porcine model. They expect the transition to Good Manufacturing Practices to be straightforward based on discussions with manufacturers of curcumin.

Dr. Robert Christy and colleagues at USAISR (Burn Repair, Project 4.6.8, USAISR) isolated human adipose-derived stem cells (ASCs) using a point-of-care device. They developed the technology to differentiate ASCs into epithelial cells for use on patients so severely burned that they do not have an autologous source of epithelial cells. They also demonstrated that stem cells can be isolated in adequate quantities from the adipose layer of discarded burn skin. They recently developed the technology to remove the cells from the amniotic membrane for epithelial differentiated ASC sheets and epithelial wound covering. They also developed a porcine model to determine the validity of skin constructs in deep partial thickness burns. The researchers plan to

Burn Repair

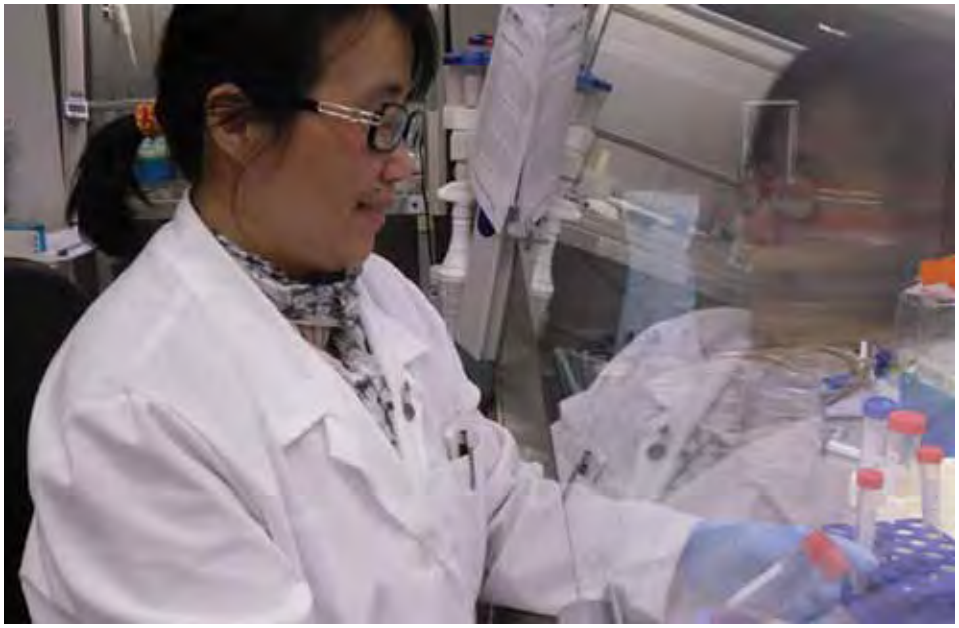
The Burn Repair focus area seeks to leverage regenerative medicine technologies to reduce morbidity and mortality in victims of severe burns, both military and civilian. In total, 15 projects received funding in Year 5. Projects span the following clinical challenge areas: Intravenous Treatment of Burn Injury, Topical Treatment of Burn Injury, Wound Healing and Scar Prevention, and Skin Products/Substitutes.

continue to use and optimize the InGeneron, Inc. point-of-care device for the isolation of ASCs. They will also develop a more clinically relevant porcine burn model, and use this model for the development of a large (up to 20%) total body surface area burn, which will provide a stringent test for their, and other AFIRM-related, skin equivalent products.

From the Laboratory to the Battlefield

AFIRM-funded researchers share a strong commitment to bringing therapies as quickly as possible to wounded warriors and the civilian sector. The conduction of clinical trials is the ultimate goal of many AFIRM-sponsored projects. Through Year 5, AFIRM investigators have advanced numerous products through clinical study planning, approval, and execution stages. Ten (10) clinical trials were open to patient enrollment during the fifth year of the program, including six Phase I or I/II, three Phase III, and one diagnostic study. Another three clinical protocols for Phase I or I/II studies were submitted to Institutional Review Boards or Human Research Protection offices.

Nontechnical summaries of the AFIRM clinical trials can be found at the end of each focus area-related chapter, and full progress reports of the clinical trials (when available) are located in the AFIRM Annual Report 2013 – Technical Progress Reports. The following are a few examples of promising translational research projects:



Hulan Shang prepares adipose-derived cells for flow cytometry.

In Project 4.7.3 (Scarless Wound Healing, RCCC/USAISR), researchers at the University of Florida (UF) are conducting a Phase I/IIa clinical trial designed to test the safety and efficacy of using autologous fat transfer (AFT) for scar prevention and remodeling. Patient enrollment began in July 2010. To date, the researchers have enrolled and treated 10 patients. The key accomplishments for the past year were the successful transition of the clinical trial to UF, and the finalization of all administrative and protocol-related logistics at both UF and USAISR/Brooke Army Medical Center, such that the trial is open to enrollment at two new sites. There are multiple companies that currently market fat tissue transplantation devices and systems. If the evidence from this trial supports the use of AFT

Compartment Syndrome

The Compartment Syndrome focus area addresses secondary sequelae of trauma. Projects in this program seek to prevent, repair, or regenerate tissue damaged by compartment syndrome, thereby enhancing the healing process for a quicker return to functionality. In total, 6 projects received funding in Year 5. Projects span the following clinical challenge areas: Cellular Therapy of Compartment Syndrome and Biological Scaffold-Based Treatment of Compartment Syndrome.

for scar remodeling, the researchers do not expect any problems regarding commercialization.

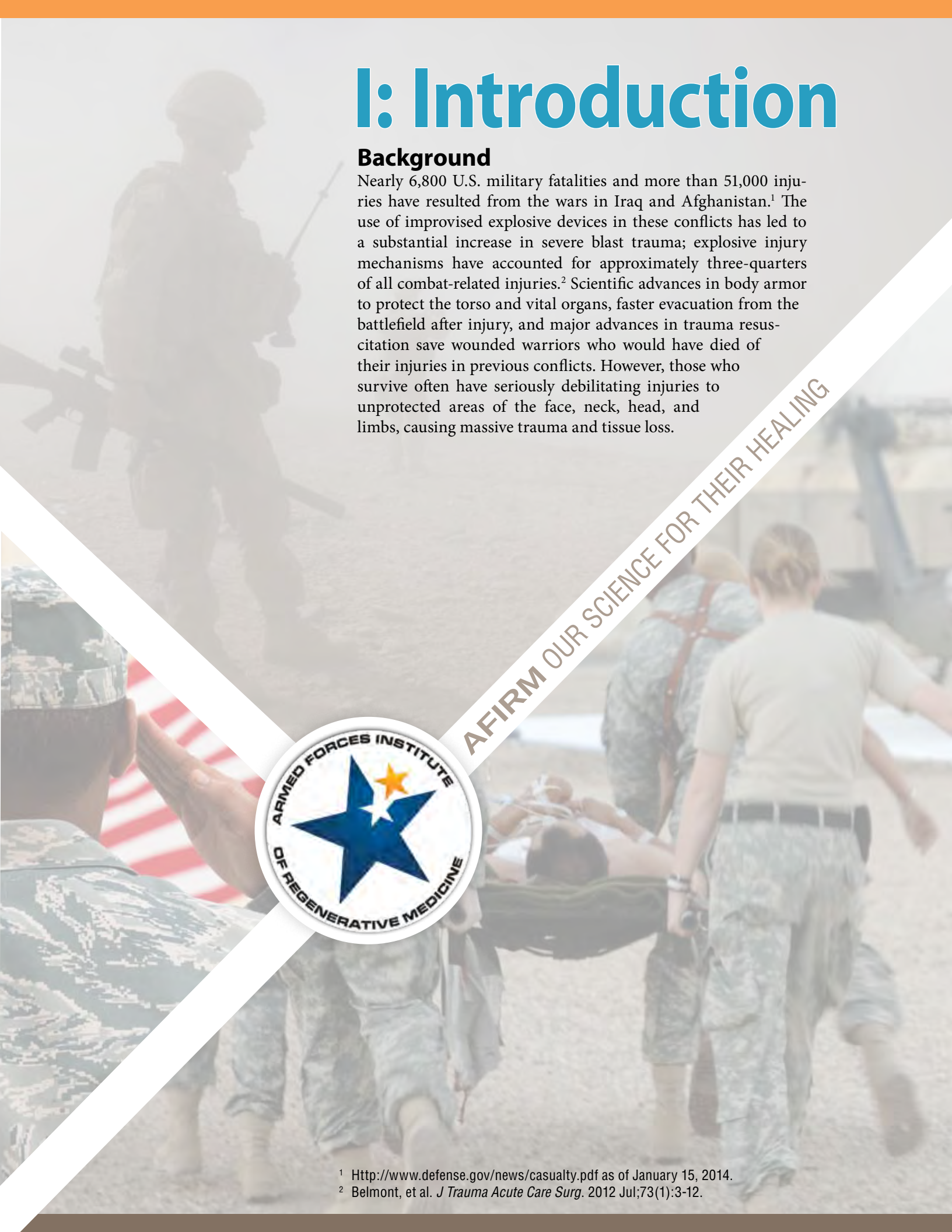
In Project 4.4.2 (Limb and Digit Salvage, WFPC), researchers at the Johns Hopkins University School of Medicine and the University of Pittsburgh are developing a protocol for hand transplantation using donor bone marrow stromal cells in combination with novel fusion proteins (the "Pittsburgh Protocol") that will minimize maintenance immunosuppressive therapy. The researchers have performed 10 successful hand/forearm transplants in six patients, including the first bilateral and first above elbow arm transplant in the U.S. All compliant transplant patients to date are being maintained on a single immunosuppressive drug at low doses, and they continue to have increased motor and sensory function of their transplanted hands, which correlates with their severity of amputation, time after transplant, and participation in hand therapy.

In Project 4.5.2 (Craniofacial Reconstruction, RCCC), researchers at the University of Michigan are using a tissue-engineering/regenerative medicine approach, in conjunction with the surgical technique of prelamination, to create a prevascularized composite soft tissue flap. More specifically, the researchers are using an ex vivo-produced oral mucosa equivalent for major areas of intra-oral reconstruction, and as a platform technology for the tissue engineering of a set of human lips. The technology in development is similar to the development of orphan drugs, in that the target market area is very specific: post-traumatic (explosives, burns, gunshot and motor vehicle accidents) and post-oncologic surgery.

I: Introduction

Background

Nearly 6,800 U.S. military fatalities and more than 51,000 injuries have resulted from the wars in Iraq and Afghanistan.[1] The use of improvised explosive devices in these conflicts has led to a substantial increase in severe blast trauma; explosive injury mechanisms have accounted for approximately three-quarters of all combat-related injuries.[2] Scientific advances in body armor to protect the torso and vital organs, faster evacuation from the battlefield after injury, and major advances in trauma resuscitation save wounded warriors who would have died of their injuries in previous conflicts. However, those who survive often have seriously debilitating injuries to unprotected areas of the face, neck, head, and limbs, causing massive trauma and tissue loss.



[1] Http://www.defense.gov/news/casualty.pdf as of January 15, 2014.
[2] Belmont, et al. *J Trauma Acute Care Surg*. 2012 Jul;73(1):3-12.

The emerging field of regenerative medicine holds great potential for healing military personnel with debilitating, disfiguring, and disabling injuries. Regenerative medicine focuses on (1) restoring the structure and function of tissues and organs that have been damaged, and (2) finding methods of curing previously untreatable injuries and diseases. Scientists are applying a variety of approaches to prompt the body to regenerate cells and tissues, often using the patient's own cells combined with degradable biomaterials. Use of a patient's own cells eliminates the possibility of tissue rejection. Recent years have seen the rapid development of technologies for engineering tissues. Scientists ultimately hope to deliver advanced therapies, such as whole organs and engineered fingers and limbs, to injured members of the military as well as civilians.

Research Goals

The AFIRM is a multi-institutional, interdisciplinary network of leading universities, hospitals, and private companies working to develop advanced treatment options for severely wounded warfighters. The AFIRM was designed to accelerate the delivery of regenerative medicine therapies to treat our most severely injured Service members, but in particular those coming from our theaters of operation in Iraq and Afghanistan. Clinical trials have begun for several AFIRM products, including advanced transplantation strategies and engineered skin replacement applications. The inclusion of military patients in these trials is the first step in delivering advanced technologies to wounded warriors.

RCCC researcher Glenda Evans analyzes images on bone regeneration.

The Five Major Research Programs of the AFIRM

Limb and Digit Salvage

Saving the limb, also referred to as "limb salvage," at a minimum requires (1) bridging large bony defects to restore skeletal integrity; (2) bridging soft tissues, such as muscle, nerves, tendons, and ligaments, to lend stability and enable movement; and (3) covering the injured area with healthy skin. The AFIRM Limb and Digit Salvage program is focused on developing regenerative medicine therapies to help healthcare providers save and rebuild injured limbs. The ultimate goal of this program is to enable victims of severe extremity trauma to recover rapidly, reliably, and completely so they can return to productive lives.

Craniofacial Reconstruction

Massive bone and soft tissue loss to the face and head due to blast forces is a devastating injury. Researchers funded by the AFIRM Craniofacial Reconstruction program are designing and developing therapies that healthcare providers can use to return form and function of the face, head, and neck to warfighters with severe craniofacial injuries. These therapies are expected to (1) regenerate functional bone and cartilage to levels of the face; (2) restore motor and sensate competencies through muscle, vascular, and nerve regeneration; (3) mitigate scar formation; (4) prevent infection; and (5) eliminate

Rutgers graduate student Koustubh Dube works in the walk-in hood used for kilogram-scale polymer synthesis at the New Jersey Center for Biomaterials.

skin coverage deficits through tissue engineering. The creation and delivery of new polymers and tissues will preserve and regenerate bone and soft tissue capable of administering stem cells, growth factors, bone derivatives, and therapeutic drugs.

Scarless Wound Healing

Severe military trauma burns often heal with large scars that may impair normal function and cause significant disfigurement. Scars are the result of the body's complex series of wound-healing processes that begin at the onset of injury and can continue for months. The AFIRM Scarless Wound Healing program is focused on investigating all phases of wound healing and scar formation to find new treatment strategies to prevent and mitigate scars.

Burn Repair

Although there have been many advances in medical care, severe burns are still associated with substantial morbidity and mortality. The AFIRM Burn Repair program is leveraging regenerative medicine technology to (1) prevent wound infection, (2) prevent burn inflammation and injury progression, (3) speed the generation of a viable wound bed and reduce the reharvest time of autograft donor sites, (4) improve skin substitutes for burn wound grafting when autografts are not immediately available, and (5) prevent and manage scars.

Compartment Syndrome

Compartment syndrome is often a secondary sequela resultant from blast injuries, severe blunt or penetrating trauma, fractures, and vascular injuries. Muscles are encased in compartments of nonyielding tissue called fascia. Bleeding or tissue swelling within a muscle compartment raises the pressure in the compartment and, if unchecked, this pressure can become high enough that blood flow into the compartment is reduced or completely stopped. Prolonged interruption of blood flow can destroy the nerves and muscles within the compartment. Restoration of these damaged or destroyed tissues has no satisfactory solution with current surgical options. The AFIRM Compartment Syndrome program focuses on advancing effective therapies to stabilize tissue and reduce the onset of late effects of nerve and muscle damage.

History

In 2005, Dr. Anthony Atala presented some of the latest advances in the field of regenerative medicine at the Advanced Technology Applications in Combat Casualty Care Conference. This talk alerted the combat casualty care research community to the near-term potential for regenerative medicine products that could make a substantial difference in the care of our wounded warriors. In 2006, the Army's Director of the Combat Casualty Care Research Program, COL Bob Vandre, developed the idea of a regenerative medicine institute similar to the Department of Defense (DoD) Multidisciplinary University Research Initiative, but aimed at near-term, translational research. Soon thereafter, COL Vandre received approval from the U.S. Army Medical Research and Materiel Command (USAMRMC) to pursue funding for this project. He briefed the DoD Technology Area Review and Analysis panel, which reviews medical research and development for the DoD, and the panel highly approved the concept.

In 2007, USAMRMC, the Office of Naval Research (ONR), the U.S. Air Force Office of the Surgeon General, the National Institutes of Health (NIH), and the Veterans Health Administration of the U.S. Department of Veterans Affairs (VA) agreed to co-fund the new institute. These funds, along with $10 million (M) from the 2007 War Supplemental bill, provided $8.5M per year in funding for the AFIRM, which was deemed sufficient to proceed. A Program Announcement was released in August 2007, and seven proposals were received in October 2007. Two finalists were selected for oral presentations in December 2007. Both received scores of "excellent," and one was selected for funding. White House staffers learned of the AFIRM and invited representatives from USAMRMC to meet and discuss the new institute.

Cell Therapy technicians at Lonza Walkersville, Inc. perform cell culture in a Lonza current Good Manufacturing Practices-compliant facility.

After two meetings, and upon hearing that funding was available for only one AFIRM finalist, the DoD was tasked to provide funding for the second AFIRM finalist. Within 1 week, an additional $8.5M per year was transferred to USAMRMC's budget lines. Both AFIRM finalists signed USAMRMC cooperative agreements in March 2008.

Funding: A Six-Way Partnership

The AFIRM is financed with basic research through exploratory development funds and is expected to make major advances in the ability to understand and control cellular responses in wound repair and organ/tissue regeneration. The program is managed and funded through USAMRMC with funding from the following organizations:

- U.S. Army
- U.S. Navy, ONR
- U.S. Air Force, Office of the Surgeon General
- Veterans Health Administration
- Defense Health Program
- NIH

Total funding for the first 5 years of the AFIRM amounts to more than $300M:

- $100M from U.S. government funding (Army, Navy, Air Force, VA, and NIH).
- $80M from matching funds received from state governments and participating universities.
- $109M from pre-existing research projects directly related to deliverables of the AFIRM from the NIH, Defense Advanced Research Projects Agency, congressional special programs, the National Science Foundation, and philanthropy.
- $25M in additional funds provided by the Defense Health Program.

AFIRM was recently extended for a sixth year with no additional funds. A new effort, referred to as AFIRM II, will build on the success of the AFIRM. Following an open competition, the Wake Forest University School of Medicine (Wake Forest Baptist Medical Center) was selected to lead AFIRM II. Funded through a cooperative agreement with USAMRMC, ONR, NIH, Air Force Medical Service, VA Office of Research and Development, and Office of the Assistant Secretary of Defense for

Health Affairs, AFIRM II is scheduled to begin in fiscal year 2013. AFIRM II will encompass five key research areas, including extremity regeneration, craniomaxillofacial regeneration, skin regeneration, composite tissue allotransplantation and immunomodulation, and genitourinary/lower abdomen reconstruction.

Structure

The AFIRM is composed of two independent civilian research consortia working with the U.S. Army Institute of Surgical Research (USAISR) at Fort Sam Houston, Texas. USAISR, which includes the San Antonio Military Medical Center – North (formerly Brooke Army Medical Center), serves as the AFIRM's primary government component and is home to the DoD's only burn unit. The two AFIRM research consortia are responsible for executing the management of overall therapeutic programs and individual projects within their consortia. One consortium is led by Rutgers, the State University of New Jersey, and the Cleveland Clinic, and the other is led by the Wake Forest Institute for Regenerative Medicine and the McGowan Institute for Regenerative Medicine in Pittsburgh. Each of these civilian consortia is itself a multi-institutional network, as described below.

Rutgers–Cleveland Clinic Consortium

The Rutgers–Cleveland Clinic Consortium (RCCC) is directed by Joachim Kohn, PhD, Director of the New Jersey Center for Biomaterials and Board of Governors Professor of Chemistry at Rutgers University, and co-directed by Linda Graham, MD, Staff Vascular Surgeon at the Cleveland Clinic and Professor in the Department of Biomedical Engineering at the Lerner Research Institute at Case Western Reserve University.

The RCCC consists of the following member institutions:

- Rutgers, the State University of New Jersey/New Jersey Center for Biomaterials
- Cleveland Clinic
- Brigham and Women's Hospital
- Carnegie Mellon University
- Case Western Reserve University
- Cooper Medical School of Rowan University
- Dartmouth Hitchcock Medical Center/Thayer School of Engineering
- Massachusetts General Hospital/Harvard Medical School
- Massachusetts Institute of Technology
- Minnesota Medical Research Foundation
- Mayo Clinic College of Medicine
- Northwestern University
- Stony Brook University
- University of Cincinnati
- University of Florida
- University of Medicine and Dentistry of New Jersey
- University of Minnesota
- University of Michigan
- University of Pennsylvania
- University of Virginia
- Vanderbilt University

Wake Forest–Pittsburgh Consortium

The Wake Forest–Pittsburgh Consortium (WFPC) is directed by Anthony Atala, MD, Director of the Wake Forest Institute for Regenerative Medicine and Professor and Chair of the Department of Urology at Wake Forest University, and co-directed by Rocky Tuan, PhD, Director of the Center for Cellular and Molecular Engineering at the University of Pittsburgh.

The WFPC consists of the following member institutions:

- Wake Forest Institute for Regenerative Medicine/Wake Forest University
- McGowan Institute for Regenerative Medicine/ University of Pittsburgh
- Allegheny-Singer Research Institute
- Carnegie Mellon University
- Georgia Institute of Technology
- Institute for Collaborative Biotechnologies (includes University of California, Santa Barbara; Massachusetts Institute of Technology; and California Institute of Technology)

- Johns Hopkins University School of Medicine
- Oregon Health and Science University
- Rice University
- Sanford-Burnham Medical Research Institute/ University of California, Santa Barbara
- Stanford University
- Tufts University
- University of California, Berkeley
- University of Chicago
- University of Texas Health Science Center at Houston
- University of Wisconsin
- Vanderbilt University

Additional Collaborators to the AFIRM

AFIRM researchers have established a wide variety of both national and international partnerships with academia and industry, which has contributed to the success of the program to date. These have included collaborations with:

- Allegheny-Singer Research Institute
- Armand Trousseau Hospital (France)
- Arizona Burn Center
- Arteriocyte Medical Systems, Inc.
- Avita Medical, LLC
- Axonia Medical
- Baylor All Saints Medical Center
- Baylor College of Medicine
- Baylor University Medical Center
- Biogeneral, Inc.
- Biologics Consulting Group
- Biosafe-America
- BioStat International, Inc.
- BonWrx, Inc.
- Buffalo General Hospital
- CVPath Institute, Inc.
- Cynvenio Biosystems
- Emory University
- Evonics

RCCC researcher Jarred Nesbitt performs post-operative care in the animal care facility.

- Fidia Advanced Biopolymers (Italy)
- GID Group, Inc.
- Glycosan BioSystems, Inc.
- Healthpoint Biotherapeutics, Ltd./ DFB Bioscience
- ImageIQ, Inc.
- InGeneron, Inc.
- Integra Spine/Integra LifeSciences
- Intercytex Ltd.
- Intrinsix Corp.
- Jefferson University Hospital
- Johann Wolfgang University (Germany)
- Kensey Nash Corporation
- KeraNetics, LLC
- Kinetic Concepts, Inc.
- Lexmark, Inc.
- LifeCell Corporation
- LifeNet Health
- Lonza Walkersville, Inc.
- Louisiana State University

Christine Miller, a National Research Council postdoctoral fellow at the U.S. Army Institute of Surgical Research, prepares Ribonucleic acid (RNA) samples for sequencing to identify RNA-based therapeutics for the treatment of drug-resistant biofilm pathogens commonly associated with chronic wounds.

- Loyola University Medical Center
- Maricopa Integrated Health Systems
- Massachusetts Eye and Ear Infirmary
- MedDRA Assistance, Inc.
- Medical University of South Carolina
- Medtronic, Inc.
- Montefiore Medical Center
- Morgridge Institute for Research
- Neodyne Biosciences
- NeoMatrix Formulations, Inc.
- New York University
- Nitinol Development Corporation
- Norman Noble, Inc.
- North Carolina State University
- NOVOTEC
- NovoPedics, Inc.
- Numia Medical
- Ohio State University
- Oregon Biomedical Engineering Institute
- Oregon Medical Laser Center
- Organogenesis, Inc.
- Orlando Regional Medical Center
- Osteotech, Inc.
- Pennington Biomedical Research Center, Louisiana State University
- PeriTec Biosciences, Ltd.
- Philadelphia University
- Proxy Biomedical
- Queensland University of Technology (Australia)
- Radboud University of Nijmegen Medical Centre (The Netherlands)
- ResearchPoint Global
- Resonetics
- Rockefeller University
- Royal Perth Hospital (Australia)
- St. Barnabas Medical Center
- San Antonio Military Medical Center – North (formerly Brooke Army Medical Center)
- Shanghai 9th People's Hospital (China)
- SimQuest, LLC
- Spaulding Rehabilitation Hospital
- Special Operations Medical Command-Fort Bragg
- Stratatech Corporation
- Stryker Corporation
- Texas Tech University
- Tolera Therapeutics, Inc.
- Trident Biomedical, Inc.
- University Hospitals, Cleveland
- University of Alabama at Birmingham
- University of California, Davis
- University of California, Los Angeles
- University of Colorado Hospital

RCCC researcher Joseph Morand performs peripheral nerve microsurgery under a microscope at the University of Pennsylvania.

- University of Indiana
- University of Kentucky
- University of Massachusetts, Lowell
- University of Michigan
- University of North Carolina at Chapel Hill
- University of South Florida/Tampa General Hospital
- University of Tampere (Finland)
- University of Tennessee Health Science Center
- University of Texas at Arlington
- University of Texas at Austin
- University of Texas, San Antonio
- University of Texas Southwestern Medical Center
- University of Utah
- University of Washington-Harborview Medical Center
- University of Wisconsin–Madison
- Virginia Commonwealth University
- Washington Hospital Center (Washington, DC)

Programs and Projects

Research activities are organized into programs within each consortium. While some of the con-sortia programs are directly comparable to the AFIRM's major research programs (e.g., RCCC's CranioMaxilloFacial Program), other consortia programs consolidate expertise (e.g., WFPC's Extremity Injury Program, which integrates experts in the areas of limb and digit salvage and compartment syndrome). A scientist or clinician Program Leader coordinates each program, which consists of multiple projects. One or more Project Leaders directs each project, which can vary in size from a single laboratory to a collaboration spanning multiple institutions.

Consortium members evaluate all levels of the operation on an annual basis to monitor progress and guide the consortium's activities. Active project management by each consortium has reshaped the programs, leading to the termination or reduced funding of some projects and the addition of projects that are more promising for accelerated development. The consortia also engage external scientific program and product development consultants who provide advice regarding clinical trials, product development plans, and other recommendations for commercialization. Additionally, information for the public, including clinical trial opportunities, has been made available through websites developed and maintained by the consortia.

In addition to the three core groups (RCCC, WFPC, and USAISR), intramural researchers from the NIH

and/or the Veterans Health Administration can participate in the AFIRM program, although none have chosen to do so as of yet. With the approval of a program leader, these intramural researchers can lead projects.

Management and Oversight

The day-to-day execution of the AFIRM's research portfolio is managed by the AFIRM Project Management Office (PMO), which is located within the U.S. Army Medical Materiel Development Activity at Fort Detrick, Maryland. The AFIRM PMO works as part of an integrated project management team, across the AFIRM consortia, to incorporate the strategic, developmental, and tactical aspects of product management. The AFIRM PMO also functions as an accountability model to ensure execution of the AFIRM portfolio.

The AFIRM is guided by a Board of Directors (BOD) and an Integrated Project Team (IPT), which contains a Steering Group. A Program Synergy Group is responsible for research coordination and communication between the three components of the AFIRM. The roles and membership of each of these entities are described below.

Board of Directors

The AFIRM's BOD is chaired by the Commanding General of USAMRMC, and its members are flag-level representatives from the Army, Navy, Air Force, NIH, VA, Office of the Assistant Secretary of Defense for Health Affairs, TRICARE Management Activity, and the Uniformed Services University of the Health Sciences. The Principal Assistant for Research and Technology of USAMRMC serves as the Deputy Chair of the BOD. The main purpose of the BOD is to provide high-level guidance for the AFIRM by presiding over the IPT and the Program Synergy Group.

Integrated Project Team

The AFIRM's IPT is chaired by the Director of the USAMRMC's Clinical and Rehabilitative Medicine Research Program (CRMRP). IPT membership consists of a group of experts who represent the interests of the funding agencies, experts in military needs, external scientists knowledgeable in regenerative medicine, and specialists in contracting and product development. The overall function of the IPT is to ensure that the AFIRM meets military needs, funds superior science, and is well managed.

The specific responsibilities of the IPT are to:

- Approve the annual report and program plans that are presented to the BOD.
- Ensure that all AFIRM research projects are aligned with military requirements.
- Monitor and evaluate the activities and progress of the AFIRM programs and management, and provide recommendations based on their expertise.

Bridget Ford, a postdoctoral fellow in the Extremity Trauma and Regenerative Medicine task area at USAISR, prepares adipose-derived stem cells for use in tissue engineered scaffolds for wound-healing applications.

- Facilitate the military's evaluation and purchasing of products developed by the AFIRM.
- Assist consortia directors and management teams in internal communication within the DoD, and in understanding and meeting DoD regulation and reporting requirements relative to AFIRM performance.
- Facilitate the leveraging of AFIRM resources by coordinating with other funding agencies that support closely related research.

The IPT's Steering Group has day-to-day decision-making authority over the AFIRM and recommends major changes in research direction or funding to the voting members of the IPT. This group is chaired by the AFIRM Project Director and also includes the USAISR Commander, the Combat Casualty Care Senior Scientist, the Contracting Officer, and the Directors and Co-Directors of the RCCC and the WFPC. Among other activities, the Steering Group ensures that all AFIRM research projects are aligned with military requirements, reviews AFIRM research allocation, establishes decision points and continuation criteria, assesses project and program achievements in relation to milestones and timelines, and recommends continuation or termination of programs and individual projects to the IPT.

The IPT has additional members from the Army, Navy, Air Force, and VA (one representative from each of these organizations), three representatives from the NIH (sharing one vote), and four external scientists. The IPT also has ex officio advisors from the Judge Advocate General, the DoD Human Research Protection Office, a commercialization expert, and a regulatory expert appointed by the CRMRP.

The Steering Group and the additional IPT members are voting members of the IPT. They are assisted by the ex officio members of the IPT and the Program Synergy Group to ensure that the AFIRM is progressing toward solutions for militarily relevant injuries.

Program Synergy Group

The Program Synergy Group includes representatives from each of the major programs in each of the consortia, members of the NIH or VA intramural research programs (as deemed appropriate), and USAISR. The Program Synergy Group is chaired by one of the consortia Co-Directors. It serves as a conduit for information exchange among the cores and seeks to build bridges between the programs and projects. It identifies and promotes opportunities to share or combine best practices and to accelerate existing projects or initiate new projects to bring therapies to our wounded Service members. The Program Synergy Group reports its findings and recommendations twice a year to the Steering Group.

II: Limb and Digit Salvage

Background

Warfighters in combat often suffer severe trauma to the extremities, and these injuries can result in large areas of tissue loss, disrupting function of the distal limb and interfering with wound healing. The primary goals of AFIRM's Limb and Digit Salvage Program are to preserve and restore damaged or missing tissue following injury, reduce amputation rates, reduce the time and risks involved in recovery, and enable the warrior's return to an independent, fully functional life and, ideally, return to duty.

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In light of this, researchers at Rutgers–Cleveland Clinic Consortium (RCCC) are working on integrated projects to develop tissue-engineering solutions for bone, nerve, blood vessels, menisci, and skeletal muscle. These researchers are collaborating with investigators in AFIRM's Craniofacial Reconstruction Program to minimize immunosuppression in limb and face transplantation for those cases when salvage is not possible. RCCC researchers are also aligned with ongoing work in AFIRM's Burn Repair Program to develop therapies for restoration of massive partial to full thickness skin loss in limbs following burn injury.

Concurrently, Wake Forest–Pittsburgh Consortium (WFPC) researchers are pursuing an interdisciplinary, multipronged approach to the functional reconstruction/replacement of limb and digit tissue, including transplantation (composite tissue allografts), epimorphic regeneration, tissue regeneration by traditional tissue-engineering approaches, and enabling technologies that may be applied broadly across many types of defects in limb and digit reconstruction.

To help expedite these medical products and procedures, AFIRM researchers have established numerous industry partnerships in anticipation of commercialization, which is a critical step in delivering products to wounded warriors.

Unmet Needs

Today's modern battlefield medicine is helping to save wounded warfighters who would not have survived in previous wars. As severe injuries to extremities are almost never limited to one tissue, failure to address any one tissue can result in functional or actual loss of the limb. Therefore, projects in the Limb and Digit Salvage Program are targeted to make specific advances in these areas of critical unmet needs, which include bone, nerve, artery, and soft tissue, as well as composite tissue injury repair and transplantation.

Bone loss presents a significant challenge for restoration of limb function. Current techniques of bone restoration most often employ autogenous (patient's own) cancellous bone graft, which provides a bone matrix, along with autogenous osteogenic (bone-forming) cells; however, the use of autogenous bone causes additional pain, bleeding, and scars as a result of the multiple surgeries required to both harvest and implant the bone material. Furthermore, the amount of bone that can be harvested from a patient is limited in cases of severe critical-size defects. As an alternative to autogenous bone, recombinant bone morphogenetic protein-2 (BMP-2) has been used in combination with scaffolds to regenerate bone. Unfortunately, while innovative, none of these strategies has achieved rapid and predictable bone regeneration.

The loss of soft tissue presents a significant challenge in the care of a severely damaged limb. These areas of soft tissue, which include muscle, tendon, ligament, fascia and cartilage, have limited restorative capabilities, yet are critical to the function of the salvaged limb. Blood vessel repair for hemorrhage control and rapid restoration of blood supply to an injured extremity remain critical factors in the survival of a wounded warrior and the preservation of limb function. Additionally, restoring vascular function is critical as the previous restorative strategies are dependent on restoring blood flow to the injury site.

Severe extremity injuries may involve extensive nerve loss. Nerve allografts (the use of cadaveric nerve tissue from another individual) represent a promising option for restoration or regeneration of lost nerves; however, allografting requires the patient to endure approximately 18 months of immunosuppressive therapy as endogenous nerves grow across the allogeneic conduit. Due to this extended duration of healing, the patient may be placed at risk of developing infections or other complications. Therefore, a need exists to develop allografts that can repair nerve defects in injured soldiers without the need for long-term immunosuppression. An added critical need exists for synthetic nerve scaffolds that can support consistent regeneration across large nerve gaps.

Limb salvage is possible for many injured warriors; however, when the salvage attempt fails, it is critical that an identically functional replacement is provided through engineering or transplantation. Accurate control of the recipient's immune response poses a significant challenge to the more widespread use of limb transplantation. Therefore, improving immunomodulation techniques to reduce the obstacles to composite tissue transplantation remains a critical unmet need.

Areas of Emphasis

AFIRM researchers are pursuing a complementary mix of research projects focused on various aspects of limb and digit salvage. As shown in **Table II-1**, projects are grouped into five "clinical challenge" topic areas: Bone Repair and Regeneration, Soft Tissue Repair and Regeneration (excluding nerve), Nerve Repair and Regeneration, Composite Tissue Injury Repair, and Epimorphic Regeneration.

Table II-1. Projects funded by RCCC and WFPC per clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Bone Repair and Regeneration	RCCC	4.2.1a	Advanced 3-D Scaffolds for Large Segmental Bone Defects: Non-Load Bearing Tyrosine-Derived Polycarbonate Scaffolds
		4.2.1b	Advanced 3-D Scaffolds for Large Segmental Bone Defects: Partial Load Bearing Poly(Propylene Fumarate) Scaffolds
		4.2.2	Optimizing Cell Sources for Repair of Bone Defects
		4.2.2a	Point of Care Autologous Stem Cell Concentrate for Bone Defect Repair
		4.2.3	Advancing Bone Repair Using Molecular Surface Design (MSD): Biodegradable Scaffolds with Tethered Osteoinductive Biomolecules
		4.8.1	Improved Preclinical Model for Orthopedic Trauma
Soft Tissue Repair and Regeneration (excluding nerve)	RCCC	4.3.2	Development of Tissue (Peritoneum) Lined Bioabsorbable and Fracture-Resistant Stent Graft for Vessel Trauma
		4.3.2a	Construction of Tissue Engineered Blood Vessels
		4.4.3a	Functional Scaffolds for Musculoskeletal Repair and Delivery of Therapeutic Agents
		4.4.3b	Functional Scaffolds for Soft Tissue Repair and Joint Preservation
	WFPC	4.4.6	Oxygen-Generating Biomaterials for Large Tissue Salvage
		4.5.8	Isolation and Expansion of Native Vascular Networks for Organ Level Tissue Engineering
Nerve Repair and Regeneration	RCCC	4.4.1	Repair of Segmental Nerve Defects: A Theragnostic System Solution for Optimal Nerve Repair – Prevention of Muscle Atrophy
		4.4.2	Repair of Segmental Nerve Defects
		4.4.2a	Cells and Bioactive Molecules Delivery in Peripheral Nerve Restoration
		4.4.2b	Repair of Peripheral Nerve Injury Using Tissue-Engineered Nerve Grafts Encased in Biodegradable Nerve Guidance Tubes
	WFPC	4.4.4	Peripheral Nerve Repair for Limb and Digit Salvage
		4.4.5	Modular, Switchable, Synthetic Extracellular Matrices for Regenerative Medicine
Composite Tissue Injury Repair	WFPC	4.4.3	Spatial and Temporal Control of Vascularization and Innervation of Composite Tissue Grafts
Epimorphic Regeneration	WFPC	4.4.1	Epimorphic, Non-Blastemal Approach to Digit Reconstruction

Nontechnical summaries of the projects are presented after the summary table, and technical progress reports for each project can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

Bone Repair and Regeneration

Studies at RCCC

RCCC's Limb Salvage and Transplantation Program aims to provide injured warriors with new clinical methods that significantly improve the regeneration of bone non-unions or large bone defects (more than 3 cm) in a reliable and timely manner. In **Project 4.2.1a**, the **Ortiz and Kohn group** at the New Jersey Center for Biomaterials at Rutgers University is developing advanced scaffolds to treat large segmental bone defects. The researchers synthesized four calcium phosphates (CaPs) with varying solubilities and then produced composite scaffolds by incorporating the CaPs into a tyrosine-derived polycarbonate (TyrPC) polymer. They tested the CaP-containing scaffolds in rabbit skull and radius (forearm) defect models and found that several of the composite scaffolds regenerated bone as well as or better than a commercially available bone scaffold. All treatment groups had demonstrated the formation of a direct interface between native bone and scaffold. The researchers found that the scaffolds containing octacalcium phosphate performed better than any of the others. They also found that the addition of bone marrow aspirate (BMA) to their TyrPC/CaP scaffolds enhanced bone regeneration. The researchers plan to pursue a pivotal large animal goat skull defect model study in the upcoming year. They have designed a Phase I clinical trial. They plan to file a 510(k) application for the first-generation bone regeneration scaffold. The researchers have an exclusive license with Trident Biomedical, Inc. for the library of TyrPCs, and work with a separate industrial partner for the large-scale current Good Manufacturing Practices (cGMP) synthesis of TyrPCs.

RCCC graduate student Koustubh Dube prepares solution in the laboratory.

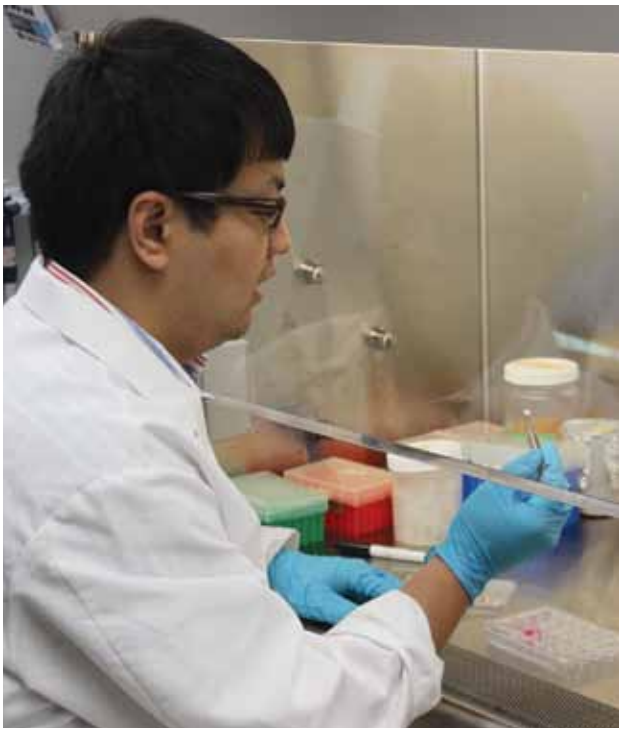
In **Project 4.2.1b**, the **Yaszemski team** at the Mayo Clinic fabricated poly(propylene fumarate) (PPF) scaffolds for analysis in various bone defect animal models. The researchers loaded the scaffolds with recombinant human BMP-2 (rhBMP-2) via a collagen-based hydrogel for controlled delivery, and quantified the delivery kinetics of the rhBMP-2 from the hydrogels in the scaffold pores. The research team increased bone formation in a rabbit skull defect model via a combination of CaP surface coating on the scaffold and rhBMP-2-controlled delivery from the scaffold. Their histology results indicated that bone is formed inside the pores of the scaffold and is distributed throughout the defect. During the upcoming year, the researchers plan to evaluate the CaP-coated PPF scaffolds with controlled rhBMP-2 delivery in a rat femoral (leg) defect model. Following the completion of these studies, the researchers will test the composite scaffolds in the minipig mandible (jaw) and goat tibia (lower leg) segmental defect models (in collaboration with Project 4.8.1). If these preclinical animal studies are successful, the researchers will proceed to a first-in-human pilot safety study of the scaffold implant, and perform cGMP scale-up, fabrication, sterilization, and pre-commercialization work for an Investigational Device Exemption (IDE)/510(k) submission.

Bone-forming stem/progenitor cells are severely depleted in the region of a large bone defect. The **Muschler group (Project 4.2.2)** at the Cleveland

Clinic is addressing the need to define and competitively assess promising methods for harvesting, processing, and transplanting osteogenic (bone-forming) connective tissue progenitor cells (CTP-O). The researchers are exploring three basic methods for cell processing: Density separation (DS) (using a centrifuge to remove red blood cells and serum), selective retention (SR) (regarding the fact that CTP-O attach very readily to some surfaces as a means for concentration and selection), and magnetic separation (magnetically labeling CTP-O and then preferentially collecting them with a magnet). The researchers have determined that magnetic separation based on hyaluronan, as a marker for CTP-O, increased bone regeneration in the canine femoral multi-defect model. They attempted to validate the chronic caprine (goat) tibial defect (CCTD) model as a viable tool for advanced assessment of cell harvest and processing strategies, but found large differences in the yield and quality of cells between the proximal humerus, pelvis and sternum. Only the sternum proved to be a suitable model approximating the harvest of bone and marrow from human or canine bone. During the upcoming year, the researchers will complete a comparison of SR processing and DS using mineralized cancellous bone allograft in the canine femoral multi-defect model. The most effective sourcing and processing methods will be advanced into appropriate assessment in the CCTD model. The researchers are working with the Project 4.8.1 team to develop a more stringent CCTD model. The researchers will evaluate the most effective methods for cell sourcing alone and in combination with advanced scaffolds, surgical methods, and/or drug delivery systems to identify the most promising therapies to advance into clinical trials.

Due to the limitations of the currently available therapies, there remains an unmet need for a therapy that can regenerate bone in large gap defects. The **Barnes team (Project 4.2.2a)** at Arteriocyte, Inc. is investigating the effects of concentrated versus non-concentrated BMA on the regeneration of long bone. They are testing whether the concentrated BMA (cBMA) produced with the Magellan® MAR01™ system results in superior defect repair when combined with graft materials in a rabbit segmental defect model. This therapy is expected to accelerate fracture healing while reducing the likelihood of serious complications (e.g., non-union and osteomyelitis). The researchers successfully accomplished graft implantation on all 60 treatment animals and assessed the ability of the Magellan® System to concentrate BMA-derived nucleated cells and mesenchymal stem cells (MSCs). Preliminary data demonstrated that the Magellan® MAR01™ system could significantly enhance the concentration of MSCs and cBMA-derived nucleated cells. During the upcoming year, the researchers will conduct a variety of assessments to determine the efficacy of cBMA in improving bone repair.

The **Bushman and Kohn group (Project 4.2.3)** at Rutgers University is using molecular surface design (MSD) to enhance the survival and performance of transplanted cells. MSD is a biomaterials strategy that involves tethering a growth factor or signaling molecule onto the surface of an implant to change the way that cells interact with the material. This can change cell attachment, migration, proliferation, differentiation, or survival in a controlled manner. During the past year, the researchers tested the efficacy of scaffolds tethered with rhBMP-2 in



RCCC researcher Dr. Zheng Zhang holds a biphasic scaffold composed of a soft cartilage-forming layer overlaying a rigid bone-forming layer for use in the treatment of osteochondral defects.

RCCC Postdoc Dr. Divya Bhatnagar operates the extruder instrument in the laboratory to prepare filaments that can be used in braided nerve regeneration conduits.

the rabbit skull defect model. They recently harvested scaffolds from the rabbits and will use several methods to gauge the efficacy of the MSD method for tethering. The researchers also focused in Year 5 on osteochondral (bone/cartilage) regeneration. They synthesized a small library of candidate polymers that induce adult stem cells to form cartilage and then conducted assays to select the best one(s). They found one polymer to be superior to all of the others. The researchers then developed a fabrication technique to mesh a bone-generating polymer scaffold with their newly identified cartilage-generating polymer. This technique allowed them to produce a "biphasic" scaffold capable of generating both bone and cartilage. The researchers next hope to obtain proof-of-principle data on their biphasic scaffold in a relevant animal model. With respect to the researchers' bone regeneration scaffolds with tethered rhBMP-2, they hope to confirm efficacy in the rabbit skull defect model. Positive indications would lead to expansion of the technology and testing in larger animal models.

In **Project 4.8.1**, the **Pluhar team** at the University of Minnesota, the **Bechtold team** at Minnesota Medical Research Foundation, and the **Muschler team** at the Cleveland Clinic are seeking to develop a large animal model that is sensitive enough to allow for the testing of novel bone regeneration technologies before moving into Phase I or II human trials. The researchers created a bone defect in the tibia (lower leg) of Spanish-Boer goats. They performed surgeries on 30 goats with 12 weeks of radiographic follow-up that demonstrated little to no bone formation in the defects treated with the current standards of practice, fresh autograft, or morsellized allograft bone. The addition of fresh bone marrow aspirate to the allograft did not result in improved healing. However, there was a significant enhancement of new bone formation when the researchers added rhBMP-2 to allograft with or without bone marrow aspirate. Overall, the researchers have shown that this model may be sensitive enough to detect differences in healing among current bone grafting technologies. All procedures are well tolerated by the goats, and researchers at other institutions can perform the surgeries by following the standard operating procedure developed by the research team. During the upcoming year, the researchers will use the model to test new bone regenerative technologies that are being developed by other AFIRM research teams.

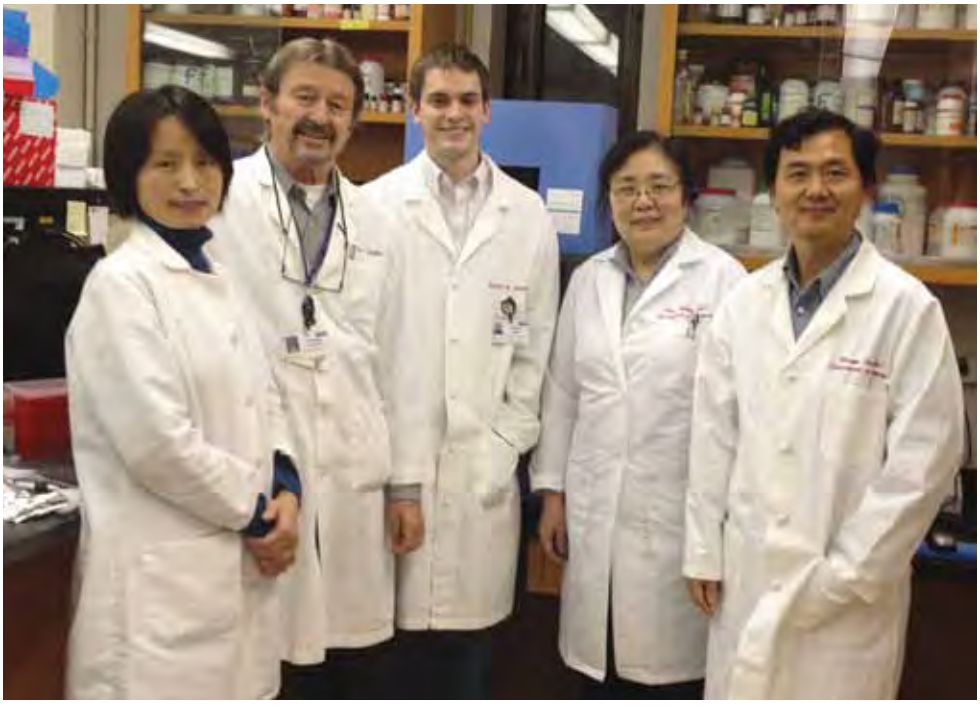
Soft Tissue Repair and Regeneration (Excluding Nerve)

Studies at RCCC

Current metal stents are designed for the treatment of late-stage peripheral vascular disease, leave young patients at risk for graft failure due to fracture or the recurrence of stenosis (narrowing of a blood vessel), and are not designed to last the lifetime of the individual. The **Sarac group (Project 4.3.2)** at the Cleveland Clinic is developing bioabsorbable and fracture-resistant, tissue-lined stent grafts that contain either polylactic acid or polydioxanone for minimally invasive treatment of arterial and venous trauma in young patients. In October 2012, the researchers held a successful pre-IDE meeting with the U.S. Food and Drug Administration (FDA) for the fracture-resistant stent design; a follow-up meeting was held in March 2013. The researchers chose Resonetics, Inc. to cut their stents with an athermal laser. They found that the thickness of the material did not allow for enough radial force, and was also too thin to be able to be sewn to tissue. The

researchers subsequently identified and manufactured appropriate dimensions for the stent graft that included a thickness that allowed for enough radial force and could support sewn tissue. They were also able to successfully crimp the stent. In conjunction with Peritec Biosciences, the researchers are now progressing to design verification and freeze. The research team has obtained Institutional Animal Care and Use Committee and Animal Care and Use Review Office approval for the evaluation of their stent design in the dog model, which will occur during the upcoming year.

The **Tulenko team (Project 4.3.2a)** at Cooper University Hospital is using a human "endothelial progenitor-like" cell line, derived from human adipose tissue, to seed the inner surface of a biological tubular scaffold for segmental vascular repairs. This technology could potentially reduce the long-term risk of vascular repair failures due to infection, re-stenosis, and re-injuries. During the past year, the researchers developed a method of activating endothelial nitric-oxide synthase (eNOS) (an essential endothelial gene) in adipose-derived stem cells that had been differentiated into an endothelial phenotype in cell culture. They also developed two types of tissue-engineered blood vessels (TEBVs) for arterial segmental repair: cell-free TEBVs and cell-lined TEBVs. The cell-free TEBVs were coated with a small molecule that is believed to encourage autogenous (one's own) cells to differentiate into endothelium (though the induction of eNOS). The researchers implanted these scaffolds into a dog carotid artery model. Preliminary analysis at 28 days post-implantation suggests that the cell-free TEBV is patent and functioning as a competent arterial segment. They will complete the in vivo evaluation of the cell-free TEBV in the dog carotid model during the upcoming year. If results following testing in a second animal species (rabbit) are positive, the researchers will file an FDA application for testing this device in humans, and will submit a clinical protocol for approval by the local Institutional Review Board (IRB).



RCCC researchers are developing a TEBV. From left to right: Drs. Shaohua Chang and Thomas Tulenko, Ben Jones, Drs. Ping Zhang and Zhengyu Wei

Dermal (skin) grafts are commonly used in clinical practice to repair abdominal wall defects and hernias. However, the implanted grafts tend to lose their integrity and strength over time, which results in complications such as bulging and hernia recurrence. Engineered improvements to biologic grafts are therefore needed to make them more suitable for abdominal wall repair and reconstruction. The **Derwin group (Project 4.4.3a)** at the Cleveland Clinic seeks to provide injured warriors with biological materials for reconstruction of large abdominal defects through the generation of a fiber-reinforced biological graft. The researchers have developed biological dermal grafts using a proprietary fiber reinforcement technique that are significantly stronger and stiffer than native dermis grafts. During the past year, the research team downselected scaffold design and transitioned to a semi-automated scaffold manufacturing process to scale up

scaffold generation. They established a clinically relevant large animal (pig) model for abdominal wall repair and initiated a pilot study using two pigs. They developed a non-invasive method for post-operative monitoring of repair of bulging/hernia recurrence in the pigs. The researchers also established the protocol for retrieval, mechanical testing and analysis of the pig abdominal wall. They will continue technology validation and product development together with prospective industrial collaborators during the upcoming year. As the device moves into product development and a regulatory path is defined, the FDA will be engaged to enable the execution of a clinical trial.

Due to limited healing capabilities, injuries to the meniscus (internal cartilage) of the knee are often treated with surgical removal of the damaged tissue. While this treatment generally leads to early symptom relief, it frequently leads to the development of degenerative arthritis of the knee. The **Gatt/Dunn group (Project 4.4.3b)** at the University of Medicine and Dentistry of New Jersey is developing an off-the-shelf, tissue-engineered meniscus scaffold that can be used to replace a moderately to severely damaged meniscus in an injured service member. The scaffold is composed of an anatomically designed, fiber-reinforced scaffold and a collagen-based extracellular matrix (ECM) similar to the native meniscus. The research team implanted their second-generation meniscus scaffolds in a functional sheep model and recovered all 16- and 32-week implants. All implants were found fully intact with no ruptures or fixation failures, which was a problem with the first generation scaffold. The researchers found that the tensile strength of the second generation implants at both 16 and 32 weeks was five times higher than the normal functional loads of the native meniscus. The implants exhibited excellent tissue adherence and incorporation at both time points, and blood vessels were found throughout them. Importantly, the meniscus scaffold was found to protect the articular (joint) surface. The researchers made minor modifications to the second generation scaffold before beginning a 52-week sheep implantation study, which they will complete in the upcoming year. Transition to clinical trials will be dependent on the results of the 52-week implantation study and planned longer term studies through 2 years post-implantation. The research team is in discussions with several companies who have expressed interest in partnering or co-developing the technology.

Studies at WFPC

The **Harrison group (Project 4.4.6)** at Wake Forest University is developing an injectable biomaterial capable of generating oxygen—a particulate oxygen generator (POG)—that would allow the delivery of oxygen in controlled amounts to engineered tissue scaffolds or pre-existing tissue. The researchers have established an in vivo model for evaluating the applicability of the POGs. This model involved the creation of ischemia (oxygen starvation) to the rat hind limb via arterial ligation (tying up a key artery). The researchers demonstrated that the POGs can preserve skeletal muscle structure and function in vivo in an ischemic environment. Overall, the research team's results suggest that its oxygen delivery system could be used as a readily available treatment to delay the onset of additional tissue damage resulting from compromised blood flow. The team's primary focus in Year 5 will be to optimize the model and delivery protocol for POGs

WFPC graduate student John McQuilling working with oxygen regenerating biomaterials.

to demonstrate physiologically relevant improvement in skeletal muscle structure and function under clinically relevant experimental conditions. Because organs are composed of multiple cell types, the researchers will continue to analyze several different tissues systems, including skeletal muscle, bone, nerve, and skin.

Numerous tissue-engineering strategies begin with the implantation of cells onto matrices followed by the attempt to create a de novo vascular system. However, researchers have fallen short in creating a viable vascular network outside of the body due to the complexities of blood vessel network formation in the living organism. The **Gurtner team (Project 4.5.8)** at Stanford University has developed novel strategies that utilize preformed native circulatory networks that can be supported outside the body during organ fabrication, matured using stem cell-based techniques, and then readily integrated into the systemic circulation. The researchers have successfully validated and optimized their protocol for the isolation and maintenance of explantable microvascular beds (EMBs) based on the rat superficial inferior epigastric vessels on an ex vivo bioreactor system. They have developed a decellularization protocol that achieves tissue decellularization while preserving matrix architecture and macroscopic vascular structure. The group successfully re-anastomosed the EMB into the native vascular circulation and established the ability to track the biomass non-invasively long-term. They have also successfully seeded decellularized rat tissue flaps with human and rat adipose-derived stem cells. Going forward, the researchers will seek to further improve long term engraftment and functional cellular differentiation for organ-level tissue engineering.

Nerve Repair and Regeneration

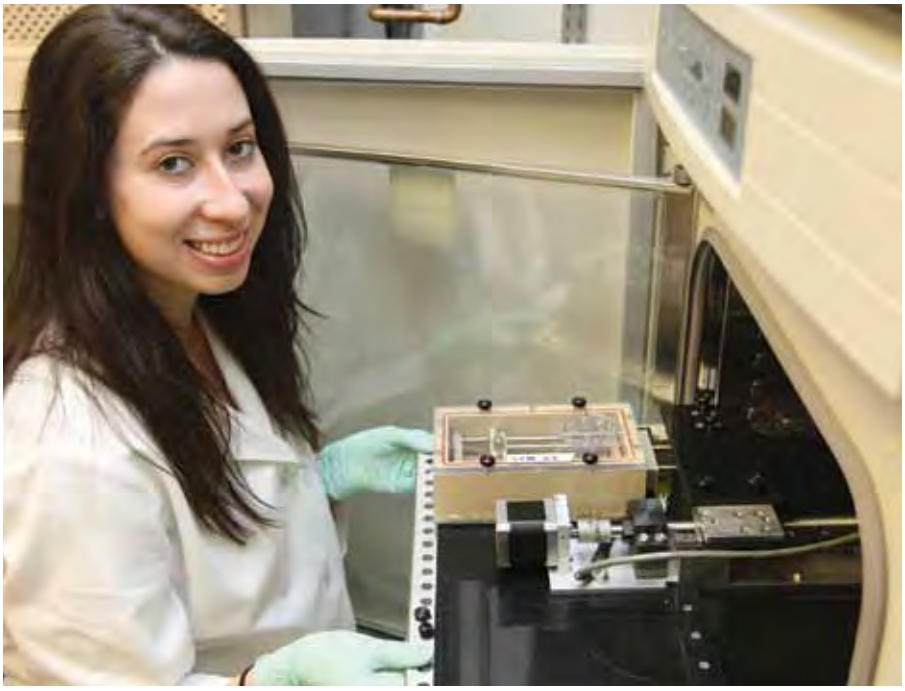
Studies at RCCC

A muscle that loses its nerve supply quickly undergoes atrophy, which can significantly affect functional recovery. The **Langer/Anderson group (Project 4.4.1)** at the Massachusetts Institute of Technology has developed an implantable, organic, stretchable microelectrode array (termed "OSMEA") that can electrically stimulate injured muscles in real time to keep them from degenerating. The researchers developed the OSMEA using biocompatible materials so that FDA approval can be obtained in the future. The researchers also set up a new animal experiment platform using electrical stimulation for treating muscle atrophy following loss of nerve supply. Data from up-to-date evaluations on the materials and the OSMEA has been very encouraging for the intended application. During the past year, the researchers synthesized a conducting polymer for the OSMEA, and conducted electrical and mechanical testing using this polymer. Their results confirmed the electrical and mechanical advantages of using this polymer in the OSMEA. The researchers are currently focusing on conducting preclinical animal studies that are aimed at proving the safety and efficacy of the proposed approach. These studies are expected to take at least 3 years to complete. Once proof-of-concept is demonstrated in a defined animal model, the researchers will submit an IDE application and design a human clinical trial. The researchers note that the OSMEA is a platform technology that is also applicable to many other neural interfacing applications, including spinal cord surface stimulation, electrocorticogram-based brain-computer interfaces, and retinal prostheses.

WFPC researcher Dr. Zuhaib Ibrahim evaluating protocol skin biopsies after swine hind limb transplantation.

In **Project 4.4.2**, the **Windebank/Yaszemski team** at the Mayo Clinic and Rutgers University is developing braided biodegradable polycaprolactone fumarate (PCLF) conduits that promote improved nerve regeneration over current commercially available tubes both in motor function recovery and in gap length. During the past year, the researchers identified electrophysiologic, histologic, and molecular parameters of muscles that can potentially serve as sensitive prognostic markers for functional outcomes after nerve reconstruction, and as targets of intervention for enhancing regeneration. They also delineated the time course and characteristics of muscle atrophy due to denervation. The researchers developed and optimized the protocols for isolation and expansion of Schwann cells from adult nerve sources. They also optimized the coating method to reduce the pore size of their braided conduits. This was accomplished by applying a secondary hydrogel and fibrin glue coating as well as an airbrushed polymer coating to the conduits. They found that the braided conduits could be effectively sterilized by a validated ethylene oxide sterilization method. They tested the braided conduits for endotoxins, and found endotoxin levels to be within the limits specified by the FDA. During the upcoming year, the researchers will complete the additional preclinical testing requested by the FDA in response to the prior IDE submission. They will prepare and file an investigator-initiated IDE with the FDA. They will also obtain IRB approval to conduct a Phase I clinical trial to assess the safety of the PCLF conduits in patients with a 6 cm nerve defect resulting from a sural nerve biopsy (see **Project 4.4.1a** in "Clinical Trials").

Bone marrow stromal cells (BMSC) comprise a mixed population of cells that contribute to the regeneration of multiple body tissues. Notably, BMSC can be placed in an individual without the need for suppression of the immune system. The **Siemionow group (Project 4.4.2a)** at the Cleveland Clinic seeks to improve nerve regeneration by enhancing the performance of epineural nerve sheath conduits with the addition of BMSC. Briefly, the researchers create empty epineural tubes from sheep median nerves. They fill the tubes with fluorescently labeled BMSC (isolated from sheep bone marrow using a protocol developed in their laboratory) to create a conduit, which is then transplanted into recipient animals that have a 6 cm median nerve gap. The conduit creates an ideal microenvironment for nerve regeneration as it not only protects against local fibrotic and inflammatory insults, but it also provides a source of growth factors crucial to effective nerve regeneration. Nine sheep were euthanized six months after transplantation of an epineural conduit. The researchers did not observe any signs of inflammation, rejection, or conduit leakage. The integrity and shape of the cross-section of the conduits were preserved. Good functional results were obtained (as



RCCC researcher Laura Struzyna checks a custom-built mechano-bioreacter in a tissue culture incubator. These mechano-bioreactors are used to generate "stretch-grown" axonal constructs that are used as living aligned scaffolds to promote nerve regeneration.

RCCC researcher Dr. Huan Wang prepares for animal surgery to study the performance of novel nerve conduits on peripheral nerve regeneration.

determined by somatosensory evoked potential analyses prior to euthanization). During the upcoming year, the researchers plan to investigate different methods for the long-term storage of the epineural conduits. They will also perform human cadaver studies to optimize the best method for collecting and preparing the conduits for human studies. Their ultimate goal is to characterize, optimize and make a clinically applicable bio-conduit built of naturally occurring human epineural sheath filled with human MSCs, and to test its regenerative potential in the clinical setting. This application may be used in patients with motor neuron disease, cerebral infarct, degenerative nerve disease and peripheral nerve injury.

Tissue-engineered nerve grafts (TENGs) are transplantable living nervous tissues generated in culture that contain two separate neuron populations connected by long integrated tracts of axons. TENGs recapitulate one of the most favorable environments for robust and long-distance axon growth and regeneration. TENGs represent a potentially transformative solution for the functional repair of substantive nerve trauma, as well as an option for reconstruction of entire nerve branches following massive tissue loss. The **Smith/Cullen team (Project 4.4.2b)** at the University of Pennsylvania is using advanced biomaterials and three-dimensional (3D) fabrication methods (made available through other AFIRM projects) to select and optimize custom nerve guidance tubes (NGTs) in which to encase TENGs in order to repair segmental nerve defects. During the past year, the researchers demonstrated accelerated axonal regeneration across TENGs encased in NGTs compared to experimental AFIRM NGTs alone or commercially available (FDA-approved) NGTs alone. They also determined that TENGs possess a novel, and in many ways superior, mechanism of action compared to NGTs and autografts. Electrophysiological assessments and behavioral assessments revealed that functional recovery (based on stimulated foot twitch, compound nerve action potential, and compound muscle action potential) was observed in some animals as early as 10 weeks following repair with TENGs or autografts, but not with NGTs alone. The mechanism of action and early-stage results support the premise that TENGs may enable regeneration of nerves across critical, 5 cm or larger, peripheral nerve lesions. In conjunction with Axonia Medical, the researchers have initiated testing of TENGs encased in commercially available and custom NGTs in a clinically relevant large animal model of peripheral nerve injury.

Studies at WFPC

Following trauma, incomplete nerve regeneration and permanent demyelination (damage to the protective sheath surrounding nerves) may lead to lifelong disability. In **Project 4.4.4**, the **Marra group** at the University of Pittsburgh, the **Kaplan group** at Tufts University, and the **Smith group** at Wake Forest University are developing biodegradable nerve conduits containing silk fibroin, polycaprolactone, or collagen that deliver chemical cues (e.g., growth factors) and biophysical cues (e.g., surface patterning) to regenerating peripheral nerves. They established a nonhuman primate (NHP) model of critical median nerve deficits (5 cm) using autograft and cell-free nerve grafts. They completed an NHP study of noncritical median nerve defects (1 cm) using keratin gel-filled collagen conduits. They completed the examination of polymer-based tubes containing glial cell-line derived neurotrophic factor

embedded in double-wall microsphere, in a critical rat sciatic nerve defect model (1.5 cm). The researchers completed a quantitative characterization of the mechanical properties of their silk nerve conduits. They improved the gradient design and delivery of neurotrophic factors from both polymer conduits and silk conduits. They implanted silk nerve conduits including neurotrophic factors and enzymes into a critical rat sciatic nerve defect (1.5 cm). They submitted a pre-IND data package to FDA and held a pre-IND meeting with Center for Drug Evaluation and Research/FDA to review preclinical data and clinical trial study design for keratin gel filler. During the upcoming year, the research teams plan to complete the NHP median nerve gap (5 cm) studies and proceed into clinical trials. Additional silk conduits will continue to be tested in the rat sciatic nerve defect model, and a basic science pipeline will continue to provide innovative ideas to the project.

Functional limb and digit tissue restoration involves a hierarchically defined process that often requires precise spatial and temporal coordination among multiple biological systems and processes. The **Tirrell group (Project 4.4.5)** at the University of California, Berkeley, is pursuing an approach to induce peripheral nerve growth following traumatic amputation by modulating components of the naturally occurring ECM (e.g., fibronectin and laminin). The researchers have developed a peptide-based hydrogel system as injectable ECMs with nanofibrous structures. They can incorporate bioactive peptide sequences and/or growth factors into the hydrogels. The 3D matrix system of the hydrogels also allows for mammalian cell growth. The researchers' data show that their hydrogels span a range of physiologically relevant stiffnesses and may be useful in many regenerative medicine applications due to their fibrous, ECM-mimicking structure. The concentration of the hydrogels, which is directly linked to stiffness, can be tuned to promote the spreading, proliferation, and migration of a model cell type, Schwann cells. In Year 5, the researchers will choose the hydrogel concentration that performs best in their in vitro models and will use that concentration in animal studies.

Composite Tissue Injury Repair

Studies at WFPC

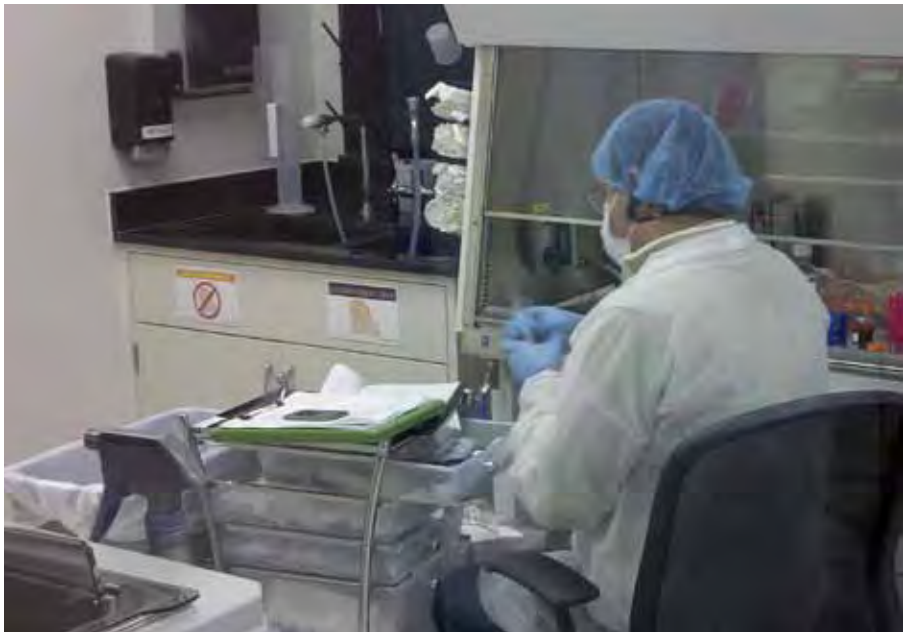
The **Guldberg group (Project 4.4.3)** at the Georgia Institute of Technology seeks to develop and test technologies that will enable the restoration of limb function following composite tissue trauma. The researchers have established promising strategies using nanofiber mesh spatial guidance and sustained delivery of a clinically approved inductive protein, BMP-2, for regeneration of bone, nerve, and muscle. They have developed composite injury animal models that simulate bone/nerve, bone/vascular, and bone/muscle injuries. They have used these models to test their nanofiber mesh/hydrogel BMP-2 delivery systems (patents pending), which provide spatial and temporal cues to guide improved bone and nerve regeneration. During the past year, the researchers completed a pivotal 6-month sheep segmental defect study (initiated in AFIRM Year 4), which was conducted with leveraged funding from the Australia Research Council. They found that the hybrid BMP delivery system in the sheep could effectively regenerate human scale (3 cm) segmental bone defects. The researchers will next complete optimization studies of hydrogel composition and degradation characteristics. They will also focus on overcoming the challenges of chronic non-union with or without adjacent concomitant soft tissue injury or mechanical instability. When proof-of-concept has been fully demonstrated in the sheep model, the researchers plan to initiate a pilot human clinical trial.

Epimorphic Regeneration

Studies at WFPC

The **Badylak group (Project 4.4.1)** at the University of Pittsburgh is investigating mechanisms for recruiting large populations of stem cells to the site of limb and digit injury, and then developing strategies to induce the formation of functional limb and digit tissue to replace the damaged or missing structures. The researchers have shown that injury sites in non-regenerating mammalian systems recruit endogenous multipotent stem cells, representing a step toward non-blastemal epimorphic regeneration. They developed a digit

amputation mouse model and used it to definitively show that endogenous multipotent stem cells are recruited to the site of injury following injection of ECM. This accumulation of cells is termed a "multipotent cell cluster" or MCC. They have continued to further define the population of cells involved in the formation of the MCC, and to examine the ability of those cells to differentiate into different functional tissues. The researchers believe that control of the "microenvironment niche" is necessary to direct complex tissue regeneration; to that end, they are developing a Biomechanical Interface for Optimized Delivery of MEMS Orchestrated Mammalian Epimorphosis (BIODOME) device. They have completed several design improvements for the BIODOME, including better flexibility to allow for digit swelling, smaller profile for greater mouse mobility, and shape changes that allow for easier removal and re-use, as well as better preservation of the healing digit. Optimization of the mouse designs is ongoing and will inform future designs for a BIODOME device for large animal models and, eventually, a human pilot study. Studies in mice and canines will also continue as the researchers introduce different bioactive molecules in a controlled fashion to the injury site microenvironment.



WFPC researcher adding cells to a tissue scaffold.

Clinical Trials

Several AFIRM Limb and Digit Salvage technologies have advanced to the human clinical trial stage. The status of each clinical trial is summarized in **Table II-2**, and additional details on these trials follow the table. Detailed clinical trial progress reports, when available, can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

While composite tissue allografts (e.g., hand transplants) are now a clinical reality and have been performed in multiple centers worldwide, the procedure has not reached widespread clinical use because recipients require lifelong, high-dose multidrug immunosuppression to prevent graft rejection. The **Lee group (Project 4.4.2, WFPC)** at the Johns Hopkins University School of Medicine and the University of Pittsburgh is developing a protocol for hand transplantation using donor BMSCs in combination with novel fusion proteins (the "Pittsburgh Protocol") that will minimize maintenance immunosuppressive therapy. The researchers

Table II-2. AFIRM-funded Limb and Digit Salvage projects with pending or active clinical trials.

Project Title	Consortium	Project No.	Trial Phase	Current Status
Hand Transplantation for Reconstruction of Disabling Upper Limb Battlefield Trauma – Translational and Clinical Trials	WFPC	4.4.2	Phase I	Open
A Clinical Trial to Assess the Safety of a Novel Scaffold Biomaterial	RCCC	4.4.1a	Phase I	Submitted IDE to FDA

have shown that their fusion protein can prolong the survival of allografts in a hind limb transplantation model in swine. They have performed ten successful hand/forearm transplants in six patients, including the first bilateral and first above elbow arm transplant in the United States. All compliant transplant patients to date are being maintained on a single immunosuppressive drug at low doses and continue to have increased motor and sensory function of their transplanted hands, which correlates with their severity of amputation, time after transplant, and participation in hand therapy. During the upcoming year, the researchers plan to optimize their strategy, combining targeted immunomodulation and BMSC/fusion protein induction to further reduce maintenance immunosuppression and allow weaning of systemic drug therapy. They note that this regimen might enable tolerance induction and reduce side effects related to high-dose immunosuppression, hopefully enabling widespread clinical application of hand transplantation for the reconstruction of upper extremity amputations.

In **Project 4.4.1a** (RCCC), the **Yaszemski/Windebank group** at the Mayo Clinic is developing novel biodegradable polymer nerve conduits that are suitable to repair nerve defects longer than 3 cm. More specifically, their goal is to repair post-biopsy sural nerve defects with a PCLF synthetic nerve conduit. Since the sural nerve is located just below the skin, potential morbidity from the conduit will be readily identifiable, and the device can be easily removed and analyzed. This model will provide a platform for determining safety and an indication of efficacy of scaffolds for nerve repair. The researchers had been working with BonWrx, Inc., to complete this study. During the past year, they validated an FDA-approved sterilization method for their PCLF conduits that involves the use of ethylene oxide (EtO) with a 12-hour cycle and an 11-hour purge. The EtO-treated tubes supported nerve regrowth when used to bridge nerve gaps, and there was no observable cytotoxicity. The researchers obtained IRB approval to initiate a separate feasibility clinical trial and follow 10 patients with sural nerve biopsy but without repair. They completed procedures, product drawings and specifications, and production documentation for their PCLF conduits to be manufactured for clinical trial use. The researchers also completed and filed an IDE, which is currently under review by the FDA. They provided nerve conduits for testing by third parties to support a second IDE submission. They plan to conduct a Phase I clinical trial to assess the safety of the PCLF conduits in patients with a 6 cm nerve defect resulting from a sural nerve biopsy. The researchers will use leveraged funding from the Mayo Clinic to conduct the clinical trial, and results of the trial will be reported to the Department of Defense.

III: Craniofacial Reconstruction

Background

During the current conflicts, more than 25% of wounded warriors treated in U.S. military facilities have sustained maxillofacial injuries.[1,2] Ranging from simple fractures to extensive bone defects, severe burns, and soft tissue avulsion, these wounds can greatly alter the life of the injured victim. A soldier with a significant craniofacial injury loses his/her interface with the world; when one's face is gone, so goes one's identity. A severe facial injury directly impacts the ability to communicate—speech may be difficult to understand and facial expressions nonexistent—and possibly the ability to eat. Unfortunately, a significant disfigurement often portrays a person as a mangled, contracted, or absent face, which typically leads to grave embarrassment and/or separation from society. As these soft tissue and bony injuries to the face and cranium are excruciatingly painful and may carry devastating psychological impact, restoring form and function to these traumatized warriors is critical to their rehabilitation.

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[1] Lew TA, et al. J Oral Maxillofac Surg. 2010 Jan; 68(1):3-7.
[2] Owens BD, et al. J Trauma 2008 Feb; 64(2):295-299.

III: Craniofacial Reconstruction

The primary focus of AFIRM's Craniofacial Reconstruction Program is to help wounded warriors with devastating, disfiguring facial injuries return to fully functional lives, which includes their reintegration into society through the application of regenerative medicine technology. Comprised of a multidisciplinary, multi-institutional collaborative research team, the Craniofacial Reconstruction Program manages projects ranging from single-tissue regeneration to complete face transplants—from emerging products in the early stages of bench research to those entering clinical trials. Drawing on the strengths of each team member, an optimal set of complementary technologies have been identified to achieve hard and soft tissue regeneration. Ultimately, the Craniofacial Reconstruction Program helps to provide wounded warriors with a new face to present to the world (**Figure III-1**).

Unmet Needs

Craniofacial injuries suffered in combat are highly unique and often massive, and most of the current treatments in craniomaxillofacial reconstruction are too inadequate to treat these deficits resulting from blast injuries. Today, massive bone loss to the craniofacial complex incurred in combat is reconstructed with nonresorbable synthetic materials, bone implants, or metallic devices, which provide limited restoration of anatomical form and function. Therefore, readily available or readily generated replacement tissue for craniofacial bone, muscle, nerve, and skin are unmet needs for craniomaxillofacial reconstruction of severely wounded warriors.

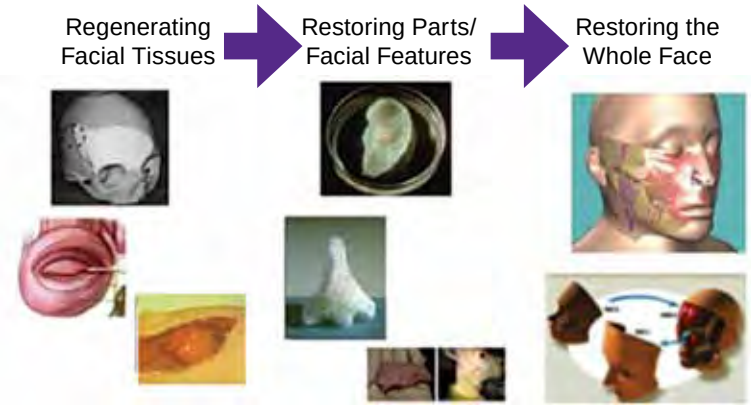


Figure III-1. Goals and products of the AFIRM Craniofacial Reconstruction program.

Tissue loss is quite massive in the most severe facial injuries, and in such cases, the agreed-upon surgical approach for facial replacement is transplantation. However, vascularized composite tissue allografts (VCAs) that constitute the face are highly antigenic and require aggressive immunosuppression regimens to prevent rejection of the transplanted tissue. The host is placed at substantial risk with immunosuppression, both from the toxicity of the immunosuppressive agents and from opportunistic infections, and this leads to significantly shortened life expectancy for all transplant recipients. In light of this, to provide adequate treatment to severe facial injuries, it is necessary to develop a technique or therapy that provides modulation of the immune response to allografts without indiscriminate immunosuppression.

Areas of Emphasis

Rutgers–Cleveland Clinic Consortium (RCCC), Wake Forest–Pittsburgh Consortium (WFPC), and U.S. Army Institute of Surgical Research (USAISR) researchers are pursuing a complementary mix of research projects focused on various aspects of Craniofacial Reconstruction. As shown in **Table III-1**, projects can be grouped into three clinical challenge topic areas: Bone Regeneration, Soft Tissue Regeneration, and Cartilage Regeneration (with a focus on the ear). Nontechnical summaries of the projects are presented after the summary table, and technical progress reports for each project can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

Bone Regeneration

Studies at WFPC

In **Project 4.1.2**, the **Mikos/Kasper group** at Rice University and the **Wong group** at the University of Texas Health Science Center at Houston (UTHSC) are developing porous poly(methyl methacrylate) (PMMA)-based space maintainers, with and without antibiotics and growth factors, that will maintain the proper anatomical relationship of the tissue adjacent to a craniofacial defect. The researchers demonstrated in a rabbit jaw defect model that animals receiving porous implants, fabricated

Table III-1. Projects funded by RCCC, WFPC, and USAISR per clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Bone Regeneration	WFPC	4.1.2	Space Maintenance, Wound Optimization, Osseous Regeneration, and Reconstruction for Craniomaxillofacial Defects
	RCCC	4.5.1a / 4.5.7	Regeneration of Bone in the Cranio-Mandibulo-Maxillofacial Complex Using Allograft Bone/Polymer Composites (4.5.1a) / Expedited Commercialization of an Injectable Bone / Allograft Composite for Open Fractures (4.5.7)
		4.5.1b	Regeneration of Bone in the Cranio-Mandibulo-Maxillofacial Complex Using Pre-Formed Tyrosine Derived Polycarbonates
		4.5.6	Vascular Tissue Engineering
		4.5.8	Accelerating the Development of Bone Regeneration Scaffolds Based on Tyrosine-Derived Polycarbonate
	USAISR	4.5.1c	Preclinical Animal Model Development for Bone Regeneration Studies
Soft Tissue Regeneration	WFPC	4.1.5	Injectable and Implantable Engineered Soft Tissue for Trauma Reconstruction
		4.1.6	Bioreactors and Biomaterials for Tissue Engineering of Skeletal Muscle
	RCCC	4.1.2	Develop Innervated, Vascularized Skeletal Muscle
		4.3.1c	Composite Tissue Allograft Transplantation without Life-Long Immunosuppression
		4.3.1d	Portable Perfusion System to Increase Preservation Time of Isolated Limbs for Transplantation
		4.3.1e	Vascularized Composite Allograft Transplantation with Topical Immunosuppression
		4.5.2b	Development of Human Lips for Facial Reconstruction
Cartilage Regeneration (Focus: Ear)	WFPC	4.1.1	Engineered Cartilage Covered Ear Implants for Auricular Reconstruction
	RCCC	4.5.4	Engineering a Replacement Autologous Outer Ear Using a Collagen / Titanium Platform

at the site of injury, experienced enhanced soft tissue healing and coverage compared to animals receiving nonporous implants. During the past year, they completed in vitro studies characterizing the physicochemical properties of the porous PMMA-based space maintainers. They also incorporated microparticles loaded with several common antibiotics into the PMMA-based space maintainers and completed comparative in vitro studies with respect to morphology, entrapment efficiency, release kinetics, and activity against bacteria. Constructs demonstrated sustained release up to at least 28 days with retained antibiotic activity. The researchers also completed an in vivo study evaluating the efficacy of colistin-releasing PMMA-based space maintainers in mitigating a bacterial infection in a rabbit composite tissue defect. They will continue to work in partnership with Synthasome, Inc. toward the development of the Good Manufacturing Practices (GMP)-compliant manufacturing process of the porous PMMA-based space maintainer technology. The researchers have received approval for a protocol they submitted to the Institutional Review Board (IRB) of UTHSC to initiate a randomized,

prospective clinical study of their space maintainer technology against clinical standards, evaluating safety and efficacy (see Clinical Trials section of this chapter for more information).

Studies at RCCC

The **Guelcher team (Projects 4.5.1a/4.5.7)** at Vanderbilt University is developing and evaluating allograft bone/polymer composites for treating trauma-related bone defects. The researchers are pursuing two related projects in collaboration with researchers at the USAISR and Medtronic.

In Project 4.5.1a, they are developing injectable, settable LV® bone grafts for the repair of long bone defects. In Project 4.5.7, they are developing injectable, settable LV bone grafts augmented with recombinant human bone morphogenetic protein-2 (rhBMP-2) for the repair of craniofacial and long bone defects. The researchers have demonstrated that their LV grafts support bone remodeling and healing in a rabbit leg bone defect model. They also found that delivery of rhBMP-2 from LV grafts enhanced new bone formation in a rat skull defect model. During the past year, the researchers determined that LV grafts supported bone remodeling in a sheep femoral defect model and passed ISO10993 biocompatibility tests. They also found that delivery of rhBMP-2 from LV/MasterGraft® (LV-MG) formulations enhanced new bone formation in a rat critical-size calvarial defect model. During the upcoming year, the LV-MG formulation will move forward to more extensive testing in larger animal models of craniofacial bone regeneration (e.g., preclinical canine and nonhuman primate models of alveolar ridge augmentation). Pending 510(k) regulatory clearance of the LV bone graft and successful completion of the preclinical studies, preparation for a clinical trial in patients requiring ridge augmentation will begin.

In **Project 4.5.1b**, the **Kohn/Ortiz group** at Rutgers University and the **Hollinger group** at Carnegie Mellon University are developing biodegradable scaffolds containing tyrosine-derived polycarbonate (TyrPC) enhanced with calcium phosphate (CaP), which may provide compelling therapeutic solutions for the regeneration of craniofacial bone. The researchers' imaging and histological data suggested that the synthetic TyrPC+CaP scaffolds were

RCCC lab technician, Barry Cunningham, prepares the Chemspeed SLT100 for the high-speed polymer synthesis of polycarbonates at the New Jersey Center for Biomaterials.

biocompatible and osteoconductive (i.e., guides the reparative growth of natural bone). They found that the addition of a minimal dose of rhBMP-2 to the TyrPC+CaP scaffolds led to significantly enhanced new bone formation in a rabbit critical-size defect (CSD) skull model at 16 weeks, which illustrates the effectiveness of adding bioactive factors into synthetic scaffolds. They determined that the TyrPC scaffolds were gradually resorbed and replaced with new bone. During the past year, the researchers optimized the fabrication method for preparing scaffolds coated with CaP for the rabbit CSD skull and radius models. They completed 60 percent of safety and efficacy studies of TyrPC+CaP scaffolds in these models. They determined that treatment with ethylene oxide is the most suitable sterilization method for TyrPC bone regeneration scaffolds. They have obtained Institutional Animal Care and Use Committee approval for the evaluation of TyrPC-containing bone regeneration scaffolds in the goat CSD skull model. In the upcoming year, the researchers will evaluate the two best performing TyrPC-based scaffolds in the goat model. The surgeries will be conducted at the Allegheny Singer Research Institute, and microCT and histology

will be used to assess new bone formation 16 weeks post-surgery.

Large, biologically compatible scaffolds are needed to treat sizable bone defects, and the success of long-term engraftment of engineered tissues in humans depends on the ability of grafted tissue to connect with host blood vessels (i.e., formation of an integrated vascular network). In **Project 4.5.6**, the **Anderson/Langer team** at the Massachusetts Institute of Technology is developing a novel biodegradable and bioactive scaffold system that can support stem cell growth and produce vascularized bone tissues. The researchers have developed a biodegradable scaffold that can dually support the growth of endothelial (vessel-producing) and osteoblastic (bone cell-forming) cells. They have demonstrated the ability to graft growth factors onto the surface of a poly(ether sulfone) (PES) vascularizing membrane. They have also demonstrated in vivo the ability to increase blood vessel growth and decrease fibrosis by changing the surface geometry of the vascularizing membrane. As an alternative to protein and growth factor use, they have incorporated new potentially therapeutic surface chemistries onto the membranes by grafting additional polymers to the PES surface via synthetic organic chemistry. As a proof-of-concept, the researchers' material will be used to encapsulate islets to treat a diabetic mouse. After demonstrating that transplanted islet cells in the PES membranes can replace the function of diseased islets in the mouse model of diabetes, the researchers will confirm the results in rats and nonhuman primates. Following the success of nonhuman primate trials, clinical trials will be scheduled.

The **Iovine/Kohn group (Project 4.5.8)** at Rutgers University is pursuing a 510(k) submission (used to prove the safety and efficacy of a device) with the U.S. Food and Drug Administration (FDA) for a TyrPC bone fixation pin. The researchers developed a robust bone pin fabrication process, involving the use of injection molding and the incorporation of zinc stearate, a processing aid. During the past year, the researchers scaled up the TyrPC polymerization process to the kilogram (kg) scale and initiated the production of 3 kg of polymer at a third-party manufacturer. They standardized the scaffold characterization techniques and established preliminary specifications. They standardized the bone pin injection molding process, and had more than 200 bone pins injection molded by a third-party fabricator. The pins were submitted for evaluation by an external venue. Future efforts will be focused on completing the process development study for the TyrPC bone regeneration scaffold. Specifically, all the remaining process steps associated with the CaP coating as well as the sterilization and packaging of the final product will be investigated. A manufacturing transfer report will be generated, and this information will be used to engage third party GMP scaffold fabricators in commercial discussions. The researchers anticipate that their synthetic, degradable bone fixation pins will have a positive impact on warfighters due to the large number of bone fractures sustained by military personnel during training and combat.

Studies at USAISR

In **Project 4.5.1c**, the **Brown Baer/Hale group** at USAISR is developing preclinical small and large animal models for bone regeneration studies by the **Guelcher team (Projects 4.5.1a/4.5.7)** and the **Yaszemski team** (**Project 4.2.1**; see Limb and

Post-doctoral fellow Tricia Van Laar of the USAISR's Dental and Trauma Research Detachment purifies bacteria DNA for sequencing analysis.

Digit Repair, Chapter II). The researchers determined that the notch defect was not feasible as a CSD model in both the rabbit mandible at 12 weeks and in the pig mandible at 16 weeks. They are now investigating a full thickness segmental defect of 2 cm in the pig mandible to develop a CSD that does not heal within 20 weeks and can be used to evaluate the efficacy of novel biomaterials-based therapies for bone healing. In addition to creating a challenging animal model that mimics the battle injuries of our wounded warriors, the researchers will continue to develop novel ways to quantify bone regeneration using clinical computer tomography studies in preclinical bone regenerative models as a therapy planning tool.

Jesse Wu, a research laboratory technician at USAISR's Dental and Trauma Research Detachment, analyzes porcine skin graft and scar tissue pathology.

Soft Tissue Regeneration

Studies at WFPC

In **Project 4.1.5**, the **Rubin/Marra group** at the University of Pittsburgh, the **Kaplan group** at Tufts University, and the **Yoo/Lee group** at Wake Forest University are seeking to develop and deliver a clinically useful, engineered soft tissue replacement that can serve as a stand-alone therapy, or be integrated with composite tissue regenerative medicine therapy of burns, craniofacial injuries, and extremity injuries. The researchers are generating implantable silk-based scaffolds and injectable hydrogels that contain autologous (the patient's own) adipose-derived stem cells (ASC), combined with carrier biomaterials, to achieve vascularized soft tissues. The researchers completed numerous preclinical studies and found that implanted silk scaffolds maintained their volumes through an 18-month period in a rat model. They observed mature adipose tissue in groups pre-seeded with ASC or lipoaspirate (source of stem cells, including ASC). Small animal studies are underway in which various formats of silk injectables (e.g., hydrogels, sponges, and foams) are being tested in physiologically relevant models.

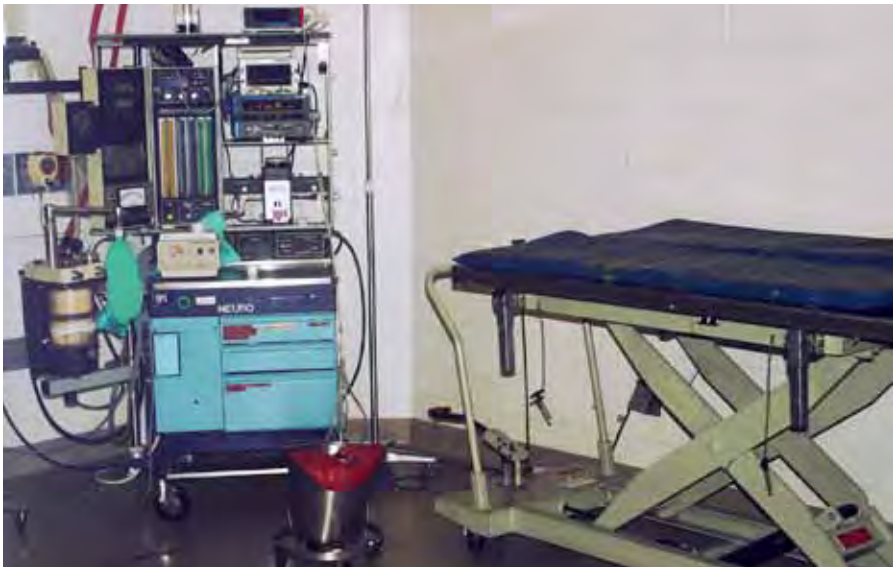
The researchers have also evaluated the silk biomaterials in a large animal (horse) model, with and without autologous lipoaspirate, in terms of cellular response, vascularization of the graft, and volume retention. Going forward, the researchers will develop a composite function tissue through the integration of the multiple tissue components. Silk scaffolds will follow the pre-Investigational Device Exemption (IDE) FDA pathways, and the integrated organ printing system will be pursued under present FDA regulations for 510(k) device. A clinical trial focused on the clinical testing of soft tissue replacement for small defects has begun via the University of Pittsburgh (see the Clinical Trials section for more information).

The **Christ group (Project 4.1.6)** at Wake Forest University is developing a technological tool that preconditions and accelerates muscle tissue maturation and function. More specifically, the researchers are creating an implantable tissue-engineered muscle repair (TEMR) construct capable of restoring clinically relevant force/tension following volumetric muscle loss (VML) injury. They have completed in vivo pilot studies on the first and second generations of controllable silk scaffolds for tibialis anterior (TA) VML injury that showed ~47% and ~65% functional recovery at 2 months post implantation, respectively. The researchers held a

pre-Investigational New Drug (IND) conversation with the FDA during the past year, which prompted the design of a definitive toxicology and proof-of-concept study in collaboration with a Contract Research Organization. Histological characterization of TEMR scaffolds made using muscle progenitor cells sourced from human skeletal muscle biopsies illustrated the formation of multinucleated and aligned myotubes after bioreactor preconditioning. A major goal for the future is to conduct the aforementioned proof-of-concept study, which will consist of five healthy patients with secondary unilateral cleft lip who are willing to participate in the use of bioengineered muscle constructs for surgical correction of aesthetic and functional deformities that have been incompletely corrected by current methods. In addition, the researchers will continue to develop and optimize a silk-based TEMR scaffold for both rodent TA and lattisimus dorsi VML injury models to further expand the potential clinical applications of the TEMR technology. They will also strive to achieve FDA guidance and approval for a second generation bladder acellular matrix-based TEMR scaffold for the treatment of secondary cleft lip.

Studies at RCCC

More than 10% of blast injury survivors have significant eye or eyelid injuries. Damage to the orbicularis oculi muscle prevents eyelid closure, which can result in blindness. The replacement of damaged orbicularis oculi muscles with engineered muscle will restore eyelid function, prevent blindness, and restore facial aesthetics. The **Sundback/ Vacanti group (Project 4.1.2)** at Massachusetts General Hospital (MGH) is collaborating with researchers at Rutgers University and the Massachusetts Eye and Ear Infirmary to engineer skeletal muscle with physiological connections to the host's neurovascular (nerve and blood vessel) network using biodegradable polymer scaffolds. The researchers engineered three-dimensional (3D) skeletal muscle similar to immature skeletal muscle and established protocols for the development of functional blood vessels and nerves in the tissue. During the past year, the researchers (1) demonstrated uniform innervation of engineered muscle constructs; (2) determined that electrical stimulation with neural-like signals increased the engineered muscle's contractile force; (3) found that engineered endothelial networks within the muscle constructs rapidly merged with the host's vasculature, and blood perfusion from the host supported the implanted construct; and (4) developed polymeric sleeves (Rutgers team) for scale-up of the muscle constructs. During the upcoming year, the researchers will implant muscle constructs bundled in sleeves into immunocompromised rodents and innervate the implants with a host nerve. The researchers will characterize the innervation, vascularization, and muscle function of the implants. Future studies will demonstrate the use of engineered muscle constructs to replace orbicularis oculi function in immunocompetent rats or rabbits. Given the complexity of this product, the project team will submit a Request for Designation to the FDA Office of Combination Products (OCP) to determine appropriate regulatory path.



Operating room at the Transplantation Biology Research Center at MGH where skin graft surgeries are performed to study the effects of topical immunosuppression on the prolongation of graft survival.

VCAs—large segments of complex, vascularized tissue—differ in their immunological responses, presenting challenges to transplant immunologists. In Project 4.3.1c, the **Siemionow group** at the Cleveland Clinic hopes to transform standards for clinical modulation of the immune system, making transplantation of VCAs safer and more widely available to victims of disease and traumatic injury. The researchers are exploring the use of fused donor–recipient "chimeric" cells as potential immunomodulators. They have proven that the fusion of cord blood cells from two unrelated donors is feasible. During the past year, the researchers studied many properties of the chimeric cells and found that: (1) these cells do not secrete inflammatory cytokines in vitro; (2) there were few dead cells following cell fusion; and (3) the cells proliferate. The research team characterized the cells' genotype and phenotype before and after culturing, and animal model testing demonstrated that the cells migrated into blood, bone marrow, and lymphoid organs. During the upcoming year, the research team plans to test the safety of human chimeric cell therapy; the phenotypic and genetic stability of human chimeric cells; and cryopreservation methods of human chimeric cells. Following confirmation of safety, stability and cryopreservation of human chimeric cells, the research team will apply for IRB approval to test the immunomodulatory effects of bone marrow-derived human fused chimeric cells as a supportive therapy for living kidney and liver donor transplantation. Tolera Therapeutics, Inc. will support the transition of chimeric cell therapy to clinical trials. Overall, the introduction of cell-based therapies to the field of VCA transplantation represents a novel, regenerative medicine approach, which will minimize or reduce the need for lifelong immunosuppression.

In **Project 4.3.1d**, the **Pomahac team** at Brigham and Women's Hospital is seeking to attenuate the effects of ischemia/reperfusion injury in isolated limbs by developing and validating an extracorporeal perfusion device capable of oxygenating, sustaining, and preserving amputated limbs. If successful, the results of this study would promote wider practice of upper limb replantation and composite tissue allotransplantation. After successful (delayed) execution of the award in August 2012, the Pomahac team actively sought partnerships with individuals and groups able to provide programming support for the development of the extracorporeal machine perfusion device. In April 2013, after vetting several candidates, the Pomahac team paired with Numia Medical (Newport, VT), who produced the first working prototype device. Using the functional prototype device, the team intends to pursue animal studies in a pig model to determine the perfusion parameters that must be used to best preserve limb tissues, based on observed chemical and histological signs of cell injury and death. The researchers then intend to use these determinants to pursue a series of transplant experiments in pigs, analyzing the survival and quality of transplanted tissue after reperfusion. After optimization of the isolated limb perfusion device has been achieved and proof-of-principle has been established in pigs, the Pomahac team intends to pursue FDA IDE approval for a transition to human studies.

In **Project 4.3.1e**, the **Sachs/Cetrulo group** at MGH and the **Kohn group** at Rutgers University are conducting a feasibility study of the topical delivery of cyclosporine and tacrolimus to reduce the need for the systemic delivery of these powerful

RCCC researcher Christopher Mallard cleans skin grafts prior to delivering topical immunosuppression for prolonged graft survival.

immunosuppressant medications. The xenogeneic skin graft has been selected as the model system because the skin of hand and face transplants is usually the primary site of immunologic rejection, but the wider impact of this work will be the possible reduction of the need for systemic immunosuppression for allogeneic limb and face transplant patients as well as for recipients of allogeneic or xenogeneic skin grafts. During the past year, the research team successfully formulated TyroSphere™ dressings that provided sustained release of cyclosporine and tacrolimus. These drug-loaded TyroSpheres™ were stable over a range of temperatures, penetrated the dermis of cadaveric skin, and released the drug over seven days from a gel. In addition, the researchers developed a novel clinical application of the TyroSpheres™—direct application to the wound bed prior to graft placement. The application of tacrolimus-loaded TyroSphere™ dressings both topically to the graft and directly to the wound bed significantly reduced inflammation in the skin grafts and wound beds in a baboon model. The project team plans to conduct safety and efficacy studies in Good Laboratory Practices (GLP) guidance, submit an IND, and initiate the design of a clinical trial. Future work will focus on the use of a different model (a skin flap with an established blood supply) and different immunosuppressive agents, which have different mechanisms of action in vivo and offer promise as topical immunosuppression dressings.

Normal lip structure is required for activities such as eating, drinking, talking, and social gestures. Avulsion of the lips is a survivable injury, but without functional lip reconstruction, life for injured individuals is burdened by drooling, food spillage while eating, unintelligible speech, and social rejection. Functional reconstruction of the lips is so important that when more than 50% of the lips are avulsed, face transplantation under lifetime immunosuppression becomes an option. In **Project 4.5.2b**, the **Feinberg team** at the University of Michigan is developing a mucocutaneous (M/C) junction with mouth and skin cells (oral and epidermal keratinocytes) grown together into 3D patterns that can be used in a staged reconstruction of the lips. During the past year, the researchers fabricated a 3D tissue structure with the key morphological features of a lip: epidermal skin, vermillion, and oral mucosa. This tissue-engineered mucosa-and-skin equivalent was manufactured using human oral and skin cells to produce similar anatomic and handling properties as native human lips. The researchers also developed an animal (rat) model for in vivo grafting of the lip construct (made of mucosa and skin components) and for testing functional muscle performance. In the future, the researchers plan to (1) extend these studies to the use of athymic rats and use of a cellular M/C construct to fabricate the pre-laminated flap; (2) extrapolate these techniques to tissue engineering of an anal sphincter; and (3) submit an IND to the FDA to perform a human clinical trial for lip reconstruction.

Cartilage Regeneration (Focus: Ear)

Studies at WFPC

The **Yoo group (Project 4.1.1)** at Wake Forest University is pursuing the development of an engineered cartilage-covered ear implant. The researchers have fabricated a flexible ear scaffold using an integrated organ printing technology that was developed in their laboratory. During the past year, they optimized their isolation and expansion protocols, which yielded an increased production of human chondrocytes (cartilage cells). They developed and fabricated a flexible and complex shaped ear scaffold using an integrated 3D organ-printing system. Their implanted 3D bioprinted ear constructs resulted in the formation of cartilage tissue in vivo in animals. They demonstrated the structural and functional integrity of the engineered cartilage covered ear implants in vivo. During the upcoming year, the researchers will continue to optimize the customized ear implants using the 3D bioprinting system. They plan to contact the OCP at the FDA to confirm the classification of their 3D bioprinter and, if needed, file a Request for Designation (RFD) with OCP in accordance with 21 CFR Part 3.

Studies at RCCC

The **Sundback/Vacanti group (Project 4.5.4)** at MGH is also developing a permanent, implantable external ear. The researchers' primary approach to ear regeneration utilizes a cartilage/titanium technology platform. They seed cartilage cells on a

human ear-shaped collagen scaffold, and an integrated titanium wire support embedded within the collagen matrix maintains the dimensions and complex central features of the ear. The researchers' alternative approach to ear regeneration utilizes resorbable polymers and stem cells. During the past year, they expanded chondrocytes successfully and sufficiently without losing the cells' ability to form neocartilage. They engineered high-quality stable cartilage in both immunocompromised rodents and large immunocompetent animals, and made ear-shaped neocartilage from chondrocytes in sheep. The ear construct's size and shape were largely maintained after 12 weeks implantation in the animal models. The team developed analytical non-invasive methods for assessing 3D changes, which suggest that the wire framework properties could be improved to eliminate shape changes due to wire sliding. Going forward, the long-term stability of cartilage engineered from extensively expanded chondrocytes and retention of the size and shape of ear-shaped scaffolds will be assessed in an immunocompetent animal model. The researchers plan to submit an RFD to the FDA to determine the regulatory path for the engineered ear. In preparation for clinical trials, they will perform a GLP preclinical trial in sheep to demonstrate the safety and efficacy of the engineered ear. They will develop a protocol for a pilot clinical trial, and submit it to the local IRB.

Clinical Trials

Several AFIRM Craniofacial Reconstruction technologies have advanced to the human clinical trial stage. The status of each clinical trial is summarized in **Table III-2,** and additional details on these trials follow the table. Detailed clinical trial progress reports, when available, can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

In **Project 4.3.1a**, the **Siemionow group** at the Cleveland Clinic is performing composite tissue allograft face transplantation, which provides a single-stage reconstructive procedure for patients with severe cranial facial injuries and spares them from multiple surgical procedures over many years. The researchers performed the first U.S. face transplant prior to the start of AFIRM. Based upon 52 months of data collected and analyzed from this individual to date, face transplantation appears to be a safe and effective, single-stage treatment procedure for subjects with severe facial deficit and deformity. Through objective testing, face transplantation has been shown to provide a return of mastication, the ability to speak clearly, smell, smile, frown, and kiss. In addition, an improved self-image has been shown. Potential serious adverse events, such as infection and rejection, which may occur with any transplant, have been successfully treated. During the past year, the research team completed five detailed evaluations on potential face transplant subjects enrolled in the study and approved one

Table III-2. AFIRM-funded Craniofacial Reconstruction projects with pending, active, or closed clinical trials.

Project Title	Consortium	Project No.	Trial Phase	Current Status
Clinical Trial – Composite Tissue Allograft Transplantation (Face)	RCCC	4.3.1a	Phase I	Open
Clinical Trial – Anti-TCR Monoclonal Antibody (TOL-101), for Prophylaxis of Acute Organ Rejection in Patients Receiving Renal Transplantation	RCCC	4.3.1b	Phase I/II	Closed; moving into Phase III
Clinical Trial – Use of Tissue Engineered Human Oral Mucosa for Large Soft-Tissue Intra-Oral Defects	RCCC	4.5.2	Phase I/II	Protocol approved by IRB
Injectable and Implantable Engineered Soft Tissue for Trauma Reconstruction	WFPC	4.1.5	Phase I	Open
Space Maintenance, Wound Optimization, Osseous Regeneration, and Reconstruction for Craniomaxillofacial Defects	WFPC	4.1.2	Phase I	Protocol approved by IRB

patient for listing for face transplantation. The two major goals for the upcoming year are to (1) perform one face transplant and (2) officially list a second patient for face transplantation. Patient safety and monitoring regulations will be followed according to IRB and Human Research Protection Office (HRPO) regulations. All IRB and HRPO reporting and accreditation requirements will be met.

The **Siemionow group** in **Project 4.3.1b** is using a therapeutic antibody, TOL-101, as a conditioning agent prior to transplantation to enhance allograft tolerance. The researchers are testing the safety and tolerability of TOL-101 in patients who are undergoing their first kidney transplantations. To date, a total of 36 subjects (nine cohorts) have been enrolled in the study, and a total of 60 biopsies were sent to the Cleveland Clinic Pathology Core for analysis. A Clinical Study Report was prepared by Tolera Therapeutics, Inc. and presented to the FDA. Drug-related adverse events were limited to a transient rash with pruritus (one subject) in cohort 9. Twenty-seven (27) serious adverse events have been reported, with only one (pneumonia) possibly related to the study drug. The data demonstrated that although the total number of patients who have received TOL101 to date is relatively small, the benign safety profile and easily assessed pharmacodynamic target allows a dosing regimen to be selected for Phase III testing from the Part A phase of the Phase I/II study.

In **Project 4.5.2**, the **Feinberg team** at the University of Michigan is using a tissue-engineering/regenerative medicine approach, in conjunction with the surgical technique of prelamination, to create a prevascularized composite soft tissue flap. More specifically, the researchers are using an ex vivo-produced oral mucosa equivalent (EVPOME) for major areas of intra-oral reconstruction, and as a platform technology for the tissue engineering of a set of human lips. In the first year of funding, they have (1) developed a manufacturing process to fabricate large-sized EVPOMEs for a clinical trial, (2) successfully fabricated a large EVPOME device in a cGMP facility, (3) received FDA approval of an amendment to an already existing IND, (4) obtained approval from the University of Michigan's IRB for the clinical protocol, and (5) submitted the clinical protocol to the HRPO. The technology in development is similar to the development of orphan drugs, in that the target market area is very specific: post-traumatic (explosives, burns, gunshot and motor vehicle accidents) and post-oncologic surgery.

In **Project 4.1.5**, the **Rubin/Marra group** at the University of Pittsburgh, the **Kaplan group** at Tufts University, and the **Yoo/Lee group** at Wake Forest University have begun a clinical trial at the University of Pittsburgh entitled "Autologous Adipose-Derived Stem Cell Therapy for Soft Tissue Reconstruction after Facial Trauma." A progress report is not yet available for this clinical trial.

The **Mikos/Kasper** and **Wong groups (Project 4.1.2)** at Rice University and the UTHSC has received approval by the UTHSC IRB for its protocol for a randomized, prospective clinical study of the porous PMMA-based space maintainer technology against clinical standards evaluating safety and efficacy. The study will commence once HRPO approval of the protocol has been issued, which will depend upon either FDA clearance of the product or approval of an IDE. A progress report is not yet available for this clinical trial.

IV: Scarless Wound Healing

Background

The consequences of dysregulated wound repair impact both soldiers and civilians, and this can lead to severe functional disability and disfigurement. Scar formation following injury is a major biomedical burden for the U.S. health care system, and the costs associated with treatment of fibrosis—the formation of excess fibrous connective tissue in an organ or tissue as a reparative or reactive process—are estimated to be more than $4 billion per year in the U.S. Current treatment regimens for fibrosis, which involve surgery, silicone sheeting, anti-inflammatory medications, and laser/radiation, have been disappointing due, in large part, to a lack of understanding regarding the fibrotic process. The pathophysiology of scar formation suggests the need to regulate numerous aspects of the wound environment, including cells and the surrounding extracellular matrix, wound mechanics, and biochemical signaling.

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IV: Scarless Wound Healing

The wound healing process occurs through overlapping and well-defined phases of repair, which may take months to resolve and often results in irreversible scar formation with resultant contractures and disfig-urement. To be truly successful, any therapeutic approach must be comprehensive and encompass the myriad inputs regulating wound healing. Studies of tissue regeneration have implicated the inflammatory environment, matrix components, mechanical context, and cellular players in producing a "scarless" wound profile. The AFIRM researchers utilize an approach that encompasses a broad continuum of technologies aimed at modulating the tissue response to injury. Collectively, the AFIRM projects represent a collaborative effort to address every aspect and stage of wound repair in a single research program—with the overarching aim of developing a more effective wound management paradigm.

Unmet Needs

Given the increasing survival of injured soldiers returning from the battlefield, effective strategies to promote wound regeneration and prevent scar formation are desperately needed. The burden of scarring that follows the 230 million surgical procedures performed worldwide each year is enormous. Although the exact incidence of pathologic scarring is unknown, soldiers and civilians continue to suffer from functional disabilities caused by wound contracture and severe disfigurement from hypertrophic scarring. These scars can become so thick that they limit the movement of joints and greatly restrict a patient's ability to move about.

While multiple factors influence wound repair, therapeutic modalities aimed at these targets (e.g., inflammation and oxygen tension) have been largely unsuccessful. Although biomolecules that limit fibrosis have demonstrated effectiveness in controlling scarring in vitro, a major hurdle for clinical translation has been the ability to sustain drug release and bioactivity in a complex wound environment. Also, there are insufficient effective animal models to study scar formation. Therefore, the development of more appropriate and clinically relevant animal models of hypertrophic scarring remains an unmet need.

Battlefield injury progression and/or impaired healing often occurs secondary to ischemia or repetitive ischemia/reperfusion (I/R) injury, which involves deficient blood flow to a tissue due to the injury, followed by the return of blood to the damaged area. Currently, no therapy exists to mitigate impaired wound healing due to ischemia or I/R injury. Hence, another unmet need is the limitation of impaired healing secondary to ischemia or I/R injury.

Areas of Emphasis

Rutgers–Cleveland Clinic Consortium (RCCC), Wake Forest–Pittsburgh Consortium (WFPC), and U.S. Army Institute of Surgical Research (USAISR) researchers are pursuing a complementary mix of research projects focused on various aspects of scarless wound healing. As shown in **Table IV-1**, projects can be grouped into three clinical challenge topic areas: Control of Wound Environment and Mechanics, Therapeutic Delivery to Wounds, and Attenuation of Wound Inflammatory Response. Nontechnical summaries of the projects are presented after Table IV-1, and full progress reports for each project can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

Two WFPC postdoctoral fellows work on cell culture in a hypoxia chamber.

Table IV-1. AFIRM-funded projects per clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Control of Wound Environment and Mechanics	WFPC	4.5.1	Mechanical Manipulation of the Wound Environment
Therapeutic Delivery to Wounds	RCCC	4.6.3	Therapy to Limit Injury (TLI) and Promote Non-Scar Healing After Burns and Severe Battle Trauma
		4.7.1	Adipose-Derived Therapies for Wound Healing, Tissue Repair, and Scar Management
	WFPC	4.5.2	Regenerative Bandage for Battlefield Wounds
		4.5.5	Scarless Wound Healing Through Nanoparticle-Mediated Molecular Therapies
		4.5.6	Peptide-Mediated Delivery of Therapeutic Compounds into Injured Tissues During Secondary Intervention
Attenuation of Wound Inflammatory Response	WFPC	4.5.3	Multi-Functional Bioscaffolds for Promoting Scarless Wound Healing
		4.5.4	Regulation of Inflammation, Fibroblast Recruitment, and Activity for Regeneration

Control of Wound Environment and Mechanics

Studies at WFPC

Using a mouse model of hypertrophic scarring based on increasing the skin stress of healing wounds, the **Gurtner team (Project 4.5.1)** at Stanford University found that the skin's biomechanical properties correlated with the amount of scarring that followed wounding. They have developed a pressure-sensitive, "stress-shielding" device that can modify mechanical forces to control scar formation in the red Duroc pig model. In an exploration of the molecular mechanisms underlying this process, the researchers found that fibroblast focal adhesion kinase (FAK) is a key mediator of load-induced fibrosis and scar formation. During the past year, the researchers evaluated the role of keratinocyte-specific FAK on wound healing progression and extracellular matrix composition. They identified a signaling pathway, mediated by FAK activation, leading to increased epidermal levels of matrix metalloproteinase 9. They also critically analyzed the role of epidermal FAK in wound healing, via a mouse model of keratinocyte FAK deletion. They demonstrated that keratinocyte FAK influences skin regeneration. Going forward, the researchers aim to develop therapeutic approaches to specifically target FAK to improve tissue regeneration. They are conducting a Phase III clinical trial with significant interim data for their polymeric stress-shielding device (see Clinical Trials section, Project 4.5.9). Additionally, the group will seek U.S. Food and Drug Administration (FDA) approval for small molecule FAK inhibition, and will provide an appropriate regulatory package to gain allowance for the initiation of a Phase I clinical trial.

Therapeutic Delivery to Wounds

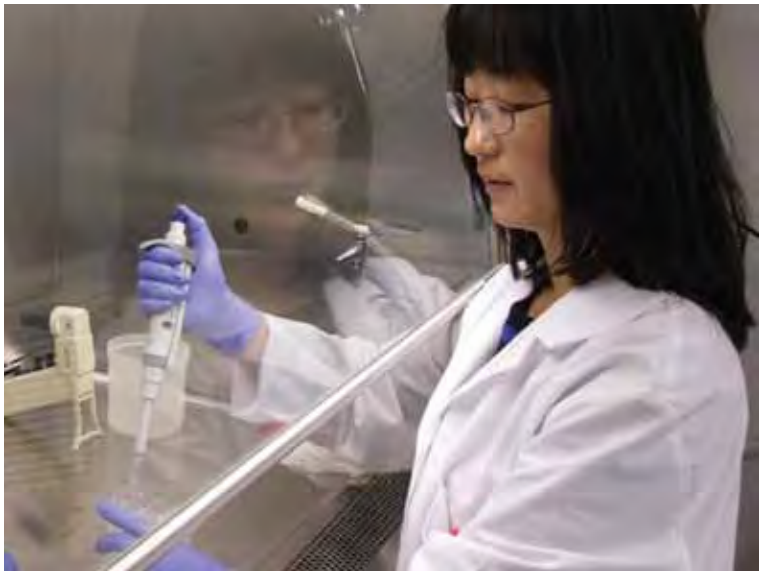
A novel approach is needed to alter the trajectory of wound healing in the initial days following injury to promote the regeneration of tissue. Such an approach could involve the delivery of cells, molecules, proteins, compounds, or genes to the wound surface.

Studies at RCCC

The **Mustoe group (Project 4.6.3)** at Northwestern University is investigating the wound-healing capability of curcumin, which is a yellow-colored component of the Indian spice turmeric. The researchers found that the intravenous delivery of curcumin accelerated healing and reduced scarring in a rabbit

ear I/R model. They also found that they could apply curcumin via tiny nanospheres to the wound site in the rabbit I/R model with no apparent toxicity. During the past year, the researchers confirmed the effectiveness of curcumin in reducing necrosis and supporting the survival of skin flaps in a porcine model. They determined that the most effective time for treatment was at the time of surgery with a single 3 µm dose. The effect was confirmed 7 and 28 days after surgery. The researchers found that treatment with curcumin 4 or 24 hours after the surgery had no statistically significant effect in rescuing injury when compared to vehicle control. They expect the transition to Good Manufacturing Practices to be straightforward based on discussions with manufacturers of curcumin.

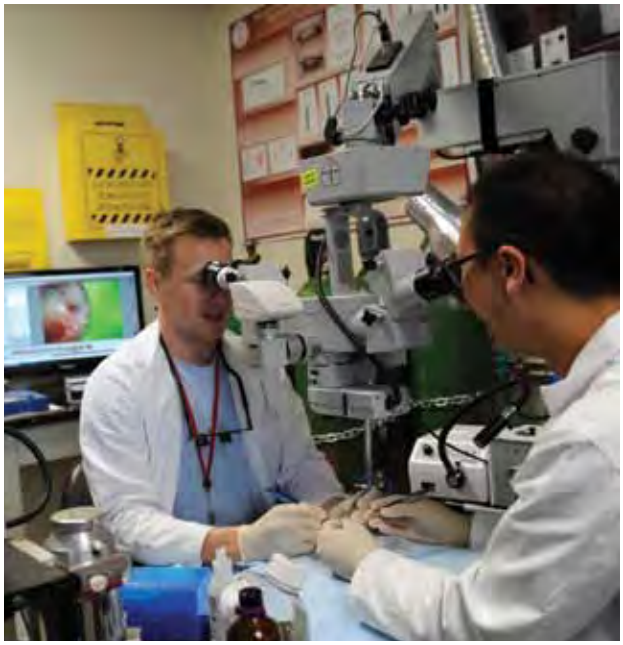
The **Katz group (Project 4.7.1)** at the University of Florida (UF) is developing regenerative therapies using adipose-derived stem cells (ASC). The researchers are developing a dermal wound paste (DWP) platform for skin repair and replacement that involves the combination of ASC (and other cells) and a cell-free dermal scaffold. They formulated DWP with cell-stabilizing solutions that maintained cell viability and growth over a 2-week period in culture. They also determined that cell-enhanced DWP reduced wound contraction in a mouse wound model more effectively than wound paste that does not contain any cells. During the past year, the researchers initiated testing and characterization of a novel device that can isolate stromal vascular fraction cells. They established fundamental quality control and potency assays that can be used to measure the reproducibility and bioactivity of the wound paste. They compared subtle but important variations in the specific formulation of the DWP. They also demonstrated that the DWP secretes numerous bioactive factors that can impact the growth of blood vessels and the movement of cells. During the upcoming year, the research team will focus efforts on completing pre-clinical animal studies that are necessary for supporting an Investigational New Drug (IND) application filing, as determined by a previous pre-IND meeting with the FDA. Upon completion of the pre-clinical studies, the research team will pursue funding for a Phase I clinical trial.



Dr. Ning Yang prepares adipose-derived cells as part of her work on an autologous "wound paste" platform.

Studies at WFPC

The **Gurtner/Longaker team (Project 4.5.2)** at Stanford University is capitalizing on the ability of wounded fetal tissue to regenerate with minimal scarring by developing a regenerative bandage that contains a fetal-like matrix and progenitor cells found in wounds. The goal is to maintain an acute wound in a pro-regenerative state and prevent the onset of scarring, fibrosis, and infection. The researchers have developed a novel, modifiable hydrogel scaffold that can deliver matrix components, cells, and/or wound-healing drugs. During the past year, they demonstrated the utility of mesenchymal stem cell (MSC)-seeded bioscaffolds for the accelerated healing and appendage formation of humanized murine excisional wounds, as compared to direct MSC injection and no treatment controls. They also evaluated the effect of hydrogel seeding on MSC survival within humanized excisional wounds and found an increase in cell viability and engraftment among hydrogel-seeded cells, compared to direct injection. In addition, they determined the beneficial effect of hydrogel seeding on MSC secretion of angiogenic cytokines in vitro is recapitulated in the in vivo wound environment. In the next phase of this project, the researchers will utilize microfluidic single-cell transcriptional analysis to identify the optimal cell source



A surgical resident and a postdoctoral fellow from WFPC perform microsurgery.

for this bandage. Following a refinement period over the next few years, the safety and regenerative capacity of the hydrogel-based regenerative bandage will be tested in a Phase I clinical trial. Phase II–III clinical testing will follow.

The **Kathju team (Project 4.5.5)** at the University of Pittsburgh is using tiny nanoparticles as a non-viral means of delivering molecules into wounds. The researchers identified a gene (chaperonin-containing T-complex polypeptide [CCT-eta]) that is normally decreased in healing fetal wounds but elevated in adult wounds. They developed a nonviral nanoparticle-mediated delivery system using small interfering RNAs (siRNAs) that can selectively decrease the expression of CCT-eta in complex adult wounds. They established new protocols that will allow them to inhibit CCT-eta more stably and for a longer period of time in a healing wound environment. They demonstrated that inhibition of CCT-eta can inhibit scar formation in an animal model of incisional wound healing, and they identified strains of bacteria that may be used as probiotic therapy for other pathogens. Finally, they demonstrated that a novel probiotic therapy can rescue mice from burn wound-induced sepsis and death, and can inhibit burn wound-induced scarring. Overall, both the researchers' CCT-eta inhibitor and their use of probiotic therapy demonstrate efficacy in pre-clinical systems and show great potential to achieve clinical translatability.

The **Ruoslahti group (Project 4.5.6)** at the Sanford-Burnham Medical Research Institute has made substantial progress in identifying peptides that home to wounds and deliver a therapeutic payload to the wounds and other injured tissues. The researchers found that treatment of mice with skin wounds with the wound-homing CARSKNKDC (CAR) peptide promotes wound healing. They generated a smaller form of the CAR peptide, tCAR, which they found to be biologically more potent than CAR. They fused the CAR peptide with the wound-homing protein decorin and found that the CAR-decorin fusion protein suppressed scar formation in mice with skin wounds. They also showed that a wound-homing peptide can deliver co-administered drugs not coupled to the peptide to injured tissue. The researchers' technology has been licensed to a biotech company for further pre-clinical studies and introduction into the clinic.

Attenuation of Wound Inflammatory Response

Following injury, an intense inflammatory response ensues and is necessary for normal wound healing. However, aberrations in this process result in chronic wounds and have been strongly implicated in fibrotic scar formation. Redirecting this process toward a regenerative outcome requires controlling the inflammatory response and is the focus of two AFIRM projects.

Studies at WFPC

The **Washburn group** (**Project 4.5.3**) at Carnegie Mellon University is developing hyaluronic acid (HA) biogels that contain monoclonal antibodies or peptides (short versions of proteins) with specific affinities for cytokines and other mediators of inflammation to absorb proinflammatory cytokines and decrease inflammation. The researchers have identified that a biogel with HA conjugated to an antibody against tumor necrosis factor-alpha (TNF-α) can effectively inhibit burn progression and control inflammation in a rat burn model. They developed a new analogue formulation that does not require covalent conjugation, which will facilitate clinical translation. During the past year, the researchers collaborated with Dr. Robert Christy's

group at USAISR to test mixtures of anti-TNF-α and HA with conjugated (anti-TNF-α)-HA to determine whether conjugation was important for activities in a burn injury model. Their results confirmed that conjugation is necessary for the potent anti-inflammatory effects (e.g., reduction in secondary necrosis) observed with these materials.

The **Hebda group (Project 4.5.4)** at the University of Pittsburgh's McGowan Institute for Regenerative Medicine is working to clarify interactions between fibroblasts and inflammatory mediators with the goal of developing novel anti-inflammatory therapies to improve the quality of healing. The researchers demonstrated that early, short-term topical treatment with the anti-inflammatory agents nimesulide and prostaglandin E2 attenuated the wound inflammatory response, which led to the promotion of healing. They also showed that early, one-time topical treatment with isogenic ASC and fetal skin fibroblasts led to reduced scarring and increased healing. During the past year, the researchers completed cell therapy studies using regenerative phenotype donor cells. They revised their combination drug therapy protocol to use FDA-approved pharmaceutical agents. They found several important differences in wound modulation to be shared by regenerative fibroblasts, supporting the promise of using a patient's own cells for regenerative (scarless) wound therapies. The research team's goal for the next phase of this research project is to design and optimize the combination pharmacologic therapy that provides a wound environment for rapid, regenerative healing with minimal scar formation, based on previous and emergent results achieved during AFIRM I.

Clinical Trials

Two AFIRM scarless wound-healing technologies have advanced to the human clinical trial stage. The status of each clinical trial is summarized in **Table IV-2**, and additional details on these trials follow the table. Detailed clinical trial progress reports, when available, can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

In **Project 4.5.9**, the **Beasley team** at Neodyne, in collaboration with the **Gurtner team** at Stanford University, is conducting a Phase III clinical trial designed to test its third-generation device capable of stress-shielding wounds. A Progress Report for this project is not available at this time.

Autologous fat transfer (AFT) is a procedure that involves removing adipose tissue from one area of a patient's body and immediately transplanting it to a different site in the same person. The **Katz group (Project 4.7.3)** at UF is conducting a Phase I/IIa clinical trial designed to test the safety and efficacy of using AFT for scar prevention and remodeling. Patient enrollment began in July 2010. To date, the researchers have enrolled and treated a total of 10 patients. The key accomplishments for the past year were the successful transition of the clinical trial to UF, and the finalization of all administrative and protocol-related logistics at both UF and USAISR/Brooke Army Medical Center (BAMC), such that the trial is open to enrollment at two new sites. The researchers plan to focus future efforts on the enrollment of patients at UF and USAISR/BAMC. There are multiple companies that currently market fat tissue transplantation devices and systems. If the evidence from this trial supports the use of AFT for scar remodeling, the researchers do not expect commercialization to be a problem.

Table IV-2. AFIRM-funded scarless wound healing projects with active clinical trials.

Project Title	Consortium	Project No.	Trial Phase	Current Status
Neodyne's Device to Actively Control the Mechanobiology during Wound Healing and Prevent Scar Formation	WFPC/ USAISR	4.5.9	III	Open
Clinical Trial – Autologous Fat Transfer for Scar Prevention and Remodeling (AFT-SPAR)	RCCC/ USAISR	4.7.3	I/IIa	Open

V: Burn Repair

Background

Over the past few decades, changes in weaponry have resulted in a higher frequency of burn injuries to warfighters, and these often occur in combination with other severe injuries. Costly in both human and economic terms, these injuries carry risks to survival, independence, and function in both the short- and long-term, as the wounds mature with scarring and movement-limiting contractures.

Currently, the standard of care for burn injuries includes early excision of the damaged tissue and autografting (i.e., transplanting healthy tissue from one part of the body to another), which is a procedure that has not changed fundamentally in more than three decades. In light of this, researchers have been tasked with discovering new solutions to overcome the challenges resulting from burn injuries. Not only will this improve outcomes for wounded warfighters suffering from burns, but it will also help in the treatment of other debilitating and disfiguring injuries where wound coverage or scarring are problematic.

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The therapies developed by AFIRM researchers are expected to benefit both the military and civilian populations of the U.S., where more than 1 million burn injury events occur annually, resulting in 900,000 hospital days, 4,500 deaths, and more than $1 billion in treatment costs and lost productivity each year.

Unmet Needs

Respiratory distress remains a critical issue immediately following burn injury; however, skin loss becomes the primary problem within the next 24 hours. This disruption of the skin barrier frequently results in fluid and heat loss—often leading to a predisposition to infection. Compounding these problems are progressive inflammation and the extension of burns during the first few days after injury. Acutely, deep second-degree burns often extend to become full-thickness, third-degree burns, which often results in increased tissue loss, longer healing times, and excess morbidity and mortality. Over the long term, burn progression typically results in increased scarring, wound contractures, and poor quality of life. Unfortunately, various therapies which utilize non-steroidal anti-inflammatory drugs (NSAIDs) and antioxidants have not shown substantial benefit in preventing injury extension. Therefore, a critical unmet need is prevention of burn inflammation and injury progression.

Within the area of the burn injury, nonviable tissue favors bacteria colonization and infection. This complication remains the most common cause of morbidity and mortality in patients with extensive burns, despite a reduced incidence of invasive infection. Although Sulfamylon®, Silvadene®, and silver nitrate are frequently used to prevent burn infections, each has disadvantages or adverse side effects. Sulfamylon is bacteriostatic for both gram-positive and -negative organisms, and it penetrates burn scabs extremely well. However, it is painful when applied to partial-thickness burns, and because it inhibits an enzyme, it can lead to the accumulation of acid in the body fluids if applied over an extensive surface. Silvadene does not induce pain or disturb acid-base balance, but it does fail to penetrate the burn scab well enough to help protect against certain types of bacteria. Furthermore, it can induce harmful conditions involving patients' blood cells. Finally, although silver nitrate is active against a broad spectrum of bacteria, it cannot penetrate the burn scab and is caustic, which results in damage to otherwise viable tissue. Clearly, new topical treatments are needed for the prevention of infection in burn patients.

Cutaneous autografts are optimal for closure after burn wound excision; however, this treatment approach mandates a viable wound bed and available donor sites. As the area in need of grafting may outsize available donor sites in patients with extensive burns, a skin substitute may be necessary. Frozen cutaneous allografts (tissue grafted from one individual to a genetically nonidentical member of the same species) and porcine cutaneous xenografts (tissue grafted from one species to an unlike species) are the two most readily available skin substitutes, but they are less adherent to the wound bed than fresh autografts, less able to control the bacterial population of the underlying wound, and usually do not become well vascularized (infiltrated with blood vessels) from the underlying wound bed. Another alternative is cultured autologous keratinocyte sheets; however, this approach is limited by a preparation time of 3–4 weeks, sheet fragility, and susceptibility to infection.

Although limited in success, synthetic skin substitutes have also been used to treat burn injury. An effective synthetic skin substitute should be compatible with a patient's own tissue, have no immune system-activating effects or toxicity, have water vapor permeability similar to that of skin, be impermeable to microorganisms, adhere to the wound, be readily vascularized, and have an indefinite shelf life. The available skin substitutes must be modified to increase their clinical usefulness by enhancing both their resistance to infection and their ability to accelerate the formation of either neodermis or granulation tissue (the fibrous connective tissue that replaces a clot in healing wounds).

The various therapies and innovations described in this chapter are proposed by AFIRM researchers to reduce wound scarring and contractures, while also preventing burn injury progression, reducing inflammation, and inducing healing following burn injury.

Areas of Emphasis

Researchers at the Rutgers–Cleveland Clinic Consortium (RCCC), Wake Forest–Pittsburgh Consortium (WFPC), and U.S. Army Institute of Surgical Research (USAISR) are pursuing a complementary mix of research projects focused on various aspects of burn repair. As shown in **Table V-1**, projects can be grouped into four "clinical challenge" topic areas: Intravenous Treatment of Burn Injury, Topical Treatment of Burn Injury, Wound Healing and Scar Prevention, and Skin Products/Substitutes. Nontechnical summaries of the projects are presented after the summary table, and full progress reports for each project can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

Intravenous Treatment of Burn Injury

Studies at RCCC

The **Lin/Clark group (Project 4.6.1)** at Stony Brook University conducted a focused screening investigation for agents that inhibit the progression of burn injury. Agents were selected for their reported ability to inhibit oxidative stress, cytokine stress, or apoptosis. The researchers found that a single intravenous infusion of either fibronectin-derived P12 or curcumin could significantly inhibit burn injury progression in the rat hot comb model. The P12 peptide also significantly limited burn injury progression in a swine hot comb model. Over the

Table V-1. AFIRM-funded projects per clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Intravenous Treatment of Burn Injury	RCCC	4.6.1	Therapy to Limit Injury Progression, Attenuate Inflammation, Prevent Infection, and Promote Non-Scar Healing After Burns and Battle Trauma
		4.6.1a	Pre-IND Studies for a Novel Cell Survival Peptide that Limits Burn Injury Progression
Topical Treatment of Burn Injury	WFPC	4.2.3	Novel Keratin Biomaterials That Support the Survival of Damaged Cells and Tissues
	RCCC	4.6.4	Polymeric, Antimicrobial, Absorbent Wound Dressing Providing Sustained Release of Iodine
		4.6.5	Topical P12 Therapy to Limit Burn Injury Progression and Improve Healing
Wound Healing and Scar Prevention	WFPC	4.2.2	Delivery of Stem Cells to a Burn Wound via a Clinically Tested Spray Device. Exploring Human Skin Progenitor Cells for Regenerative Medicine Cell-Based Therapy Using Cell Spray Deposition
		4.2.5	In Situ Bioprinting of Skin for Battlefield Burn Injuries
Skin Products/ Substitutes	WFPC	4.2.6	Fluid-Derived and Placenta-Derived Stem Cells for Burn
		4.2.8	In Vitro Expanded Living Skin for Reparative Procedures
	RCCC	4.7.2	Burn Repair with Autologous Engineered Skin Substitutes
	USAISR	4.6.8	Autologous Human Debrided Adipose-Derived Stem Cells for Wound Repair in Traumatic Burn Injuries

past year, the researchers strove to further determine the safety and efficacy of P12, and establish systemic dosing details and treatment validation to prevent burn injury progression, reduce inflammation, induce healing and inhibit scarring. They determined that intravenous delivery of P12 in concert with growth factors sustains tissue cell survival. They also demonstrated that P12 is a cryptic peptide that is exposed by the interaction of fibroblasts with fibronectin when cells are seeded. During the upcoming year, the researchers will continue to investigate the mechanism of P12 bioactivity at the molecular level in both endothelial cells and fibroblasts (supported by a National Institutes of Health R21 grant).

RCCC researcher, Ganesh Subramanian, prepares for a release study using Starch-Iodine polymers for potential use in patients to control wound infection.

The **Clark group (Project 4.6.1a)** at NeoMatrix Formulations, Inc., are extending the research completed by the Lin/Clark group at Stony Brook University to develop the P12 peptide as a first-in-class therapeutic for burn injury progression. The researchers have obtained orphan drug designation for P12, thus creating access to potential funding through the U.S. Food and Drug Administration (FDA) as well as non-government sources to support clinical trials and commercialization. During the past year, they demonstrated that an infusion of P12 limits burn injury progression and speeds re-epithelialization of burn injury in the swine vertical burn injury model. During the upcoming year, the researchers will continue to execute pre-Investigational New Drug (IND) studies of P12 treatment to prevent burn injury progression. The group intends to file an IND based on the activities supported by this award by the end of 2015.

Topical Treatment of Burn Injury

Studies at WFPC

Keratins are tough, fibrous structural proteins found in structures that grow from the skin (e.g., hair and nails). The **Van Dyke group (Project 4.2.3)** at Wake Forest University School of Medicine is exploiting the thermoprotective properties of keratins to ameliorate the progression of injury immediately following a burn. The researchers have completed a mechanistic study and a pivotal preclinical study in swine. The swine study showed promising results for faster wound closure and no scarring. However, more testing of the tissue salvage capabilities of keratin biomaterials is required in a critical-size swine burn model (i.e., one that does not spontaneously heal and would require grafting to completely close). The researchers have completed pre-Investigational Device Exemption (IDE) activities with the FDA. The FDA has responded favorably, and the team is currently preparing a formal IDE application and finalizing manufacturing operations under Quality System Regulation 21 Code of Federal Regulations (CFR) 820. The commercial product being developed from this research, KeraStat™ Burn, is being considered for clinical trial evaluation under an IDE currently in preparation by KeraNetics.

Studies at RCCC

In **Project 4.6.4**, the **Iovine group** at Rutgers University initially identified a polymer system that releases molecular iodine from a wound dressing to potentially prevent infection. However, various

technical- and toxicity-related issues limited the usefulness and applicability of this system. The research team abandoned research efforts on the initial polymer system and instead created a tunable iodine delivery system in an absorbent wound dressing that releases iodine on demand and does not require frequent dressing changes, resulting in better patient comfort. During the past year, the researchers coupled the pre-polymer process with a mold/cure/cut fabrication sequence that produces a uniform, well-defined product on a very consistent basis. This process has been scaled up in the laboratory, and the researchers have shown that it is reproducible and controllable at this laboratory scale. They completed studies demonstrating the broad anti-bacterial and anti-fungal properties and sustainable slow release benefits of the starch polymer approach. Their room temperature stability studies on the packaged device indicate no change in product functionality over a 6-month period. During the upcoming year, the researchers will complete a 12-month shelf life study. They also plan to evaluate the efficacy of the iodine-releasing wound dressing in an infected animal model, and pursue discussions with potential commercialization partners.

The **Macri/Clark group (Project 4.6.5)** at Stony Brook University is engineering a drug delivery scaffold for the topical therapy of large, acute burn injuries that cannot be closed. The researchers are exploring the effects of sustained release of the fibronectin-derived peptide P12 in this scaffold model. They developed tyrosine-derived polycarbonate (TyrPC) terpolymers as fiber-based drug delivery matrices for releasing P12 at ultrafast (<24 hours) and fast (4 days) rates. During the past year, the researchers demonstrated the linear release of cyclized peptide (cP12) from both ultrafast- and fast-eroding cP12-loaded electrospun fiber mats. They determined that electron-beam irradiation with 25 kGy serves as a suitable sterilization method for cP12-loaded fiber mats. They demonstrated the in vivo safety of the cP12-loaded fiber mats. They also developed a porcine partial-thickness burn model suitable for evaluating topical therapies. During the upcoming year, the researchers will focus on the delivery of cP12 from gel matrix technologies, rather than from TyrPC, with hopes to eliminate the potential for polymer degradation product interference with cP12 bioavailability and bioactivity. They also plan to conduct safety and efficacy studies (TRL 5) in Good Laboratory Practices guidance, submit an IND application, and initiate the design of a clinical trial.

Wound Healing and Scar Prevention

Studies at WFPC

The **Gerlach group (Project 4.2.2)** at the University of Pittsburgh is finding that skin progenitor cells derived from human fetal skin tissue may provide an interesting new cell source for regenerative cell-based therapy for acute and chronic skin disease and burn patient treatment. The researchers are expanding on successful work, conducted previously in Germany, in which fetal skin cells are sprayed onto excised burn wounds with a precision device, known as a "Skin Gun." They have established methods for isolating, growing, and freezing fetal skin cells. They established an antibody marker panel for characterizing stem cell markers in fetal

WFPC researcher preparing a bioreactor.

as well as adult epidermal and dermal cells. Freshly isolated adult epidermal and dermal progenitor cells can now be provided for full-thickness burn wound healing. Fetal dermal derived fibroblasts exhibited higher wound healing activity than bone-marrow mesenchymal stromal cells. Overall, in vitro cultured fetal dermal derived stem cells show a high potential for full thickness and larger burn wound healing as an anticipated off-the-shelf product. Additionally, adult dermal progenitor cells will enhance autologous on-site cell grafting and should lead to clinical studies on advancing adult autologous skin cell grafting into the area of third-degree burn therapy.

WFPC researcher Brian Phelps designing new parts for a bioreactor.

The **Yoo group (Project 4.2.5)** at Wake Forest University is using inkjet technology to achieve the "printing" of skin onto an excised burn wound. Since bioprinting has been used successfully to fabricate other tissues, skin should be similarly amenable to this approach, especially given its minimally three-dimensional, linear spatial orientation. The researchers developed a portable skin printing device to deploy skin cells to a wound in a spatial-specific manner. This process involved design, construction, laser scanner integration, software development, and validation. The researchers also developed isolation and expansion techniques for skin keratinocytes and dermal fibroblasts. They first achieved direct delivery of skin cells onto skin defects in a mouse model. They also developed a porcine full-thickness burn wound model. They found that bioprinting of autologous skin cells enhanced the healing time of full thickness excisional wounds as well as third degree burn wounds in the porcine model. By 2 weeks post-bioprinting, the autologous cells formed an epithelial layer that covered up to 90% of the wound. This work will continue under other funding sources. The researchers will contact the Office of Combination Products at the FDA to confirm the classification of the bioprinter and, if needed, file a Request for Designation with the Office of Combination Products in accordance with 21 CFR Part 3.

Skin Products/Substitutes

Studies at WFPC

The need for a skin replacement that is instantly available and alleviates the need to take a split- or full-thickness skin graft has long been sought. The **Jackson/Furth group (Project 4.2.6)** at Wake Forest University is developing an improved off-the-shelf bioengineered skin product that uses amniotic fluid-derived stem (AFS) cells for the treatment of extensive burns. The researchers are capitalizing on the capability of AFS cells to differentiate into skin progenitor (stem) cells without forming tumors to facilitate healing of a burn wound following the introduction of cells. They established a nude mouse full-thickness wound model and bioprinted AFS cells into the wound. They demonstrated enhanced wound healing as well as increased neovascularization using AFS cells. They also combined AFC cells with mature keratinocytes, which led to enhanced wound healing. These results suggest that AFS cells bioprinted as a cell therapy may be a potentially powerful tool for burn and wound healing treatments. This work will continue under other funding sources.

The **Lee/Yoo/Holmes group (Project 4.2.8)** at Wake Forest University has developed an in vitro tissue expander system that permits a rapid increase in surface dimensions of donor skin

while maintaining tissue viability for subsequent skin transplantation. This system allows for an approximately 40 cm^2 split-thickness piece of skin to be harvested at the initial operation from a burn patient who is anticipated to require multiple graft operations. The harvested skin is then expanded in the bioreactor to approximately 100 cm^2 over 2 weeks and subsequently "grafted" back onto the patient in the standard manner, with or without meshing, at the next operation. The researchers are defining parameters that maximize the surface dimensions of skin for treating battlefield burns. They have built a new generation uniaxial bioreactor system. They also have devised a strategy to perform a clinical trial. During the upcoming year, the researchers will develop working parameters for the skin bioreactor expander system that will maximize skin expansion while maintaining tissue viability. They are working towards obtaining an IDE by the FDA for approval of a prospective, multicenter, non-randomized, uncontrolled pilot study (Feasibility/Phase I), and are currently seeking IRB approval. Based on conversations with an FDA consultant, the researchers will perform a preclinical large animal study, which is essential for FDA approval for the clinical trial.

Studies at RCCC

Engineered skin substitutes (ESSs) have been developed and tested clinically as an adjunctive treatment for burn repair. Although ESSs reduce the requirements for harvesting skin autografts, these have two major deficiencies: (1) incomplete pigmentation, which does not resolve with time, and (2) the absence of a network of blood vessels, which limits the thickness and rate of engraftment of ESSs. The **Boyce/Clark group (Project 4.7.2)** at the University of Cincinnati and Stony Brook University is designing and testing new prototypes of ESSs that restore skin color and develop vascular networks, thereby resulting in improved outcomes in recovery from life-threatening burns. They have restored skin color in an animal model using ESSs, and have developed protocols to translate research procedures into a testable therapy. During the past year, the research group demonstrated complete restoration of skin color in autologous ESS with pigment (ESS-P). Their preliminary data showed that melanin expression can be downregulated to promote cryopreservation of human melanocytes to allow multiple applications of ESS-P. Going forward, the researchers plan to develop a set of standard operating procedures to determine any variability in uniformity of pigment distribution among ESS-P containing human melanocytes from different skin donors or body sites. As a first step in the process toward a clinical trial, the researchers will prepare a Request for Designation with advice from the translational research office at WFIRM. Determination by the FDA of the regulatory identity of ESS-P as a device, biologic, drug or combination product will facilitate development of the technical, clinical and regulatory protocols needed to prepare for and perform clinical trials directed toward delivery of an advanced therapy to reduce morbidity and mortality for our nation's wounded warriors.

At the University of Cincinnati, project staff member Rachel Zimmerman prepares selective cultures of human epidermal melanocytes for transplantation to full-thickness skin wounds. Melanocytes are combined with epidermal keratinocytes and dermal fibroblasts on collagen-based scaffolds to restore normal skin color to wounds treated with the engineered skin grafts.

Studies at USAISR

Adipose-derived stem cells (ASC) have the potential to grow into cells found in human skin. The **Christy group (Project 4.6.8)** at USAISR hypothesized that a person's own ASC could be used to produce a clinically relevant tissue-engineered skin equivalent. The researchers isolated human ASC using a point-of-care device. They developed the technology to differentiate ASC into epithelial cells for use on patients so severely burned that they do not have an autologous source of epithelial cells. The team also demonstrated that stem cells can be isolated in adequate quantities from the adipose layer of discarded burn skin. During the past year, the researchers developed the technology to remove the cells from the amniotic membrane for epithelial wound covering and epithelial differentiated ASC sheets. They developed PEGylated plasma-based biomaterial products to be used for wound healing and the development of skin equivalents. They also developed platelet-free plasma to use instead of purified fibrin for vascularized biomaterial matrix. Finally, they developed a porcine model to determine the validity of skin constructs in deep partial thickness burns. The researchers plan to continue to use and optimize the InGeneron, Inc. point-of-care device for the isolation of ASCs. This will be used for the isolation of cells from debrided burn patient tissue, as well as from surgically isolated adipose tissue obtained from abdominoplasty. The researchers will continue to develop a more clinically relevant porcine burn model, and they will use this model for the development of a large (up to 20%) total body surface area burn, which will provide a stringent test for their and other AFIRM-related skin equivalent products. Finally, they are continuing to address and use FDA-approved products that are commercially available.

Clinical Trials

Several AFIRM burn repair technologies have advanced to the human clinical trial stage. The status of each clinical trial is summarized in **Table V-2,** and additional details are presented in the following paragraphs. Detailed clinical trial progress reports can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

The **Holmes group (Project 4.2.7)** at Wake Forest University is collaborating with researchers at USAISR and Avita Medical, among other institutions, to conduct a multicenter clinical trial for the ReCell® Device. The device is designed to provide a simple, safe technique for the harvesting of skin cells for enhancement of epidermal repair. The first step involves harvesting a thin, split-thickness skin sample, followed by harvesting the cells of the epidermis, dermis and epidermal-dermal junction. The separated cells and associated signaling factors are combined into a suspension containing a mixed population of live keratinocytes, melanocytes and papillary fibroblasts. The suspension is then sprayed onto the prepared wound bed. The cells migrate over the surface providing epidermal reconstruction with site-matched characteristics of color and texture. The applied cells are incorporated into the developing epidermis. The speed of re-epithelialization is very important as the "sealing" of the skin surface limits the inflammation that has been

Table V-2. AFIRM-funded burn repair projects with pending or active clinical trials.

Project Title	Consortium	Project No.	Trial Phase	Current Status
A Multicenter Comparative Study of the ReCell® Device and Autologous Split-thickness Meshed Skin Graft in the Treatment of Acute Burn Injuries	WFPC / USAISR	4.2.7	Phase III	Open
Stratatech Technology for Burns	WFPC / USAISR	4.2.9	Phase Ib	Open
Enhanced Detection of Pathogens in Burn Wounds Using a Novel Multi-primer PCR/Mass Spectrometric Assay	WFPC	4.5.5a	Phase I	Awaiting HRPO approval
Expedited Availability of Autologous Engineered Human Skin for Treatment of Burned Soldiers	RCCC / USAISR	4.7.4	Phase I	Awaiting IND approval

implicated as the pivotal factor in hypertrophic scar formation. Patient enrollment for this clinical trial began in May 2010. The researchers enrolled and treated a total of 21 subjects during the past year, bringing total enrollment to date to 85 (of 106) subjects. Thirty-six (36) subjects have been followed through 52 weeks. Eight (8) sites are actively enrolling subjects. Enrollment has been discontinued at sites with limited performance in terms of enrollment in order to focus the use of resources. Permission from the FDA and ethics approval has been secured for the addition of the Sunnybrook Health Sciences Centre to the study. Avita Medical is engaged in dialog with the FDA regarding potential changes toward expediting completion of the study and product approval. Additional sites are being considered for participation in the study to increase the rate of enrollment.

In **Project 4.2.9**, the **Holmes group** at Wake Forest University is collaborating with researchers at USAISR and Stratatech Corporation, among other institutions, to conduct a multicenter, Phase Ib clinical trial to assess the safety, tolerability, and efficacy of prolonged exposure to increasing amounts of a single application of StrataGraft® skin tissue compared to autograft in the deep partial thickness component of complex skin defects. StrataGraft tissue is a living, meshable, suturable, human skin substitute developed by Stratatech Corporation that reproduces many of the structural and biological properties of normal human skin. The research team has obtained all necessary regulatory approvals for the dose-escalation clinical trial and has performed site initiation visits at each of the six clinical sites. The researchers have maintained a continuous production stream of StrataGraft skin tissue for the clinical trial at a cGMP-compliant biomanufacturing facility. Two (2) cohorts of 10 subjects each have been fully enrolled. The second patient cohort was treated with a larger area of StrataGraft tissue than the first cohort. None of the sites in either cohort required autografting of any percentage of the StrataGraft treated site by Day 28. The researchers found no evidence of safety concerns or immunological responses to the StrataGraft tissue. The researchers' data suggest that StrataGraft provides immediate wound closure and is replaced as the patient's own cells heal the wound. Research plans for the upcoming year include completion of follow-up assessments of safety and efficacy outcomes, and preparation of a study report. Results of this study will inform the design of a Phase III registration study in patients with complex skin defects.

The diagnosis of an actual burn wound infection remains largely a clinical one, depending on such signs as surrounding cellulitis, drainage of pus, and skin graft failure, and aided by cultural microbiology obtained from swabs, biopsies, or operative specimens. However, it has recently been recognized that numerous bacteria (and fungi) exist within burn wounds in the form of biofilms (organized communities of microorganisms encased in a matrix of extracellular polymeric substance). The recent advent of a novel coupled polymerase chain reaction-mass spectrometric technology, the Ibis PLEX-ID™, offers multiple potential advantages for the molecular detection of biofilm-based and other infections compared to all previous molecular assays. The semi-quantitative Ibis assay simultaneously tests for more than 3,000 species, including virtually all known pathogens, in a multiplex fashion, eliminating the need for an a priori choice as to the likely infecting organism. The

Researchers at Lonza Walkersville, Inc. perform cell therapy manufacturing in one of several clean room suites.

Kathju group (Project 4.5.5a) at the University of Pittsburgh is preparing to conduct a clinical trial designed to evaluate the utility of the Ibis PLEX-ID system in the diagnosis of burn wound infection. The researchers have begun the process of patient accrual for the clinical trial and currently have up to 52 discrete patients enrolled. They are optimizing the sample extraction and preparation techniques and have demonstrated that sample extraction and analysis are proceeding within acceptable technical parameters. Notably, the Ibis PLEX-ID technology is already under commercial development by Abbott Laboratories.

In **Project 4.7.4**, the **Smith/Nigida/D'Souza group** at Lonza Walkersville, Inc. (LWI) has developed autologous ESSs to promptly and effectively close extensive, deep burns. LWI has licensed the technology and completed the technology transfer and product development. The researchers received an Orphan Drug Product Designation for the ESSs on June 1, 2012. They submitted an IND application to the FDA on July 26, 2012, and received a "Clinical Hold Letter" for the ESSs on September 21, 2012. They have completed all but one item in the Clinical Hold Letter and are providing all completed actions to the FDA for additional review. The researchers anticipate that upon receiving FDA approval to proceed with this IND-based study, they will enroll 14 subjects (4 for a "priming phase" and 10 for the clinical trial) at the USAISR and Harborview Medical Center in Seattle, Washington. In the upcoming year, the research team plans to develop rapid release assays and potency assays, evaluate the matrix by assessing its physical attributes, prepare for large-scale matrix manufacturing, and determine commercial packaging. In addition, the researchers are looking to increase their ESS manufacturing capability should there be a national emergency with multiple casualties or a national emergency that might include multiple treatment sites. Expansion is being explored through two paths, one being increased output per lot and the other by establishing additional manufacturing sites. Besides the aforementioned clinical trial, the researchers anticipate conducting (at a future date) a larger, Phase II trial that will include up to 40 subjects

VI: Compartment Syndrome

Background

While many consider battlefield trauma to be the visible types of injuries caused by blasts and projectiles, often the Warfighter experiences hidden complications that are a result of these injuries. Compartment Syndrome (CS) is a potentially serious medical condition in which increased pressure or swelling within a "compartment" compromises the blood supply to that area. Simply put, compartments are confined spaces throughout the human body that contain muscles, nerves, and blood vessels. While CS can result from fractures, blunt and penetrating trauma, blast trauma, injury to blood vessels, and the return of blood flow to a muscle after surgical intervention, it can also result from the use of a combat tourniquet in the field. Regardless of the cause, the diagnosis of CS is considered a medical emergency.

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VI: Compartment Syndrome

In the arms and legs, thick layers of connective tissue called fascia surround the groups of muscles within these compartments, holding them in place and protecting them. If CS is identified, release of the fascia (known as a fasciotomy) must be performed within 6 hours of diagnosis to reduce the likelihood of limb amputation and death.

As no effective treatments are available currently to overcome the complications caused by CS, military surgeons are seeking safe and effective therapies to replace and regenerate damaged cells and tissues. Service members who develop CS have prolonged recovery times and rarely regain complete muscle function, and typically they do not return to active duty at the same level of performance. Notably, most unrecognized CS injuries of the extremities result in permanent disability.

The AFIRM Compartment Syndrome Program is focused on helping to reduce the impact of CS on wounded warriors and to improve their functional recovery through the application of regenerative medicine. These projects seek to increase the salvage of injured limbs affected by CS utilizing an interdisciplinary approach based on a combination of stem cells and inductive biodegradable scaffolds for the reconstruction of functional compartment tissues.

Unmet Needs

Unfortunately, little improvement has been realized in the treatment of CS since the introduction of surgical fasciotomy more than a century ago. Lack of progress in this area is due partially to the fact that, despite decades of research attempts, no satisfactory surrogate animal models have been established to study new prospective treatments. The AFIRM is supporting the successful creation of platform animal models of small and large wounds complicated by CS. Additionally, instrumentation has been developed within the program to quantitatively assess muscle and nerve regeneration in a precise, reproducible manner in these models.

Complications of untreated CS include Volkmann's contracture, which is a permanent shortening of musculature of the hand at the wrist that results in a claw-like deformity of the hand and fingers. Advanced CS can lead to permanent paralysis due to the failure of muscles and nerves in an affected compartment to recover. At that advanced stage, amputation of the affected limb may be the patient's only remaining treatment option, and in light of this, partial replacement of the dysfunctional tissue by living engineered muscle tissue is an attractive concept. Therefore, this continues to be an unmet need. Researchers at the AFIRM are generating technologies that will provide improved functional recovery for injured soldiers through the regeneration of muscle, nerve, and blood vessels lost to CS and other battlefield wounds.

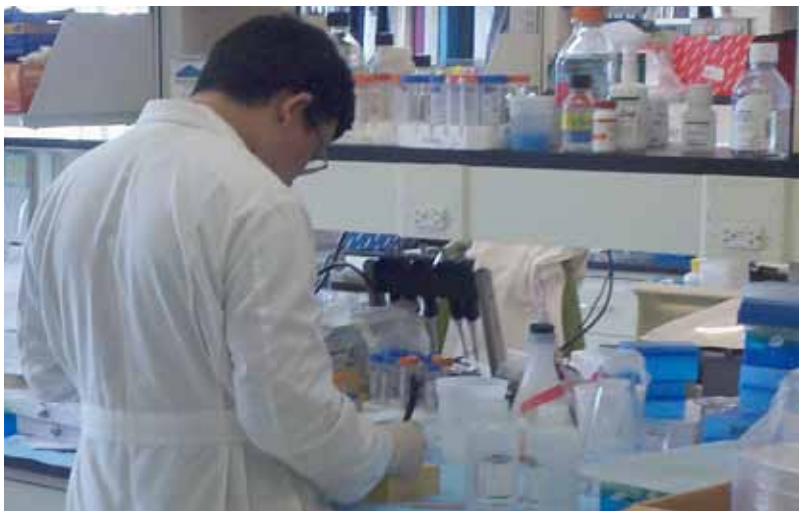
Areas of Emphasis

AFIRM researchers are pursuing a complementary mix of research projects focused on various aspects of treatment of CS. Projects can be grouped into two "clinical challenge" topic areas: Cellular Therapy of CS and Biological Scaffold-Based Treatment of CS. Nontechnical summaries of the projects are presented after **Table VI-1**, and technical progress reports for each project can be found in the AFIRM Annual Report 2013 – Technical Progress Reports.

Cellular Therapy of CS

Studies at Wake Forest–Pittsburgh Consortium

Wake Forest–Pittsburgh Consortium (WFPC) researchers are using human muscle-derived and



WFPC researcher Zhan Wang preparing samples for analysis with Dr. Shay Soker.

Table VI-1. Projects Funded by WFPC* and USAISR** per Clinical Challenge Topic Area

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Cellular Therapy of CS	WFPC	4.3.1	Cellular Therapy for Treatment and Consequences of Compartment Syndrome
		4.3.2	Use of Bone Marrow-Derived Cells for Treatment of Compartment Syndrome
Biological Scaffold-Based Treatment of CS	WFPC	4.3.3	Biodegradable Elastomeric Scaffolds Microintegrated with Muscle-Derived Stem Cells for Fascial Reconstruction Following Fasciotomy
		4.3.4	Use of Autologous Inductive Biologic Scaffold Materials for Treatment of Compartment Syndrome
		4.3.5	Material-Induced Host Cell Recruitment for Muscle Regeneration

* Wake Forest–Pittsburgh Consortium
**U.S. Army Institute of Surgical Research

bone marrow-derived stem and progenitor cells to reconstruct functional compartment tissues following the development of CS. In **Project 4.3.1**, the **Huard group** at the McGowan Institute for Regenerative Medicine (MIRM) and the **Soker group** at the Wake Forest Institute for Regenerative Medicine have developed a rat model of CS using neonatal blood pressure cuffs to apply external compression on the hindlimb. The researchers characterized anatomic and functional changes in the muscular, vascular and neural components of the hind limb muscles following compression-induced damage in their rat model, which mimics CS observed in clinical patients. They found that muscle precursor cell (MPC)-injected muscles exhibited significant functional improvement 14 days after injury, and this effect was observed for both adult and young animals. The research team also demonstrated that co-seeding of endothelial cells with MPCs led to enhanced vascularization, innervation and skeletal muscle tissue formation in vivo. They developed cell culture expansion protocols that maintained the muscle tissue-forming capability of MPCs for at least 25 cell passages. They also obtained and characterized biopsies from CS patients, and found variability among patients. The researchers used the U.S. Food and Drug Administration (FDA)-approved drug Losartan and found it to be effective in reducing the amount of fibrosis in injured skeletal muscle. Based on these findings, a two-person case study conducted at the University of Pittsburgh Medical Center showed that Losartan is an effective treatment for grade II hamstring injuries. During the upcoming year, the researchers will conduct combinatorial therapeutic strategies in the CS animal model including delivering MPCs with and without angiogenic factors, Losartan, and Platelet Rich Plasma fractions. They will also continue using Losartan to reduce fibrosis and optimize the ideal dose and time after injury to begin Losartan treatment. They are planning a larger, double-blinded, multi-center, clinical study.

The **Gregory group (Project 4.3.2)** at the Oregon Medical Laser Center is evaluating the effectiveness of autologous bone marrow mononuclear cell (BM-MNC) treatments in CS injuries. The researchers have developed an in vivo cell-tracking technique that allows them to demonstrate extremely robust cell engraftment up to 3 months post treatment. They completed a pivotal proof-of-concept preclinical study in Sinclair mini-swine to evaluate the use of BM-MNCs to treat extremity injuries complicated by CS. They observed significantly improved muscle and nerve function in animals treated with BM-MNCs compared to control animals. While control animals stopped improving clinically 6 weeks after injury, BM-MNC treated animals continued to improve clinically through the 12-week study endpoint. Significant gait improvement was observed at 3 months in the cell-treated animals compared to control animals. No adverse events or complications were associated with any cell treatments. This research demonstrates the potential of a safe, new treatment for severe extremity injury that offers injured troops an improved

WFPC researcher adding cells to a tissue scaffold.

functional recovery. During the upcoming year, the researchers will finish their randomized 6-month study comparing BM-MNC treatments (10 animals) to sham controls (10 animals) for validating safety and efficacy as a prelude to clinical trials. They will also perform a rodent study to evaluate the toxicology, biodistribution, tumorigenicity, and microbiological effects of the treatment protocol. In addition, they aim to begin a multicenter Phase I human clinical trial.

Biological Scaffold-Based Treatment of CS

Studies at WFPC

WFPC researchers are developing animal models of CS and implantable scaffolds that can be used to treat this potentially devastating condition.

The **Wagner group (Project 4.3.3)** at the MIRM is focused on developing biodegradable scaffolds with elastic properties that can be integrated with autologous muscle-derived stem cells (MDSCs) to reconstruct fascia (thick, fibrous tissue that encloses and protects the organs) after abdominal CS injury. The researchers created three scaffold designs that incorporate skin extracellular matrix (ECM) and an elastic polymer. ECM provides structural and functional support to cells. The researchers assessed the bioactivity and mechanical properties of these scaffolds in a full-thickness abdominal wall reconstruction rat model. The scaffold that performed the best consisted of outer layers of elastic polymer surrounding an inner hybrid skin ECM-polymer layer. During the past year, the researchers developed new material processing techniques to produce mechanically robust biocompatible and bioactive scaffolds. They investigated concurrent electrospinning of degradable poly(ester urethane)urea fibers with electrospraying of a saline solution to integrate MDSCs into the scaffolds. Evaluation in a rat abdominal wall defect demonstrated that such constructs were capable of producing pronounced cellularity and de novo formation of ECM. The researchers added reinforcing surface layers to this material to improve mechanical integrity. Constructs developed in this manner were found to be superior to currently clinically available materials in terms of cellularity, ECM content, and tensile mechanical properties. In Year 5, the researchers will evaluate their biohybrid scaffolds in a more clinically relevant porcine model. The developed materials will also be considered for other applications, including skin, craniofacial, and soft tissue reconstruction.

The **Badylak group (Project 4.3.4)** at the University of Pittsburgh is investigating a method for using the inductive properties of ECM as a scaffold for the recruitment of one's own stem cells and the attachment, proliferation, and spatial organization of these cells into functional tissue. The researchers are also developing methods of decellularization (removing the cells) from necrotic (dead) tissue while retaining native ECM. Notably, the decellularization of cellular material produces ECM that can be used to promote the regeneration of tissue in an animal model. During the past year, the researchers completed an evaluation of decellularization as a treatment of peripheral CS (PCS) in rabbits. They also developed a mouse model of volumetric muscle loss (VML) to investigate the mechanisms of ECM remodeling associated with massive skeletal muscle injury. Using this model, they found that resident perivascular stem cells participate in remodeling and may promote increased myogenesis (muscle formation) and reduced scarring compared to a fasciotomy and debridement (removal of dead, damaged,

or infected tissue) PCS treatment alone. Finally, the researchers began applying their optimized decellularization method to a porcine model of PCS. During the upcoming year, the research team will complete the porcine model studies, which they anticipate will provide the foundation for a Phase I clinical trial. They will continue to use the mouse model of VML to elucidate the mechanisms of ECM remodeling following skeletal muscle injury.

The **Lee group (Project 4.3.5)** at Wake Forest University is focused on developing an approach to enhance the recruitment of endogenous stem and progenitor cells to the site of CS injury to increase the regenerative response. The researchers are using biomaterials containing myogenic (muscle cell)-inducing factors that can be implanted within the injured muscle compartment. They have demonstrated that host muscle stem cells can be recruited into implanted biomaterials in situ, and that these cells can be transformed into muscle cells using myogenic-inducing factors. They have developed novel injectable and implantable scaffolding systems using heparin-conjugated gelatin microparticles and heparin-conjugated cell-free muscle matrix, respectively. The researchers demonstrated the mobilization of host stem and progenitor cells, as well as new muscle tissue regeneration in vivo in rats, using these scaffolding systems. During the upcoming year, the Lee group plans to continue to optimize and validate the injectable/implantable systems that can deploy multiple bioactive molecules in a controlled manner. To evaluate the clinical feasibility of the delivery of multiple bioactive molecules from the systems in vivo, they will use two rabbit muscle injury models, including models of muscle atrophy and VML.

WFPC researcher Manasi Vadhavkar preparing samples for histological analysis of muscle tissue.

VII: AFIRM Statistics

Introduction

The AFIRM is a large, multifaceted complex biomedical research and development consortium of collaborating scientists, engineers, and medical product development experts. Across the AFIRM consortia, hundreds of basic and clinical researchers apply their expertise in regenerative medicine research and development to find medical solutions to treat our Service members with severe combat wounds. AFIRM investigators and product developers represent nearly 60 research universities and hospitals, and 26 commercial partners at the core institutions and collaborating organizations.

VII: AFIRM Statistics

While the previous chapters of this report demonstrate the depth of the AFIRM's individual research projects, this chapter displays the research consortium as a whole to provide an overarching perspective of the program's breadth. This chapter demonstrates the extent and quality of scientific and technical expertise being applied to the problems of regenerative medicine by displaying aggregated program data. This chapter also demonstrates tangible, scientific outcomes attributable to AFIRM-supported research: inventions disclosed, patent applications filed, research or review articles published, conference and meeting presentations and posters presented, and the advancement of products through research and development stages. The AFIRM data shown in this chapter cover the 5 years of the program with particular emphasis placed on the AFIRM in Program Year 5 (PY5).

Personnel

A substantial workforce has contributed to AFIRM-funded studies to conduct research on regenerative biology and medicine. From faculty members to undergraduate students, nearly 500 researchers contributed to the AFIRM research activities in PY5.[1] The distribution of these scientists and students is illustrated in **Figure VII-1**. For example, 85 faculty members from the Rutgers–Cleveland Clinic Consortium (RCCC) and 90 faculty members from the Wake Forest–Pittsburgh Consortium (WFPC) contributed to the AFIRM in PY5. In addition, more than 100 postdoctoral associates and fellows and more than 120 scientific and technical staff contributed to the AFIRM studies in PY5.[2] Figure VII-1 also depicts the 9 research investigators and technical staff at the U.S. Army Institute of Surgical Research (USAISR) who contributed to the AFIRM through the support of the Clinical and Rehabilitative Medicine Research Program in PY5.

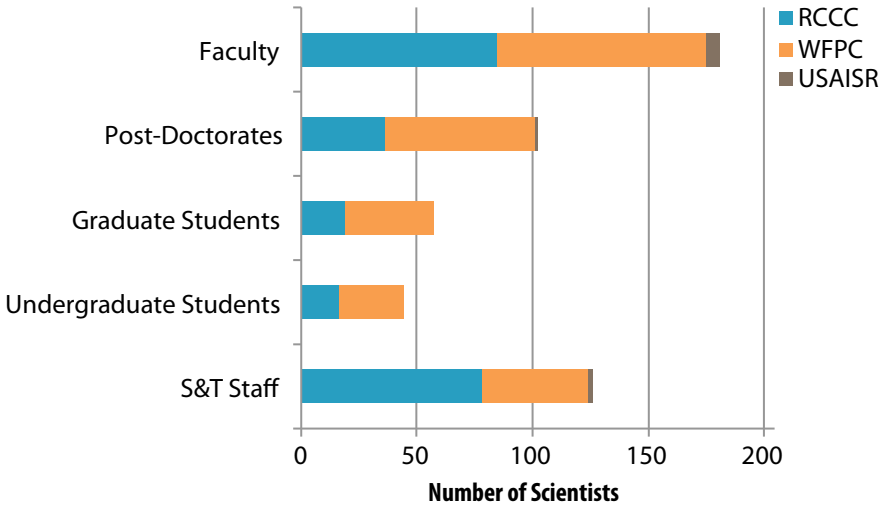


Figure VII-1. Numbers of scientists and students contributing to the AFIRM program during PY5.[3]

[1] For the purpose of this annual report, PY5 is defined as the period from June 1, 2012 to May 31, 2013. PY4 spans from June 1, 2011 to May 31, 2012; PY3 spans June 1, 2010 to May 31, 2011; PY2 spans June 1, 2009 to May 31, 2010; and PY1 spans the period from the initiation of research projects in May 2008 through the end of May 2009.

[2] The numbers may be slightly overestimated due to some individuals working on multiple projects. To minimize duplicate counts of the same individuals, the names of scientists and students provided by project investigators were cross-referenced. Anyone who contributed to more than one project was counted only once. However, not all individuals who worked on the AFIRM were named; thus, it is possible that some individuals working on two or more projects could have been included in the count for each separate project.

[3] This chart displays the number of unique individuals, both funded and unfunded by the AFIRM, who contributed to the AFIRM program during any part of PY5.

Another highlight of the AFIRM is the substantial recruitment and training of the next generation of scientists to advance regenerative medicine research and development into the future. More than 100 students (57 graduate students and 44 undergraduate students) received valuable scientific training through AFIRM-sponsored research projects in PY5 (Figure VII-1).

The numbers of AFIRM-supported graduate students who completed their degree requirements each year of the program are shown in **Figure VII-2**. During the first PY, three graduate degree recipients were supported through the AFIRM. In each of the following 4 years, between 12 and 24 graduate degree recipients had received training support through the AFIRM. For the 5-year span of the program, a total of 70 graduate students supported through the AFIRM completed their degree requirements (54 received a PhD and 16 received a master's degree).

The AFIRM program was originally organized into five research focus areas: Limb and Digit Salvage, Craniofacial Reconstruction, Burn Repair, Scarless Wound Healing, and Compartment Syndrome.[4] **Figure VII-3** depicts the proportion of all core personnel, funded and unfunded, who worked on the different program areas in PY5.[5] Two of these research focus areas account for more than half the personnel: Limb and Digit Salvage (32%) and Craniofacial Reconstruction (25%).

Honors and Achievements

The AFIRM program's faculty members are highly accomplished in their respective scientific fields. From June 2012 through May 2013, 43 honors and awards were conferred upon the AFIRM faculty. These honors include selection to membership or leadership positions in professional societies, invited presentations and lectureships, receiving honorary degrees, awards from private foundations,

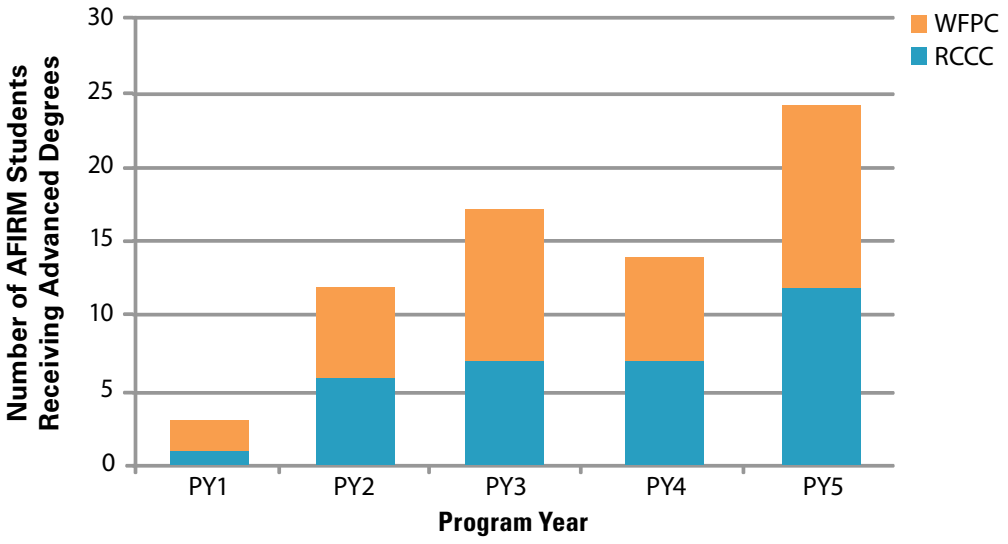


Figure VII-2. Number of graduate degrees awarded to students who received training through AFIRM projects.

[4] As the AFIRM program has matured, the overarching research focus areas have been redefined by the consortia to more accurately describe their research focus. To compare the consortia across different years, Figure VII-3 displays the percentage of personnel conducting research according to the original research focus areas.

[5] Figure VII-3 counts each person once. The few researchers who worked on projects in two or more research focus areas are only represented once in the chart according to their principal research area.

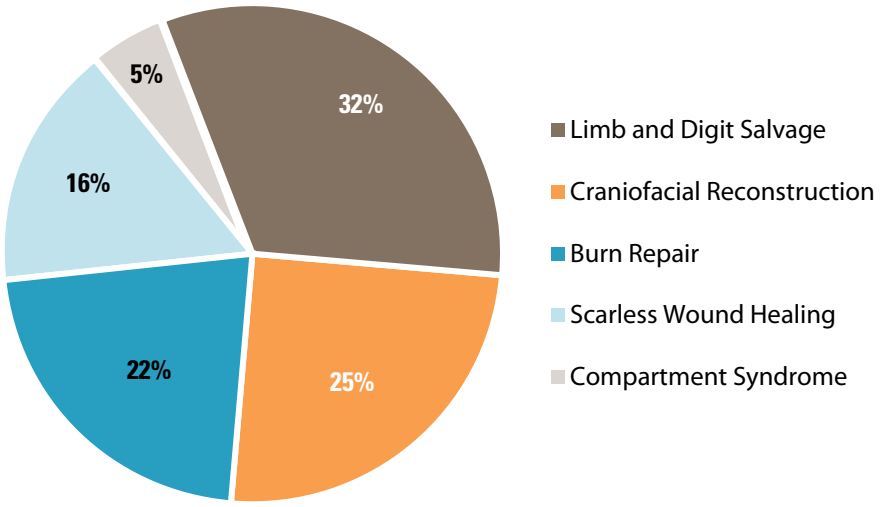


Figure VII-3. Percentage of personnel conducting research in the AFIRM program across the five research focus areas in PY5.

recognition of excellence at conferences, and faculty teaching awards. The distribution of the honors received is displayed according to the type of conferring organization in **Figure VII-4**.[6] The complete lists of honors and awards received by the AFIRM faculty during PY5 are shown in Appendix A.

In addition to awards and honors received by AFIRM researchers, many AFIRM investigators have successfully competed for new research funds. AFIRM investigators reported the submission of 45 proposals in PY5 that were under review. AFIRM investigators also reported the initiation of 52 newly awarded grants, contracts, or subcontracts in PY5.[7]

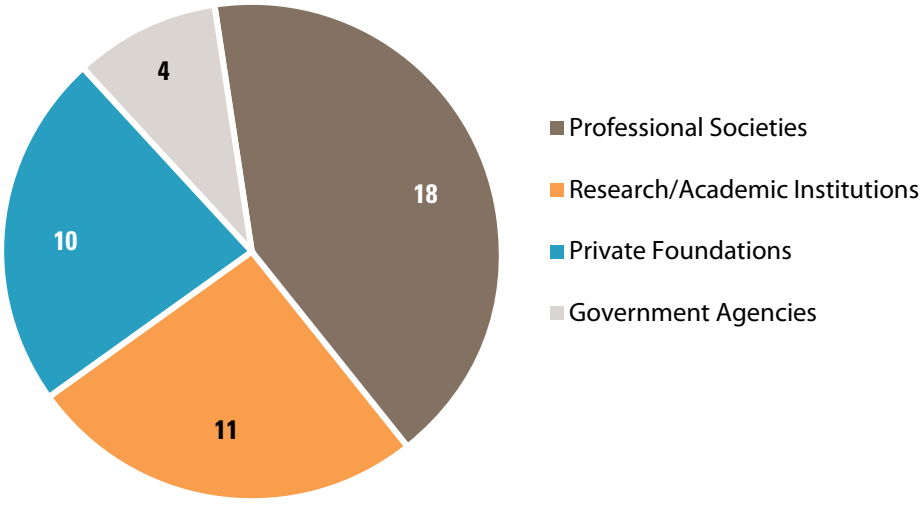


Figure VII-4. Distribution of honors and awards to the AFIRM faculty by type of conferring organization in PY5.

[6] Awards to faculty are reported by the researchers. Data exclude awards to postdoctoral fellows and students, and also exclude the awarding of competed grants and contracts.

[7] The number of proposals submitted includes those with self-reported submission dates falling within PY5 as well as proposals that were reported without submission dates. The number of new grants or contracts includes those reported with funding start dates within PY5 and those that did not report funding dates.

Publications and Presentations

The presentation and publication of research findings are the most immediate gauges of the accomplishments of AFIRM-supported researchers.

For the purposes of this report, the following definitions have been applied for consistency:

Non-Peer-Reviewed Publications and Presentations

Meeting symposia, invited talks, oral presentations, and posters delivered or accepted are included in the PY numbers regardless of the review process for accepting a presentation or the eventual publication of an abstract in a scientific journal. Additionally, editorial comments, letters, non-peer-reviewed book chapters, and other types of non-peer-reviewed published works are included.

Peer-Reviewed Publications

Research or review articles accepted to, in press, or published in peer-reviewed journals or peer-reviewed edited books are included for each PY. Research or review manuscripts in preparation or submitted to a journal but not yet accepted are not included in this annual report.

The number of non-peer-reviewed publications and presentations resulting from AFIRM-sponsored research by WFPC and RCCC investigators in PY5 was 70, and the number has ranged from 125 in PY1 to 188 in PY3 (**Figure VII-5**).[8] In addition, 86 peer-reviewed manuscripts were published in PY5 by the WFPC and RCCC investigators, and the number has ranged from 62 in PY1 to 104 in PY3. The complete lists of AFIRM researchers' publication and presentation citations from PY5 are shown in Appendix B.

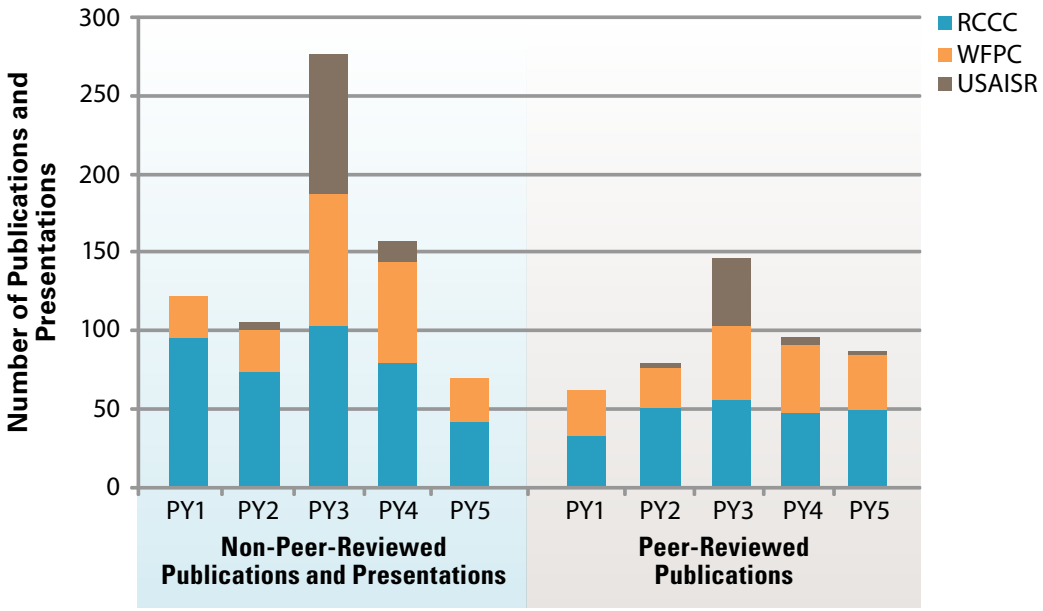


Figure VII-5. Dissemination of AFIRM-sponsored research findings to the scientific community through presentations and publications.

[8] Articles published or accepted, and presentations and posters delivered or accepted during the PYs were included in the count for only one PY even if the acceptance and publication dates spanned two PYs.

Inventions, Patent Applications, and Patents

The successful development of tangible products or inventions can be tracked across three milestone phases: (1) an invention disclosure is filed by a researcher with his/her institutional technology licensing office, (2) a patent application is submitted to the government patent office (e.g., U.S. Patent and Trademark Office [USPTO]), and (3) a patent is awarded by the USPTO or another government patent office for the intellectual property.

Many of the AFIRM program's principal investigators were already developing regenerative medicine-related research products at the time the program was initiated. Products developed before the AFIRM program existed are not recognized as AFIRM program outcome accomplishments.[9] However, products initially developed prior to AFIRM support but refined during the AFIRM program period are considered AFIRM program outcome accomplishments as are all newly disclosed intellectual property.

Through the first 4 years of the program, a combined 75 invention disclosures and/or patent applications were made,[10] of which more than 60 patent applications were filed with government patent offices. The distributions of invention disclosures and patent applications are shown in **Figure VII-6**. In PY5, 8 patent applications were filed, which are also indicated in Figure VII-6 as invention disclosures. The complete lists of patent applications filed and inventions disclosed in PY5 that are attributable to AFIRM-sponsored research are shown in Appendix C.

Developmental Accomplishments and Milestones

Technology Readiness Levels (TRLs) of Products

Research progress can be measured in terms of the transition of products through research and development stages.[11] Biomedical research and development activities funded through the U.S. Army

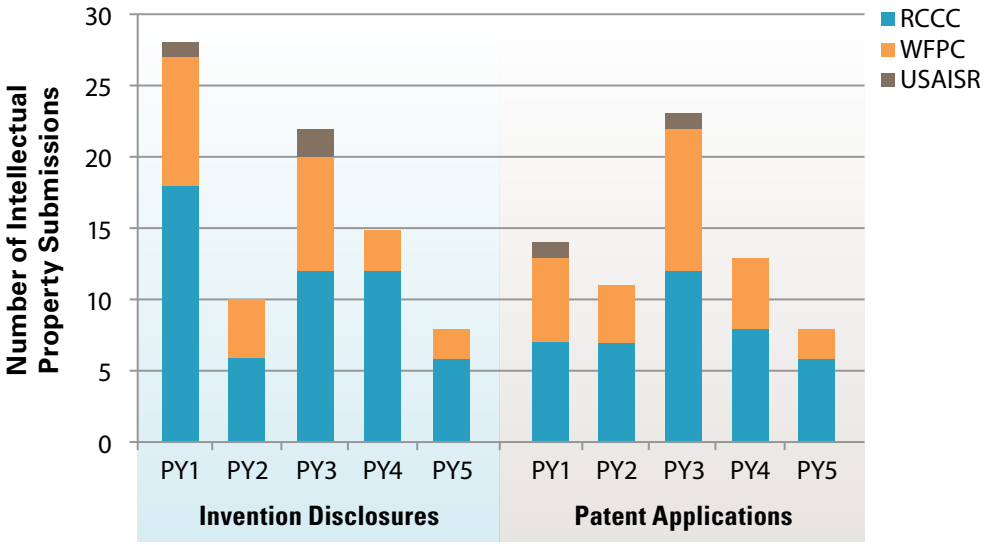


Figure VII-6. AFIRM-attributable invention disclosures and government patent applications filed.

[9] Definitions of AFIRM-attributable inventions, patent applications, and patents were developed to standardize the self-reported intellectual property and are described in Appendix C.

[10] The number of invention disclosures includes self-reports of invention disclosures to institutional technology offices and self-reported patent applications filed that were not previously reported as "invention disclosures."

[11] A given research project can develop one or more products. Each developed product is separately assigned a TRL; therefore, a given project may have one or more products, each of which would be designated a separate TRL. For the TRL analysis, the term "product" can describe either the project (if no specific product has resulted from basic research) or product (if a specific product is being further developed).

Medical Research and Materiel Command are categorized by TRLs.[12] TRLs identify a given product's research and development stage along a 9-point scale, which for therapeutic products extends from basic research at TRLs 1 and 2 to proof-of-concept studies (TRLs 3–4), preclinical (TRL 5) and clinical (TRLs 6–8) technology development stages, and post-marketing surveillance (TRL 9).[13]

Figure VII-7 shows the AFIRM's overall research and development progression from predominantly basic research to proof-of-concept and technology development stages.[14] At the start of the AFIRM program, 58 of 63 research and development products (92%) were distributed across TRLs 1, 2, and 3; the other 5 products were initially at TRLs 4 and 5.[15] By the end of PY2, most of the products (56 of 78) were at TRL 2 or 3, and 19 of the other 22 products were distributed at TRLs 4 and above. By the end of PY5, approximately one-third of the products were at TRLs 2 and 3, one-third at TRL 4, and one-third above TRL 4. The 15 products at TRL 5 and the 8 products at TRLs 6 and above demonstrate the increasing translation of products into preclinical studies and human clinical studies, respectively.

While Figure VII-7 displays the status of all products' TRL stages at annual intervals, it does not track the transition of products over time. Most products in the AFIRM portfolio at the end of PY5 have been part of the portfolio since the program began, while other projects have been added to the AFIRM's portfolio in subsequent years. **Figure VII-8** summarizes the transition of the 74 products being developed by AFIRM investigators at the end of PY5 that have been part of the AFIRM portfolio for 2 or more years. AFIRM investigators report that 34 products (48%) advanced one TRL

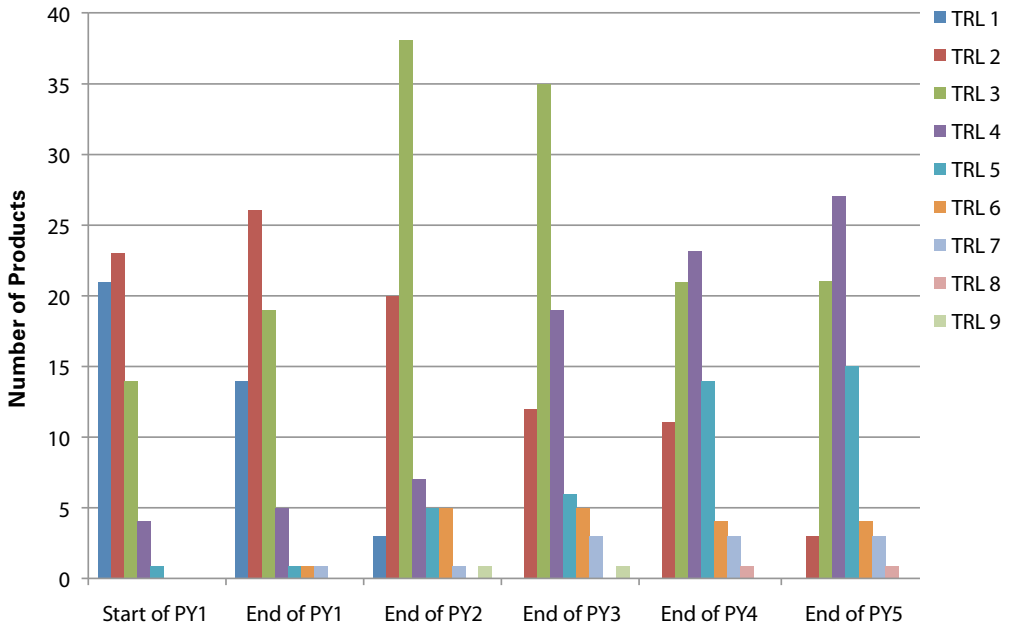


Figure VII-7. AFIRM research products' progression through research and development stages.

[12] For simplicity, the term TRL is used in this report to generically refer to both TRL and knowledge product readiness level (KRL). The KRL is intended for tracking the development of practical medical and health performance knowledge (e.g., clinical practice guideline) and is analogous to the TRL, which is used to track the development of pharmaceuticals, biologics, devices, or other materiel medical and health products.

[13] See Appendix D for more detailed descriptions of biomedical TRLs.

[14] As the program progressed, AFIRM investigators better understood TRL definitions, and some previously self-reported TRL values have been revised. The TRL values in Figure VII-7 reflect the most recent assessment of past and present TRL values and may not be consistent with previously reported TRL values.

[15] The AFIRM is a dynamic program, and investigators make programmatic decisions about the continuation or termination of projects or the initiation of new projects. As such, the portfolio of AFIRM research and development products will change from year to year.

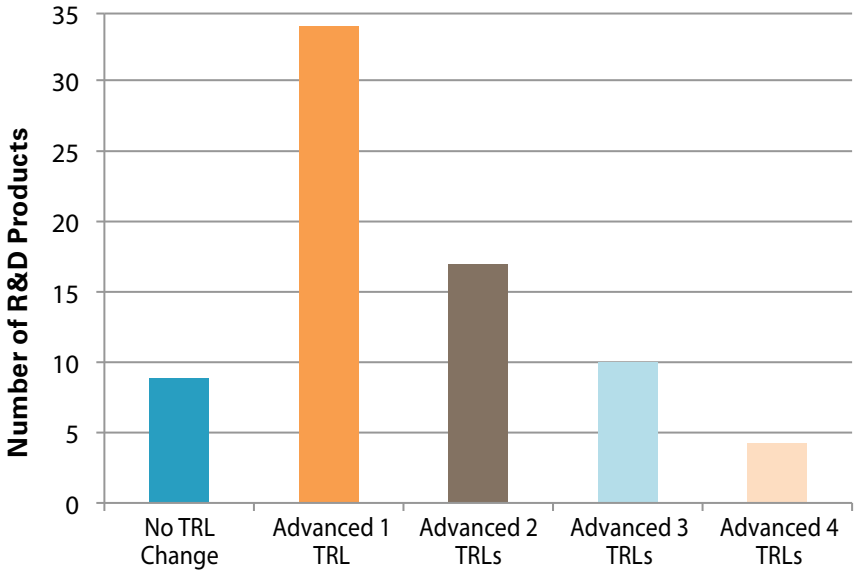


Figure VII-8. Advancement of AFIRM products through TRL stages.

and another 31 products (42%) advanced two or more TRLs.

Preclinical Models and Clinical Studies

Products at TRL 4 or 5 are being tested in in vivo animal models, some of which had to be newly developed or validated for the purpose of testing the products in an appropriate injury model. In PY5, AFIRM researchers completed the development and/or validation of 9 experimental models for studying injury mechanisms, developing therapeutic approaches, and conducting the necessary preclinical studies to demonstrate the safety and efficacy potential of therapeutic products to enable regulatory submissions.

Products at TRLs 6 and above are being evaluated in human clinical studies that require federal regulatory approval and approval through local institutional review boards (IRBs). Medical devices and therapeutics require federal regulatory approval by the appropriate U.S. Food and Drug Administration (FDA) center prior to conducting studies with human subjects and again prior to marketing the device or therapeutic. For medical devices, the FDA's Center for Diagnostics and Radiological Health (CDRH) reviews Investigational Device Exemption (IDE) applications prior to product testing in human subjects, and the CDRH evaluates the Premarket Approval application for approval to market the device or the 510(k) application for clearing a device as " 510(k) exempt" prior to commercial marketing of the device. For clinical therapeutics, the FDA's Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER) are responsible for reviewing the Investigational New Drug (IND) application for sponsors seeking to test the drug or biologic in humans. After clinical testing is completed, sponsors submit a New Drug Application or Biologics License Application, respectively, to CDER or CBER for the approval to market the drug or biologic.

Over the 5 years of the AFIRM, sponsors have introduced medical devices and therapeutics across a wide range of developmental stages, including conducting investigations with products that had previously been approved by the FDA. In PY5, sponsors affiliated with the RCCC submitted one IDE application, one 510(k) exemption application, and two IND applications.[16] Other sponsors have initiated pre-IND and pre-IDE meetings with the FDA and have begun to prepare IND or IDE applications.

[16] Prior to PY5, IND and IDE applications submitted to the FDA for medical devices and therapeutics under investigation by the AFIRM were not reported.

Through PY5, AFIRM investigators advanced numerous products through clinical study planning, approval, and execution stages. Nine clinical trials were open to patient enrollment in PY5, including three Phase III, three Phase I/II, and three Phase I protocols.[17] An additional two clinical protocols for Phase I studies and one clinical protocol for a Phase I/II study had been submitted to IRBs or human research protection offices. One diagnostic study was also open to enrollment in PY5. **Figure VII-9** shows the number of unique products undergoing clinical evaluations by the most advanced stage of development.

Commercialization Plans

Commercial partnerships are important and necessary to complete the final development and fielding of medical materiel products. The collaboration of AFIRM investigators with commercial partners will enable clinical trials to be conducted as commercial and venture capital is leveraged with government funds. Furthermore, commercial partners can provide expertise and facilities for Good Manufacturing Practices-compliant product manufacturing, product testing and validation, clinical study design and execution, and filing regulatory submissions for approval to market the products. The formal agreement between an investigator and an industry partner is also a surrogate measure of the potential utility of the product being developed. In PY5, 35 commercial organizations were involved in the AFIRM program in a variety of capacities. Of these, 26 are AFIRM members or collaborators with the AFIRM, while the other 9 partners provided materials or services under contracts or agreements.[18]

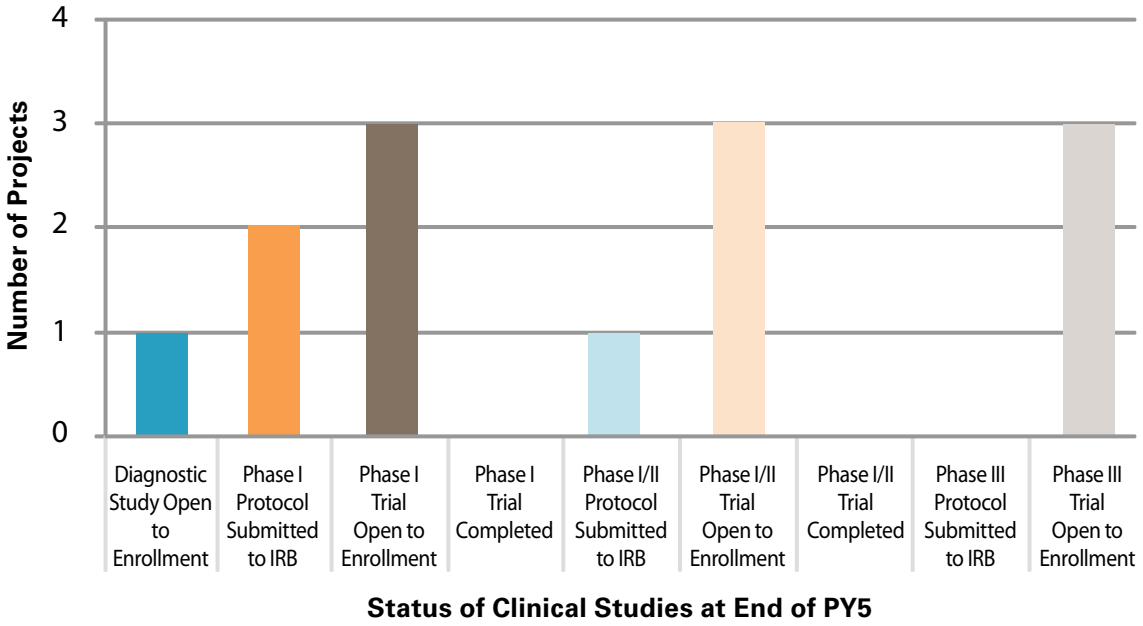


Figure VII-9. A snapshot of the clinical protocol stages of AFIRM products at the end of PY5.

[17] See Appendix D for descriptions of clinical study phases.

[18] Some of the commercial partnerships preceded the start of the program, including some investigations of off-the-shelf products or products licensed by the partnering company.

Appendices


AFIRM OUR SCIENCE FOR THEIR HEALING
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Appendix A: Honors and Awards to AFIRM Faculty

During the reporting period from June 2012 through May 2013, 43 honors or awards were received by AFIRM-supported faculty, as reported by the investigators. The honors and awards are listed below by the recipient faculty member. Awards to postdoctorate fellows, students, and staff are not presented. Honors and awards reported in previous years are not repeated.

Rutgers–Cleveland Clinic Consortium

Anderson, D (Massachusetts Institute of Technology): Goldblith Career Development Professorship, MIT.

Cwykiel, J (Cleveland Clinic Foundation): Postgraduate Poster Award, Case Western Reserve University's National Center for Regenerative Medicine 4th Annual Retreat.

Langer, R (Massachusetts Institute of Technology): Wilhelm Exner Medal (Austria), Oesterreichischer Gewerbeverein.

Langer, R (Massachusetts Institute of Technology): Fellow, American Institute of Chemical Engineers.

Langer, R (Massachusetts Institute of Technology): National Academy of Inventors (Charter Fellow), National Academy of Inventors.

Langer, R (Massachusetts Institute of Technology): Feodor Lynen Award, Nature Biotechnology.

Langer, R (Massachusetts Institute of Technology): Scientist of the Year, R&D Magazine.

Langer, R (Massachusetts Institute of Technology): Perkin Medal, Society of Chemical Industry, America International Group.

Langer, R (Massachusetts Institute of Technology): United States National Medal of Technology and Innovation, U.S. Patent and Trademark Office at the Department of Commerce.

Langer, R (Massachusetts Institute of Technology): Distinguished Investigator Award, American College of Clinical Pharmacology.

Langer, R (Massachusetts Institute of Technology): Chemical Pioneer Award, American Institute of Chemists.

Langer, R (Massachusetts Institute of Technology): Honorary Degree, Ben Gurion University.

Langer, R (Massachusetts Institute of Technology): Honorary Degree, Boston University.

Langer, R (Massachusetts Institute of Technology): Medal for Innovations in Healthcare Technology, IEEE.

Langer, R (Massachusetts Institute of Technology): Medal, Industrial Research Institute.

Langer, R (Massachusetts Institute of Technology): Founders Award, Society of Biomaterials.

Langer, R (Massachusetts Institute of Technology): Honorary Degree, Tel Aviv University.

Langer, R (Massachusetts Institute of Technology): Wolf Prize in Chemistry, Wolf Foundation.

Pomahac, B (Brigham and Women's Hospital): Transplant Foundation Award, Czech Transplant Foundation.

Pomahac, B (Brigham and Women's Hospital): Award for "Excellence in Design" Education Exhibit, Radiological Society of North America.

Sarac, T (Cleveland Clinic Foundation): Cleveland Clinic Innovator Award, Cleveland Clinic.

Siemionow, M (Cleveland Clinic Foundation): Honorary Guest, 75th Annual Pulaski Day Parade Banquet, New York City, 75th Annual Pulaski Day.

Siemionow, M (Cleveland Clinic Foundation): SAPIENTI SAT Medal, Ministry of Education of Poland.

Siemionow, M (Cleveland Clinic Foundation): President, American Society for Reconstructive Transplantation.

Siemionow, M (Cleveland Clinic Foundation): Best Paper Award, Clinical Category, American Society of Maxillofacial Surgeons.

Siemionow, M (Cleveland Clinic Foundation): Nomination Award, Honorary Citizen of Krotoszyna, Mayor of Krotoszyna.

Siemionow, M (Cleveland Clinic Foundation): International Member of the Polish Academy of Arts and Science, President of the Republic of Poland.

Windebank, A (Mayo Clinic): Educator of the Year Award, Mayo Clinic Center for Translational Science Activities.

Yaszemski, M (Mayo Clinic): William W. Tipton, Jr., MD, Leadership Award, American Academy of Orthopedic Surgeons Annual Meeting.

Wake Forest–Pittsburgh Consortium

Atala, A (Wake Forest School of Medicine): The Research Excellence Award, Wake Forest School of Medicine.

Christ, G (Wake Forest School of Medicine): Innovation Award, Wake Forest Innovations.

Guldberg, R (Georgia Institute of Technology): Host of the TERMIS-AM 2013 meeting, Tissue Engineering and Regenerative Medicine International Society – Americas Chapter (TERMIS-AM).

Hebda, P (University of Pittsburgh): Medical Student Research Mentoring Merit Award, University of Pittsburgh.

Ibrahim, Z (Johns Hopkins University School of Medicine): JK Hardesty Award, Plastic Surgery Research Council.

Kasper, FK (Rice University): Young Alumnus Award, The Alumni Association of Case Western Reserve University.

Kasper, FK (Rice University): Young Investigator Award, Society for Biomaterials.

Marra, K (University of Pittsburgh): TERMIS Educational Award, TERMIS.

Mikos, AG (Rice University): Founding Fellow, TERMIS.

Mikos, AG (Rice University): Member, Institute of Medicine of the National Academies.

Ruoslahti, E (UC Santa Barbara): Citation Laureate, Thomson Reuters.

Tirrell, M (University of Chicago): Polymer Physics Prize, American Physical Society.

Wagner, WR (McGowan Institute): Chairman, 2013 Annual Meeting, Biomedical Engineering Society.

WFIRM (Wake Forest School of Medicine): Game Changer, Science/Medical Section, Edison Awards.

Appendix B: Publications and Presentations

Peer-reviewed journal articles are defined as research articles and review articles accepted to, "in press," or published in scientific and technical journals from June 2012 through May 2013. Additionally, book chapters are included as peer-reviewed publications when indicated by investigators. Peer-reviewed publications recognized in previous AFIRM Annual Reports are not repeated here. The publications shown in Tables B-1a, B-1b, and B-1c were self-reported by the AFIRM investigators.

Table B-1a. Peer-Reviewed Publications: Rutgers–Cleveland Clinic Consortium

Alghoul M, Mendiola A, Seth R, Rubin B, Zins J, Calabro A, Siemionow M, Kusuma S. The Effect of Hyaluronan on Fat Graft Survival, *Aesthetic Surgery Journal*, 32(5):622-633. 2012 Jul 1.
Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ. A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials* 33(32):8034-8039, 2012.
Baldini EH, Lapidus MR, Wang Q, Manola J, Orgill DP, Pomahac B, Marcus KJ, Bertagnolli MM, Devlin PM, George S, Abraham J, Ferrone ML, Ready JE, Raut CP. Predictors for Major Wound Complications Following Preoperative Radiotherapy and Surgery for Soft-Tissue Sarcoma of the Extremities and Trunk: Importance of Tumor Proximity to Skin Surface. *Ann Surg Oncol*. 2012 Dec 15. [Epub ahead of print]
Bassiri Gharb B, Rampazzo A, Altuntas SH, Madajka M, Cwykiel J, Stratton J, Siemionow M. Effectiveness of Topical Immunosuppressants in Prevention and Treatment of Rejection in Face Allotransplantation. *Transplantation*. 2013 Mar 25. [Epub ahead of print]
Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, Redl H, Rubin JP, Yoshimura K, Gimble JM. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of IFATS and ISCT. 2013 Jun;15(6):641-8. DOI: 10.1016/j.jcyt.2013.02.006. [Epub 2013 Apr 6]
Boyce ST, RK Rice, KC Lynch, AP Supp, VB Swope, RJ Kagan, and DM Supp. Assessment of replication rates of human keratinocytes in engineered skin substitutes grafted to athymic mice. *Wound Repair Regen* 20(4):544-51. 2012. PMID:22672265.
Bozkurt M, Klimczak A, Nasir S, Zor F, Krokowicz L, Siemionow M. Composite Osseomusculocutaneous Sternum, Ribs, Thymus, Pectoralis Muscles, and Skin Allotransplantation Model of Bone Marrow Transplantation. *Microsurgery*, 33(1):43-50, Jan 2013. [Epub 23 Jul 2012]
Bueno EM, Diaz-Siso JR, Sisk GC, Chandawarkar A, Kiwanuka H, Lamparello B, Caterson EJ, Pomahac B. Vascularized composite allotransplantation and tissue engineering. *J Craniofac Surg*. 2013 Jan;24(1):256-63.
Caralla T, Joshi P, Fluery S, Luangphakdy V, Shinohara K, Pan H, Boehm C, Bryan JA, Vasanji A, Hefferan TE, Walker EA, Yaszemski M, Hascall V, Zborowski M, Muschler G. In vivo transplantation of autogenous marrow-derived cells following rapid intraoperative magnetic separation based on hyaluronan to augment bone regeneration. *Tissue Eng Part A*. 2013 Jan;19(1-2):125-34.
Carty MJ, Hivelin M, Dumontier C, Talbot SG, Benjoar MD, Pribaz JJ, Lantieri L, Pomahac B. Lessons Learned from Simultaneous Face and Bilateral Hand Allotransplantation. *Plast Reconstr Surg*. 2013 Apr 11. [Epub ahead of print]
Carty MJ, Zuker R, Cavadas P, Pribaz JJ, Talbot SG, Pomahac B. The Case for Lower Extremity Allotransplantation: Lower Extremity Transplantation. *Plast Reconstr Surg*. 2013 Feb 14. [Epub ahead of print]
Caterson EJ, Diaz-Siso JR, Bueno EM, Shetye P, Soga S, Rybicki FJ, Pomahac B. Craniofacial Principles in Face Transplantation. *J Craniofac Surg*. 2012 Sep;23(5):1234-8.
Caterson EJ, Lopez J, Medina M, Pomahac B, Tullius SG. Ischemia-Reperfusion Injury in Vascularized Composite Allotransplantation. *J Craniofac Surg*. 2013 Jan 13. [Epub ahead of print]
Chang G and Pomahac B. Psychosocial Changes Six Months after Face Transplantation. *Psychosomatics*. 2012 Nov 27.

Appendix B

Table B-1a. Peer-Reviewed Publications: Rutgers–Cleveland Clinic Consortium (cont.)

Cloud BA, Ball BG, Chen BK, Knight AM, Hakim JS, Ortiz AM, Windebank AJ. Hemisection spinal cord injury in rat: The value of intraoperative somatosensory evoked potential monitoring. *J Neurosci Methods*. 2012;211:179-84.

Coffman KL, Siemionow MZ. Face Transplantation: Psychological Outcomes at Three-Year Follow-Up. *Psychosomatics*. 2013 Jan 22. DOI:pii: S0033-3182(12)00195-8. 10.1016/j.psym.2012.10.009 [Epub ahead of print]

Cwykiel J, Klimczak A, Krokowicz L, Siemionow M. Pre- and Postischemic Pulsed Acoustic Cellular Expression Conditioning Modulates Expression of Inflammation Factors in Cremaster Ischemia/Reperfusion Injury Model. *Microsurgery*. 33(2):134-40, Feb. 2013. [Epub 14 Nov 2012]

Dadsetan M, Taylor KE, Yong C, Bajzer Z, Lu L, Yaszemski MJ. Controlled release of doxorubicin from pH-responsive microgels. *Acta Biomater.* 2013 Mar;9(3):5438-46.

Diaz-Siso JR, Bueno EM, Sisk GC, Marty FM, Pomahac B, Tullius SG. Vascularized composite tissue allotransplantation – state of the art. *Clin Transplant*. 2013 Apr 14. [Epub ahead of print]

Donnelly EM, Madigan NN, Rooney GE, Knight A, Chen B, Ball B, Kinnavane L, Garcia Y, Dockery P, Fraher J, Strappe PM, Windebank AJ, O'Brien T, McMahon SS. Lentiviral vector delivery of short hairpin RNA to NG2 and neurotrophin-3 promotes locomotor recovery in injured rat spinal cord. *Cytotherapy*. 2012; 14(10):1235-44.

Edrich T, Cywinski JB, Colomina MJ, López IJ, Xiong L, Sedaghati A, Pomahac B, Gilton A. Perioperative management of face transplantation: a survey. *Anesth Analg*. 2012 Sep;115(3):668-70. DOI: 10.1213/ANE.0b013e3182554493. [Epub 2012 Apr 20]

Gastman B, Djohan R, Siemionow M. Extending the Cordeiro Maxillofacial Defect Classification System for Use in the Era of Vascularized Composite Transplantation. *Plast Reconstr Surg*. 130(2): 419-422, Aug 2012.

Grisold W, Cavaletti G, Windebank AJ. Peripheral neuropathies from chemotherapeutics and targeted agents: diagnosis, treatment, and prevention. *Neuro Oncol*. 2012 Suppl 4:iv45-54. DOI: 10.1093/neuonc/nos203.

Hahn JM, Glaser K, McFarland KL, Aronow B, Boyce ST, and Supp DM. Keloid-derived keratinocytes exhibit an abnormal gene expression profile consistent with a distinct causal role in keloid pathology. *Wound Repair Regen*. 2013 Jul-Aug;21(4):530-44. DOI: 10.1111/wrr.12060.

Harsono K, Finelt N, Castano EB, Tonnesen MG, Clark RAF. Curcumin myths or wonders? – a systematic analysis of in vitro studies. *Wound Repair Regen* 21:335-336, 2013.

Hirth DA, McClain SA, Singer AJ, Clark RAF. Endothelial necrosis at 1h post-burn predicts progression of tissue injury. *Wound Repair Regen*. 2013 Jul-Aug;21(4):563-70. DOI: 10.1111/wrr.12053. [Epub 2013 Apr 29]

Hirth DA, Singer AJ, Clark RAF, McClain SA. Histopathogic staining of low temperature cutaneous burns: comparing biomarkers of epithelial and vascular injury. *Wound Repair Regen* 20:918-927, 2012.

Knoll BM, Hammond SP, Koo S, Issa NC, Tullius SG, Baden LR, Pomahac B, Marty FM. Infections Following Facial Composite Tissue Allotransplantation-Single Center Experience and Review of the Literature. *Am J Transplant*. 2012 Dec 27. DOI: 10.1111/ajt.12013. [Epub ahead of print]

Lehman RA Jr, Huddleston P, Yaszemski M. Axial spine injuries in the current conflicts in Iraq and Afghanistan. *J Am Acad Orthop Surg*. 2012;20 Suppl 1:S13-7.

Luangphakdy V, Walker E, Shinohara K, Pan H, Hefferan T, Bauer TW, Stockdale L, Saini S, Dadsetan M, Runge MB, Vasanji A, Griffith L, Yaszemski M, Muschler GF. Evaluation of osteoconductive scaffolds in the canine femoral multi-defect model. *Tissue Eng* Part A. 2013 Mar;19(5-6):634-48.

Ma M, Guo L, Anderson DG, Langer RS. Bio-inspired Polymer Composite Actuator and Generator Driven by Water Gradients. *Science*. 339(6116); 186–189, Jan, 2013.

McGee-Lawrence ME, Bradley EW, Dudakovic A, Carlson SW, Ryan ZC, Kumar R, Dadsetan M, Yaszemski MJ, Chen Q, An KN, Westendorf JJ. Histone deacetylase 3 is required for maintenance of bone mass during aging. *Bone*. 2013 Jan;52(1):296-307.

McIlhenny SE, Zhang P, Tulenko T, Comeau J, Fernandez S, Policha A, Ferroni M, Faul E, Bagameri G, Shapiro I, Dimuzio P. eNOS transfection of adipose-derived stem cells yields bioactive nitric oxide production and improved results in vascular tissue engineering. *J Tissue Eng Regen Med*. 2013 Jan 14. DOI: 10.1002/term.1645. PMID:23319464. [Epub ahead of print]

Nijhuis TH, Bodar CW, van Neck JW, Walbeehm ET, Siemionow M, Madajka M, Cwykiel J, Blok JH, Hovius SE. Natural conduits for bridging a 15-mm nerve defect: Comparison of the vein supported by muscle and bone marrow stromal cells with a nerve autograft. *J Plast Reconstr Aesthet Surg*. 66(2):251-9, Feb 2013. [Epub 10 Oct 2012]

Table B-1a. Peer-Reviewed Publications: Rutgers–Cleveland Clinic Consortium (cont.)

Pan Z, Ghosh K, Hung V, Macri L, Einhorn J, Bhatnagar D, Simon M, Clark RA, Rafailovich MH. Deformation gradients imprint the direction and speed of en masse fibroblast migration for fast healing. *J Invest Dermatol*. 2013 Oct;133(10):2471-9. DOI: 10.1038/jid.2013.184. [Epub 2013 Apr 17]

Peramo A, Marcelo CL, Feinberg SE. Tissue Engineering of Lips and Muco-Cutaneous Junctions: In Vitro Development of Tissue Engineered Constructs of Oral Mucosa and Skin for Lip Reconstruction. *Tissue Engineering*. 18:273-282 (2012) PMID: 22067042.

Pomahac B, Bueno EM, Sisk GC, Pribaz JJ. Current Principles of Facial Allotransplantation: The Brigham and Women's Hospital Experience. *Plast Reconstr Surg*. 2013 May;131(5):1069-76.

Pomahac B, Pribaz JJ, Bueno EM, Sisk GC, Diaz-Siso JR, Chandawarkar A, Westvik TS, Malin EW, Eriksson E. Novel Surgical Technique for full face transplantation. *Plast Reconstr Surg*. 2012 Sep;130(3):549-55.

Shen FH, Werner BC, Liang H, Shang H, Yang N, Li X, Shimer AL, Balian G, Katz AJ. Implications of adipose-derived stromal cells in a 3D culture system for osteogenic differentiation: an in vitro and in vivo investigation. *The Spine Journal*. 2013, Jan;13(1):32-43.

Siemionow M, Klimczak A. Chimerism-Based Experimental Models for Tolerance Induction in Vascularized Composite Allografts: Cleveland Clinic Research Experience. *Clinical and Developmental Immunology*. 2013; 2013.831410. DOI: 10.1155/2013/831410. Review [Epub 2013 Mar 14]

Siemionow M, Madajka M, Cwykiel J. Application of Cell Based Therapies in Facial Transplantation. *Ann Plast Surg*. 69(5):575-9, Nov 2012.

Siemionow M, Uygur S, Ozturk C, Siemionow K. Techniques and Materials for Enhancement of Peripheral Nerve Regeneration: A Literature Review. *Microsurgery*. 2013 Apr 9. DOI: 10.1002/micr.22104. [Epub ahead of print]

Singer, AG, DH Ahrenholz, P Chang, RAF Clark, R Fey, P Fidler, W Garner, N Gibran, D Greenhalgh, S Honari, L Jones, R Kagan, J Kirby, J Leggett, N Meyer, C Reigart, K Richey, L Rosenberg, J Weber, B Wiggins, and ST Boyce. 2013. Consensus metrics for healing of burn wounds. *J Burn Care and Res*, In Press.

Sisk GC, Kumamaru KK, Schultz K, Bueno EM, Diaz-Siso JR, George E, Redjaee MM, Mitsouras D, Steigner ML, Pomahac B, Rybicki FJ. Cine computed tomography angiography evaluation of blood flow for full face transplant surgical planning. *Eplasty*. 2012;12:e57. [Epub 2012 Dec 18]

Soga S, Pomahac B, Kumamaru K, Wake N, Schultz K, Powers SL, Prior RF, Steigner ML, Mitsouras D, Bueno EM, Rybicki FJ. CT Angiography for Surgical Planning in Face Transplantation Candidates. *AJNR Am J Neuroradiol*. 2012 Aug 9. PubMed PMID: 22878008. [Epub ahead of print]

Sriwiriyanont P, Lynch KA, McFarland KL, Supp DM, Boyce ST. Characterization of hair follicle development in engineered skin substitutes. *PLoS One*. 2013 Jun 17;8(6):e65664. DOI: 10.1371/journal.pone.0065664.

Sriwiriyanont P, Maier EA, Lynch KA, Supp DM, and Boyce ST. Dermal papilla cells stimulate trichogenesis in engineered skin substitutes. *Exp Dermatol*. 21(10):783-785; 2012. PMID:23078401.

Ta LE, Schmelzer JD, Bieber AJ, Loprinzi CL, Sieck GC, Brederson JD, Low PA, Windebank AJ. A novel and selective poly (adp-ribose) polymerase inhibitor ameliorates chemotherapy-induced painful neuropathy. *PLoS One*. 2013; 8(1):e54161. [Epub 2013 Jan 10]

Wu P, Spinner RJ, Gu Y, Yaszemski MJ, Windebank AJ, Wang H. Delayed repair of the peripheral nerve: A novel model in the rat sciatic nerve. *J Neurosci Methods*. 2013 Mar 30;214(1):37-44. DOI: 10.1016/j.jneumeth.2013.01.003. [Epub 2013 Jan 11]

Yang SY, O'Cearbhaill ED, Sisk GC, Park KM, Cho WK, Villiger M, Bouma BE, Pomahac B, Karp JM. *Nat Commun*. 2013 Apr 16;4:1702.

Yoshii T, Hafeman AE, Esparza JM, Okawa A, Gutierrez G, Guelcher SA. Local injection of lovastatin in biodegradable polyurethane scaffolds enhances bone regeneration in a critical-sized segmental defect in rat femora. *J Tissue Eng Regen Med*. 2012 Jun 20. DOI: 10.1002/term.1547. [Epub ahead of print]

Appendix B

Table B-1b. Peer-Reviewed Publications: Wake Forest–Pittsburgh Consortium

Atala A, Kasper FK and Mikos AG. Engineering Complex Tissues. *Science Translational Medicine*, 4(160):160rv12, 2012.

Bellas E, Panilaitis B, Glettig DL, Kirker-Head CA, Yoo JJ, Marra KG, Rubin JP, Kaplan DL. Sustained volume retention in vivo with adipocyte and lipoaspirate seeded silk scaffolds. *Biomaterials* 2013 Apr; 34(12):2960-8.

Boerckel JD, Kolambkar YM, Stevens HY, Dupont KM, Guldberg RE. Mechanical Loading Enhances Segmental Bone Defect Repair by Altering Bone Formation and Distribution. *Journal of Orthopaedic Research*. 30(7):1067-75, 2012.

Brandacher G, Lee WPA, Schneeberger S. Minimizing Immunosuppression in Hand Transplantation. *Expert Rev Clin Immunol*. 2012 Sep;8(7):673-84.

Brandacher G. Composite Tissue Transplantation. In Andrea A. Zachary and Mary S. Leffell (eds.), Transplantation Immunology: Methods and Protocols, Second Edition, *Methods in Molecular Biology*, vol. 1034, pp. 103-115. DOI 10.1007/978-1-62703-493-7_5, © Springer Science+Business Media, LLC 2013.

Criswell TL, Corona BT, Wang Z, Zhou Y, Niu G, Xu Y, Christ GJ, Soker S. The role of endothelial cells in myofiber differentiation and the vascularization and innervation of bioengineered muscle tissue in vivo. *Biomaterials*. 2013 Jan;34(1):140-9. DOI: 10.1016/j.biomaterials.2012.09.045. [Epub 2012 Oct 8]

Ekenseair AK, Kasper FK and Mikos AG. Perspectives on the Interface of Drug Delivery and Tissue Engineering, Advanced Drug Delivery Reviews, 65(1):89-92, 2013.

Fong ELS, Watson BM, Kasper FK and Mikos AG. Building Bridges: Leveraging Interdisciplinary Collaborations in the Development of Biomaterials to Meet Clinical Needs. *Advanced Materials*, 24(36):4995-5013, 2012.

Friedrich EE, Sun LT, Natesan S, Zamora DO, Christy RJ, Washburn NR. Effects of hyaluronic acid conjugation on anti-TNF-α inhibition of inflammation in burns. *J Biomed Mater Res A*. 2013 Jun 13. DOI: 10.1002/jbm.a.34829. PMID:23765644. [Epub ahead of print], In press.

Guldberg RE, Awad HA, Vunjak-Novakovic G, Donahue H, Das A. Principles of Engineering Tissue Regeneration, IBMS Bonekey, In Press.

Hronik-Tupaj M, Raja WK, Tang-Schomer M, Omenetto FG, Kaplan DL. Neural Responses to Electrical Stimulation on Patterned Silk Films. *J Biomed Mater Res A*. 2013 Sep;101(9):2559-72. DOI: 10.1002/jbm.a.34565. [Epub 2013 Feb 11]

Hutmacher DW, Woodruff MA, Shakesheff K, Guldberg RE. Direct Fabrication as a Patient-Targeted Therapeutic in a Clinical Environment. *Methods Mol Biol*. 868:327-40, 2012.

Hwang CM, Ay B, Kaplan DL, Rubin JP, Marra KG, Atala A, Yoo JJ, and Lee SJ. Assessments of injectable alginate particle-embedded fibrin hydrogels for soft tissue reconstruction. *Biomed. Mat*. 2013;8(1): 014105.

Ibrahim Z, Cooney DS, Schneeberger S, Shores JT, Bonawitz S, Sacks JM, Lee WPA, Brandacher G. A modified hetrotopic swine hind limb transplant model for translational Vascularized Composite Allotransplantation (VCA) research. *JOVE* 2013, In Press.

Järvinen TAH, Ruoslahti E. Targeted antiscarring therapy for tissue injuries. *Adv Wound Care*, 2(2):50-54, Mar, 2013. DOI:10.1089/wound.2011.0299.

Järvinen TAH. Design of Target-Seeking Antifibrotic Compounds. Meth Enzymol 509: 243-261, 2012.

Johnen C, Chinnici C, Triolo F, Plettig J, Bräutigam K, Amico G, Young M, Over P, Esteban-Vives R, Schmelzer E, Conaldi PG, Turner M, Thompson R, Zeilinger K, Rubin P, Vizzini G, Gridelli B, Gerlach JC. Phenotypical characterization of 6-21-week gestational age human dermis and epidermal cell isolation methods for in vitro studies on epidermal progenitors. *Burns*. 2013 Mar;39(2):300-10. DOI: 10.1016/j.burns.2012.05.025. [Epub 2012 Jun 30]

Kathju S, Gallo PH, Satish L. Scarless integumentary wound healing in the mammalian fetus: molecular basis and therapeutic implications. *Birth Defects Res C Embryo Today*. 2012 Sep;96(3):223-36.

Kinard LA, Chu C, Tabata Y, Kasper FK and Mikos AG. Bone Morphogenetic Protein-2 Release from Composite Hydrogels of Oligo(poly(ethylene glycol) fumarate) and Gelatin. *Pharmaceutical Research*, DOI:10.1007/s11095-013-1077-5, In Press.

Lang RS, Gorantla VS, Esper S, Montoya M, Losee JE, Hilmi IA, Sakai T, Lee WPA, Raval JS, Kiss JE, Shores JT, Brandacher G, Planinsic RM. Anesthetic Management in Upper Extremity Transplantation: The Pittsburgh Experience. *Anesth Analg*. 2012 Sep;115(3):678-88. DOI: 10.1213/ANE.0b013e31825da401. [Epub 2012 Jun 28]

Lin Y-C, Oh SJ, Marra KG. Synergistic Lithium Chloride and Glial Cell Line-Derived Neurotrophic Factor Delivery for Peripheral Nerve Repair in a Rodent Sciatic Nerve Injury Model. *Plastic and Reconstructive Surgery*, 2013, In Press.

Table B-1b. Peer-Reviewed Publications: Wake Forest–Pittsburgh Consortium (cont.)

Nozaki M, Ota S, Terada S, Li Y, Uehara K, Gharaibeh B, Fu FH, and Huard J. Timing of the administration of Suramin treatment after muscle injury. Muscle and Nerve. *Muscle Nerve*. 2012 Jul;46(1):70-9. DOI: 10.1002/mus.23280. [Epub 2012 May 29]
Pace LA, Plate JF, Smith TL, Van Dyke M. The Effect of Human Hair Keratin Hydrogel on Early Cellular Response to Sciatic Nerve Injury in a Rat Model. *Biomaterials*. 2013, In Press.
Rutten MJ, Janes MA, Chang IR, Gregory CR, Gregory KW. Development of a functional Schwann cell phenotype from autologous porcine bone marrow mononuclear cells for nerve repair. *Stem Cells Int*. 2012;2012:738484. DOI: 10.1155/2012/738484. [Epub 2012 Jun 24]
Satish L, O'Gorman DB, Johnson S, Raykha C, Gan BS, Wang JH, Kathju S. Increased CCT-eta expression is a marker of latent and active disease and a modulator of fibroblast contractility in Dupuytren's contracture. *Cell Stress Chaperones*. 2013 Jan 6. [Epub ahead of print]
Schneeberger S, Gorantla VS, Brandacher G, Zeevi A, Demetris AJ, Lunz JG, Metes DM, Donnenberg AD, Shores JT, Dimartini AF, Kiss JE, Imbriglia JE, Azari K, Goitz RJ, Manders EK, Nguyen VT, Cooney DS, Wachtman GS, Keith JD, Fletcher DR, Macedo C, Planinsic R, Losee JE, Shapiro R, Starzl TE, Lee WP. Upper-Extremity Transplantation Using a Cell-Based Protocol to Minimize Immunosuppression. *Ann Surg*. 2013; 257(2): 345-51.
Sicari BM, Agrawal V, Sui BF, Medberry CJ, Dearth CL, Turner NJ, Badylak SF. A murine model of volumetric muscle loss and a regenerative medicine approach for tissue replacement. *Tissue Engineering, Part A*. Oct;18 (19-20):1941-8, 2012.
Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, Soker S. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl Med*. 2012 Nov; 1(11):792-802.
Stoppato M, Stevens HY, Carletti E, Migliaresi C, Motta A, Guldberg RE. The Effects of Silk Fibroin Fiber Incorporation on Mechanical Properties, Endothelial Cell Colonization, and Vascularization of PDLLA Scaffolds. *Biomaterials*, In Press.
Uhrig BA, Boerckel JD, Willett NJ, Huebsch N, Guldberg RE. Recovery from hind limb ischemia enhances rhBMP-2-mediated segmental bone defect repair in a rat composite injury model. *Bone*, In Press.
Uhrig BA, Clements IP, Boerckel JD, Huebsch N, Bellamkonda RV, Guldberg RE. Characterization of a Composite Injury Model of Severe Lower Limb Bone and Nerve Trauma. *J Tissue Eng Regen Med*. 2012 Jun 11. DOI: 10.1002/term.1537. PMID:22689452. [Epub ahead of print]
Wang L, Yoon DM, Spicer PP, Henslee AM, Scott DW, Wong ME, Kasper FK and Mikos AG. Characterization of Porous Polymethylmethacrylate Space Maintainers for Craniofacial Reconstruction. *J Biomed Mater Res B Appl Biomater*. 2013 Jul;101(5):813-25. DOI: 10.1002/jbm.b.32885. [Epub 2013 Jan 29]
Willett NJ, Li MA, Uhrig BA, Boerckel JD, Huebsch N, Lundgren TL, Warren GL, Guldberg RE. Attenuated rhBMP-2 Mediated Bone Regeneration in a Rat Model of Composite Bone and Muscle Injury. *Tissue Engineering Part C*. 19(4):316-25, 2013.
Xu T, Binder KW, Albanna MZ, et al. Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. *Biofabrication*. 2013;5(1):015001.
Xu T, Zhao W, Zhu J-M, Albanna MZ, Yoo JJ, Atala A. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials*. 2013;34(1):130-139.

Table B-1c. Peer-Reviewed Publications: USAISR

Rathbone CR, Guda T, Singleton B, Oh S, Appleford MR, Ong JL, Wenke JC. Effect of cell-seeded hydroxyapatite scaffolds on rabbit radius bone regeneration. *J Biomed Mater Res A*. 2013 Jun 15. DOI: 10.1002/jbm.a.34834. [Epub ahead of print]
Wu X, Corona BT, Chen X, Walters TJ. A standardized rat model of volumetric muscle loss injury for the development of tissue engineering therapies. *Biores Open Access*. 2012 Dec;1(6):280-90. DOI: 10.1089/biores.2012.0271.
Wu X, Walters T, Rathbone CR. Skeletal Muscle Satellite Cell Activation Following Cutaneous Burn in Rats. *Burns*. 2013 Jun;39(4):736-44. DOI: 10.1016/j.burns.2012.10.016. [Epub 2012 Nov 10]

Appendix B

Tables B-2a and B-2b list non-peer-reviewed publications and all presentations self-reported by AFIRM investigators. The non-peer-reviewed publications are defined as editorials, letters, or opinion writings that have been accepted to, "in press," or published in scientific and technical journals from June 2012 through May 2013. Books and book chapters are included here as indicated by the authors. Presentations include all invited talks, symposia, oral presentations, and posters presented at scientific research conferences and meetings regardless of the peer review process. All such presentations made and all presentations accepted from June 2012 through May 2013 are included. Presentations not specifically labeled as accepted in the researchers' progress reports were not assumed to be accepted and were not included in the following tables. Non-peer-reviewed publications and presentation citations recognized in previous AFIRM Annual Reports are not repeated for this year.

Table B-2a. Presentations and Non-Peer-Reviewed Publications: Rutgers–Cleveland Clinic Consortium

Cervantes T, Bassett E, Tseng A, Kimura A, Roscioli N, Gupta R, Randolph MA, Vacanti JP, Pomerantseva I, Sundback CA. 3D Imaging Analysis and Rapid Prototyping of Composite Scaffolds for Tissue Engineered Ear Reconstruction. MGH Scientific Advisory Committee Scientific Poster Session. March 20–21, 2013.
Cervantes T, Bassett E, Tseng A, Kimura A, Roscioli N, Gupta R, Randolph MA, Vacanti JP, Pomerantseva I, Sundback CA. Design and Analysis of Flexible Composite Scaffolds for Engineered Ear. 2013 Annual Meeting Society of Biomaterials. Boston, MA, April 11–12, 2013.
Cervantes T, Bassett E, Tseng A, Kimura A, Roscioli N, Vacanti JP, Pomerantseva I, Sundback CA. Rapid Prototyping of Flexible Structures for Tissue Engineered Ear Reconstruction. 2013 Design of Medical Devices Conference. Minneapolis, MN, April 8–11, 2013.
Chawla AS, Spinner RJ, Yaszemski MJ, Windebank AJ, Wang H. Non-invasive isometric muscle force measurement of plantar flexion in the rat sciatic nerve injury model. Annual Meeting of the American Society for Peripheral Nerve, Naples, FL, January 11–13, 2013.
Clark RAF. Bioactives and biomechanics provide key signals for dermal healing.
Cullen DK, Kameswaran N, Struzyna LA, Morand JP, Wolf JA, Ledebur H, Smith DH. Tissue Engineered Grafts with Stretch-Grown Axons Accelerate Peripheral Nerve Regeneration. Annual Hilton Head Workshop, Regenerative Medicine: Technologies Enabling Novel Therapies, Hilton Head Island, SC, March 2013.
Cwkiel J, Askar M, Siemionow M. Phenotype Characterization of Human Di-Chimeric Cells for Tolerance Inducing Protocols in Transplantation. Preliminary Study (poster presentation), 24th International Congress of The Transplantation Society, Berlin, Germany, July 16, 2012.
Cwykiel J, Askar M, Siemionow M. Characterization of Human Di-Chimeric Cells for Tolerance Inducing Protocols in Transplantation: A Preliminary Study (oral presentation), Session: Experimental and Research, 17th World Congress of the Int'l. Confederation for Plastic, Reconstructive, & Aesthetic Surgery, Santiago, Chile, February 27, 2013.
Cwykiel J, Askar M, Siemionow M. Human Di-Chimeric Cells – A New Approach for Tolerance Inducing Protocols in Transplantation: A Preliminary Study (oral presentation), Session: Basic Science and CTA papers, American Society for Reconstructive Microsurgery Annual Meeting, Naples, FL, January 14, 2013.
Cwykiel J, Askar M, Siemionow M. Human Di-Chimeric Cells – a New Approach for Tolerance Inducing Protocols in Transplantation: A Preliminary Study (poster presentation), American Society for Blood and Bone Marrow Transplantation/Center for International Blood and Marrow Transplant Research, BMT Tandem Meetings, Salt Lake City, UT, February 13, 2013.
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Appendix C: Patent Applications and Invention Disclosures

The attribution of inventions and patent applications to specific research support is subject to varying interpretations in the absence of a standard definition. Optimally, only those patents and patent applications displaying the AFIRM contract number in the Government Interest field in the U.S. Patent and Trademark Office (USPTO) patent application record should be included as directly attributable to the AFIRM program; however, this strict definition would exclude provisional patent applications left undisclosed to the public and recently filed applications not yet included in government databases. Rather than applying the more rigid validation approach outlined above, the following definitions were applied to self-reported intellectual property milestones:

- A self-reported invention disclosure filed with the inventor's institutional technology licensing office during a given program year is attributed to the AFIRM program in that program year.
- A self-reported patent application filed with a government patent office during a program year period is attributed to the AFIRM program that program year (e.g., June 2012 – May 2013 for PY5).
- A self-reported patent award is attributed to the AFIRM program when the patent application was filed after September 2008.

All self-reported patent application numbers and inventors (i.e., principal investigators) were queried against the World Intellectual Property Organization (WIPO) patent application database (http://www.wipo.int/pctdb/en/), the USPTO AppFT patent application database (http://patft.uspto.gov/), and/or Google Patent Search (http://www.google.com/patents). The database queries were used to (1) identify patent applications filed for self-reported inventions and (2) identify and validate filing dates for patent applications.

Patent Applications:

AFIRM researchers self-reported 8 government-filed patent applications that included a filing date or year; a patent priority number, serial number, or other patent application number; and/or were identified on the USPTO or WIPO databases. The reported patent applications were either new applications or continuing applications to earlier filed patent applications.

Rutgers–Cleveland Clinic Consortium

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Wake Forest–Pittsburgh Consortium

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Invention Disclosures:

Invention disclosures are not publicly reposed in standard databases; therefore, the AFIRM consortium reports are the only information source for inventions disclosed. From June 2012 through May 2013, the self-reported disclosure of inventions corresponded to the self-reported filing of patent applications; therefore, no invention disclosures are specifically cited for PY5.

Appendix D: Descriptions of Technology Readiness Levels and Clinical Study Phases

Technology Readiness Levels (TRLs) – Biomedical

TRL 1 – Maintain scientific technical watch.

TRL 2 – Research ideas and protocols are developed.

TRL 3 – Hypothesis testing and initial proof of concept is demonstrated in a limited number of in vitro and in vivo models.

TRL 4 – Proof of concept and safety of candidate product (e.g., drug formulation, biologic or vaccine construct, or device or system) is demonstrated in a defined laboratory or animal model.

TRL 5 – Pre-clinical studies, including GLP animal safety and toxicity, sufficient to support IND application. For devices, medical device review of IDE results is sufficient to begin investigation.

TRL 6 – Phase I clinical trial support proceeding to Phase II clinical trials. IND application submitted to and reviewed by FDA. For devices, Class 3 device safety demonstrated. 510(k) data demonstrates substantial equivalency to predicate device.

TRL 7 – Phase II clinical trial is completed, and Phase III clinical trial plan is approved by FDA. For devices, final product design is validated and final prototypes are produced and tested.

TRL 8 – Phase III clinical trial is completed, and FDA approves NDA or BLA. For devices, FDA approves the premarket approval for medical device or applicable 510(k) for devices.

TRL 9 – Post marketing studies and surveillance.

Biomedical TRL descriptions provide a systematic way for the science and technology community to assess and communicate the level of maturity of a particular technology or combination of technologies. The TRL descriptions above summarize the pharmaceutical product and medical device TRL descriptions accessible on the U.S. Army Medical Research and Materiel Command's Army Technology Objectives website (https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.ppae.atostat).

Clinical Study Phases

Phase 0: Exploratory study involving very limited human exposure to the drug, with no therapeutic or diagnostic goals (e.g., screening studies, microdose studies).

Phase I: Studies that are usually conducted with healthy volunteers and that emphasize safety. The goal is to find out what the drug's most frequent and serious adverse events are and, often, how the drug is metabolized and excreted.

Phase II: Studies that gather preliminary data on effectiveness (whether the drug works in people who have a certain disease or condition). For example, participants receiving the drug may be compared with similar participants receiving a different treatment, usually an inactive substance (called a placebo) or a different drug. Safety continues to be evaluated, and short-term adverse events are studied.

Phase III: Studies that gather more information about safety and effectiveness by studying different populations and different dosages, and by using the drug in combination with other drugs.

Phase IV: Studies occurring after FDA has approved a drug for marketing. These including postmarket requirement and commitment studies that are required of or agreed to by the sponsor. These studies gather additional information about a drug's safety, efficacy, or optimal use.

The description of clinical study phases is attributed to ClinicalTrials.gov, a website maintained by the National Library of Medicine at the National Institutes of Health. More information on clinical studies can be found at the link http://www.clinicaltrials.gov/ct2/about-studies/learn. Clinical study phases are the FDA categories for describing the clinical trial of a drug based on the study's characteristics, such as the objective and number of participants (http://www.fda.gov/drugs/resourcesforyou/consumers/ucm143534.htm).

Appendix E: Acronyms

3D ..... three-dimensional

AFIRM ..... Armed Forces Institute of Regenerative Medicine

AFS ..... amniotic fluid-derived stem

AFT-SPAR ..... Autologous fat transfer for scar prevention and remodeling

ASC ..... adipose-derived stem cell

BAMC ..... Brooke Army Medical Center

BIODOME ..... Biomechanical Interface for Optimized Delivery of MEMS Orchestrated Mammalian Epimorphosis

BMA ..... bone marrow aspirate

BM-MNC ..... bone marrow mononuclear cell

BMP ..... bone morphogenetic protein

BMP-2 ..... bone morphogenetic protein-2

BMSC ..... bone marrow stromal cells

BOD ..... Board of Directors

CaP ..... calcium phosphate

CAR ..... peptide CARSKNKDC

CBER ..... Center for Biologics Evaluation and Research

cBMA ..... concentrated BMA

CCTD ..... chronic caprine tibial defect

CCT-eta .... chaperonin containing T-complex polypeptide

CDER ..... Center for Drug Evaluation and Research

CDRH ..... Center for Devices and Radiological Health

CFR ..... Code of Federal Regulations

cGMP ..... current Good Manufacturing Practices

cm ..... centimeter

CRMRP ..... Clinical and Rehabilitative Medicine Research Program

CS ..... Compartment Syndrome

CSD ..... critical-size defect

CTP-O ..... connective tissue progenitor cells

DNA ..... deoxyribonucleic acid

DoD ..... Department of Defense

DS ..... density separation

DWP ..... dermal wound paste

ECM ..... extracellular matrix

EMB ..... explantable microvascular bed

eNOS ..... endothelial nitric-oxide synthase

ESS ..... Engineered skin substitutes

ESS-P ..... ESS with pigment

EtO ..... ethylene oxide

EVPOME ..... ex vivo-produced oral mucosa equivalent

FAK ..... focal adhesion kinase

FDA ..... U.S. Food and Drug Administration

GLP ..... Good Laboratory Practices

GMP ..... Good Manufacturing Practices

HA ..... hyaluronic acid

HRPO ..... Human Research Protection Office

I/R ..... ischemia/reperfusion

IDE ..... Investigational Device Exemption

IND ..... Investigational New Drug

IPT ..... Integrated Project Team

IRB ..... Institutional Review Board

ISO ..... International Organization for Standardization

kg ..... kilogram

LWI ..... Lonza Walkersville, Inc.

M ..... million

M/C ..... mucocutaneous

MCC ..... multipotent cell cluster

MDSC ..... muscle-derived stem cell

MG ..... MasterGraft®

MGH ..... Massachusetts General Hospital

MHC ..... major histocompatibility complex

MIRM ..... McGowan Institute for Regenerative Medicine

MPC ..... muscle precursor cell

MSC ..... mesenchymal stem cell

MSD ..... molecular surface design

NGT ..... nerve guidance tube

NHP ..... nonhuman primate

NIH ..... National Institutes of Health

Appendix E

NSAID ..... non-steroidal anti-inflammatory drug
OCP..... Office of Combination Products
ONR ..... Office of Naval Research
OSMEA .....organic, stretchable microelectrode array
PCLF .....polycaprolactone fumarate
PCS.....peripheral compartment syndrome
PES .....poly(ether sulfone)
PMMA..... poly(methyl methacrylate)
PMO.....Project Management Office
POG .....particulate oxygen generator
PPF .....poly(propylene fumarate)
PY .....Program Year
RCCC .....Rutgers–Cleveland Clinic Consortium
RFD..... Request for Designation
rhBMP-2 .....recombinant human bone morphogenetic protein-2
RNA ..... Ribonucleic acid
siRNA..... small interfering ribonucleic acid
SR..... selective retention
TA .....tibialis anterior
TEBV.....tissue-engineered blood vessel
TEMR..... tissue-engineered muscle repair
TENG..... tissue-engineered nerve graft
TNF-α.....tumor necrosis factor-alpha
TRL ..... technology readiness level
TyrPC .....tyrosine-derived polycarbonate
UF ..... University of Florida
USAISR.....U.S. Army Institute of Surgical Research
USAMRMC..... U.S. Army Medical Research and Materiel Command
UTHSC ..... University of Texas Health Science Center at Houston
USPTO ..... U.S. Patent and Trademark Office
VA ..... U.S. Department of Veterans Affairs
VCA.....vascularized composite tissue allografts
VML .....volumetric muscle loss
WFPC.....Wake Forest–Pittsburgh Consortium
WRNMMC..... Walter Reed National Military Medical Center
µM ..... micron

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