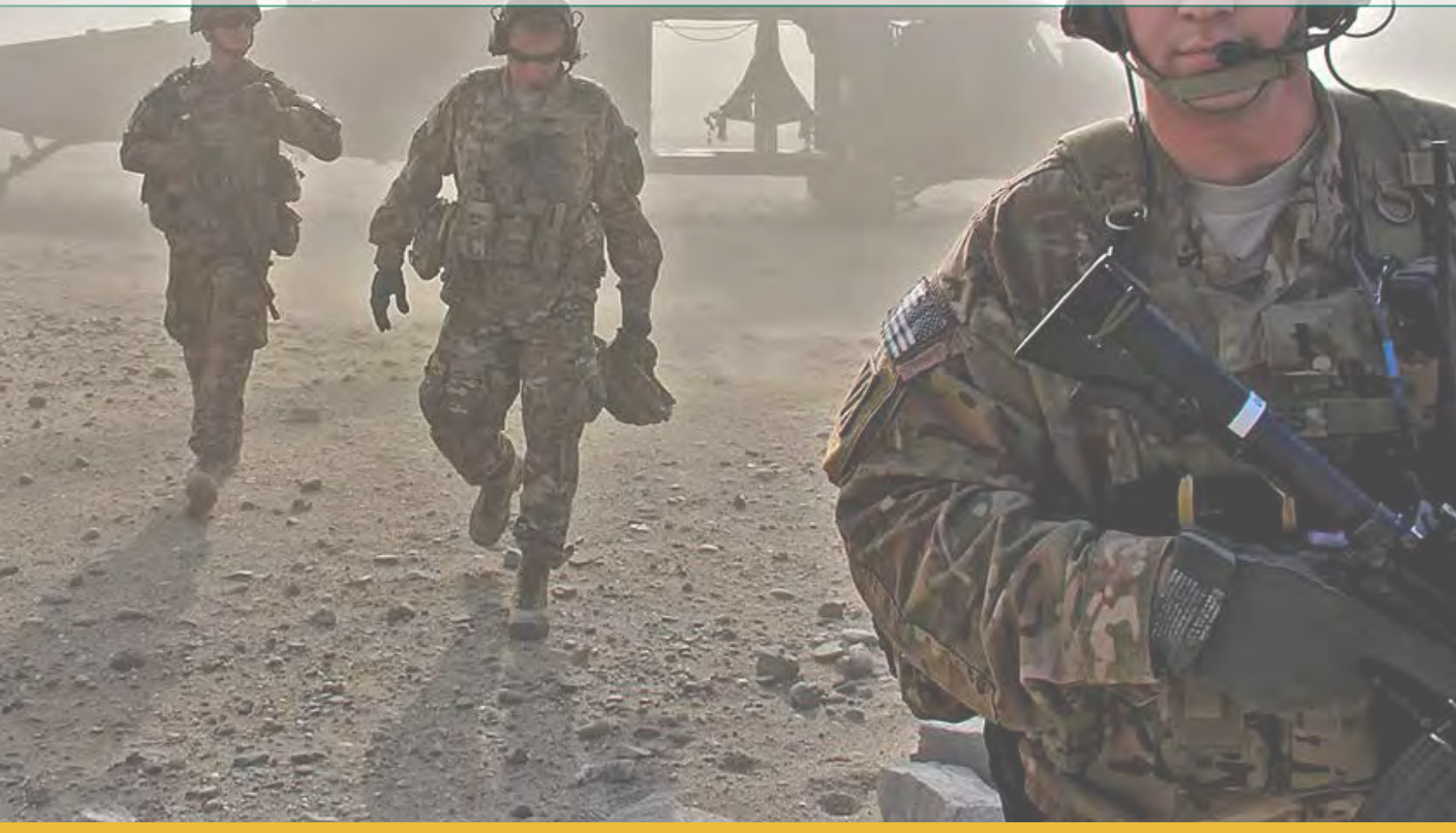
AFIRM OUR SCIENCE FOR THEIR HEALING

Armed Forces Institute of Regenerative Medicine

Annual Report 2014



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Armed Forces Institute of Regenerative Medicine Annual Report 2014

This report contains an overview of the Armed Forces Institute of Regenerative Medicine and summaries of all currently funded research projects.

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Executive Summary

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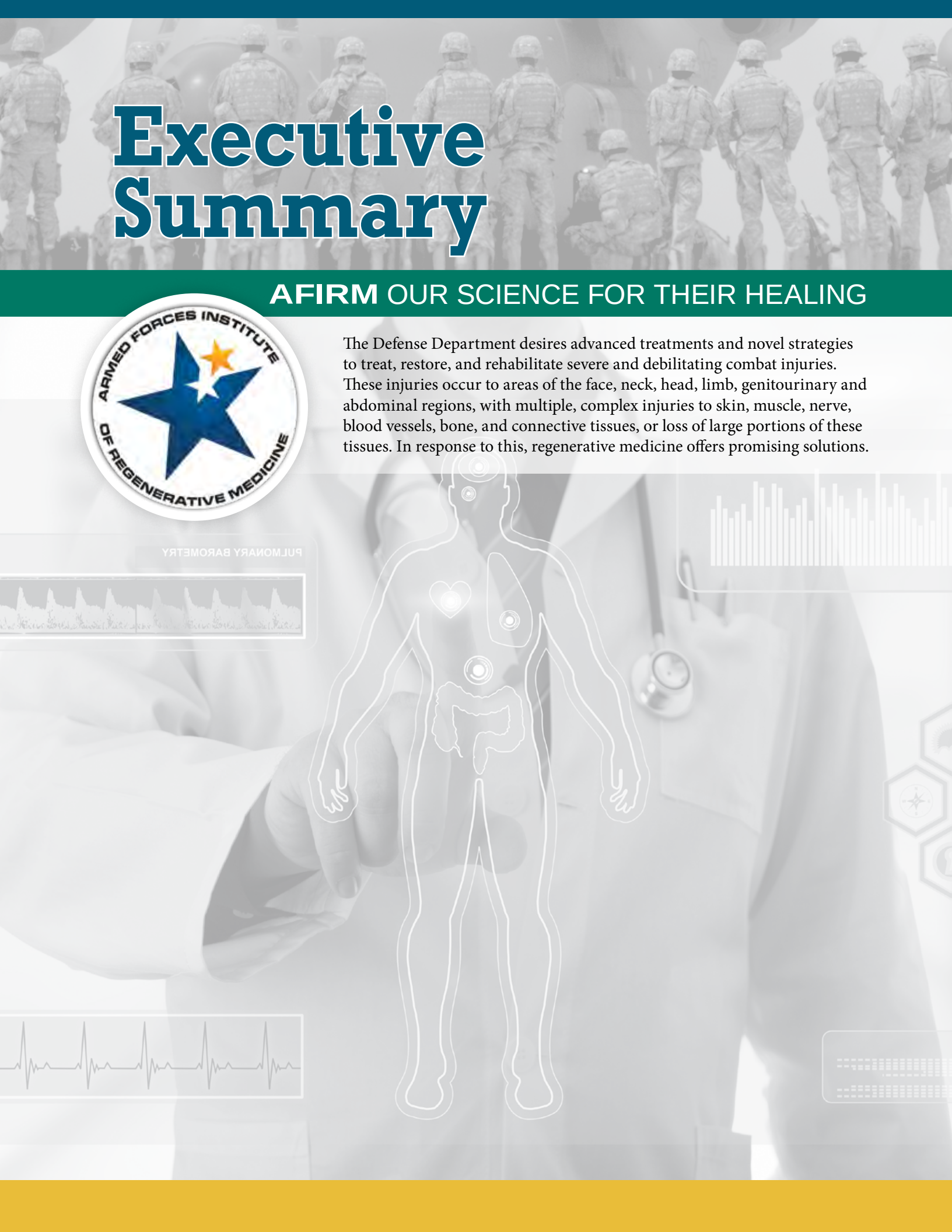
The Defense Department desires advanced treatments and novel strategies to treat, restore, and rehabilitate severe and debilitating combat injuries. These injuries occur to areas of the face, neck, head, limb, genitourinary and abdominal regions, with multiple, complex injuries to skin, muscle, nerve, blood vessels, bone, and connective tissues, or loss of large portions of these tissues. In response to this, regenerative medicine offers promising solutions.



Executive Summary

Regenerative medicine is a rapidly growing discipline that provides hope for restoring the structure and function of damaged tissues and organs, and curing previously untreatable injuries and diseases. This emerging field encompasses many novel approaches to promote the self-regenerative capacity of the body. Advanced technologies, such as tissue regeneration, novel biomaterials scaffolding, and stem cell-enabled treatments, are envisioned to replace or regenerate human cells, tissues, or organs to restore or establish normal function.

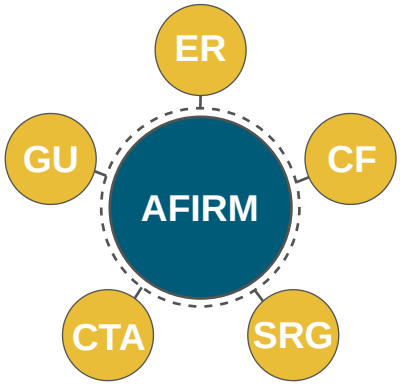
History of the AFIRM

The U.S. Army Medical Research and Materiel Command (USAMRMC) developed the idea of a regenerative medicine institute similar to the Multidisciplinary University Research Initiatives program of the Department of Defense (DoD), but aimed at near-term, translational research. In 2008, the Armed Forces Institute of Regenerative Medicine (AFIRM) was established and funded through a partnership of USAMRMC, Office of Naval Research, U.S. Air Force Office of the Surgeon General, National Institutes of Health (NIH), and Veterans Health Administration of the Department of Veterans Affairs (VA). From 2008 to 2013, the AFIRM was composed of two independent civilian research consortia: the Rutgers–Cleveland Clinic Consortium (RCCC) and the Wake Forest–Pittsburgh Consortium (WFPC). These two bodies worked closely with the U.S. Army Institute of Surgical Research (USAISR) at Fort Sam Houston, Texas. USAISR, which is home to the DoD's only burn unit, is co-located with the San Antonio Military Medical Center – North (formerly Brooke Army Medical Center) and the Center for the Intrepid, a treatment, rehabilitation, and research center for the care of severely wounded Warfighters.

In 2013, with the period of performance ending on the original agreements, the government wished to build upon the success of the first 5 years of the AFIRM program. In particular, there was interest in improving the translation of promising regenerative medicine technologies into clinical use, as well as in addressing the emerging clinical concern of treating traumatic genitourinary and abdominal injuries. A Wake Forest University School of Medicine (Wake Forest Baptist Medical Center)-led consortium, the Warrior Restoration Consortium (WRC), was selected for this next phase of AFIRM. The WRC includes members from both of the initial AFIRM consortia—RCCC and WFPC—along with new investigators. The WRC consists of over 30 member institutions. Continued collaboration with government laboratories and Medical Centers/Medical Treatment Facilities including the Army, Navy/Marine, Air Force, NIH, and the VA is expected. The WRC team creates a broad coalition of leading academic partners and companies with the resources, experience, partnerships and collaborations to advance regenerative medicine-based solutions for clinical evaluations and ultimately to usage by the Armed Forces.

Major Focus Areas

The research of the AFIRM is currently divided into five focus areas representing the critical clinical challenges that need advanced solutions for wounded warriors: Extremity Regeneration (ER), Craniomaxillofacial Regeneration (CF), Skin Regeneration (SRG), Composite Tissue Allotransplantation and Immunomodulation (CTA), and Genitourinary/Lower Abdomen Reconstruction (GU). Each focus area addresses the major tissues affected by trauma. The research addresses both restoring and regenerating tissue at the component and complex integrated structure levels. Strategies employing degradable polymers, composites, pharmacologic agents, devices, bone marrow, stem cells, tissue constructs, and immune and cell growth modulation, as well as means to monitor and assess tissue restoration/regeneration, are among the many candidate solutions under exploration. The goals of the research programs are to restore not only form and cosmetic appearance to the injured areas, but also to provide full functional recovery.



Research Highlights

AFIRM-sponsored researchers are making substantial contributions to the scientific knowledge base, including presentations, publications, and novel patentable discoveries in the field of regenerative medicine. In the sixth year of the program alone, the investigators published more than 50 articles in peer-reviewed journals and produced over 80 presentations and non-peer-reviewed publications. During the first six years of the AFIRM, more than 110 invention disclosures were reported by investigators, of which more than 90 patent applications were filed with government patent offices, and the U.S. Patent and Trademark Office has granted 14 patents.

Some of the recent research accomplishments include:

- Using molecular surface design to tether bioactive molecules, such as biotinylated recombinant human bone morphogenetic protein-2, to bone repair scaffolds to control tissue responses and regeneration.
- Demonstrating eNOS competent endothelial-like cells lining tissue engineered blood vessels promotes long-term implantation success.
- Developing bioengineered muscle constructs that have skeletal muscle-like structure, integrate with and are perfused by the host vasculature, become innervated, and respond to electrical stimulation with neural-like signals.
- Bioprinting of three-dimensional tissue constructs, including bone, cartilage, and muscle, with corresponding structural elements formed and cellular proliferation and differentiation observed.
- Developing a patient conformable, highly absorbent, sustained iodine releasing wound dressing with in vitro release characteristics superior to commercially available devices and anti-bacterial and anti-fungal activity.
- Demonstrating the ability to bioprint skin cells directly on wounds and third-degree burns in a large animal model, with subsequent tissue repair and healing.
- Combining human Leydig, Sertoli, and spermatogonial stem cells in spheroid culture to produce compact organoids with cellular organization and structure similar to a human testis tubule.
- Developing and initial validating of a rabbit model of partial sphincterectomy to serve as a model for studies of treatments for injured anus and passive incontinence in humans.
- Showing a portable perfusion system, as a means to increase preservation time of isolated limbs, could reliably perfuse a limb for 12 hours, saturate the perfusion system with oxygen, and maintain some tissue viability without muscle fiber damage in the limb.
- Establishing in a rat model the ability of chimeric cells to migrate, become engrafted, and survive in vivo, and thus potentially form the basis of a novel cellular therapy to eliminate the need for lifelong immunosuppression with vascularized composite allograft transplantation.

Translating Promising Solutions

Sponsors have advanced to or near the clinical evaluation phase for a number of products including novel therapeutics and products previously approved by the U.S. Food and Drug Administration (FDA). In the sixth year of the program, sponsors submitted 4 Investigational New Drug (IND; drug and biologic products) and Investigational Device Exemption (IDE) applications for approval to conduct clinical studies; and 4 INDs and 2 IDEs were approved. Other sponsors have initiated pre-IND and pre-IDE meetings with the FDA to discuss regulatory and clinical trial strategies, and have begun to prepare IND or IDE applications. Eight (8) studies have been submitted to Institutional Review Boards or human research protection offices for review. Also in the sixth year of the program, six clinical trials were open to patient enrollment.

Some of the potential solutions under or pending clinical evaluation include:

- A polycaprolactone fumarate biodegradable polymer-based nerve conduit for guiding nerve regeneration across large gaps.
- Conversion of skin cells to palmo-plantar skin at the prosthetic-stump interface in order to reduce pain, discomfort, and skin breakdown.
- An Ex Vivo Produced Oral Mucosal Equivalent for the repair of large intraoral defects, and potentially other mucosal structures (e.g., lips, eyelid, and anterior nares).
- An implantable and injectable vascularized soft tissue, comprised of connective tissue and fat, for craniofacial reconstruction.
- A novel coupled polymerase chain reaction-mass spectrometric technology for detecting a wide spectrum of pathogens as well as antibiotic resistance markers in burn wounds.
- StrataGraft® (Stratatech) skin tissue as an alternative to autografting for full thickness skin defects in complex areas of the body such as the hands, face, and feet.
- An anti-TCR monoclonal antibody, TOL-101 from Tolera Therapeutics, to enhance tolerance to transplantation.
- Stromal vascular fractions of white adipose tissue for promoting healing and modulating immune response to treat graft rejection episodes in hand transplants.

Looking Forward

Critical to the success of the AFIRM will be the continued integrated and collaborative network of academic and industry members central to WRC. Collaboration and partnering with the military research and clinical communities, as well as others, will also be instrumental in advancing the AFIRM's mission. With the maturation of many areas of regenerative medicine research, the AFIRM is expected to generate a robust pipeline of potential solutions and translate these into clinical evaluations in the future.

I: Introduction

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Background

Nearly 6,900 U.S. military fatalities and more than 52,000 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 areas of the face, neck, head, limb, genitourinary and abdominal regions, with multiple, complex injuries to skin, muscle, nerve, blood vessels, bone, and connective tissues, or loss of large portions of these tissues.

[1] As of August 7, 2015; *http://www.defense.gov/news/casualty.pdf*.
[2] Belmont, et al. *J Trauma Acute Care Surg*. 2012 Jul;73(1):3-12.

I: Introduction

Therapies developed by the award recipients of the Armed Forces Institute of Regenerative Medicine program (AFIRM) are intended to aid these traumatically injured wounded warriors. Regenerative medicine encompasses many novel approaches to the treatment of damaged tissues and organs by promoting the self-regenerative capacity of the body. Furthermore, integrating cells with other technologies may create engineered tissues and organs for therapeutic use. These innovations are possible because of several key advances, including the development of systems that reliably induce progenitor and stem cell proliferation and differentiation, the discovery of growth factors that control tissue morphogenesis, and derivation of biomaterials and scaffolds that guide tissue regeneration and reconstruction.

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. His message 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 at the U.S. Army Medical Research and Materiel Command (USAMRMC), COL Bob Vandre, developed the idea of a regenerative medicine institute similar to the Multidisciplinary University Research Initiatives program of the Department of Defense (DoD), but aimed at near-term, translational research.

In 2007, USAMRMC, the Office of Naval Research, the U.S. Air Force Office of the Surgeon General, the National Institutes of Health (NIH), and the Veterans Health Administration of the Department of Veterans Affairs (VA) agreed to co-fund the new institute. Following a request for proposals, two finalists were selected for oral presentations in December 2007. Both received scores of "excellent," and one was selected for funding. White House staffers heard about the AFIRM and invited representatives from USAMRMC to meet and discuss the new institute. After two meetings, and upon hearing that there was funding for only one AFIRM finalist, the DoD was tasked to provide funding for the second AFIRM finalist. Within one week, additional funding was transferred to USAMRMC's budget lines. Both AFIRM finalists signed USAMRMC cooperative agreements in March 2008.

In September 2008, the Clinical and Rehabilitative Medicine Research Program (CRMRP) was officially established in recognition of the need to expand the USAMRMC's traditional research focus to include definitive and rehabilitative care innovations required to "reset" the terms of duty performance and quality of life of wounded Soldiers. It is the lead organization for program development and oversight of the AFIRM.

From 2008 to 2013, the AFIRM was composed of two independent civilian research consortia: the Rutgers–Cleveland Clinic Consortium (RCCC) and the Wake Forest–Pittsburgh Consortium (WFPC). These consortia worked closely with the U.S. Army Institute of Surgical Research (USAISR) at Fort Sam Houston, Texas. USAISR, which is home to the DoD's only burn unit, is co-located with the San Antonio Military Medical Center – North (formerly Brooke Army Medical Center) and the Center for the Intrepid, a treatment, rehabilitation, and research center for care of severely wounded warfighters.

Current Structure

In 2013, with the period of performance ending on the original contracts, the government wished to build upon the success of the first 5 years of the AFIRM program. In particular, there was interest in improving the translation of promising regenerative medicine technologies into clinical use, as well as in addressing the emerging clinical concern of treating traumatic genitourinary and abdominal injuries. Therefore, the government released a Request for Proposal to create "AFIRM II." Following an open competition, the proposal from the Wake Forest University School of Medicine (Wake Forest Baptist Medical Center)-led consortium was selected. This next phase of AFIRM established the Warrior Restoration Consortium (WRC), which included members from both of the initial AFIRM consortia—RCCC and WFPC—along with new investigators. This team creates a broad coalition

of leading academic partners and companies that have the resources and experience to deliver on the promises made in the AFIRM II proposal. The WRC has cemented a series of partnerships with other researchers that add further breadth and depth to the project teams. The management of the WRC capitalizes on the successful approaches learned during the original AFIRM award, and incorporates important lessons learned.

The WRC consists of the following member institutions:

- Brigham and Women's Hospital
- Cleveland Clinic Foundation
- Georgia Institute of Technology
- Jewish Hospital and St. Mary's Healthcare
- Johns Hopkins University School of Medicine
- Livionex, Inc.
- Massachusetts General Hospital
- Mayo Clinic and Foundation, Rochester
- New York University School of Medicine
- Northwestern University
- Oregon Health and Science University
- Rutgers, State University of New Jersey
- Southwest Research Institute
- Stanford University
- State University of New York, Stony Brook
- Stratatech Corporation
- University of California, Los Angeles
- University of Cincinnati
- University of Colorado, Denver, Anschutz Medical Campus
- University of Connecticut, Storrs
- University of Florida
- University of Illinois, Chicago
- University of Maryland School of Medicine
- University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School
- University of Michigan
- University of Pittsburgh
- University of Virginia
- Vanderbilt University
- Wake Forest Institute for Regenerative Medicine
- Wake Forest School of Medicine
- Wake Forest University Health Sciences

The mission of the WRC is **to deliver to the Armed Forces regenerative medicine-based technologies that will lead to functional and aesthetic recovery from injuries incurred in military service,**

while its vision is **to become the international leader in developing restorative therapies for battlefield trauma.**

Research Goals

The AFIRM combines the efforts of the nation's leading experts in regenerative medicine into a team whose work spans from research and development to clinical translation, implementation, and commercialization. The AFIRM is focused on addressing combat trauma, with therapies aimed at treating extremity and craniofacial trauma, skin and genitourinary injuries, and vascularized composite allotransplantation. Clinical trials have begun for several AFIRM products, including advanced transplantation strategies, skin replacement technologies, and scar prevention/remodeling.

By partnering with health professionals in the Armed Forces, AFIRM acts as a catalyst to bring advances from the nation's leading regenerative medicine laboratories to the warfighter. The AFIRM integrates its expertise with military clinical research efforts to form teams that develop and implement solutions for tissue deficits that restore function and, where relevant, cosmetic appearance. AFIRM intends to serve as: 1) the military's source for regenerative medicine technologies, 2) a training ground to prepare experts for the treatment of trauma with regenerative medicine, 3) a resource where military health care professionals can find expertise and collaborators to engineer solutions to identified needs in tissue replacement, and 4) a nexus through which industrial and academic partners in the field of regenerative medicine can pair with AFIRM and military professionals to develop and implement healing strategies.

Programs and Projects

The overall goal of the AFIRM is to provide technologies and devices that will lead to the functional and aesthetic recovery of the wounded warrior. With this in mind, one of the aims of the AFIRM is to position promising technologies and therapeutic/restorative practices for entrance into human clinical trials, with the ultimate goal of helping the warfighter. This will be done through translational regenerative medicine research and development, including basic scientific research, development of animal models, preclinical studies required for initiation of U.S. Food and Drug Administration-regulated human clinical trials, and Phase I–II human clinical trials. To do this, an expert team from multiple academic institutions and industry partners was assembled to satisfy the objectives of the AFIRM. This experienced, diverse team of medical and scientific professionals will operate in an outcome-driven environment that efficiently designs, tests, compares, and evaluates discoveries leading to the selection of promising technologies for translation to investigational human studies. It is envisioned that integration of basic science, translational, and clinical research efforts will be necessary to advance effective regenerative medicine into treatments for combat-related injuries. It is also envisioned that the AFIRM will continue to collaborate with government laboratories and Medical Centers/Medical Treatment Facilities including the Army, Navy/Marine, Air Force, NIH, and the VA to advance promising technologies to the wounded warrior. The AFIRM addresses the five focus areas identified as critical areas of need to help the wounded warrior.

The Major Focus Areas

Extremity Regeneration

The Extremity Regeneration focus area is a multi-institutional, multi-disciplinary collaborative effort spanning basic science, translational research, and clinical trials, with the goal of enabling those who have sustained severe extremity trauma to recover rapidly, reliably, and completely, so they can return to duty and live productive lives. This focus area is developing a broad spectrum of approaches that can be used as a single intervention or in combination to treat a large spectrum of severe extremity injuries to bone, muscle, nerve, and vasculature, where current therapeutic approaches fail to provide adequate functional recovery. All approaches move the technologies from research to commercialization and clinical deployment in a practical, product-oriented, efficient, leveraged, and cost-effective tactical strategy.

Craniomaxillofacial Regeneration

Defects of the face, in particular, have a devastating impact on the quality of life, emotional health, and socioeconomic opportunities of patients. The overarching goal of the craniomaxillofacial regeneration focus area is to develop strategies and tools to aid in craniofacial reconstruction for the wounded warrior with greater fidelity and fewer procedures. The technologies being developed by this program will benefit not only wounded warriors, but also civilians suffering from traumatic injury, cancer, congenital defects, and other debilitating diseases. The current portfolio includes innovative strategies—synthetic polymers, ceramics, decellularized extracellular matrix, composites, and others—to regenerate craniofacial bone, muscle, soft tissue, and non-articular cartilage and dentition. Investigators are advancing strategies such as three-dimensional printing, implantable in vivo bioreactors, wound infection treatment, and the concurrent regeneration of multiple tissue types. These materials and strategies are being tested in a variety of animal models, and if successful, may be followed by approved clinical trials in humans.

Skin Regeneration

Functional outcome and quality of life following burns or other traumatic skin injuries depends on the quality of healing, degree of scarring, and cosmetic appearance of the healed wounds. Developing effective therapies for Soldiers with complex battlefield wounds requires a multifaceted approach that promotes tissue regeneration while mitigating fibrosis. Given the dire unmet need, a broad approach has been taken that encompasses biotech, pharma, and device approaches to both mitigate skin injury and encourage true regenerative healing, such as growth factor/cytokine modulation, improved cell-based therapies, better scaffolds, novel skin substitutes, and new skin replacement techniques.

Genitourinary/Lower Abdomen Reconstruction

The Genitourinary/Lower Abdomen Reconstruction focus area philosophy is dedicated to repairing battlefield injuries through the use of regenerative medicine technologies. The main goal of this focus area is to place promising regenerative medicine technologies on a path to human clinical trials. The projects focus on urethral, bladder, penis, testes, and anal reconstruction to address the types of injuries sustained by dismounted troops encountering improvised explosive devices. Not only will the results from the studies contribute to the treatment of wounded warriors, but these will provide a much wider contribution to the health and well-being of the civilian population that suffer from debilitating genitourinary dysfunction due to injuries and disease.

Composite Tissue Allotransplantation and Immunomodulation

The investigators in the Composite Tissue Allotransplantation (CTA) and Immunomodulation focus area are comprised of scientists and clinicians from the leading CTA centers in academic institutions. The proposed projects range from basic science research, through the translational investigations, to clinical trials, and aim to achieve optimal graft conditions for transplantation, develop highly sensitive, non-invasive methods of rejection surveillance, immunomodulation, and tolerance induction with the overall goal of minimizing or eliminating long-term immunosuppressive treatment, and improve the safety and outcomes in CTA. The investigators will utilize bone marrow and stem cell-based immunomodulatory strategies, novel

technologies to monitor the immune response post transplantation, and treatment protocols that abrogate the adverse effects of long-term immunosuppression. The focus area portfolio consists of projects employing diverse approaches at varying stages of scientific development ranging from hypothesis development and proof of concept, to data accumulation and treatment implementation.

Funding – A Federal Partnership

The AFIRM is financed with basic research through advanced technology development funds. The program is managed by the USAMRMC, and funded through a federal partnership (**Figure 1**). Total funding for the first 5 years of the AFIRM ultimately amounted to more than $300 million (M). This figure includes core program funds from the U.S. government; matching funds received from state governments and participating universities; funds from pre-existing research projects; and additional funds provided by the Defense Health Program.

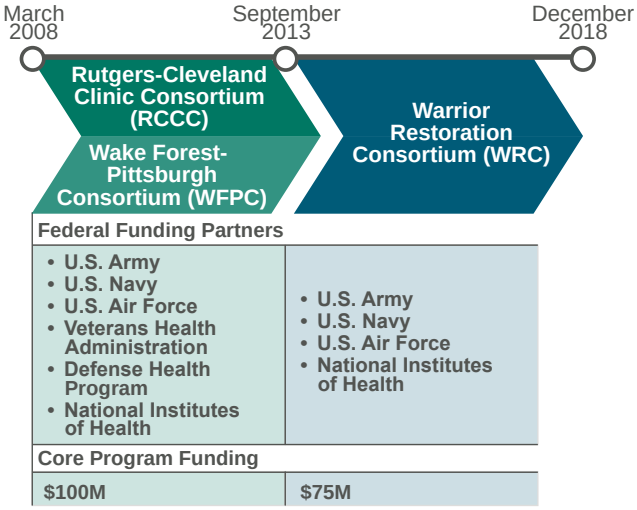

Figure 1. AFIRM Funding Structure

Management and Oversight

Our wounded warriors need regenerative medicine technologies to accelerate healing after severe injuries. In addition, the U.S. government's extraordinary financial commitment to establishing this capability brings with it the responsibility for world-class stewardship, responsiveness, and management. Management of the WRC differs from traditional models of university-based inter-institutional collaboration because of the requirement of a broad array of research and clinical advancements within a relatively short time. The needs of the Armed Forces often outpace academic timelines, and for this reason, a unique organizational structure has been established that maximizes the value of experience and demonstrated skill sets. The structure is designed to provide exemplary oversight of the programs and procedures, transparent processes for selection and de-selection of research programs, and, most importantly, protection for the human subjects who will bravely volunteer to participate in clinical trials.

The AFIRM program's direction is led by the CRMRP office. Oversight of the AFIRM projects is conducted by the Tissue Injury and Regenerative Medicine (TIRM) Project Management Office (PMO). Guidance by a Board of Directors (BOD) and an Integrating Integrated Project Team (IIPT), which contains a Steering Group, is part of the overall management plan. The roles and membership of each of these entities are described as follows.

Clinical and Rehabilitative Medicine Research Program

The CRMRP is the lead for program development and oversight of the AFIRM, a multi-institutional, interdisciplinary network focused on developing advanced treatment options for severely wounded Service members. The CRMRP also more tightly links the USAMRMC research and development community with the clinical investigations community of the U.S. Army Medical Command and the Military Health System.

Tissue Injury and Regenerative Medicine Project Management Office

The day-to-day execution of the AFIRM's research portfolio is managed by the TIRM PMO, which is located within the U.S. Army Medical Materiel Development Activity at Fort Detrick, Maryland. The TIRM PMO functions as an accountability model to ensure execution of the AFIRM portfolio.

Board of Directors

The AFIRM's BOD is chaired by the Commanding General, 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 IIPT.

Integrating Integrated Project Team

The AFIRM's IIPT is chaired by the Director of the CRMRP. IIPT 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 IIPT is to ensure that the AFIRM meets military needs, funds superior science, and is well managed.

The specific responsibilities of the IIPT 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.
- 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 IIPT's Steering Group has day-to-day decision-making authority over the AFIRM, and it recommends major changes in research direction or funding to the voting members of the IIPT. 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 AFIRM. 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 IIPT.

The IIPT 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 IIPT also has ex officio advisors from the Judge Advocate General, the DoD Human Research Protections Office, a commercialization expert, and a regulatory expert appointed by the CRMRP.

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

AFIRM I: Leadership of Rutgers–Cleveland Clinic Consortium and the Wake Forest–Pittsburgh Consortium

The RCCC was 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 WFPC was 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.

AFIRM II: Leadership of the Warrior Restoration Consortium

The Directors and Officers managing the WRC include the following:

- Consortium Director and Principal Investigator: Anthony Atala, MD
- Consortium Co-Director: Rocky Tuan, PhD
- Chief Scientific Officers: James Yoo, MD, PhD, and William Wagner, PhD
- Chief Operating Officer: Benjamin Harrison, PhD

In addition to these key members, there is an oversight cabinet composed of the Directors and additional experienced members Joachim Kohn, PhD, and Linda Graham, MD, former directors of the RCCC. Program leadership and team leaders play a significant role for each program/focus areas. See **Figure 2** below for the organizational structure of the AFIRM-WRC.

Building Relationships with the Military

Critical to the success of the AFIRM are the partnerships and relationships between the academic and industry members of the WRC and the military research and clinical communities. The Regenerative Medicine Traveling Exchange Program, established by the TIRM PMO, will facilitate relationships by having military investigators travel to various regenerative medicine sites funded through USAMRMC, including those in the WRC, to enhance information exchange and build partnerships.

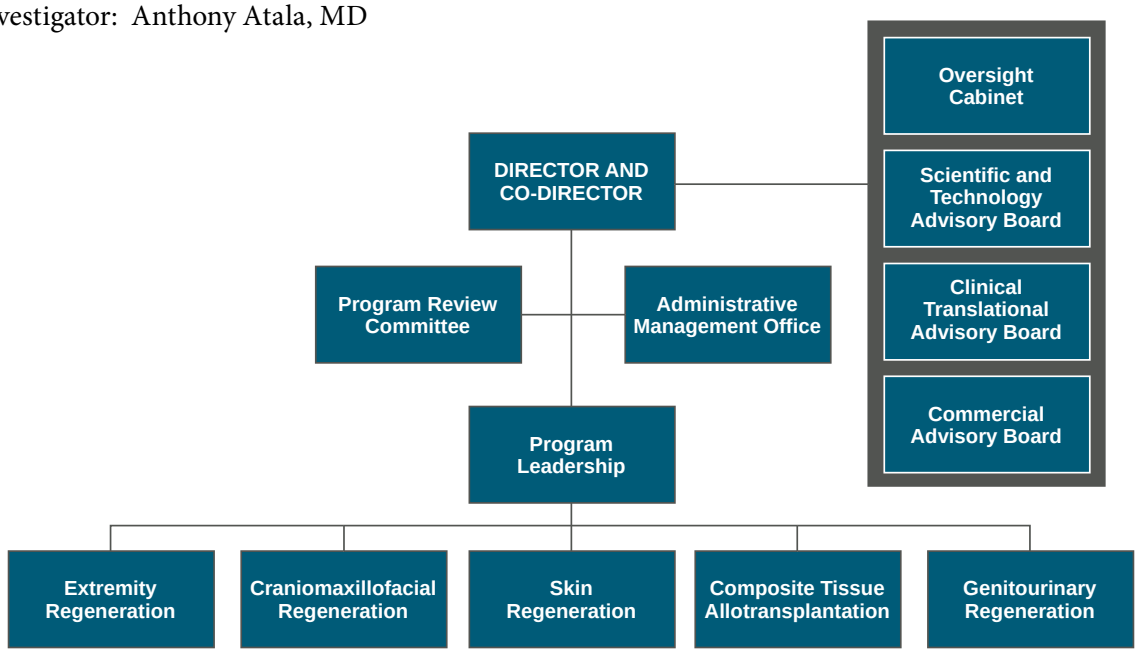

Figure 2. AFIRM-WRC Organization Structure

II: Extremity Regeneration

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Background

Warfighters in combat often suffer severe trauma to the extremities, and this can result in large areas of tissue loss as well as complex injuries involving multiple tissue types. Injuries may involve bone, nerve, blood vessels, cartilage, and skeletal muscle. The healing and regenerative properties of these tissues may be compromised in such catastrophic injuries. Loss of tissue volume, scarring, and atrophy impairs or prevents the functioning of the extremity following wound healing. In addition to functional loss, attempts at limb salvage may be unsuccessful and result in subsequent amputation of the non-functioning or poorly healing limb. Using autologous (patient's own) tissue from other body sites requires additional surgeries and has post-surgical effects (e.g., pain, bleeding, scarring, and possibly functional loss), and sufficient tissue may not be available for harvest to restore severe trauma such as significant bone loss. Further, such surgeries may not be possible in traumatically injured patients. Despite the current advanced surgical, restorative, and rehabilitative care capabilities, these injuries frequently lead to an inability for Warfighters to return to active duty, and they often create life-long disability.

II: Extremity Regeneration

The AFIRM's Extremity Regeneration Program addresses the restoration of all types of extremity tissue. Additionally, the program seeks to limit the progression of injury due to insufficient blood flow (ischemia) in traumatic injuries and preserve the extremities. Recovery of the complex functioning of extremities is an important objective, and the program is investigating means to not just restore structures but to ensure that they become functionally integrated with the surrounding tissues. Advances from the program are expected to preserve and restore damaged or missing tissue following injury, reduce amputation rates, reduce time and risk involved in healing, and enable full functional recovery.

Areas of Emphasis

The goal of the AFIRM's Extremity Regeneration Program is to develop and advance regenerative medicine-based approaches to restore tissue and function to the traumatically injured extremities. The AFIRM researchers are focusing on a broad spectrum of approaches, as single or combination interventions. The current portfolio of projects investigates tissue engineering approaches to repair tissue loss such as the stimulation of native regenerative properties of tissue, seeding wounds with regenerative cells (stem and progenitor cells), tissue scaffolds, controlling fibrosis, modulating growth factors and oxygen in the wound environment, preventing infection, and spatially guiding regeneration through structural and biochemical means. The researchers are testing these novel strategies and materials in small and large animal models, including newly developed models that may better mimic human healing processes, as well as clinical trials of promising therapies. As shown in **Table II-1**, projects are grouped into six clinical challenge topic areas: bone, muscle, nerve, blood vessel, joint, and compartment syndrome. A summary of each project is provided. Clinical trials are not included in Table II-1 and are instead presented in a separate section of this chapter.

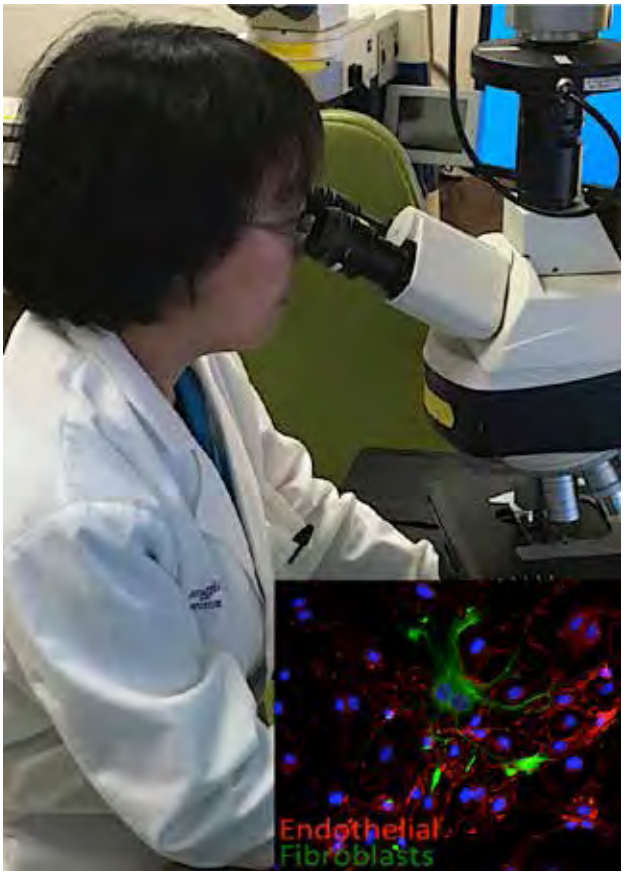


Table II-1. Projects funded by RCCC, WFPC, and WRC by clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Bone	RCCC	4.2.1	Advanced 3D Scaffolds for Large Segmental Bone Defects: Non-Load Bearing Tyrosine-Derived Polycarbonate Scaffolds
		4.2.2	Optimizing Cell Sources for the Repair of Bone Defects
		4.2.2	Point of Care Autologous Stem Cell Concentrate for Bone Defect Repair
		4.2.3	Advancing Bone Repair using Molecular Surface Design: Biodegradable Scaffolds with Tethered Osteoinductive Biomolecules
		4.8	Improved Preclinical Model for Orthopaedic Trauma
	WRC	ER-02	Spatiotemporal Regenerative Strategies to Restore Function to Severely Injured Extremities
		ER-09	Functionalized Allograft for Large Scale Bone Defect Healing
		ER-10	Enhancement of Bone Regeneration through Cell-Based Strategies
Muscle	WRC	ER-06	In Situ Influence of Cell Fate for Functional Soft Tissue Reconstruction
		ER-12	Tissue Preservation of Ischemic Skeletal Muscle Using Oxygen Generating Biomaterials
Nerve	RCCC	4.4.1	Repair of Segmental Nerve Defects: A Theragnostic System Solution for Optimal Nerve Repair – Prevention of Muscle Atrophy
		4.4.2a	Cells and Bioactive Molecules Delivery in Peripheral Nerve Restoration
		4.4.2c / 4.4.2d	Repair of Peripheral Nerve Injury using Tissue-engineered Nerve Grafts Encased in Biodegradable Nerve Guidance Tubes
	WFPC	4.4.4	Peripheral Nerve Repair
	WRC	ER-03	Biodegradable Conduits for Large Extremity Nerve Injuries
		ER-04	Tissue Engineering for Repair of Critical Nerve Gaps
Blood Vessel	RCCC	4.3.2	Development of Tissue (Peritoneum) Lined Bioabsorbable and Fracture-Resistant Stent Graft for Vessel Trauma
		4.3.2	Construction of Tissue Engineered Blood Vessels
		4.5.6	Vascular Tissue Engineering
	WRC	ER-07	Engineered Small Diameter Blood Vessels for Limb Reconstruction
Joint	RCCC	4.4.3b	Functional Scaffolds for Soft Tissue Repair and Joint Preservation
	WFPC	4.4.5	Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes
	WRC	ER-13	Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes
		ER-14	Development of a Novel Medical Device to Restore Knee Function & Prevent Osteoarthritis
Compartment Syndrome	WFPC	4.3.2	Use of Bone Marrow Derived Stem Cells for Treatment of Compartment Syndrome
	WRC	ER-05	Development of Biological Approaches to Improve Functional Recovery after Compartment Syndrome Injury

Clinical Challenge Bone

The AFIRM researchers are focused on developing strategies for the repair of large bone defects. They are focused on materials for scaffolds to support the regeneration of bone, as well as ways to modify the scaffold surface to enhance bioavailability of osteoconductive molecules. Additionally, researchers are optimizing the harvesting and isolation of cells from bone marrow and other areas to seed scaffolds and enhance bone reconstruction. In order to provide a model with better predictive value for results in human, researchers developed the chronic caprine tibial defect (CCTD) model.

RCCC 4.2.1

Advanced 3D Scaffolds for Large Segmental Bone Defects: Non-Load Bearing Tyrosine-Derived Polycarbonate Scaffolds

Team Leader: *Shuang Chen, PhD Candidate, Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University; New York University, NYU*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project seeks to identify the most promising materials for advanced scaffolds for treating bone defects. A "tournament" design, comparing pairs of scaffolds, is employed to enable rapid downselection of material designs for advancement using established animal models.

Accomplishments: Using a hierarchy of animal models, the team evaluated five scaffold substrate materials: poly(glycolic-co-lactic acid), poly(lactide-cocaprolactone), tyrosine-derived polycarbonate (TyrPC), poly(propylene fumarate), and tri-calcium phosphate (TCP) ceramic granules. Also assessed were methods for three-dimensional (3D) scaffold fabrication, including Three Dimensional Printing™, laser stereolithography, and porogen-leaching. Early results demonstrated that adding calcium phosphate (CaP)-based minerals enhanced the performance of TyrPC scaffolds significantly, and the project was extended to optimize the mineral component. The team synthesized four CaP variations with varying solubility and incorporated these into TyrPC polymer to create composite scaffolds. They screened the E1001(1k) bone regeneration scaffolds containing different CaP formulations in a rabbit calvaria defect model. While the final evaluation is pending, the results show the TyrPC+CaP were biocompatible and regenerated bone, as assessed by microCT.

Future: The team will complete the rabbit calvaria model studies of TyrPC+CaP scaffolds and the goat calvaria critical size defect model studies of TyrPC+CaP+BMP. This project has transitioned to the AFIRM-WRC program. Under that effort, TyrPC+CaP scaffolds will be fabricated, characterized, and evaluated using a sheep tibia segmental defect model.

RCCC 4.2.2

Optimizing Cell Sources for the Repair of Bone Defects

Team Leader: *George Muschler, MD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *Cleveland Clinic*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: The goal of this project is to assess promising methods for the harvesting, processing, and transplanting of osteogenic connective tissue progenitors (CTP-O; cells that are capable of making new bone) to influence bone regeneration. The comparison of methods used both the canine femoral multidefect (CFMD) and the CCTD model.

Accomplishments: The team compared three different methods for marrow processing: Density separation (DS) (using a centrifuge to remove red blood cells and serum), selective retention (SR) (based on the fact that CTP-Os attach very readily to some surfaces as a means for concentration and selection), and magnetic separation (MS) (magnetically labeling CTP-Os and then preferentially collecting them with a magnet). All of these methods can increase CTP-O concentration. SR and MS processing can also increase CTP-O prevalence (i.e., reduce the number of non-CTPs). The team used the CFMD model to compare bone scaffold materials in the early phase of this project, and a mineralized cancellous allograft (MCA) scaffold was selected for use in comparing the marrow processing methods. Cells processed using each of these methods resulted in excellent reconstitution of bone and marrow tissue in a bone defect site when transplanted on an MCA scaffold. The team completed efforts which demonstrated that all three methods improve cell and CTP concentration, and support excellent reconstitution of bone and marrow tissue in a bone defect site when transplanted on an MCA scaffold. SR or MS processing also improves CTR prevalence. The team concluded that all three methods are viable for further study in a more stringent animal model. Work under other AFIRM projects (4.2.2 and 4.8) involves development of a CCTD model and has suggested the goat sternum as a better source of osteogenic bone and marrow than humerus or pelvis. The CCTD model could be used to further study the DS, SR, and MS cell-sourcing methods.

Future: This RCCC project has closed. The next steps would involve further refinement and assessment of the cell-sourcing methods in the CCTD model, including combinations with advanced scaffolds, surgical methods, and/or drug delivery systems.

RCCC 4.2.2

Point of Care Autologous Stem Cell Concentrate for Bone Defect Repair

Team Leader: *Brian Barnes, PhD (Arteriocyte, Inc.)*

Team/Collaborating Partner Institution: *Arteriocyte, Inc.; USAISR; Rutgers University*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project is evaluating the use of concentrated bone marrow aspirate (BMA) for regenerating bone. The Magellan®MAR01™ system is used to concentrate BMA-derived nucleated cells and mesenchymal stem cells (MSCs), and studies are being conducted in a rabbit radial segmental defect model.

Accomplishments: The investigators evaluated the use of CBMA in a rabbit radial segmental defect model using microCT and histological analysis. They tested three types of bone graft materials with cBMA: demineralized bone matrix (DBM) material plus gel MARO-Match™/MARO-Fuse™ (Arteriocyte), collagen-beta TCP (bTCP) composite Mozaik™ scaffolds (Integra Lifesciences), and TyrPC E1001(1K) scaffolds (Rutgers University). Bone marrow was harvested from donor rabbits, filtered, and concentrated with Magellan® MAR01™ System. Preliminary data suggests the system provided a 4.4-fold enrichment in MSCs, and 1.8-fold enrichment in nucleated cell concentration in the cBMA fraction, over their respective baseline values. Animals were implanted for 8 weeks prior to evaluation of the defect. In both microCT and histomorphometry analyses, while the DBM overall had a better mean % bone formation compared to the other materials tested, there was no statistical difference in %BV between BMA and cBMA use in all three scaffolds. Further evaluation in more stringent models with larger defects, such as a sheep critical size tibial defect model, is needed to determine if cBMA is beneficial.

Future: This RCCC project is closed. Under AFIRM-WRC, the investigators will evaluate the allogeneic bone chips (i.e., DBM) with an antibiotic and cBMA.

RCCC 4.2.3

Advancing Bone Repair using Molecular Surface Design: Biodegradable Scaffolds with Tethered Osteoinductive Biomolecules

Team Leader: *Zheng Zhang, PhD, Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University*

Target Clinical Application: *Reconstruction of Bone/ Cartilage Defects*

Goals: This project is investigating the use of molecular surface design to tether a bioactive molecule, such as biotinylated recombinant human bone morphogenetic protein-2 (b-rhBMP-2) via streptavidin, to the surface of bone repair scaffolds to control tissue responses and regeneration. The effort involves developing scaffold materials and studies in a rabbit calvarial defect model.

Accomplishments: The team conducted a rabbit calvarial defect study of E1001(1k)-β-TCP (E1001(1k)- β-TCP) scaffolds tethered with b-rhBMP-2 via Streptavidin. Assessments after 4 weeks of regeneration included microCT analysis, histological assessment, and histomorphometry. MicroCT and histological data showed the woven bone formation was similar in tethered versus adsorbed b-rhBMP-2 groups. The team is also developing a flexible and elastic polymer for use as the cartilage-forming layer of a biphasic scaffold – previously used as a technique to fabricate biphasic (bone- and cartilage-generating layers). The team synthesized flexible polymers of DTE-DT-PTMC (DTE = desaminotyrosyl tyrosine ethyl ester; DT = desaminotyrosyl tyrosine) and conducted mechanical testing to evaluate the tensile stress-strain properties and elasticity. By tuning the polymer composition, they can obtain a range from rigid to viscous in nature – flexible copolymers with 27% PTMC were elastic. The team conducted 3-week and 3-month in vivo degradation and biocompatibility studies in rats. In vivo degradation (molecular weight loss) was slower than in vitro due to less fluid exposure. Fibrous capsules formed around the implants and was considered a normal tissue response. Use of a tethering approach will allow for a potentially lower dose of b-rhBMP-2 and prevent diffusion into the cartilage-forming layer or other tissue areas.

Future: The team will complete the evaluation of the in vivo degradation of the cartilage-forming polymers. Under AFIRM-WRC, the team will continue development of biphasic scaffolds.

RCCC 4.8

Improved Preclinical Model for Orthopaedic Trauma

Team Leader: *G Elizabeth Pluhar, DVM, PhD (University of Minnesota), Joan Bechtold, PhD (Minnesota Medical Research Foundation), George Muschler, MD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *University of Minnesota; Minnesota Medical Research Foundation; Cleveland Clinic*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project developed the CCTD model as a relevant large animal model that, similar to humans, does not heal after grafting with the existing standard of care, and thus may be better predictor of human results than other preclinical models. The effort involved developing and confirming the model, establishing surgical standard operating procedures, and testing various scaffold and graft strategies under development.

Accomplishments: The model created by the team involves a bone defect in the tibia (lower leg) of Spanish-Boer goats. They confirmed that bone did not form when treated with the current standards of practice (fresh autograft or morsellized allograft bone). Using radiographs and microCT information, they demonstrated that defects treated with MCA bone has the least bone formation while treatment with MCS+ BMA + recombinant human bone morphogenetic protein-2 (BMP-2) was the greatest. The addition of BMP-2 to allograft bone, with or without BMA, increased the amount of new bone formation. Additionally, BMA and cancellous bone graft harvested from the sternum had a higher prevalence of osteogenic cells compared to the proximal humerus and ilium, and bone graft harvested from the sternum resulted in a greater amount of bone formation in the defects. The membranes induced by polymethylmethacrylate spacers are composed of fibroblast-like cells in a loose collagenous matrix with few to no inflammatory cells seen; and there was some variation in membrane weight and cellularity among animals. Analysis of the membrane data is ongoing. The analysis of the gene expression/biochemical properties of the induced membranes included measures of relative concentrations of BMP-2, hyaluronan synthase, platelet derived growth factor, and epidermal growth factor via ELISA assay. Polypropylene fumarate implants (developed by Mayo Clinic) with rhBMP-2 added were assessed in the model, and complete bone bridging was noted in some animals by radiography and microCT.

Future: The team will continue to characterize the membranes induced by the spacers under an award from the Congressionally Directed Medical Research Programs Peer Reviewed Orthopaedic Research Program.

WRC ER-02

Spatiotemporal Regenerative Strategies to Restore Function to Severely Injured Extremities

Team Leader: *Robert Guldberg, PhD (Georgia Institute of Technology)*

Team/Collaborating Partner Institution: *Georgia Institute of Technology; University of Queensland*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: The goal of this project is to understand the biology of non-union and harness this knowledge to develop tissue-engineered treatment strategies for prevention and treatment of non-union in large bone defects and composite tissue limb injuries. The study will look at the interactions between vascular status, age, BMP-2 dose, and spatiotemporal variations in bone regeneration, and investigate the use of a heparin microparticle-based carrier system for growth factors (BMP-2 and vascular endothelial growth factor [VEGF]).

Accomplishments: The team began characterizing the regenerative microenvironment profile in healing and non-healing conditions using three rat models: chronic non-union (from large, critically sized segmental defects), mechanical instability (from mechanical perturbations), and composite non-union (from large adjacent soft tissue loss). Gene expression and immunohistochemistry were the primary characterization methods. Femoral segmental defects in rats were treated either with the hybrid delivery system with 5ug BMP-2 or with crushed femoral diaphyseal autografts. Autograft-treated defects showed lower new bone formation, localized mainly around the grafts, and inferior mechanical properties. A pilot study using the chronic non-union model is ongoing to characterize bone formation after delayed treatment (no- or sub-healing doses of BMP-2). Preliminary data suggested differences in early inflammatory and osteogenic gene expression based on age (8- and 10-week old animals) . Characterization of early gene expression in segmental defects in ischemic limbs was performed to understand the paradoxical improvement in bone healing in ischemic limbs, but while osteogenic and angiogenic gene expression increased over time, there was not significant effect of ischemia. Expression of osteogenic genes was reduced in the absence of BMP-2. Early cell populations in healing (2.5ug BMP-2) and non-healing (untreated) segmental bone defects were investigated to determine differences in osteoprogenitor cells (MSCs) and inflammatory cells (macrophages) in the defects. The results suggest that healing defects may recruit osteoprogenitor cells and resolve acute inflammation faster, and switch to a pro-healing environment. The team is also working on developing a spatiotemporal cytokine (BMP-2 and VEGF) delivery system to combat non-union. They are evaluating three strategies: spatiotemporal control of growth factor release, spatial localization of bone formation, and treatment of non-union by usage of high-dose BMP-2; as well as the effects of heparin and amnion in spatiotemporal control. They investigated the retention and release of growth factors from heparin methacrylate microparticles and the ability of amniotic membrane (sheet or micronized form) to localize and augment bone formation. Variations in binding affinity for BMP-2 and VEGF may be exploitable for providing temporal variations in angiogenic and osteogenic growth factors. The use of micronized suspension of amnion (with or without BMP-2) and sheet form (without BMP-2) showed poor bone formation. Regardless of BMP-2 dose, bone defects treated with amnion sheet had similar amounts of bone volume as defects treated with BMP-2 in alginate. Amnion sheets wrapped around the femoral bone defect treated with high-dose BMP-2 (30ug) showed better bone formation in the defect center and lower heterotopic ossification, suggesting that amnion sheets may function to contain BMP-2 within the defect space. The team also conducted a pilot study to investigate the co-delivery of amnion particles and stem cells, with BMP-2, in bone regeneration in femoral segmental defects. The approach did not have a positive effect on bone regeneration and the defect was not bridged, possibly due to spatial isolation of the particles from the cells. The team studied bone regeneration in a composite model with a femoral bone defect and quadriceps volumetric defect, treated with microvascular collagen constructs around the bone defect containing alginate and BMP-2 (2ug). While a higher vascular volume was noted at 1 week, there was reduced mineral volume later (4 and 8 weeks), which suggests either the revascularization was not sufficiently robust to aid bone healing or that conditions may be removing BMP-2 and reducing chemotaxis. The results presented are preliminary or pilot in nature, as the studies are ongoing.

Future: The team will continue studies and the development of strategies supporting all three aims: pathophysiology of non-union, spatiotemporal control of cytokine delivery, and cell/vascular recruitment to augment BMP-2-induced bone formation.

WRC ER-09

Functionalized Allograft for Large Scale Bone Defect Healing

Team Leader: *Yusuf Khan, PhD (University of Connecticut)*

Team/Collaborating Partner Institution: *University of Connecticut; University of Connecticut Health Center*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: The goal of this project is to evaluate the potential of localized delivery of growth factors, VEGF and BMP-2, from polymer-coated devitalized trabecular bone allografts to stimulate angiogenesis, osteoclastic resorption, and bone formation. The effort involves developing techniques to coat the allografts with variable-rate degradable polymers incorporating growth factors, and to evaluate the constructs in a rabbit bone defect model.

Accomplishments: The team focused on establishing a technique for fully coating the trabecular allografts with a micron-scale thick layer of degradable polymer. A uniformly thin coating of the degradable poly (lactide-co-glycolide) polymer that does not occlude the open pore structure is critical to allow for bone ingrowth and nutrient/waste exchange. The allografts appear coated at a macroscale level; however, scanning electron microscopy (SEM) analysis indicates that the smaller pores do become occluded with the polymer coating, and that the viscosity of the polymer may require adjustment. The team used microcomputed tomography and experimented with varying concentration of iodine as a contrast agent to differentiate bone and polymer in order to evaluate the 3D structure. The ability to determine coverage and polymer volume, and thus the desired polymer concentration for coating, was not achieved. The team determined that full scans of the coated allografts will need to be conducted and serial sections characterized to examine extent of coverage, using side-by-side comparisons of the same allograft sample prior to and after polymer coating.

Future: The team will continue to develop techniques to evaluate polymer coverage and optimize the coating process. Once established, they will characterize the release kinetics, bioactivity, and efficacy of the BMP-2 and VEGF coated allografts. Studies will then be conducted in a rabbit ulnar segmental defect model.

WRC ER-10

Enhancement of Bone Regeneration through Cell-Based Strategies

Team Leader: *Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University; Arteriocyte; USAISR*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: The goal of this project is to evaluate the performance of a bone regeneration scaffold, TyrPC/CaP and BMP-2, against a commercially available product in support of a regulatory filing. The project will also investigate the effects of an antibiotic on wound healing and bone-marrow derived cells. The sheep tibia defect model will be used for the studies.

Accomplishments: In preparation for studies in the sheep tibia defect model, the team mainly focused on fabricating and characterizing the TyrPC/CaP scaffolds. They characterized the pore structure of the scaffolds using SEM, which demonstrated a bimodal, highly porous scaffold architecture (micropores 10-20 μm; macropores 100-200 μm) with a wide distribution of interconnected pores and a highly organized, self-aligned microporous (< 20 μm) structure around the macropores. An open and highly interconnected porous structure may promote cellular infiltration and enhance the diffusion rates of nutrients and waste products, and the microporous structure may improve angiogenesis and osteoconduction. Cell attachment to the scaffolds was also confirmed using pre-osteoblasts MC3T3-E1 cells. The team completed development and characterization of MAR0Mycin™ (Arteriocyte). This material is an allogeneic DBM-based material designed to provide local antibiotic delivery in a sustained and controlled manner. They optimized this for a 6-day vancomycin release. By varying the particle size, effect of material processing, and the type of allograft, a DBM+Gel (Arteriocyte) was chosen for its osteoinductive properties and DBM+Gel is expected to provide a more efficient retention and release of vancomycin in the bone defect. Vancomycin retention and release from these materials was quantified, and the initial pharmacokinetics results demonstrated a high dose of vancomycin retention and a sustained release at desired doses.

Future: The team will complete the characterization of the TyrPC/CaP/BMP-2 bone void fillers and prepare for animal studies. They will conduct studies in the sheep tibia segmental defect model to assess the scaffolds and also antibiotic effects on healing.

Clinical Challenge Muscle

AFIRM investigators are studying new ways to control the wound environment in muscle in order to promote better healing. They are researching factors that could modulate the cellular remodeling during the healing process and reduce scarring. Investigation is also ongoing into preventing ischemic damage in muscle by injecting oxygen-generating materials.

WRC ER-06

In Situ Influence of Cell Fate for Functional Soft Tissue Reconstruction

Team Leader: *Stephen Badylak, DVM, PhD, MD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh*

Target Clinical Application: *Soft Tissue Reconstruction*

Goals: This project will identify factors, such as oxygen tension or pH, which may be important in controlling the cellular remodeling that occurs as wounds heal. The effort will use a Biodome device to aid in sampling the wound environment in a canine volumetric muscle loss model to identify critical factors, and these factors then will be manipulated to study wound healing and identify candidate treatment approaches.

Accomplishments: The team used 3D printing technologies to create three generations of Biodomes using medical-grade materials, with each generation refined based on adjustments to the animal model, anatomical considerations at the implant site, and investigator feedback. Multiple materials, port designs, and shapes were evaluated. The preferred composition was a Parylene C-coated elastomer body with a polyurethane rubber septa. Based on evaluations in cadaveric leg, however, the port designs were still difficult to locate under the skin and were likely to bend under the overlying skin. Design of a fourth-generation device that incorporates a single large port and palpation bumps has begun. In preparation for future studies, the team has been optimizing immunostaining conditions in canine tissue. Markers of interest include macrophage phenotypes (M1 and M2, as M2 macrophages are critical to tissue remodeling), perivascular stem cells, blood vessel, and progenitor, stem, nerve, and muscle cell. Specificity and binding of antibodies for M1, M2, and pan-macrophage markers, as well as markers of stem cells and blood vessels, was evaluated in formalin-fixed and sectioned tissue. Optimization for additional markers is ongoing using alternative methods of antigen retrieval and frozen tissue preparations.

Future: The team will finalize the design and fabricate Biodomes, and begin implantations and tissue analysis. Additionally, the immunostaining protocols will be determined and optimized.

WRC ER-12

Tissue Preservation of Ischemic Skeletal Muscle Using Oxygen Generating Biomaterials

Team Leader: *Benjamin Harrison, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest University; WFIRM*

Target Clinical Application: *Prevention of Ischemic Injury in Muscle*

Goals: The goal of this project is to develop particulate oxygen generators (POGs) as a solution for providing oxygen at injury sites lacking sufficient vascular supply to prevent long-term ischemic damage and tissue necrosis. The effort will entail optimizing the POG chemistry and evaluating POGs in rodent and porcine ischemic limb models.

Accomplishments: Prior work by the investigators demonstrated the feasibility of using sodium percarbonate (SPO), a POG, to delay the onset of ischemia and extend the functional window of injured muscle in a rat ischemic injury model. The investigators are evaluating different POGs, and refining the ischemic injury model and measures. In addition to SPO, they are evaluating calcium peroxide (which degrades slower) and the use of antioxidants with POGs. Following ischemic injury and POG treatment, functional measures are recorded and the tibialis anterior muscle tissue is harvested for histological analysis. Because functional testing is time-consuming and there are non-responders in the treatment groups, the investigators are looking at the use of magnetic resonance imaging (MRI) as a means to measure ischemic injury and assess functional deficits. Preliminary experiments showed a correlation between a decrease in muscular function and elevated T2 relaxation times.

Future: The investigators will continue to refine the oxygen-generating materials, dosage and treatment schedule, and the ischemic injury model. Following studies in the rodent model, they will translate the effort into a porcine ischemic limb model and begin discussions with the FDA on the regulatory path.

Clinical Challenge Nerve

The AFIRM researchers are focused on developing strategies for the repair of large nerve gaps. The technologies under exploration are nerve conduits to guide regenerating nerves. The approaches use a variety of allogenic, tissue-engineered, and composite technologies in combination with cellular and other factors to improve nerve regeneration. Additionally, a means to prevent muscle atrophy during the time course of nerve repair using a novel electrode technology is under development.

RCCC 4.4.1

Repair of Segmental Nerve Defects: A Theragnostic System Solution for Optimal Nerve Repair – Prevention of Muscle Atrophy

Team Leader: *Robert S. Langer, ScD, Daniel G. Anderson, PhD (Massachusetts Institute of Technology [MIT])*

Team/Collaborating Partner Institution: *MIT; Mayo Clinic*

Target Clinical Application: *Prevention of Muscle Atrophy During Nerve Repair*

Goals: This project is developing an organic stretchable microelectrode array (OSMEA) technology, based on a new conducting polymer composite, for the prevention of muscle atrophy during nerve repair. The effort involves developing the OSMEA and demonstrating the ability of the OSMEA to both record and stimulate.

Accomplishments: The team developed a stretchable multi-electrode array for neural interfacing, using a new PolyPyrrole/PolyCaprolactone-block-polyTetrahydrofuran-block-polyCaprolactone (PPy/PCTC) composite film as the sole conductor for both electrodes and leads. Diameters of the electrode surface micro-grains were in the range of 1–5 µm and could be controlled, and stretchability was in the range of 22% with minimal loss in electrical conductivity. The OSMEA's mechanical and electrical performance was characterized. They are more suitable for recording local field potentials, such as electromyograms (EMG), electrocardiograms, electrocorticograms, and electroencephalograms, which demonstrated in an acute experiment the ability of the OSMEA to acquire multichannel EMGs from the lateral gastrocnemius muscle in a rat model. The characteristics of PPy/PCTC electrodes are also sufficient to safely activate a denervated muscle epimysially. Additionally, pilot in vitro and in vivo biocompatibility experiments had favorable results. Long-term stability of two types of the conducting polymer in PBS at 37°C for one month was evaluated, polymer instability was investigated, and improved material designs are being tested. Finally, a branched DNA-based protocol to better characterize the phenotype of neuronal cells in contact with OSMEA materials was developed.

Future: This RCCC project has been closed. Development of the OSMEA is expected to continue under the DoD's Joint Warfighter Medical Research Program. The team will focus on preclinical animal studies regarding safety and effectiveness.

RCCC 4.4.2a

Cells and Bioactive Molecules Delivery in Peripheral Nerve Restoration

Team Leader: *Maria Siemionow MD, PhD (Cleveland Clinic)*
Team/Collaborating Partner Institution: *Cleveland Clinic*
Target Clinical Application: *Repair of Nerve Defect*

Goals: The team is developing allogenic epineural tubes supplemented with bone marrow stromal cells (BMSCs) as a treatment strategy for nerve gap repair. The effort involves evaluating the strategy in rat and large animal (sheep) models.

Accomplishments: The naturally available epineural sheath conduit constitutively expresses laminin B2, an extracellular matrix component crucial for axonal growth, and has low immunogenicity due to a lack of Schwann cells. The addition of BMSC as a source of eurotrophic factors is proposed to further support nerve regeneration. The team tested the epineural sheath/BMSC conduit in a rat model involving a 2 cm defect in the sciatic nerve. Assessments included BMSC migration through the conduit via PKH-dye staining, and quantitative and qualitative measures of axonal regeneration. While the allogenic epineural tube alone promotes regeneration, the addition of BMSCs improved maturation (larger fibers) of regenerated nerve fibers. The team next evaluated the conduit in a sheep model comprising a 6 cm defect. They established a procedure for collecting and culturing sheep BMSC, and harvested epineural sheaths from median nerve. Macroscopic examination at 6 months post-implantation showed no signs of inflammation, rejection, or conduit leakage, and the conduit integrity and characteristics were maintained. Saline-filled epineural conduits showed similarity in the localization, distribution, and the size of the fascicle-like structures compared to the autograft control, and this result may indicate correct structural nerve organization and growth.

Future: This RCCC project has been closed. Next steps include optimizing the source and dosage of BMSCs and improving the long-term storage, characterization, and optimization of the epineural sheath conduits, as well as additional studies in the sheep model.

RCCC 4.4.2c/d

Repair of Peripheral Nerve Injury using Tissue-engineered Nerve Grafts Encased in Biodegradable Nerve Guidance Tubes

Team Leader: *D. Kacy Cullen, PhD, Douglas H. Smith, MD (University of Pennsylvania); Joachim Kohn, PhD (Rutgers)*
Team/Collaborating Partner Institution: *University of Pennsylvania; Rutgers; Axonia Medical, Inc.*
Target Clinical Application: *Repair of Nerve Defect*

Goals: The goal of this project is to develop a combination nerve guide tube (NGT) with encased tissue engineered nerve graft (TENG) to treat nerve defects. The effort involves translating the TENG technology into a porcine model, assessing TENG facilitation of nerve regeneration and functional recovery following nerve defect repair, and evaluating potential NGTs for TENG encasement.

Accomplishments: TENGs are lab-grown nervous tissue comprised of long axonal tracts spanning two neuronal populations, generated via continuous mechanical tension or "stretch growth." This in vitro process results in a highly organized parallel orientation of large axonal tracts in a very short time frame, and these tracts are embedded in a 3D matrix and removed en masse for transplantation within an NGT. Upon implantation, host axons grow directly along the tissue-engineered axonal tracts in a process called "axon-facilitated axon regeneration". The team manufactured NGTs using TyrPC, including melt extrusion, braiding (up to 5.4 cm long), hydrogel coating, and sterilization. TENGs encased in NGTs (TyrPC or commercially available collagen NGTs) were used to repair 1.0 cm defect of a motor (deep peroneal) or sensory (superficial peroneal) nerve in pigs. TENGs accelerated acute axon regeneration and Schwann cell infiltration versus empty NGTs (3- to 4-fold faster) and similar to autografts. TyrPC NGT performance was equivalent to commercially available, FDA-approved, collagen NGTs. The team then used TENGs encased in collagen NGTs to repair 5 cm defects of the peroneal nerve in pigs. As early as 3 months post-repair, robust axonal regeneration, restored gross nerve structure, and reconstituted CNAPs (compound nerve action potential) were observed, and at 7 and 10 months post-repair, stimulation of the repaired nerve evoked muscle activation observed via a "hoof twitch" and robust CMAPs (compound muscle action potential). Axon regeneration to the target muscles and a high density of myelinated axons in nerve cross-section were determined by histology. The team successfully translated the TENG technology from a rat to porcine model. A Request for Designation was submitted to FDA, and TENGS are a combination product (device and biological component) that will be reviewed and regulated by the Center for Biologics Evaluation and Research for pre-market review and regulation.

Future: This RCCC project has been closed. Based on the results, TENG development will be accelerated. Efforts to utilize TyrPC NGTs will be advanced under the DoD's Joint Warfighter Medical Research Program.

WFPC 4.4.4

Peripheral Nerve Repair

Team Leader: *Kacey Marra, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh*

Target Clinical Application: *Repair of Nerve Defects*

Goals: The team is developing a composite nerve repair conduit consisting of a combination of an outer layer of slowly degrading poly(caprolactone) (PCL) containing double-walled microspheres releasing glial cell line-derived neurotrophic factor (GDNF) with an inner lumen containing decellularized nerve allograft. The effort involves evaluation of the composite conduit, as well as nerve allograft, in a non-human primate (NHP) model.

Accomplishments: The team developed a PCL-based nerve conduit that contains double-walled microspheres encapsulating GDNF, and it also established a NHP median nerve defect model (5- and 7-cm NHPs were obtained for studies and trained in a manual dexterity task [retrieval from a Kluver board] to be used for assessments), underwent surgery to create the median nerve defect, and then underwent repair (i.e., decellularized nerve graft, autograft, PCL, PCL/GDNF, and the composite guide). Results indicated the median nerve was regenerating across the gap for the decellularized nerve allograft. Decreased stimulation thresholds when compared to autograft in both motor and sensory responses were also observed for the decellularized allograft. There was no significant difference in the number of migrating Schwann cells in the decellularized nerve allograft versus autograft at explant, and larger number of smaller fibers are seen in regenerating nerve through decellularized nerve allograft. Preliminary functional results from the Kluver board suggest that PCL/GDNF conduits have a beneficial impact functional recovery. The team optimized fabrication and sterilization of 5- and 7-cm PCL conduits, and bioactivity of encapsulated GDNF after sterilization confirmed. Composite guides were successfully fabricated by inserting a decellularized nerve allograft into the lumen of PCL conduit containing GDNF microspheres. In summary, the team collected electrophysiology, histology, and functional testing data in support of an FDA submission for the PCL/GDNF conduit in a clinically relevant model.

Future: The team is beginning the process of transitioning from pre-clinical to clinical studies, and it has had discussions with FDA. Work on the nerve conduits is also continuing under AFIRM-WRC.

WRC ER-03

Biodegradable Conduits for Large Extremity Nerve Injuries

Team Leader: *Kacey Marra, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh; AxoGen, Inc.; MedGenesis Therapeutix, Inc.*

Target Clinical Application: *Repair of Nerve Defects*

Goals: The goal of this project is to develop a nerve scaffold incorporating neurotrophic factors to repair nerve defects. The team is evaluating multiple scaffold approaches including PCL, decellularized nerve allograft, autograft, and a composite guide, and it will conduct studies in NHPs.

Accomplishments: Previously, the investigators had developed a PCL-based nerve conduit that contains double-walled microspheres encapsulating GDNF, and also established a NHP median nerve defect model (5 cm and 7 cm). The team optimized fabrication and sterilization of 5- and 7-cm PCL conduits and confirmed bioactivity of encapsulated GDNF after sterilization. The team has successfully fabricated composite guides by inserting a decellularized nerve allograft into the lumen of PCL conduit containing GDNF microspheres. NHPs were obtained for studies and trained for a manual dexterity task that will be used as one of the assessment measures. Following intraoperative electrophysiology measures, 5-cm median nerve defects were created and repaired using one of the strategies (i.e., decellularized nerve graft, autograft, PCL, PCL/GDNF, and the composite guide). Electrophysiology, histology, and functional task measured are used to assess the strategies. Studies to assess nerve regeneration and functional recovery are in process. Preliminary functional results suggest the PCL/GDNF conduits have a beneficial impact on return to function.

Future: The team will conduct studies of the composite guide in a rat model and then in the established 5-cm NHP median nerve defect model. They will complete the NHP studies with the decellularized nerve graft, autograft, PCL, PCL/GDNF, and the composite guide. The team will also seek guidance from FDA.

WRC ER-04

Tissue Engineering for Repair of Critical Nerve Gaps

Team Leader: *Anthony Windebank, MD (Mayo Clinic)*

Team/Collaborating Partner Institution: *Mayo Clinic; University of Puerto Rico; The Miami Project to Cure Paralysis*

Target Clinical Application: *Repair of Nerve Defects*

Goals: The goal of this project is to develop procedures for seeding a PCL fumarate (PCLF) scaffold with autologous human Schwann cells (ah-SC) in order to bridge nerve gaps, thereby inducing and guiding regeneration. The effort will include preclinical development and testing in rats, development of GMP manufacturing for ah-SC, and regulatory submission to FDA for a clinical trial.

Accomplishments: The team has developed a protocol for isolation and expansion of SC from adult rat nerves. Sciatic and sural nerves were harvested, and the purity of the resulting cell cultures was above 90% for SCs. The bioactivity of the SCs was assessed by evaluating the extent of differentiation of PC12 cells co-cultured with the SCs. Measures included the percentage of neurite bearing cells, average number of neurites per cell, and neurite length. Results for SCs from sural nerves were comparable to those from sciatic nerves – important in that the sciatic nerve (a major nerve) could not be harvested from clinically, whereas sural nerve could. While there was a gradual decrease in purity of the SCs with expansion of the cell culture, bioactivity of the sural nerve derived SCs was not affected. For obtaining human SCs, the team has established the protocol for harvesting and expansion of cells from human nerve samples, including retrieval from the operating room to the laboratory. The Mayo Clinic Institutional Review Board (IRB), Research Biospecimens Subcommittee, and Tissue Request Acquisition Group have all approved the protocol, and Human Research Protection Office approval is pending. The team also prepared for future studies by confirming the ability to fabricate the combined PCLF/SC conduit and implant in a rodent critical size nerve defect model.

Future: The team will develop protocols and procedures for harvest and expansion of ah-SCs, and characterize the isolated cells. The PCLF/SC conduit's ability to support nerve regeneration will be tested in the rodent nerve defect model.

Clinical Challenge Blood Vessel

Research teams are designing and developing tissue-engineered blood vessel (TEBV) replacement products. The approaches use polymers or decellularized vasculature as the tube material. The tubes are lined with cells to create a structure similar to vasculature. The designs are being tested in animal models. One team found that endothelial-like cells producing nitric oxide synthase was key to long-term implant success. Researchers also investigated material surface modifications such as the addition of growth factors to induce vascular networks to support reconstruction of large wounds.

RCCC 4.3.2

Development of Tissue (Peritoneum) Lined Bioabsorbable and Fracture-Resistant Stent Graft for Vessel Trauma

Team Leader: *Timur Paul Sarac, MD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *Cleveland Clinic; Nitinol Development Corp.; Biogeneral Incorporated; Evonics; Resonetics, Inc.; LABiomed/ UCLA Harborview*

Target Clinical Application: *Reconstruction of Blood Vessel*

Goals: The team is developing a biodegradable tissue-lined stent graft made of polydioxanone (PDO) or polylactic acid (PLA) for blood vessel reconstruction. The effort involves design and fabrication of the stents, followed by studies in animals.

Accomplishments: The team's previous work has focused on the design of a stent graft with a nested architecture to allow for flexibility and durability once placed in a blood vessel. Initial prototypes were constructed from nitinol, and the stent graft performed exceptionally in an animal model. Based on 2012 and 2013 pre-Investigational Device Exemption (IDE) meetings with FDA, they expected to begin the IDE study of the nitinol stent in January 2014. The stent design was translated into PDO and PLA versions, and then minor adjustments were made to manufacturing to improve radial force and ability to support the sewn tissue lining. Minor modifications were also made to improve the delivery system. Animal implants were performed. Tissue swelling (stent increased in size) was observed post-implantation and after storage, which impacted delivery of the stent graft. All animals had pulses throughout the entire study. However, the stents dissolved before they could heal to maintain lumen integrity.

Future: This RCCC project has been completed. In order to improve storage capabilities and obviate the need to readjust the delivery system, future work by the team will focus on one polymer, the development of a dry tissue technology, and a revised stent design that accommodates both a proximal and distal flair.

RCCC 4.3.2

Construction of Tissue Engineered Blood Vessels

Team Leader: *Thomas N. Tulenko, PhD (Cooper University Hospital)*

Team/Collaborating Partner Institution: *Cooper University Hospital; Jefferson University Hospital*

Target Clinical Application: *Reconstruction of Blood Vessel*

Goals: This project is developing TEBV and strategies to achieve endothelial nitric oxide synthase (eNOS) expression in the cells lining the lumen of the TEBV. The effort included assessment of TEBV for long-term durability and suitability in dog neck vessel implantations.

Accomplishments: The team has developed TEBV made from decellularized arterial segments seeded luminally with adipose-derived stem cells (ASCs) differentiated to an endothelial-like phenotype followed by transfection with the eNOS gene. This new method activates the essential endothelial genes (CD31, vWF, and eNOS) in ASCs differentiated into an endothelial phenotype. The team discovered that the small molecule S1P could assist in activating the expression of these genes. Procedures were developed to coat the tubular scaffold with ASCs differentiated to eNOS competent endothelial cell (EC)-like cells; and smooth muscle cell (SMC)-like ASCs on the adventitial surface. Following incubation in a bioreactor, the vessel constructs were transplanted (carotid interposition graft) into canines for 6 weeks or 6 months. The TEBV coated with eNOS competent cells showed full patency at 6 weeks, and vessels remained remarkably patent with very little intimal hyperplasia at 6 months. The team's findings demonstrate the necessity for eNOS competent EC-like cells lining the vascular conduit in order for successful long-term implantation.

Future: This RCCC project has been completed. Additional studies will expand on the in vivo testing of the TEBVs to confirm safety and efficacy. A request for designation is expected to be submitted to FDA to facilitate regulatory planning.

RCCC 4.5.6

Vascular Tissue Engineering

Team Leader: *Daniel G. Anderson, PhD, Robert S. Langer, ScD (MIT)*

Team/Collaborating Partner Institution: *MIT; Stanford*

Target Clinical Application: *Induction of Vasculature to Support the Reconstruction of Large Wounds*

Goals: The team is developing strategies to support the reconstruction of large tissue wounds using tissue regeneration scaffolds by inducing vascular networks, including a microenvironment that releases synthetic growth factor or a bioactive pro-angiogenic microenvironment within the scaffold system. The effort involves modifying surface characteristics to incorporate growth factors and evaluating the design's vascularization capability in normal rats.

Accomplishments: Poly(ether sulfone) (PES) is a material that is amenable to surface modifications and can be microfabricated into a variety of conformations. The team has developed and optimized protocols to modify the surface of PES to incorporate bioactive molecules (such as growth factors) or polymer side chains that incorporate therapeutic functional groups. Angiogenic growth factor (VEGF) was attached to the surface using an intermediate linking polymer. Vinyl monomers were also successfully polymerized to the polymer surface. Neither approach had a significant impact on implant topography. Modified PES implants were then subcutaneously implanted in normal rats for 1 month and examined for new blood vessels (mature ECs expressing Tie2 protein) and fibrosis (indicator of tissue reaction). The number of mature, stable ECs in contact with the vascularizing PES membrane was increased. Maturity and longevity of the newly formed vasculature around the PES implant is generated by the inclusion of pericytes. The team therefore identified and selected proangiogenic and anti-inflammatory growth factors for screening. Overall, the team has successfully demonstrated the ability to create PES modified with chemical and growth factor surface modifications, and has shown that surface geometry can also be used to tune angiogenesis and modulate the immune response, including implant fibrosis.

Future: This RCCC project is closed. The team will consider angiogenic and immunomodulatory applications for the modified PES materials, such as cell encapsulation and transplantation. The team will encapsulate islet cells as a strategy for diabetic treatment, and will conduct a proof-of-concept study in mice.

WRC ER-07

Engineered Small Diameter Blood Vessels for Limb Reconstruction

Team Leader: *Sang Jin Lee, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest University; WFIRM*

Target Clinical Application: *Reconstruction of Blood Vessel*

Goals: The goal of this project is to engineer small diameter blood vessels with anti-thrombogenic and compliance matching properties similar to native vessel. The engineered vessels will ultimately be evaluated in a sheep femoral arterial interposition model.

Accomplishments: The team is using electrospinning technology to fabricate a vascular scaffolding system, which will be optimized by controlling the composition, structure, and biological and biomechanical properties of vascular scaffolds. Bilayered scaffolds will be seeded with vascular cells to achieve an EC-lined lumen and SMCs-infiltrated outer layer, and the cellularized vascular scaffolds will be preconditioned to enhance functional maturation using a pulsatile bioreactor system. The team worked on optimizing the vascular cell sourcing. Sheep ECs were derived from endothelial progenitor cells (EPC) using a CD133 antibody-conjugated cell purification system developed in the team's laboratory. The EPC-derived ECs expressed EC-specific markers, had similar NO production to ECs, and exhibited anti-thrombogenic capability. SMCs were isolated from sheep femoral artery biopsy, and following expansion were characterized with SMC-specific markers. The team optimized the parameters for electrospinning of the vascular scaffolds to control fiber morphology, diameter, and alignment, and it electrospun a 1:1 (weight ratio) polymer blend of PCL and type I collagen. Bilayered scaffolds were evaluated by SEM. PCL/gelatin composite scaffolds were also prepared and showed increased elasticity and similar crosslinking and cell attachment to PCL/collagen scaffolds. Using EDC/NHS chemistry, EPC/EC-specific antibodies and heparin molecules were conjugated onto the PCL/gelatin scaffolds. Cell adherence was assessed using a flow chamber and light microscopy. The Flk-1-conjugated vascular scaffold showed higher cell attachment compared to other antibody-conjugated vascular scaffolds.

Future: The team will continue efforts to optimize the surface functionality for EC capturing using either antibodies or surface receptor immobilization. Standard operating procedures will be developed and vascular scaffolds fabricated under Good Laboratory Practices guidelines for studies in a rabbit carotid arterial interposition model.

Clinical Challenge Joint

The AFIRM investigators are developing tissue-engineered scaffolds for repairing or replacing articular cartilage (AC) and meniscus. The approaches include polymer and hydrogel approaches, and enrichment with stem cells and platelet-rich plasma (PRP).

RCCC 4.4.3b

Functional Scaffolds for Soft Tissue Repair and Joint Preservation

Team Leader: *Charles J. Gatt, Jr., MD, Michael G. Dunn, PhD (University of Medicine and Dentistry of New Jersey [UMDNJ])*

Team/Collaborating Partner Institution: *UMDNJ; Rutgers University*

Target Clinical Application: *Repair of Meniscus*

Goals: The focus of this project is to develop a tissue-engineered meniscus scaffold for the treatment of moderate to severe meniscal damage. The effort included evaluating the polymer fiber/collagen/glycosaminoglycan-based meniscus scaffold in a sheep implantation model, design and fabrication of a 3rd-generation scaffold, and 1-year implantation study.

Accomplishments: The team conducted an implantation study in sheep in which the medial menisci were removed and replaced with the implant. Implants were allowed to incorporate and remodel for 16 or 32 weeks before recovery. Upon recovery, the implants were found to be intact with no ruptures or fixation failures. The tensile strength of the recovered implants was five times higher than the normal functional loads of the native meniscus, and the stiffness of the scaffold did not significantly change the course of the study and was similar to that of the native meniscus. Confined compressive creep experiments were performed to determine the aggregate modulus and permeability of the new tissue ingrowth. The explants are 25%–40% as stiff in compression as the native meniscus and trend towards an increased modulus over time. As expected, the permeability of the scaffolds greatly decreases after implantation, with values approaching the native structure. Histological evaluation of the implants showed an expected mild inflammatory response, persistence of the polymer fiber in the body with little resorption, and vascularization throughout. In comparison to control meniscectomy knee cartilage with moderate surface damage and hypocellularity, the knees with the implants showed only some areas of minor surface hypocellularity, and areas with little or no degeneration. A 3rd-generation meniscus scaffold was designed with a new poly (DTD DD) fiber that was 80% stronger and 50% tougher than the 2nd generation implant used in the implant study. A 1-year study was begun in which 3rd-generation meniscus scaffolds were fabricated and implanted in a sheep model.

Future: This RCCC project has been completed. The team will complete the analysis of the data from the 1-year implantation study to assess effectiveness of the scaffold. They will also initiate regulatory discussions with the FDA.

WFPC 4.4.5

Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes

Team Leader: *Rocky S. Tuan, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh*

Target Clinical Application: *Repair of Articular Cartilage*

Goals: The goal of the project is to develop a point-of-care procedure for the effective repair of damaged or degenerated AC using a combination of PRP and native AC (as fine morsels or cell aggregates) for the stimulation of MSCs chondrogenic differentiation. The effort will involve in vitro studies to develop and characterize the constructs, as well as testing in a goat model.

Accomplishments: Previously in the project, the investigators determined that cartilage has a beneficial effect upon MSC chondrogenesis in vitro, and the benefits of PRP may only be realized if the timing, amount, and composition of PRP is controlled. The investigators revised the study aims to (1) test the utility of nanofibers increasing mechanical strength of the hydrogel and permitting or enhancing MSC chondrogenesis co-encapsulated with chondrocytes and cartilage fragments, and (2) evaluate the AC repair approach in a goat model rather than a rabbit model. The effort in the past year was directed at determining the scaffold, cell, and PRP content of the implants for the goat studies. The investigators evaluated the effect of adding PCL nanofibers to the hydrogels. Although photo-crosslinkable hydrogels infused with nanofibers were fabricated, the addition of the fibers did not significantly enhance the mechanical properties of the hydrogel. The utility of PRP in stem cell-based cartilage repair was investigated by assessing the effect of PRP on in vitro chondrogenesis by bone marrow-derived MSCs or ASCs. The effect of PRP on differentiated chondrocytes under mechanical stress (i.e., stretch) was evaluated as well. The investigators concluded a 20% PRP should be added to hydrogels, and that PRP had a beneficial effect based on increased gene expression for Collagen type II and Aggrecan and reduced expression of inflammatory mediators. Additionally, a chondro-protective effect of MSCs upon chondrocytes was shown post-mechanical impact of adult bovine AC plugs. Overall, the data suggested testing the application of chondrocytes and MSCs together within a chondro-supportive scaffold in the presence of PRP. The investigators began studies in a goat model, including establishing feasibility of the surgical procedures for the defects, and polymerizing the gel construct in situ.

Future: The investigators will continue the goat model studies.

WRC ER-13

Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes

Team Leader: *Rocky S. Tuan, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh*

Target Clinical Application: *Repair of Articular Cartilage*

Goals: The team is developing a photo-crosslinked hydrogel containing PRP and AC promote chondrogenesis of adult MSCs and prevent subsequent chondrocyte hypertrophy and formation of inferior neo-cartilage following repair of damaged/degenerated. Following development and in vitro testing, studies will be done in an animal model.

Accomplishments: The investigators assessed the pro-chondrogenic effects of AC fragments/chondrocytes and PRP upon ASCs in vitro. MSCs were isolated from adipose tissue and bone marrow using established protocols. MSC-specific markers and flow cytometry were used to confirm the isolation. The MSCs were encapsulated in a hydrogel to mimic the future construct and potential biochemical and biophysical stimuli of the scaffold matrix. Transwell assays were used to assess the cytokine-mediated chondrocyte effects on MSC chondrogenesis, with the following cells/tissues added as potential signal sources: ASCs, minced AC, human chondrocytes, and urea-extracted cartilage. Expression of chondrogenic markers was measured and constructs underwent histology and proteoglycan content staining. Chondrocytes consistently increased MSC chondrogenesis more than other cell types. However, ASCs were out-performed by chondrocytes. The effect of PRP on MSC chondrogenesis was evaluated using pellet cultures. Cell proliferation increased in both bone marrow- and adipose tissue-derived MSCs. Chondrogenic gene expression was assayed by real-time RT-PCR for chondrogenic markers. A decrease in bone marrow-derived MSC differentiation with increasing PRP concentrations was observed. Alcian blue staining revealed dramatic decreases in hyaline cartilage-specific glycosaminoglycan for PRP-treated cultures, and analysis of similar cultures using adipose tissue-derived MSCs are ongoing. A high cell proliferation rate induced by PRP and a pulse of PRP in the first week of culture might improve chondrogenesis. The pellets were much larger and produced more cartilage matrix, although still less than those in chondrogenic culture with TGF-ß in the absence of PRP. The investigators also began efforts to evaluate pro-chondrogenic effects of AC fragments/chondrocytes and PRP in a PCL nanofiber-infused hydrogel similar to the anticipated construct. While the nanofibers were successfully incorporated into the hydrogel, the addition of nanofibers did not significantly enhance the mechanical properties of the hydrogel.

Future: The investigators will continue in vitro studies to characterize various approaches to MSC chondrogenesis, including contact-mediated, and cytokine-mediated, leukocyte depleted PRP; and evaluate MSC differentiation in PCL nanofiber-infused hydrogels with and without PRP. They will seek approval of the animal study protocols from Institutional Animal Care and Use Committee and Animal Care and Use Review Office.

WRC ER-14

Development of a Novel Medical Device to Restore Knee Function & Prevent Osteoarthritis

Team Leader: *Michael Dunn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University; NovoPedics, Inc.*

Target Clinical Application: *Replacement or Repair of Meniscus*

Goals: The team is developing a meniscus replacement device, consisting of a collagen sponge reinforced by a network of high-strength resorbable fibers to promote neo-meniscus formation and prevent the onset of meniscectomy-associated degenerative osteoarthritis. Devices for both total meniscus replacement and partial meniscectomies are being developed and will be evaluated in sheep models.

Accomplishments: The team continued to optimize the mechanical properties of the meniscus scaffolds (developed in AFIRM I) in preparation for a long-term (2-year) implantation in a total meniscus replacement sheep model. The design was modified to add circumferential fibers through the middle of the scaffold in order to improve resistance to hoop stresses generated in the knee and borne by the scaffold after implantation. Evaluation of the new design is pending. Development and evaluation of a partial meniscectomy device has not begun.

Future: Future studies will compare the new and previous total meniscus replacement designs and will include a contact stress study (simulates the distribution of loads in a joint) and an ultimate tensile test (stiffness and strength of the scaffold). Long-term (2-year) efficacy studies will be conducted in a sheep model. For the partial meniscectomy device, woven fiber and non-woven designs will be fabricated and undergo suture retention tensile testing to determine the stronger design, suturing techniques will also be evaluated by tensile testing, and efficacy studies will be done in a sheep model.

Clinical Challenge Compartment Syndrome

Work is under way in animal models to develop treatments to restore muscle and function following compartment syndrome (CS) injury. The applications being investigated include the injection of bone marrow mononuclear cells (BM-MNC) or human muscle progenitor cell (hMPC). The hMPC therapy is combined with anti-fibrosis and neovascularization treatments. The use of BM-MNCs to treat CS transitioned into clinical studies under AFIRM II.

WFPC 4.3.2

Use of Bone Marrow Derived Stem Cells for Treatment of Compartment Syndrome

Team Leader: *Kenton Gregory, MD (Oregon Health & Sciences University [OHSU], Oregon Biomedical Engineering Institute [OBEI])*

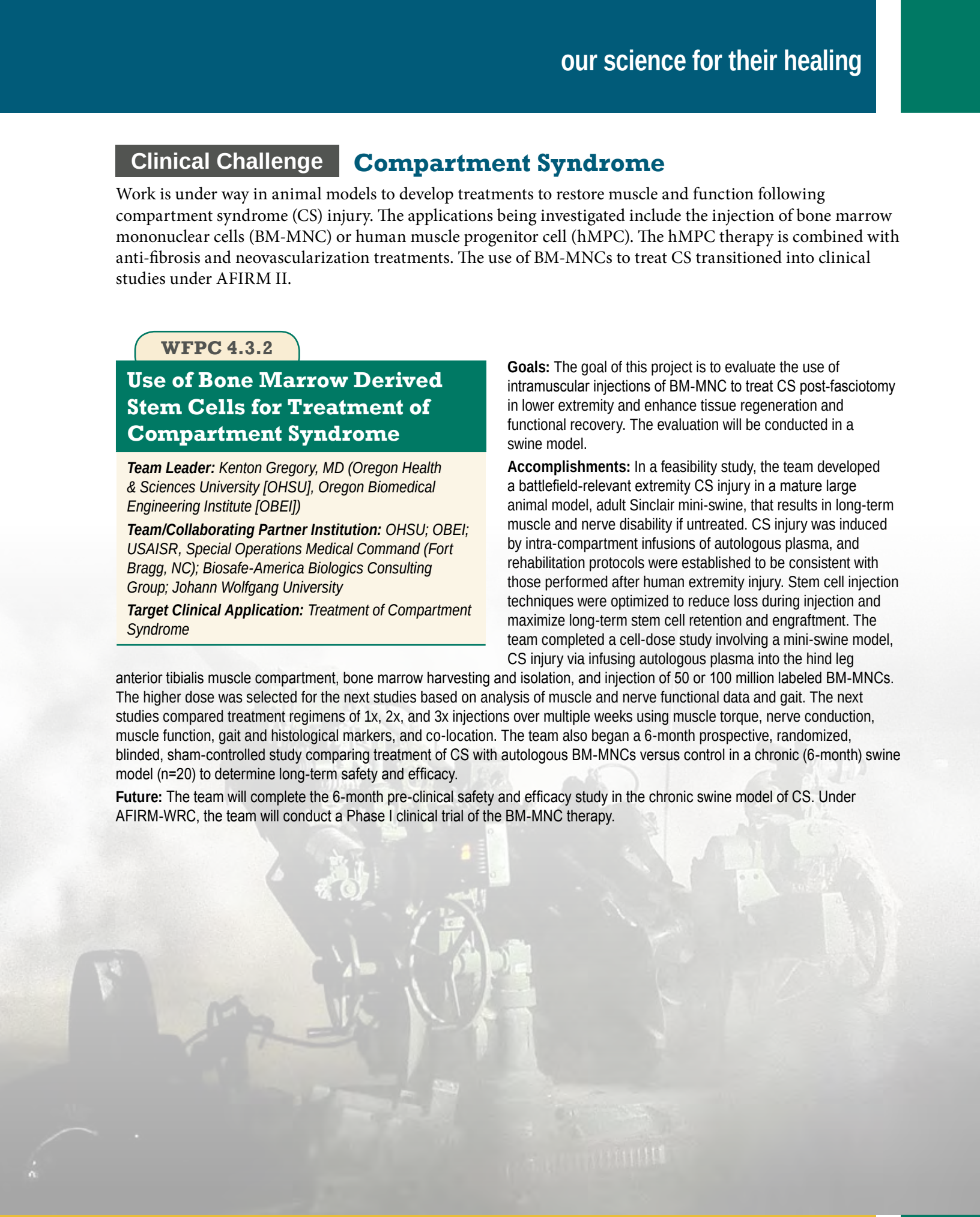
Team/Collaborating Partner Institution: *OHSU; OBEI; USAISR, Special Operations Medical Command (Fort Bragg, NC); Biosafe-America Biologics Consulting Group; Johann Wolfgang University*

Target Clinical Application: *Treatment of Compartment Syndrome*

Goals: The goal of this project is to evaluate the use of intramuscular injections of BM-MNC to treat CS post-fasciotomy in lower extremity and enhance tissue regeneration and functional recovery. The evaluation will be conducted in a swine model.

Accomplishments: In a feasibility study, the team developed a battlefield-relevant extremity CS injury in a mature large animal model, adult Sinclair mini-swine, that results in long-term muscle and nerve disability if untreated. CS injury was induced by intra-compartment infusions of autologous plasma, and rehabilitation protocols were established to be consistent with those performed after human extremity injury. Stem cell injection techniques were optimized to reduce loss during injection and maximize long-term stem cell retention and engraftment. The team completed a cell-dose study involving a mini-swine model, CS injury via infusing autologous plasma into the hind leg anterior tibialis muscle compartment, bone marrow harvesting and isolation, and injection of 50 or 100 million labeled BM-MNCs. The higher dose was selected for the next studies based on analysis of muscle and nerve functional data and gait. The next studies compared treatment regimens of 1x, 2x, and 3x injections over multiple weeks using muscle torque, nerve conduction, muscle function, gait and histological markers, and co-location. The team also began a 6-month prospective, randomized, blinded, sham-controlled study comparing treatment of CS with autologous BM-MNCs versus control in a chronic (6-month) swine model (n=20) to determine long-term safety and efficacy.

Future: The team will complete the 6-month pre-clinical safety and efficacy study in the chronic swine model of CS. Under AFIRM-WRC, the team will conduct a Phase I clinical trial of the BM-MNC therapy.



WRC ER-05

Development of Biological Approaches to Improve Functional Recovery after Compartment Syndrome Injury

Team Leader: *Shay Soker, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest University; University of Pittsburgh; USAISR*

Target Clinical Application: *Treatment of Compartment Syndrome Injury*

Goals: The goal of the project is to develop a combination approach involving anti-fibrosis, neovascularization, and hMPC for the treatment of CS and other musculoskeletal injuries. The approaches will be studied in a rat model of CS and in acute grade II or III hamstring strain patients.

Accomplishments: The team has developed and characterized a model of CS injury in rats. They characterized the endogenous regeneration of adult nude rats in order to establish a baseline for comparing therapeutically treated animals. Tissue degeneration and subsequent regeneration were followed by in vivo muscle function testing and histology. The team characterized three different sources of hMPCs in order to identify the best source of cells to use for therapy in the rat model of injury. The sources included CookMyosite (h-MPC-CM), the WFIRM Regenerative Medicine Clinical Center (hMPC-RM), and freshly isolated from cadaveric tissue in the lab (hMPC1). Based on in vitro growth characteristics, myogenic markers (histology and qPCR), and ability to form myotubes, the hMPC-RM cells may be the best source for future cell transplantation studies. Ongoing experiments are examining the ability of these different cell populations to survive and differentiate in our in vivo model of CS injury. In preliminary studies, the team investigated the potential functional improvement of ischemic injured skeletal muscle (CS-like) in rats using orally administered Losartan. The ischemic CS-like injury is created using a neonatal blood pressure cuff and a rubber band. The administration of Losartan after CS-like skeletal muscle injury yielded isometric torque improvements and histologically showed a greater number of regenerating myofibers (centronucleated myofibers) and less fibrosis.

Future: The team will complete the identification of the hMPC source for the studies and begin or continue rat studies involving hMPC transplantation and angiogenic and Losartan treatments. The team will also finalize clinical protocols for the IRB and begin subject recruitment.

Clinical Trials

AFIRM investigators are conducting clinical transplantation studies with a focus on nerve and vascular reconstruction. Additionally, investigators are evaluating a means to improve skin-prosthetic interface by conversion of skin at the stump site. The status of each clinical trial is summarized in **Table II-2**, and additional details on these trials follow the table.

Table II-2. AFIRM-funded Extremity Regeneration projects with pending or active clinical trials.

Therapy/Product	Project Title	Consortium	Project Number	Trial Phase	Current Status
Polycaprolactone Fumurate (PCLF) Nerve Conduit	Clinical Trial: Safety Assessment of a Novel Scaffold Biomaterial	RCCC	4.4.1CT	I	Pending
Bone Marrow Mononuclear Cell (BM-MNC) Treatment for Compartment Syndrome	Clinical Treatment of Severe Extremity Injury Complicated by Compartment Syndrome with Autologous Bone Marrow Mononuclear Cells	WRC	ER-01	I	Pending
Humacyte VasTech Vascular Conduit	Evaluation of Efficacy and Safety of Immediately Available Bioengineered Vascular Grafts for Extremity Reconstruction	WRC	ER-08	I	Pending
Conversion of Skin for Improved Skin-Prosthetic Interface	Enhancing Prosthetic Use via Application of Autologous Palmo/Planta Fibroblasts to Convert the Stump Site to Ectopic Palmo/Plantar Skin	WRC	ER-11	I	Pending

RCCC 4.4.1CT

Clinical Trial: Safety Assessment of a Novel Scaffold Biomaterial

Team Leader: *Anthony Windebank, MD, Michael Yaszemski, MD, PhD (Mayo Clinic)*

Team/Collaborating Partner Institution: *Mayo Clinic*

Target Clinical Application: *Nerve Gap Repair*

Goals: The team is developing PCLF biodegradable polymer nerve conduits suitable to repair nerve defects longer than 3 cm. The PCLF nerve conduits will be evaluated for safety (Phase I) in a clinical model involving sural nerve biopsy which leaves a 6-cm gap in the nerve.

Accomplishments: The team worked on designing and establishing GMP facilities and procedures for PCLF synthesis and nerve conduit fabrication. The team submitted pre-IDE materials to FDA to initiate regulatory discussions regarding a Phase I, first-in-human Early Feasibility Study.

Future: The team plans to submit an IDE, and begin subject recruitment once IDE and IRB approvals are obtained. A 2-year clinical trial is planned and will use leveraged funding from Mayo Clinic.

WRC ER-01

Clinical Treatment of Severe Extremity Injury Complicated by Compartment Syndrome with Autologous Bone Marrow Mononuclear Cells

Team Leader: *Kenton Gregory, MD (Oregon Health & Science University/Texas Health Science Center)*

Team/Collaborating Partner Institution: *Oregon Health & Science University/Texas Health Science Center*

Target Clinical Application: *Treatment of Compartment Syndrome*

Goals: The team will evaluate the use of intramuscular injections of BM-MNC to treat CS post-fasciotomy in lower extremity. A Phase I clinical trial with endpoints up to 6 months will assess muscle strength, adverse events, gait, and nerve conduction via MRI muscle fibrosis/regeneration following treatment.

Accomplishments: The primary effort during this period was work on the regulatory approval process. The team initially considered a regulatory approach based on a Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) regulatory path that would avoid an Investigational New Drug (IND) filing; however, given evolving FDA policies, it was decided that the BM-MNC treatment would not meet the HCT/P criteria for homologous use. The team is developing a detailed clinical protocol in anticipation of discussions with FDA (Type B pre-IND meeting).

Future: The team intends to hold a Type B pre-IND meeting with FDA to obtain regulatory guidance, and it will then prepare and submit IND and IRB submissions. A Data Safety Monitoring Board will be established, and training materials will be developed for the clinical sites.

WRC ER-08

Evaluation of Efficacy and Safety of Immediately Available Bioengineered Vascular Grafts for Extremity Reconstruction

Team Leader: *Jaimie Shores, MD (Johns Hopkins University)*

Team/Collaborating Partner Institution: *Johns Hopkins University*

Target Clinical Application: *Vascular Repair*

Goals: The goal of this project is to clinically evaluate a tissue-based bioengineered vascular conduit, VasTech from Humacyte, for use in vascular reconstruction.

Accomplishments: Humacyte engineered the vascular grafts by culturing banked human cells in bioreactors in the laboratory. After culturing, the vascular grafts are decellularized, to produce a mechanically strong, tissue-based graft that is non-immunogenic. The original study plan was to implant grafts for arterial reconstruction in forearms where vascular tissue was previously harvested for other procedures, and the investigators were working towards IRB and IDE approvals for this preliminary study. There is, however, existing data from trials involving elective vascular reconstruction for peripheral arterial disease and hemodialysis access, which makes the planned preliminary study unnecessary as a first evaluation of safety and effectiveness. The investigators are in the process of redesigning the study to focus on vascular reconstruction for lower extremity (and possibly large upper extremity) arterial trauma. The new study design will be directly applicable to military acute trauma.

Future: The investigators will continue efforts to refine the study design and obtain sponsor, IRB and IDE approvals for the study, and begin subject enrollment. Given the limited number of civilian penetrating trauma cases expected, an enrollment of 5 subjects in the 12-month period is anticipated once the study begins.

WRC ER-11

Enhancing Prosthetic Use via Application of Autologous Palmo/Planta Fibroblasts to Convert the Stump Site to Ectopic Palmo/Plantar Skin

Team Leader: *Luis Garza, MD, PhD (Johns Hopkins University)*

Team/Collaborating Partner Institution: *Johns Hopkins University; City of Hope Cellular Therapy Core*

Target Clinical Application: *Improvement of Skin-Prosthetic Interface*

Goals: The goal of this effort is to develop a means to improve the prosthetic-skin interface to reduce pain, discomfort, and skin breakdown. The team will conduct laboratory studies to optimize the use of skin stem cells to create “ectopic” or new palmo-plantar-type skin. The approach will be tested in a clinical trial in which autologous skin stems cells are injected to convert stump skin to palmo-plantar-type skin, with its attendant friction-resistant, irritant-resistant, and weight-bearing properties.

Accomplishments: The team received IRB and FDA approvals to proceed with the clinical study, and is in the process of ensuring compliance with DoD regulatory requirements. KRT9 presence will be used to assess differentiation of the stem cells. The team is developing an in-house polyclonal rabbit antibody for KRT9, given that commercial KRT9 antibodies are frequently non-specific. Crystal structure data was used to identify peptide sequences of KRT9, which are accessible epitopes given 3D structures of keratins. To identify unique peptide sequences of KRT9, which are not present in other suprabasal keratins such as KRT1, 1b, 2, and 10, the team performed BLAST alignment analysis on sequences of KRT9 and other keratins. A region was identified for consideration.

Future: The team will continue work to develop a quality control test for the ability to activate KRT1 as a positive control proxy for activation of KRT9, to establish optimum positive control efforts for KRT9 measurements in vitro, and to develop a polyclonal rabbit antibody for KRT9. The clinical trial is not expected to begin until later in the period of performance.

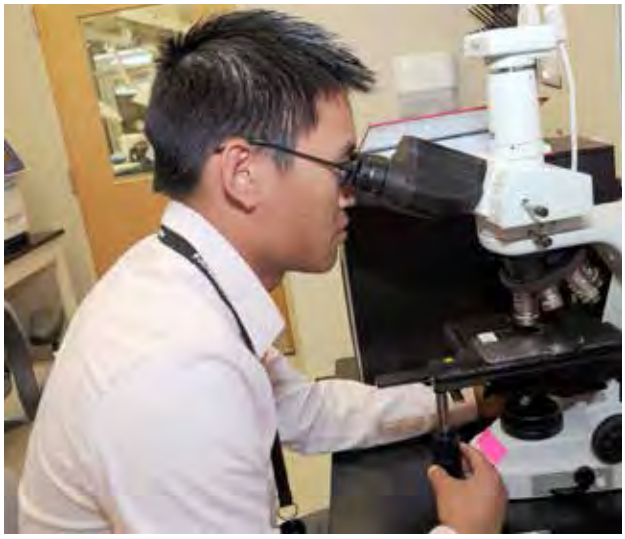
III: Craniomaxillofacial Regeneration

AFIRM OUR SCIENCE FOR THEIR HEALING

Background

Recent studies examining the battle injury patterns and resource impacts of injuries for Operation Iraqi Freedom and Operation Enduring Freedom have highlighted the significance of wounds to the head and neck. The face is largely unprotected in combat, leaving the region vulnerable to injuries from both ballistic and explosive weapons. Unfortunately, the techniques and materials currently available to repair or reconstruct delicate facial tissues are often inadequate, and the outcome from the injuries unsatisfactory. The persistent deficits in form, function, and appearance can have a devastating impact on the quality of life, emotional health, and socioeconomic opportunities of patients.

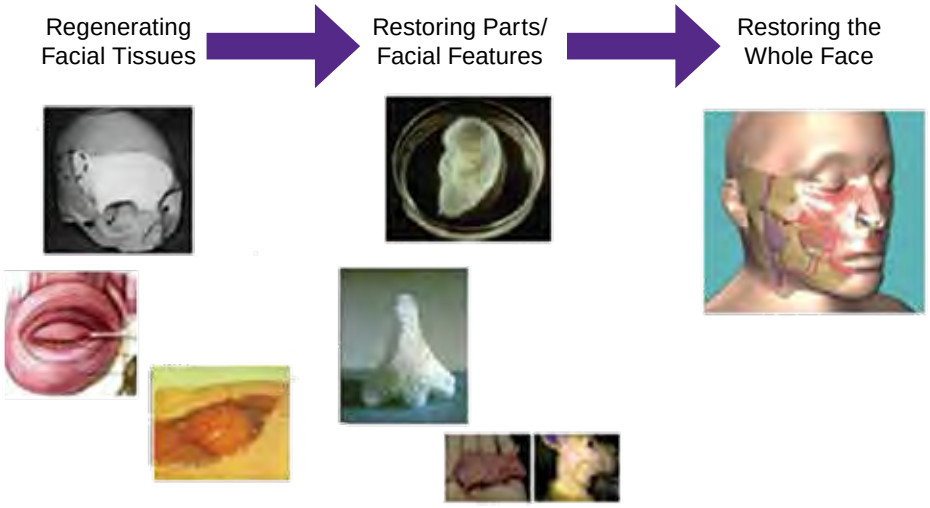
III: Craniomaxillofacial Regeneration



The AFIRM's Craniomaxillofacial Regeneration Program encompasses an array of potential solutions to craniomaxillofacial restoration challenges, from individual tissues such as bone, muscle, or skin, to composite blocks of specialized tissue for restoration of large portions of the face.

Areas of Emphasis

The goal of the Craniomaxillofacial Regeneration Program is to develop strategies and tools to aid in craniofacial reconstruction for the wounded warrior, with greater reconstructive fidelity and with fewer surgeries. The current portfolio includes innovative strategies to regenerate craniofacial bone, muscle, soft tissue, adipose tissue, cartilage, and dentition. The materials being characterized include synthetic polymers, ceramics, decellularized extracellular matrix (ECM), composites, and others. The research teams are advancing strategies such as three-dimensional (3D) printing, implantable in vivo bioreactors, wound infection treatment, and the concurrent regeneration of multiple tissue types. These materials and strategies are being tested in a variety of small and large animal models to be followed by human clinical trials. As shown in **Table III-1**, projects can be grouped into three clinical challenge topic areas: bone, muscle, and other tissues. A summary of each project is provided. Clinical trials are not included in Table III-1 and are instead presented in a separate section of this chapter.



Goals and products of the AFIRM Craniomaxillofacial Reconstruction program.

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

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Bone	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
		4.5.8	Accelerating the Development of Bone Regeneration Scaffolds Based on Tyrosine-Derived Polycarbonates
	WFPC	4.1.2	Space Maintenance, Wound Optimization, Osseous Regeneration and Reconstruction for Craniomaxillofacial Defects
	WRC	CF-01	Space Maintenance, Wound Optimization, Osseous Regeneration and Reconstruction for Composite Craniomaxillofacial Defects
		CF-02	Injectable, Settable Bone Grafts for Reconstruction of Weight-Bearing Craniofacial Bone Defects with Augmentation Using Recombinant Human Growth Factors
		CF-03	Craniomaxillofacial Tissue Engineered Bone Grafts
		CF-05	Novel Strategies for Repair and Restoration of Calvarial Bone Defects in Wounds Compromised By Infection and Scarring
		CF-06	Development of Resorbable Calcium Phosphate Cement for Regeneration of Cranial and Midface Bones
		CF-10	Biofabrication of Complex Tissue Components for Craniomaxillofacial Reconstruction
Muscle	RCCC	4.1.2	Develop Innervated, Vascularized Skeletal Muscle
	WRC	CF-07	Creating Innervated Vascularized Muscle Flaps from Elastic, Cellularized Biocomposites Developed In Situ for Facial Muscle Reconstruction
		CF-08	Bioreactors and Biomaterials for Tissue Engineering of Skeletal Muscle
		CF-09	Injectable and Implantable Soft Tissue for Craniofacial Reconstruction
		CF-12	In Vivo Functional Muscle Regeneration Utilizing an Implantable Modular Bioreactor
		CF-13	Hybrid Muscle-Nerve Tissue Integration System (MuNTIS) for Functional Restoration of Larger Volumetric Muscle Loss Injuries
Other Tissue	RCCC	4.5.4	Engineering of a Replacement Autologous Outer Ear Using a Collagen/ Titanium Platform
	WRC	CF-04	Bioengineered Alveolar Bone and Tooth Constructs

Clinical Challenge Bone

The research teams have made significant progress in the generation of bone for craniofacial reconstruction. Approaches to reconstruct different craniofacial defect geometries include porous polymers to encourage cell attachment and infiltration, resorbable bone grafts capable of delivering osteogenic growth factors, and the bioprinting of high-fidelity 3D scaffolds. The teams have utilized studies in large animals, such as sheep and canine, to validate several of these strategies.

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)

Team Leader: *Scott A. Guelcher, PhD (Vanderbilt University)*

Team/Collaborating Partner Institution: *U.S. Army Institute of Surgical Research (USAISR), Vanderbilt University, Medtronic*

Target Clinical Application: *Reconstruction of bone defects*

Goals: The team is pursuing two related projects for developing and evaluating allograft bone/polymer composites for treating trauma-related bone defects. In Project 4.5.1a, they are developing injectable, settable low-viscosity (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.

Accomplishments: The team has 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. They determined that LV grafts supported bone remodeling in a sheep femoral defect model. During the past year, final cured grafts have undergone ISO10993 biocompatibility testing, and the team has identified packaging requirements on stability testing. They also found that delivery of rhBMP-2 from LV/MasterGraft® (LV-MG) formulations enhanced new bone formation in multiple models, including rat critical-size calvarial defect model, canine mandibular ridge saddle defect, and porcine mandibular continuity defect models. LV grafts remodel and heal when injected into rabbit and sheep femoral condyle plug defects. In preparation for studies in large animal models and future clinical trials of ridge augmentation, the supplier for the polyester polyol component has been identified, and current Good Manufacturing Practices (cGMP) raw materials used to prepare the grafts have been manufactured.

Future: This RCCC project has been completed. Further development is expected to continue under the AFIRM-WRC program to progress the technology towards clinical trials. Specifically, the next steps are additional safety and efficacy studies in pre-clinical models of ridge augmentation and mandible continuity defects.

RCCC 4.5.1b

Regeneration of Bone in the Cranio-Mandibulo-Maxillofacial Complex Using Pre-Formed Tyrosine Derived Polycarbonates

Team Leader: *Shuang Chen, PhD Candidate, Joachim Kohn, PhD, Lauren Macri, PhD (Rutgers University), and Jeffrey Hollinger, DDS, PhD (Carnegie Mellon University)*

Team/Collaborating Partner Institution: *Carnegie Mellon University, Rutgers University, ImageIQ, Inc., USAISR*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project involves developing biodegradable scaffolds containing tyrosine-derived polycarbonate (TyrPC) enhanced with calcium phosphate (CaP) to be used as substitutes for bone grafts in the calvaria and upper face. The effort is focused on optimizing the scaffold design and demonstrating safety and efficacy in a goat calvaria critical-size defect model.

Accomplishments: The project previously fabricated TyrPC+CaP scaffolds containing a minimal dose of rhBMP-2 and demonstrated new bone formation in a rabbit critical-size defect skull model using. TyrPC scaffolds were gradually resorbed and replaced with new bone. The team also determined that treatment with ethylene oxide is the most suitable sterilization method for TyrPC bone regeneration scaffolds. In the past year, the team fabricated and characterized TyrPC scaffolds containing CaP and bone morphogenic protein (BMP-2) for evaluation in a goat model. The team characterized pore size (micropores <20µm and macropores 200-400µm), pore organization, and CaP and mechanical properties of the scaffolds. Additionally, implantation studies were begun in a goat calvaria critical-size defect model. Initial results suggest no adverse tissue responses. MicroCT, for assessing new bone formation, and histological/histomorphometric analyses are pending. In addition, the team maintains a database containing information about microCT, histology, and histomorphometry analyses of all rabbit calvaria and radius scaffold studies performed during AFIRM I efforts. Analysis of the data suggests that: 1) the surgical handling properties of TyrPC-based scaffolds were much better than commercially available bone graft substitutes, which were friable and particulate; 2) the in vivo biocompatibility, biodegradability, and osteoconductivity of TyrPC-based scaffolds were similar to commercially available bone graft substitutes; 3) the incorporation of a minimal dose of growth factor into TyrPC-based scaffolds significantly increased new bone formation; and 4) the best performing TyrPC-based scaffolds were identified.

Future: The team will complete evaluations of the performance of TyrPC+CaP scaffolds containing CaP with and without rhBMP-2 in the goat calvaria critical-size defect model. Under the AFIRM-WRC program, the team anticipates additional evaluations in a sheep tibia critical-size defect model.

RCCC 4.5.8

Accelerating the Development of Bone Regeneration Scaffolds Based on Tyrosine-Derived Polycarbonates

Team Leader: *Carmine P. Iovine, MS, MBA, Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University, and Howard Schrayer (Independent Contractor, Manufacturing Process Advisor)*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project seeks to develop a TyrPC-based bone pin implant for bone reconstruction that is osteoconductive (i.e., promotes attachment and bone growth at the interface). The focus is on advancing the implant fabrication methods.

Accomplishments: The team has developed a robust bone pin fabrication process, involving the use of injection molding and the incorporation of zinc stearate, a processing aid. Previously, the team successfully scaled up the TyrPC polymerization process to the kilogram scale, standardized the scaffold characterization techniques, and established preliminary specifications. During the past year, the team focused on developing a scalable process suitable for the transfer to a commercial manufacturing site to produce bone regeneration scaffolds for human use. The manufacturer has completed the scale-up of the target tyrosine-based polycarbonate synthesis to a 22-liter scale using a validated and reproducible process. Further, the team optimized the injection molding process for TyrPC-based small bone fixation pins, and more than 200 bone pins were successfully produced. Laboratory studies and scale-up of the TyrPC-based bone regeneration scaffold manufacturing process have been completed for all steps prior to the CaP coating process. The team has optimized conditions for reproducible scaffold production, standardized measurement techniques, and established preliminary manufacturing specifications and standard operating procedures (SOPs) for the base scaffold formulation.

Future: This RCCC project has been completed. Under RCCC project 4.5.1b, TyrPC scaffolds augmented with CaP and BMP-2 will be evaluated in the goat calvaria critical-size defect model. Further development, including evaluations of the technology in a sheep tibia critical-size defect model, is expected to continue under the AFIRM-WRC program.

WFPC 4.1.2

Space Maintenance, Wound Optimization, Osseous Regeneration and Reconstruction for Craniomaxillofacial Defects

Team Leader: *Antonios G. Mikos, PhD (Rice University), Mark E. Wong, DDS (University of Texas Health Science Center at Houston [UTHSC]), F. Kurtis Kasper, PhD (Rice University)*

Team/Collaborating Partner Institution: *Rice University, UTHSC, Synthasome Inc.*

Target Clinical Application: *Reconstruction of Bone Defects*

Goals: This project seeks to facilitate staged reconstruction of large osseous defects by developing: (1) porous poly(methyl methacrylate) (PMMA)-based product for osseous space maintenance and (2) porous PMMA-based product releasing antibiotic(s) in a controlled manner to mitigate wound infection in osseous space maintenance applications. The focus is on conducting pre-clinical studies in support of, and preparing, the 510(k) U.S. Food and Drug Administration (FDA) application for the space maintainer technology.

Accomplishments: The team initiated pre-clinical studies based on prior interaction with FDA regarding the regulatory pathway for their product. Physiochemical properties of various formulations of porous PMMA were determined, including mechanical properties, residual monomer release, and setting temperature, and found to be acceptable for space maintenance applications. The team successfully completed lot-to-lot reproducibility studies using the criteria of axial screw pullout strength and bulk porosity. Other studies completed included sterilization and biocompatibility validations. The team also established product specification, and the manufacturing process was designed and initiated. Additional preparations for a future 510(k) submission included establishing a Device Master Record of manufacturing methods and validation studies, reviewing predicate devices, and assembling a preliminary 510(k) file.

Future: This WFPC project has been completed. Further development is expected to continue under the AFIRM-WRC program to advance the technology and prepare the 510(k) submission, including studies in animal models and humans.

WRC CF-01

Space Maintenance, Wound Optimization, Osseous Regeneration and Reconstruction for Composite Craniomaxillofacial Defects

Team Leader: *Mark Wong, DDS (UTHSC), Antonios Mikos, PhD (Rice University)*

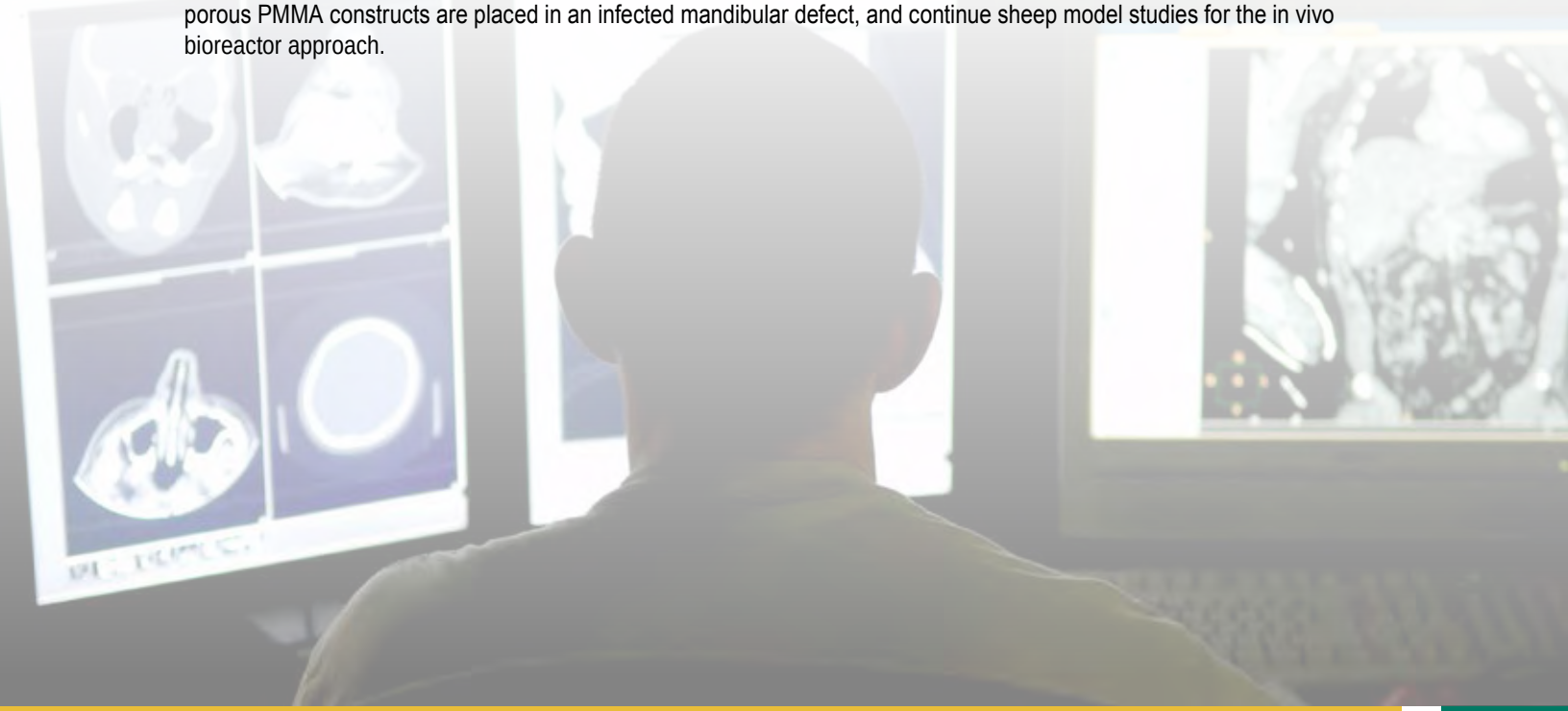
Team/Collaborating Partner Institution: *Rice University, UTHSC, Synthasome Inc.*

Target Clinical Application: *Reconstruction of large osseous defects*

Goals: This project seeks to facilitate staged reconstruction of large osseous defects by developing: (1) PMMA-based materials for osseous space maintenance and (2) porous PMMA-based materials releasing antibiotic(s). In parallel, an "in vivo bioreactor" approach will be advanced for the fabrication of autologous vascularized bone away from the site of injury that may be used as donor tissue for second-stage reconstructive surgeries. The effort includes pre-clinical activities in support of a 510(k) submission such as animal model studies, and initiation of a clinical trial.

Accomplishments: The team completed several studies to provide information regarding the mechanical properties, sterility, and biocompatibility of the PMMA-based porous bone space maintainer (PBSM) to support FDA regulatory approval. Electron beam sterilization was validated and also found to enhance the mechanical properties of the PBSM. UTHSC Institutional Review Board (IRB) approval was received, but the clinical trial will be delayed based on discussions with FDA, the industrial collaborator, and AFIRM II leadership. In support of developing an antibiotic-releasing PBSM, the team evaluated the sustained release characteristics of various antibiotic-loaded poly(lactic-co-glycolic acid) (PLGA) microparticles. Clindamycin was selected for future studies, although other antibiotics are viable alternatives. Additional release kinetics studies were undertaken in preparation for studies using an infected rabbit mandibular defect model. For studying the in vivo bioreactor approach, a sheep model is used in which a vascularized bone graft for mandibular reconstruction is grown in a chamber implanted on the rib for 9 weeks. This model was used to conduct a pilot study in which bone growth in chambers containing varying ratios of autograft and synthetic graft was investigated, and regeneration of the mandible using these explanted and transferred flaps was evaluated. Histological evaluation showed robust bone growth and integration with native bone in both the 0% and 100% synthetic graft groups. Use of synthetic bone grafts versus autografts would reduce donor site morbidity. The team has received UTHSC and Animal Care and Use Review Office (ACURO) approval for a sheep study that will elucidate the effects of bone chamber fill composition on the generation of bone, and how the use of a flap affects integration with the surrounding bone compared to grafts.

Future: For the next year of this project, the team plans to submit to the Department of Defense Human Research Protection Office (HRPO) for evaluation of the PBSM, determine through in vitro release studies the optimal loading of antibiotic containing PLGA microspheres embedded within porous PMMA constructs, complete the rabbit study in which these antibiotic-releasing porous PMMA constructs are placed in an infected mandibular defect, and continue sheep model studies for the in vivo bioreactor approach.



WRC CF-02

Injectable, Settable Bone Grafts for Reconstruction of Weight-Bearing Craniofacial Bone Defects with Augmentation Using Recombinant Human Growth Factors

Team Leader: *Scott Guelcher, PhD (Vanderbilt University)*

Team/Collaborating Partner Institution: *Vanderbilt University, UTHSCA*

Target Clinical Application: *Reconstruction of craniofacial bone defects*

Goals: This purpose of this project is to design and develop an injectable, settable, and compression-resistant LV bone graft augmented with rhBMP-2 for healing of mandibular bone defects. The compression-resistant LV graft is hypothesized to maintain space without the use of protective membranes (necessary for absorbable collagen sponge [ACS]-based approaches). The product will be validated in pre-clinical models of ridge augmentation and mandibular continuity defect representative of combat injuries.

Accomplishments: The team completed the canine mandibular ridge saddle defect study, in which bioactive glass and LV-MG bone graft formulations augmented with rhBMP-2 bone graft were screened. After 16 weeks, bone growth was present in all groups and bone morphometry was assessed using histomorphometry and µCT images. The results suggest that there were no differences in the quality of the bone in the defect area at the two doses of rhBMP-2. The high dose of BMP-2 enabled the graft to maintain ridge width and height, but did not enhance the quality of new bone. LV-MG+BMP-H was identified as the lead candidate graft. The team has also initiated the Canine Lateral Ridge Augmentation Study, in which chronic dental disease in humans with tooth loss with significant mandibular atrophy is simulated. Following creation of the defect, a second procedure simulates the lateral ridge mandibular bone grafting procedure (the first step in preparing the mandible for dental implants). Bilateral ridge defects will be treated with LV-MG +BMP (430 or 215 µg rhBMP-2/ml) and compared to mesh-protected ACS+BMP (430 µg rhBMP-2/ml) to test the ability to promote ridge augmentation. Additionally, for a Porcine Mandibular Continuity Defect Study, the Dental & Trauma Research Detachment at USAISR performed a pilot pig study in which mandibular defects were created and treated with LV-MG+100 µg rhBMP-2/ml. Initial computed tomography (CT) images showed extensive new bone formation, and 5 out of 6 specimens showed unions at 12 weeks. Histological, histomorphometric, and µCT analyses are pending.

Future: The team will focus on completing the canine lateral ridge augmentation pilot and full studies, and also the clinical control (ACS + rhBMP-2) group in the porcine mandibular continuity defect study. The team also plans to submit several of the completed studies for publication.

WRC CF-03

Craniomaxillofacial Tissue Engineered Bone Grafts

Team Leader: *David Dean, PhD (The Ohio State University)*

Team/Collaborating Partner Institution: *The Ohio State University, University of Akron, Case Western Reserve University*

Target Clinical Application: *Patient-specific tissue engineering bone grafts*

Goals: This project seeks to validate the process for fabricating patient-specific tissue engineered maxillary and/or mandibular grafts in a canine cranial implant model. The external shape of these implants would be produced in proprietary Computer Aided Design software using both the patient's 3D CT image and a skull surface template. Implant internal pore geometry would also be designed to facilitate bone marrow-derived mesenchymal stem cell seeding and culture within a bioreactor. The team will also obtain a regulatory pathway designation from FDA.

Accomplishments: The effort in the past year has been to design and fabricate (i.e., 3D print) porous, resorbable implants. The team designed the external shape and internal porous geometry of these implants. Pore geometries were selected to optimize cell seeding, bioreactor nutrient and growth factor flow, and finally, in vivo tissue and vascular infiltration and the resorption of these polymeric implants (i.e., scaffolds). The investigators have also begun testing the scaffolds for the pre-culturing of bone progenitor cells in a bioreactor. An Institutional Biosafety Committee exemption was received to obtain human bone marrow-derived mesenchymal stem cells and use them to seed these scaffolds in a bioreactor.

Future: The next steps for the team are to begin growing and characterizing bone ECM in a bioreactor, and to conduct biological, histological, and mechanical analysis post-culture. Studies involving the implantation of these "tissue engineered bone grafts" in a canine mandibular segmental defect model will begin. Future efforts include Good Laboratory Practices-compliant culturing of human mandibular and maxillary implants, and obtaining the FDA designation.

WRC CF-05

Novel Strategies for Repair and Restoration of Calvarial Bone Defects in Wounds Compromised By Infection and Scarring

Team Leader: *Joseph Losee, MD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh, Carnegie Mellon University*

Target Clinical Application: *Reconstruction of infected calvarial bone defects*

Goals: The goal of this project is to develop a calvarial reconstruction strategy that will induce bone regeneration in defects that have been complicated by infection. The effort involves three stages. The first stage will use a novel strategy of inkjet bio-patterning in degradable matrices to print different biologics (BMP-2, vascular endothelial growth factor [VEGF]) or cytokines (IL-10) in specified spatial configurations. A second stage will establish the role of different sources of stem cell therapy to heal calvarial bone, either by exogenous delivery to the defect site (ASC, bone marrow stromal cells) or by induced homing of endogenous hematopoietic progenitors using the drug AMD3100. Lastly, the synergistic or complementary efficacy of combination biologic and cellular therapies will be established. The team will conduct studies in a rabbit calvarial defect model.

Accomplishments: The team successfully created the bioprinted scaffolds for implantation. These implants were bioprinted with BMP2, IL-10, or a mixture of BMP2 and IL-10. About 50% of the procedures that comprise the 3-stage surgical rabbit model have been completed. This model consists of: 1) primary surgery to create and induce infection of a calvarial defect, 2) surgical debridement and systemic antibiotic therapy, and 3) final procedure for scar debridement and secondary reconstruction of the scarred wound, when the printed constructs are implanted in vivo to induce bone repair. Final time points have not yet been reached for the animals, so results are pending.

Future: During the next year, the team will complete all experiments related to identifying a combination of growth factors and/or cytokines that will effectively and reproducibly form bone within calvarial bone defects complicated by infection and scarring. This includes testing the effects of bio-patterned BMP-2 and IL-10 in compromised skull defects. Future studies will investigate the effects of bio-patterned BMP-2 and VEGF in skull defects.

WRC CF-06

Development of Resorbable Calcium Phosphate Cement for Regeneration of Cranial and Midface Bones

Team Leader: *John Jansen, DDS, PhD (Radboud University Nijmegen Medical Center)*

Team/Collaborating Partner Institution: *Radboud University Nijmegen Medical Center, Rice University*

Target Clinical Application: *Reconstruction of cranial and midface bones*

Goals: The main objective is to develop fully resorbable, injectable, calcium phosphate cements (CPCs) for clinical application in the craniomaxillofacial region. The team intends to conduct pre-clinical studies of a first generation resorbable CPC-PLGA formulation (CPC-PLGA) and perform a Phase I clinical trial. In parallel, the team will develop a 2nd generation resorbable CPC with accelerated degradability (by varying the amount of polymer content or changing the type of porogen) and evaluate in a Phase I clinical trial. Inclusion of antibiotics will also be considered.

Accomplishments: Working on regulatory issues, including ethical approval, concerning the pre-clinical study with CPC-PLGA was one of the major activities in the past year. The preclinical study design includes use of a rabbit femoral condyle defect model and evaluations such as descriptive histology and quantitative histomorphometry on bone formation and implant degradation. For the Phase I clinical trial with CPC-PLGA, the team developed the protocol and is in the process of obtaining regulatory approval from the Dutch Medical Ethical Commission. Clinical safety and efficacy of the implants will be evaluated in sinus floor elevation surgery patients.

Future: The team plans to conduct the rabbit femoral condyle defect model study and the Phase I clinical trial of CPC-PLGA once the appropriate regulatory approvals are received. For the development of 2nd generation resorbable CPC, the team will focus on the use of gelatin microparticles of designer degradability for use as porogen and conduct in vitro and in vivo studies of CPC-gelatin microparticle composites.

WRC CF-10

Biofabrication of Complex Tissue Components for Craniomaxillofacial Reconstruction

Team Leader: *Thomas Shupe, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Reconstruction of bone, muscle and cartilage defects*

Goals: This project aims to create complex tissue components, including bone, cartilage, and muscle, which are critical for restoring craniomaxillofacial injuries. These tissue components will be fabricated by a novel 3D bioprinting system, which can control the composition, structure, and biological and mechanical properties of tissue constructs. The 3D bioprinted tissue constructs will be evaluated in an in vivo autologous animal model.

Accomplishments: The team has developed a cell carrier material that is suitable for 3D bioprinting that includes fibrinogen, cell culture media, gelatin, glycerol, and hyaluronic acid. For printing, the material is mixed with a pellet of cells and printed at temperatures within the gel phase of the gelatin concentration. The printed, gelled solution is treated with thrombin to stabilize bioprinted tissue constructs. To achieve the goal of formulating a printable material that promotes bone formation, poly(caprolactone) (PCL) is being blended with nano-powders of tri-calcium phosphate (TCP) and hydroxyapatite ceramics. The team successfully printed using a formulation of PCL/TCP, but not with formulations incorporating hydroxyapatite. PCL/TCP scaffolds were also evaluated for water uptake ability and mechanical stability. To examine osteogenesis in vitro, the printed bone scaffolds were seeded with human amniotic fluid-derived stem cells and cultured in osteogenic differentiation media. Cell proliferation and osteogenic differentiation were observed. To determine whether the bioprinting system could be used to generate complex 3D tissue constructs, a human-sized external ear was chosen to model using a CT image to develop the printing program, and constructs were printed using three hydrogels. Rabbit ear chondrocytes were mixed with the composite hydrogel. A human ear cartilage construct was successfully fabricated. After 5 weeks in the culture medium, histological staining of the tissue constructs showed the production of a new cartilaginous matrix, and cells in the newly formed tissue demonstrated similar morphological characteristics to those in native ear cartilage. The team also designed a muscle-like fiber bundle structure composed of fibers of 500 µm width with PCL pillars placed on both ends of the structure. The pillars were incorporated to induce compaction of fibrinogen that can cause aligned cell morphology along with fiber direction. The designed structure was fabricated with 3D bioprinting technology by mixing the carrier material with C2C12 and printed with PCL. Following hydrogel induction, the structure was cultured in medium. The printed cells started stretching along the longitudinal axis of the printed fiber structures at Day 3 in culture, and the printed muscle structures induced the compaction phenomenon, keeping the fiber taut during cell differentiation. After 7 days of cell differentiation, the team observed a muscle-like structure with aligned myotube formation.

Future: The team will work on developing SOPs for the printing process for all tissue components and for autologous cell isolation and expansion. Additionally, the in vivo evaluations of printed tissue constructs will be initiated using a rabbit model.

Clinical Challenge Muscle

Another major goal of the Craniomaxillofacial Regeneration Program is to generate muscle tissue for the restoration of facial contours and function. The research teams optimized naturally derived scaffolds, such as decellularized muscle tissue and silk-based polymers, for muscle regeneration. Strategies to vascularize and innervate these muscle constructs have been developed to maximize functionality. The teams are also exploring engineering of muscle constructs to replace ocular muscle.

RCCC 4.1.2

Develop Innervated, Vascularized Skeletal Muscle

Team Leader: *Cathryn Sundback, ScD, Joseph Vacanti, MD (Massachusetts General Hospital)*

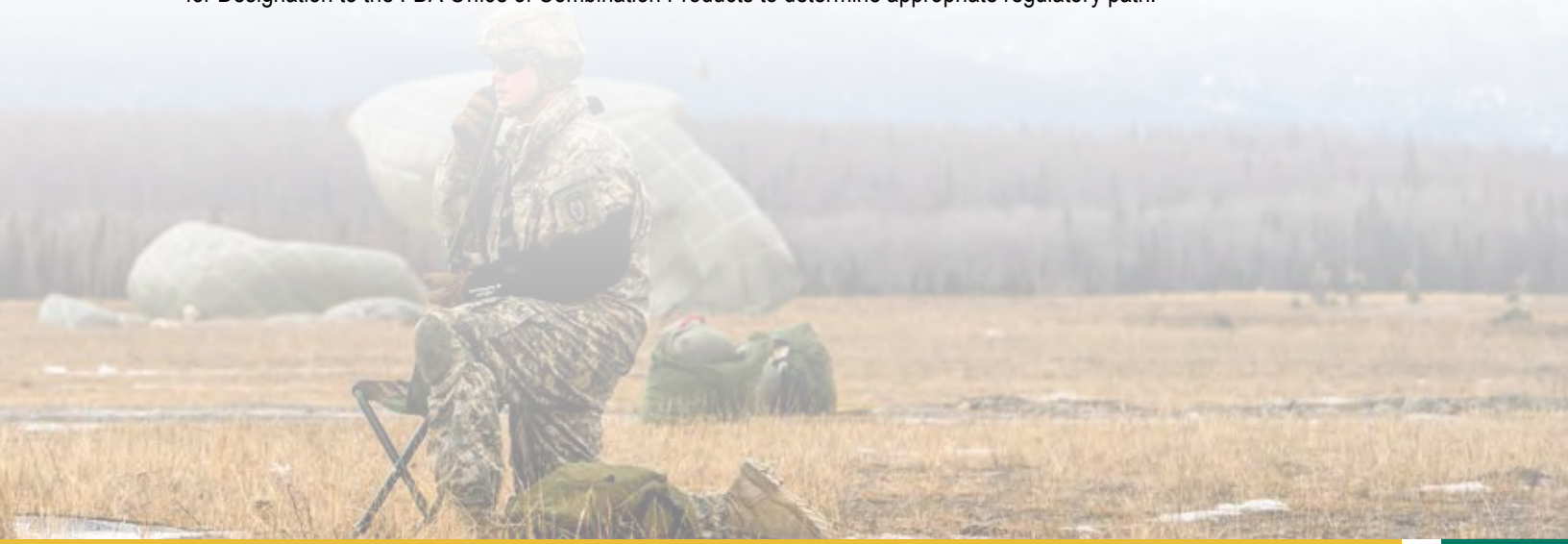
Team/Collaborating Partner Institution: *Massachusetts General Hospital, Massachusetts Eye and Ear Infirmary, Children's Hospital Boston*

Target Clinical Application: *Replacement of Skeletal Muscle*

Goals: The goal of this project is to develop a bioengineered muscle construct, using biodegradable polymer scaffolds, that is capable of integrating with nerve and blood vessels, for replacing orbicularis oculi with the intent to restore eyelid function and facial aesthetics. The effort is focused on developing the fabrication methodology and evaluating the constructs in a mouse model.

Accomplishments: The team has previously shown that myotubes, differentiated from a co-culture of mouse myoblasts and mouse fibroblasts, self-assemble to form a 3D muscle on the size scale of a fascicle (500–1000 μm diameter). The resulting construct has the complex multi-nucleated muscle architecture of native neonatal skeletal muscle and, using a vasculogenesis approach, a prevascular network is established within the engineered muscles in vitro. The team's main focus of the past year was optimizing the assembly of the muscle construct and the engineering of the endothelial network. The use of unfiltered fibrinogen improved the assembly process and the quality of the resulting muscle constructs. For the optimized muscle constructs, the team (1) demonstrated innervation of the constructs; (2) determined that electrical stimulation with neural-like signals increased the engineered muscle's contractile force; and (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. The team also conducted in vivo studies to define implantation parameters. Muscle constructs were subcutaneously implanted into a variety of immunodeficient mouse models. Constructs implanted into NOD SCID mice best retained muscle morphology and viability after three weeks implantation, and NOD SCID mice will thus be used in future studies. Finally, the team is evaluating scale-up methods for achieving muscle constructs the size of the human orbicularis oculi, and this scale-up is envisioned to be done by bundling self-assembled muscles. The team began testing an approach to combine muscle constructs, engineered with mouse myoblasts and fibroblasts, without a polymeric scaffold.

Future: The team will continue to perform studies to characterize innervation, vascularization, and muscle function. Future studies will evaluate the use of engineered muscle constructs to replace orbicularis oculi function. The team will submit a Request for Designation to the FDA Office of Combination Products to determine appropriate regulatory path.



WRC CF-07

Creating Innervated Vascularized Muscle Flaps from Elastic, Cellularized Biocomposites Developed In Situ for Facial Muscle Reconstruction

Team Leader: *William Wagner, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh*

Target Clinical Application: *Reconstruction of Facial Muscle*

Goals: The aim of this study is to generate a proto-muscle flap from an elastic biocomposite scaffold comprised of a microfibrillar biodegradable elastomer integrated with ECM digest and precursor cells. The proto-flap will be developed and evaluated in a rat model and then transferred to a rabbit model.

Accomplishments: The team has completed the in vitro synthesis and characterization of the scaffold, and mechanical and histological assessment of the constructs in vitro. A novel fabrication method is used to generate the proto-flap system in which the polyester-urethane urea (PEUU) polymer is electrospun while the ECM gel +/- stem cells are concurrently electrosprayed. Scaffolds made included small intestinal submucosa (SIS)-PEUU bi-layer scaffolds, and dermal ECM (DECM)-PEUU and urinary bladder matrix (UBM)-PEUU scaffolds. Visual inspection showed the ECM gel properly accumulated on top of the PEUU rich layer and the stiffer direction of the scaffold which corresponded to the circumferential direction of the mandrel, and Masson's trichrome staining confirmed ECM content and the bi-layer structure. Mechanical characterization was performed by biaxial tensile testing and included strain energy quantification and membrane tension vs. stretch characteristic under equi-stress conditions. The SIS-PEUU bi-layer scaffolds showed lower strain energy at a peak tension of 60 N/m when compared to both the DECM-PEUU and UBM-PEUU scaffolds. In terms of mechanical anisotropy, the SIS- and DECM-based scaffolds showed mechanics comparable to the native rat abdominal wall, whereas the UBM-based construct was characterized by a smaller difference in compliance between the circumferential and longitudinal direction axes. IACUC and ACURO approvals were received on the protocols for the in vivo studies. The acellular scaffold studies have begun. Initial results at the 2-week time point show no marked differences between the groups based on visual inspection and histological assessment. The team saw comparable morphology, cell infiltration of the scaffold ECM rich layer, and host cell recruitment with all three ECM types. Mechanical and structural assessments at longer time points are planned.

Future: The team will next focus on completing the acellular scaffold assessments on the 8- and 16-week explants, fabricating and implanting the best ECM + cells groups, completing cell integrated scaffold explant assessments, and developing a load bearing proto-flap model in the rat.

WRC CF-08

Bioreactors and Biomaterials for Tissue Engineering of Skeletal Muscle

Team Leader: *George Christ, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM, Tufts, UTHSC*

Target Clinical Application: *Functional restoration of volumetric muscle loss injuries*

Goals: The overall goal of this effort is preclinical development and pilot clinical testing of scalable, implantable Tissue Engineered Muscle Repair (TEMR) constructs capable of cosmesis and functional restoration for treatment of volumetric muscle loss (VML) injuries. The approach will involve development and optimization of silk-based TEMR scaffolds in rat tibialis anterior (TA) and mouse latissimus dorsi (LD) VML injury models, and a pilot clinical study of a 2nd generation bladder acellular matrix-based TEMR scaffold.

Accomplishments: The team has optimized the scaffold configuration for cell infiltration following implantation in TA VML injury, and design, formulation, and analysis of LD scaffolds is underway. The team evaluated cell infiltration in a rat model involving subcutaneous implantation, and the results indicated that the addition of fibrin enhanced cell infiltration, and channels perpendicular to alignment improved cell infiltration and matrix production. Addition of VEGF silk particles enhanced cell infiltration near the spot of the injection, but not throughout the construct. The team also assessed the ECM composition in skeletal muscle (rat anterior tibialis) using LC-MS/MS and peptide analysis to determine if collagen collected from the digestion of rat-tail tendon would be appropriate ECM mimic for this tissue. Addition of collagen isolated from rat tail tendon will provide proper integrin binding for initial studies, and will be used to generate scaffolds for in vivo analysis. With regard to the pilot clinical study, the team has obtained FDA guidance to finalize the plan for preclinical toxicology studies and is working to revise the clinical study protocol based on prior guidance. An Investigational New Drug (IND) application submission to FDA is planned for use of TEMR for the treatment of secondary revision of cleft lip in patients 18-30 years of age. The team initiated preclinical studies and completed technology transfer to the CRO (cell culture, scaffolds, cell seeding techniques, bioreactor use and sterilization, VML surgery, TEMR implantation, etc.). Subject recruitment has begun, with 3 of 5 subjects identified.

Future: The team will continue work to optimize the Gen III (TA) and Gen I (LD) silk scaffolds, and evaluate the scaffolds via in vitro analysis and in vivo implantation studies. The pilot clinical study effort will continue recruitment of subjects, and the team will revise the protocol and submit the IND to FDA.

WRC CF-09

Injectable and Implantable Soft Tissue for Craniofacial Reconstruction

Team Leader: *Peter Rubin, MD (University of Pittsburgh)*

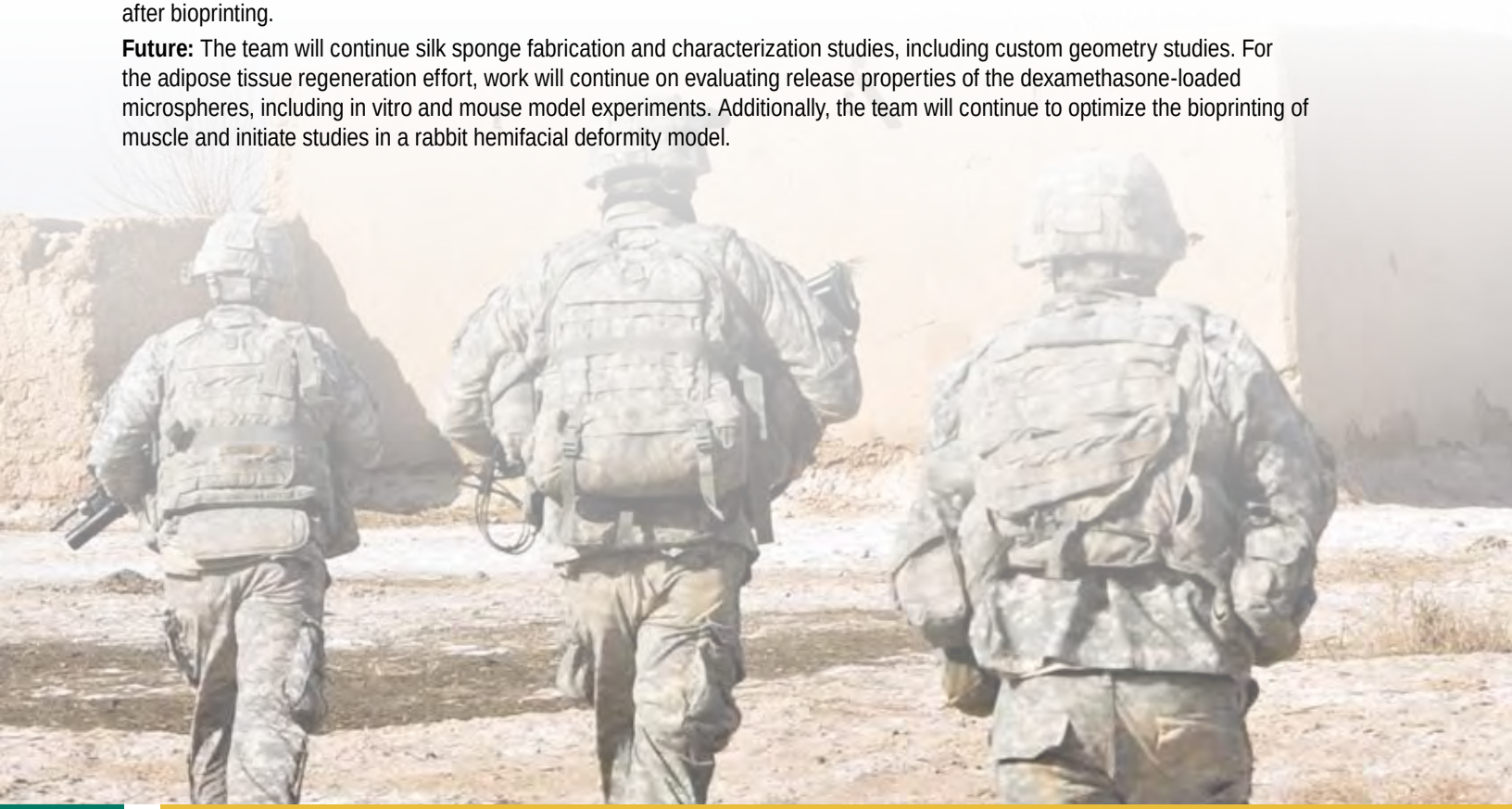
Team/Collaborating Partner Institution: *University of Pittsburgh, WFIRM, Tufts, USAISR*

Target Clinical Application: *Reconstruction of soft tissue*

Goals: The goal of the project is to develop soft tissue constructs that can be implanted using a minimally invasive approach for craniofacial soft tissue. The specific focus is to optimize controlled release drug delivery system and perfusion delivery system based on adipose stem cells and biomaterial scaffolds, optimize injectable scaffolds for soft tissue regeneration, and incorporate nerve and muscle regeneration enhancing strategies into the soft tissue constructs. The effort will involve in vitro characterization of the various technologies, as well as studies in animal models.

Accomplishments: The team fabricated biodegradable porous silk tubes for the delivery of therapeutics (inner diameter of 300-400 µm and outer diameter of 700-800 µm). The team is developing animal studies for implantation, and future work will look at controlling the in vivo degradation rate of the silk tubes, and optimizing pore size and wall thickness for material transit through the walls. The team also worked to create polymer microspheres containing adipogenic factors, specifically dexamethasone was encapsulated within the core of PLGA (poly[lactic-co-glycolic acid]) surrounded by shell of PLLA (poly-L-lactic acid). The team evaluated microsphere wall structure by SEM microscopy, and encapsulation efficiency and loading capacity by UV-absorbance spectrometry for drug released from degrading microspheres. The team performed mechanical characterization of porous silk sponges incorporating a plasticizer to tune the swelling properties, stiffness, and morphology. Following compression and expansion in PBS, sponges recovered to near initial volume with less deformation, and had stiffness values (7.5 kPa to 810 kPa) suitable for reconstructing a variety of soft tissues, pore diameters (100-200µm) large enough for cell infiltration, and uncompromised pore size and shape. The team is also working to optimize the bioprinting of muscle constructs. Constructs of varying cell densities were evaluated for cell viability, cell apoptosis, and skeletal muscle development. To improve muscle development and promote neuromuscular junction formation, bioprinted muscle constructs containing neural cells (NCs) and endothelial cells were also fabricated and evaluated. Mytotube formation was evident in the constructs containing NCs, and enhanced AchR cluster formation on the myofibers was induced. The expression of neurofilament in the constructs showed the integration of NCs and myofibers. Overall, the results indicate that NCs can improve the muscle cell viability and development after bioprinting.

Future: The team will continue silk sponge fabrication and characterization studies, including custom geometry studies. For the adipose tissue regeneration effort, work will continue on evaluating release properties of the dexamethasone-loaded microspheres, including in vitro and mouse model experiments. Additionally, the team will continue to optimize the bioprinting of muscle and initiate studies in a rabbit hemifacial deformity model.



WRC CF-12

In Vivo Functional Muscle Regeneration Utilizing an Implantable Modular Bioreactor

Team Leader: *Robert Galiano, MD (Northwestern University)*

Team/Collaborating Partner Institution: *Northwestern University, UTARI*

Target Clinical Application: *Reconstruction of muscle*

Goals: This goal of this project is to develop a novel strategy using an implanted bioreactor, decellularized muscle scaffolds, myogenic stem cells, and neurotization strategies to engineer functional vascularized muscles in vivo. The approach is to optimize the decellularized muscle scaffold and stem cell bioreactor system in a rodent model, assemble bioreactors robotically, test strategies for neurotization in rodents, and advance to larger animal studies.

Accomplishments: The team focused primarily on screening of decellularization methodologies and characterization of these constructs using rat muscle tissues. Based on the hypothesis that negative pressure would open pores, permitting the more efficient penetration of decellularization solutions, negative pressure-assisted decellularization was tried and found to improve the efficacy of decellularization by gross appearance, and the methods did not significantly alter tissue microstructure. The team is continuing to work on further fabrication methodologies to improve hydrogel production. Tensile testing of decellularized scaffolds from rat soleus was conducted and found to be of higher stiffness and yield strength that native muscle tissue, possibly due to the loss of native ECM components such as elastin. The team attempted to efficiently measure multiple growth factors using mass spectrometric proteomic analysis, but the results were inconsistent due to the tight associations of the growth factors with the insoluble ECM components. Future studies will employ enzyme-linked immunosorbent assays. The team also attempted to seed cells onto decellularized matrices in vitro with myoblasts and myogenic precursors; however, success was limited to the thickness of the graft. In particular, direct injection methods resulted in the formation of seeded cell islands and cell death, while centrifuge-assisted recellularization resulted in one-sided penetration of seeded cells. The team has refined literature techniques for the isolation of ASCs. Protocols have also been developed for muscle generation using an in vivo static bioreactor. One strategy involves a gradual layer-by-layer construction of vascularized muscle tissue using sheets of decellularized muscle scaffold, avoiding the nutritional diffusion limit and permitting angiogenesis to occur in a step-wise manner. The other strategy involves muscle regeneration on a neurovascular pedicle with the presence of existing muscle tissue in order to use the myogenic properties of extant muscle tissues to promote de novo formation of tissue on a decellularized scaffold with incorporated progenitor cells.

Future: The team will focus on optimizing current decellularization techniques to produce suitable hydrogels for bio-fabrication and also conduct in vitro characterization studies. To improve cell engraftment approaches, future efforts will focus on utilizing thin sheets of scaffold materials (100-500 µm) to promote full-thickness recellularization while overcoming nutritional diffusion distances. The team will continue efforts to optimize conditions for differentiation of ASCs into myoblasts for muscle regeneration. Finally, the team is revising the design and manufacturing of 3D-printed bioreactors based on clinical feedback.

WRC CF-13

Hybrid Muscle-Nerve Tissue Integration System (MuNTIS) for Functional Restoration of Larger Volumetric Muscle Loss Injuries

Team Leader: *George Christ, PhD (Wake Forest University)*

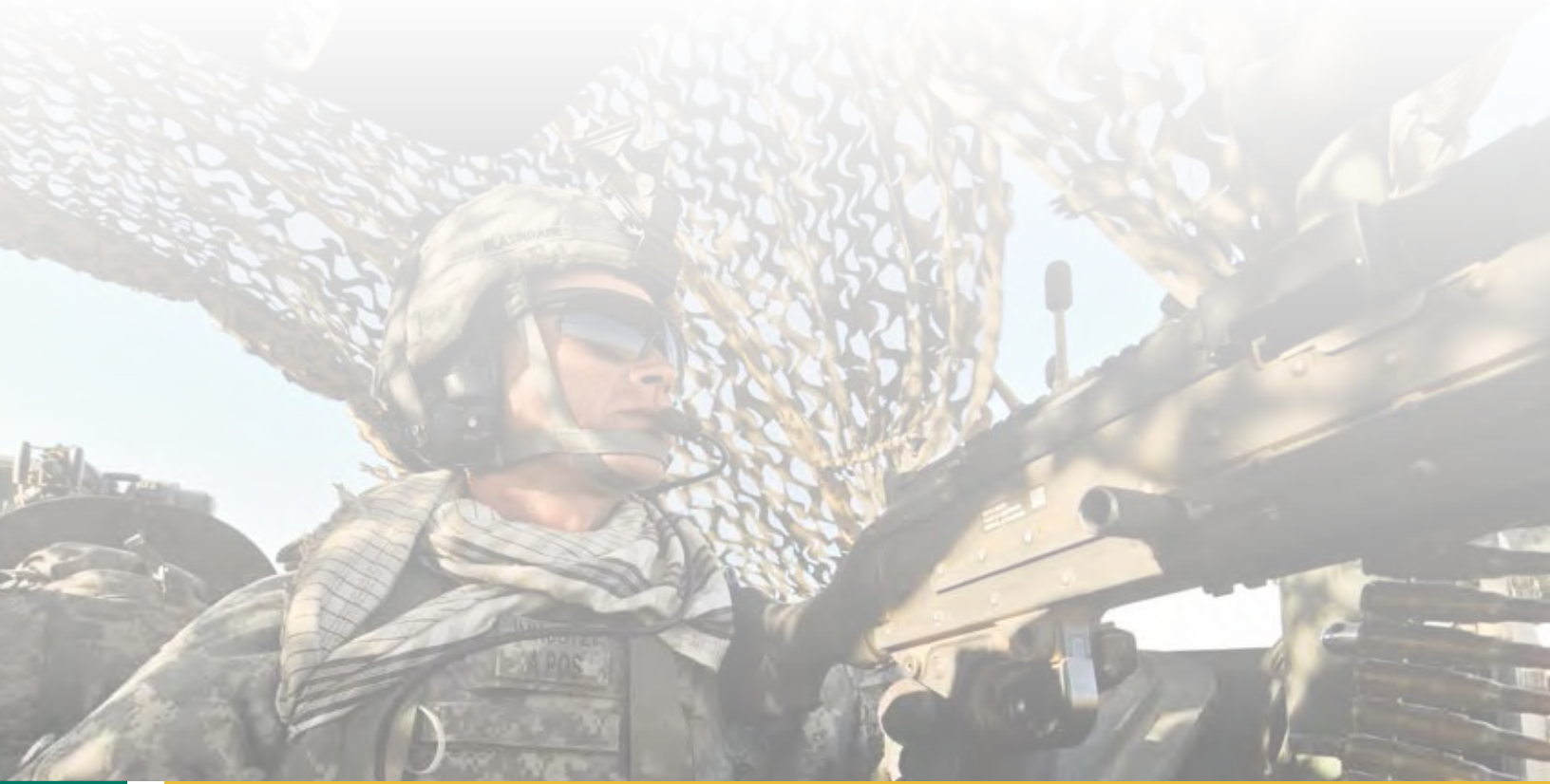
Team/Collaborating Partner Institution: *WFIRM, Tufts, University of Pittsburgh*

Target Clinical Application: *Reconstruction of muscle*

Goals: The objective of this project is to restore muscle–nerve interfaces through complex biomaterial designs. This approach will be integrated with an existing bioreactor pre-conditioning technology to promote formation of more fully functional bioengineered muscle constructs adapted to include a hybrid Muscle–Nerve Tissue Integration System (MuNTIS). The MuNTIS will be tested in rat and canine tibialis anterior-volumetric muscle loss injury models.

Accomplishments: The team conducted in vitro studies to verify the applicability and similarity of rat antigen presenting cells (APCs) to human APCs for cell seeding of nerve conduit for use in preclinical evaluations. The team successfully isolated rat ADCs from fat and plated on rat-tail collagen coated flasks, and then allowed these to adhere overnight. Rat adipose-derived stem cells (ASCs) displayed typical spindle-shaped phenotype and proliferated well in culture. The team used flow cytometry and antibodies to evaluate CD marker expression. Rat ASCs showed typical CD marker expression of adipose-derived stem cells and were CD 45- (a marker for hematopoietic cells), as well as CD29+ and CD90+ (mesenchymal stem cell markers). Early flow cytometry data suggests the CD marker expression is similar to human ASCs. Studies for functional testing in rat peroneal nerve defect model have just begun, with surgical protocols/methods for in vivo testing having been developed. The MuNTIS scaffold design and prototype construction effort to create an improved prototype silk-based system where APCs and mesenchymal progenitor cells can be co-cultured together is progressing. The APCs should be seeded within isotropic silk scaffolds (blue), and the mesenchymal progenitor cells will benefit from both ECM components and alignment. The team formed initial molds to fit the design, which will allow for culturing the cells individually and then suturing the parts together.

Future: The team will confirm the multipotent nature of isolated rat ASCs with osteogenic, chondrogenic, and adipogenic differentiation studies. Further refinement and optimization of the MuNTIS scaffold is expected.



Clinical Challenge Other Tissue

The Craniofacial Program is also focused on the regeneration of other tissues found in the craniofacial complex, such as the dentition, cartilage, adipose, and soft tissue. The researchers developed an implantable ear construct, and this is progressing towards initiating clinical trials. Another effort characterized composite scaffolds and demonstrated these support human dental stem cell (hDSC) differentiation into alveolar bone. In addition, an optimal scaffold and cell source for bioprinting cartilage was established. Lastly, a team synthesized an off-the-shelf soft tissue replacement technology employing GMP standards, and they are currently involved in regulatory discussions.

RCCC 4.5.4

Engineering of a Replacement Autologous Outer Ear Using a Collagen/Titanium Platform

Team Leader: *Cathryn Sundback, ScD, Joseph P. Vacanti, MD (Massachusetts General Hospital)*

Team/Collaborating Partner Institution: *Massachusetts General Hospital, Massachusetts Eye and Ear Infirmary, Massachusetts Institute of Technology, Kensey Nash Corp*

Target Clinical Application: *Reconstruction of Outer Ear*

Goals: The goal of this project is to develop a permanent, implantable external ear in which cartilage cells are seeded on a human ear-shaped collagen scaffold with an embedded titanium wire support. The effort focused on developing fabrication methods and studies in animals.

Accomplishments: The team designed a composite scaffold comprised of porous collagen and embedded titanium framework. They demonstrated size and shape maintenance in immunocompromised rodents and in an immunocompetent animal. The researchers developed a non-invasive analytical method for assessing 3D changes in ear-shaped implants using high-resolution CT scans. The team developed and validated an in vitro pre-implantation culture procedure to initiate neocartilage maturation. High quality mature and stable elastic neocartilage was demonstrated in sheep, both in a simple disk shape and in an ear shape. To address the challenge of obtaining sufficient number of autologous chondrocytes, the team explored several approaches to expand chondrocytes without loss of differentiation. Ear-shaped cartilage was engineered in sheep using extensively expanded chondrocytes and composite scaffolds with embedded titanium frameworks consistent with the previously reported data. This is the first demonstration of ear-shaped implant engineered with extensively expanded chondrocytes in an immunocompetent animal.

Future: This RCCC project has been completed. The team anticipates continuing to develop clinically relevant cell sources for the engineered ear, conducting long-term stability studies in an immunocompetent animal model, and submitting a Request for Designation to the FDA to determine the regulatory path. In preparation for clinical trials, the researchers expect to perform a Good Laboratory Practices preclinical trial in sheep to demonstrate safety and efficacy, and to develop the protocol for a pilot clinical trial.

WRC CF-04

Bioengineered Alveolar Bone and Tooth Constructs

Team Leader: *Pamela Yelick, PhD (Tufts University)*

Team/Collaborating Partner Institution: *Tufts University, Rutgers University*

Target Clinical Application: *Repair of jaw and dental defects*

Goals: The goal of this project is to fabricate biphasic, biodegradable scaffolds to facilitate the repair and regeneration of alveolar jaw bone and teeth to treat jaw and tooth defects. The effort is focused on fabricating scaffolds, demonstrating hDSC viability on scaffolds using in vitro and in vivo methods, and demonstrating alveolar bone formation on in vivo hDSC seeded CaP containing scaffolds in a rat model.

Accomplishments: In the past year, the team fabricated CaP containing scaffolds made of poly(DTE-co-10% DT-co-1% PEG1K), abbreviated E1001(1k), with and without 30% beta-TCP (βTCP). The morphology of E1001(1K) and E1001(1K)/βTCP scaffolds were examined using light and electron microscopic analyses. The team observed a bimodal, highly porous scaffold architecture (micropores 10-20μm; macropores 100-200μm) with a wide distribution of interconnected pores, and a highly organized, self-aligned microporous (< 20μm) structure around the macropores. These structural features may promote cellular infiltration, enhance nutrient and waste product diffusion, and improve angiogenesis and osteoconduction. The team devised histological methods to examine unseeded scaffolds, and histological analyses of DSC seeded scaffolds cultured in vitro for up to 3 weeks showed that DSCs adhered to and proliferated on the porous surfaces of the scaffold and also extended across scaffold pores. CaP containing scaffolds supported the viability, proliferation, and differentiation of hDSCs in an in vitro model. Immunofluorescent histochemical analyses showed that hDSC seeded scaffolds exhibit high proliferation on E1001(1K)/βTCP scaffolds. The researchers also devised histological methods to analyze hDSC differentiation when seeded onto CaP containing scaffolds and cultured in vitro in osteogenic media. The following conclusions were drawn from the studies: (1) cells can penetrate through both types of scaffolds, (2) scaffolds with βTCP support hDSC cell growth better than those without, (3) scaffolds with βTCP do not support pDE cell growth (E1001[1K] scaffolds do support DE cell growth), (4) scaffolds, especially the scaffolds with βTCP, become brittle after 3 weeks in vitro culture, (5) phalloidin staining results on sections suggest cells spread along the surface of scaffolds and also proliferate in the macropores, desirable for optimal tissue formation, and (6) cells may help maintain the integrity of scaffolds, allowing better control of size and shape of bioengineered tissues.

Future: Over the next year, the team plans to optimize the scaffold fabrication/design for in vivo human DSC differentiation to alveolar bone and perform in vivo testing of scaffold designs in a subcutaneous rat implant model.

Clinical Trials

Several AFIRM craniomaxillofacial 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.

Table III-2. AFIRM-funded Craniomaxillofacial Reconstruction projects with pending or active clinical trials.

Therapy/Product	Project Title	Consortium	Project Number	Trial Phase	Current Status
Repair of intraoral soft tissue defects / Ex Vivo Produced Oral Mucosa Equivalent (EVPOME)	Clinical Trial – Tissue Engineering Complex Soft Tissues for Repair of CMF Injuries	RCCC	4.5.2CT	I/II	Active
Soft tissue reconstruction / Autologous stromal vascular fraction cells admixed with lipoaspirate grafts	Autologous Adipose Derived Stem Cell Therapy for Soft Tissue Reconstruction after Facial Trauma	WFPC	4.1.7	I	Active
Soft tissue reconstructions / Acellular adipose tissue (AAT)	Pilot Clinical Testing of a Novel Soft Tissue Reconstruction Solution	WRC	CF-11	Pilot	Pending

RCCC 4.5.2CT

Clinical Trial – Tissue Engineering Complex Soft Tissues for Repair of CMF Injuries

Team Leader: *Stephen E. Feinberg, DDS, PhD (University of Michigan)*

Team/Collaborating Partner Institution: *University of Michigan, Carnegie Mellon University, LifeCell Corporation*

Target Clinical Application: *Repair of intraoral soft tissue defects*

Goals: This project seeks to develop an Ex Vivo Produced Oral Mucosa Equivalent (EVPOME) for the treatment of large intra-oral defects, using tissue engineering in conjunction with the surgical technique of prelamination, to create a prevascularized composite soft tissue flap. The team will conduct a Phase I/II clinical trial to assess safety and efficacy.

Accomplishments: The development of a tissue engineered human oral mucosa will facilitate intraoral reconstruction of the oral cavity and other mucosal structures such as the lips, eyelid, and anterior nares. During prior years in preparation for the trial, the team has developed a manufacturing process to fabricate large-sized EVPOMEs for a clinical trial, successfully fabricated a large EVPOME device in a cGMP facility, and received FDA, IRB, and HRPO approval of the protocol. In the past year, the case report forms were finalized and the study was activated. The team has screened and enrolled the first subject, and performed the first graft placement surgery. Continuing review approval from the IRB and HRPO has also been received.

Future: The team will continue to expand subject recruitment efforts.

WFPC 4.1.7

Autologous Adipose Derived Stem Cell Therapy for Soft Tissue Reconstruction after Facial Trauma

Team Leader: *Peter Rubin, MD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest University, University of Pittsburgh, Louisiana State University*

Target Clinical Application: *Reconstruction of Soft Tissue*

Goals: The goal of this project is to develop implantable and injectable vascularized soft tissue composed of connective tissue and fat. The technology will be assessed in two related clinical trials, one for craniofacial reconstruction and one for amputation stump pain and poor prosthetic fit.

Accomplishments: The team has initiated clinical trials involving the use of autologous stromal vascular fraction cells admixed with lipoaspirate grafts to achieve vascularized soft tissues at either a craniofacial site to achieve volume retention, or an amputation stump site for alleviating pain and improving prosthetic fit. While initially a manual method was considered, going forward, the team is enrolling subjects for treatments that involve the use of an automated machine, Tissue Genesis Isolation System, to isolate ASCs. For the craniofacial application, facial appearance and persistence of treatment effect will be assessed using aesthetic grading scales, state-of-the-art 3D photography, and high-resolution CT scanning with 3D reconstruction; and subjects will be followed for 9 months after treatment to define long-term outcomes. The team will characterize ASCs function in each subject, correlate biologic properties of the stem cells with clinical outcomes, and expand and bank cells under GMP conditions for future therapy. Quality of life in subjects after treatment will be assessed using validated psychosocial measures. Seven subjects have been enrolled, 5 have completed the surgical intervention, and long-term follow-up is ongoing. In the amputation stump study, the enriched fat grafts are placed to provide additional subcutaneous tissue padding over bony structures and nerve trunks in subjects with pain at an amputation site that limits function and/or interferes with the ability to use a prosthetic. Limb anatomy and healing of the graft over time, along with stability/persistence of the new tissue, will be assessed by high-resolution CT scanning with 3D reconstruction, and subjects will be followed for 6 months after treatment to define long-term outcomes. The team will assess biologic properties of the cells within the fat graft and correlate with clinical outcomes. Quality of life will be measured in subjects after treatment using validated psychosocial measures. Six subjects have been enrolled, 5 have completed the surgical intervention, and long-term follow-up is ongoing.

Future: The team will continue the clinical trials to complete the long-term follow-up and perform data analysis.

WRC CF-11

Pilot Clinical Testing of a Novel Soft Tissue Reconstruction Solution

Team Leader: *Jennifer Elisseeff, PhD (Johns Hopkins University)*

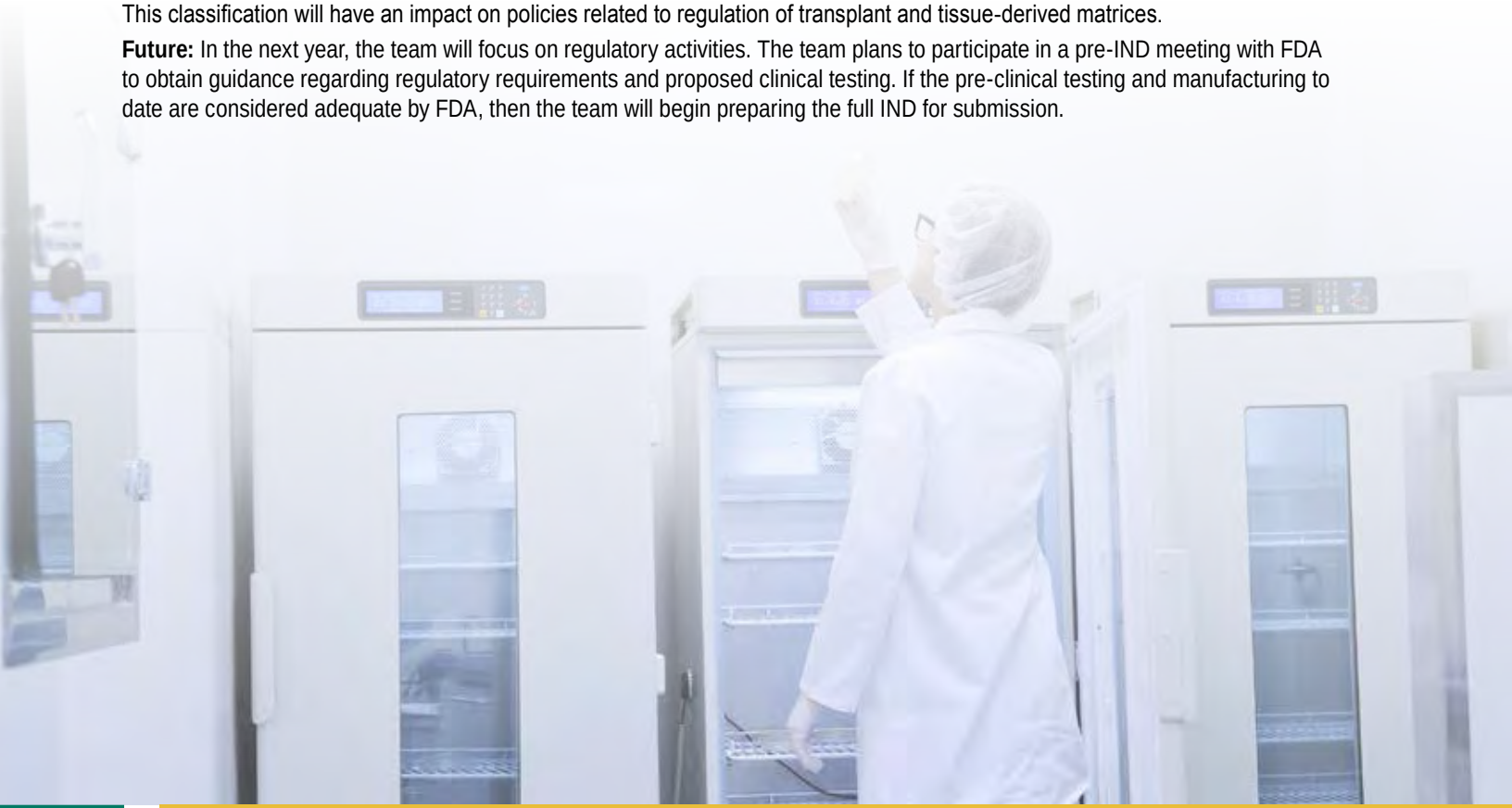
Team/Collaborating Partner Institution: *Johns Hopkins University, Aegeria*

Target Clinical Application: *Reconstruction of soft tissue defects*

Goals: A predictable, "off-the-shelf" material that retains the mechanical and biological properties of adipose tissue was previously developed by the Elisseeff laboratory. The goal of this pilot clinical study is to evaluate the safety and efficacy of an off-the-shelf soft tissue replacement technology for treating soft tissue defects. Project milestones include manufacture of clinical grade material, regulatory approvals from FDA and the IRB for the study, and conduct of the pilot clinical study.

Accomplishments: Acellular adipose tissue (AAT) is composed of structural proteins and ECM components derived from adipose tissue harvested from cadaver donors. The product is manufactured and delivered to provide a viscoelastic consistency for optimal injection and maintenance of volume after implantation. For evaluation of the manufacturing process, the team (1) evaluated intermediates in AAT processing and the resulting final product with respect to morphology and mechanical properties, and (2) characterized tissue samples during processing to confirm minimal alterations and consistency within the process. The findings indicated that 1) lipids can be removed from adipose tissue without altering the basic ECM organization, 2) ultrastructure of tissue is maintained during processing and after cutting into smaller pieces, 3) rheological (mechanical) properties of the processed adipose are nearly identical to lipoaspirate, 4) thermal properties of the adipose tissue shift significantly when lipids are removed but further processing and sterilization produces minimal changes (suggesting minimal changes to the AAT structure), and 5) proteomic analysis of the ECM proteins reveals a complex mixture of structural proteins typical of a extracellular tissue matrix. The team evaluated the in vivo behavior of the AAT in multiple animal models including mouse, rat, and swine in comparison to the current clinical gold standard of autologous fat grafting. In the large animal studies, the team also piloted additional techniques to monitor implant volumes and surface contours using novel imaging strategies. Overall, the preclinical studies demonstrated biocompatibility of the processed AAT in mice, rats, and pigs; volume maintenance of AAT implants in all animal models; and reduction in calcification and cysts that occur from autologous fat grafting (due to an inflammatory response to released intracellular lipids). In response to a Request for Designation, the FDA classified the AAT as a biologic, thus requiring an IND application prior to initiating human clinical trials. This classification will have an impact on policies related to regulation of transplant and tissue-derived matrices.

Future: In the next year, the team will focus on regulatory activities. The team plans to participate in a pre-IND meeting with FDA to obtain guidance regarding regulatory requirements and proposed clinical testing. If the pre-clinical testing and manufacturing to date are considered adequate by FDA, then the team will begin preparing the full IND for submission.



IV: Skin Regeneration

AFIRM OUR SCIENCE FOR THEIR HEALING

Background

More than 45,000 U.S. Soldiers have been wounded in Operations Enduring Freedom, Iraqi Freedom, and New Dawn. Combat wounds range from superficial to extensive injuries involving skin and underlying tissues. Burn injuries comprise 5%–20% of wartime casualties and cause 500,000 U.S. civilians to seek medical attention annually. Extensive skin injuries can have devastating outcomes. Infection is a grave short-term risk; disability and disfigurement pose greater challenges over the long term due to scarring, contractures, and suboptimal healing. Strategies to address skin repair are of critical importance. A broad approach encompassing burn mitigation, small molecule and cell-based therapies, tissue scaffolds, and "off-the-shelf" skin is needed to successfully address combat wounds

IV: Skin Regeneration

Areas of Emphasis

Developing effective therapies for Soldiers with complex battle wounds requires a multifaceted approach that promotes tissue regeneration while mitigating fibrosis. The projects in the Skin Regeneration focus area address both near-term improvements in care and longer-term, potentially transformative technologies for treating these injuries. The approaches include growth factor/cytokine modulation, improved cell-based therapies, better scaffolds, novel skin substitutes, and new skin replacement techniques. Early successes include the discovery that statins, already in wide clinical use, inhibit a crucial mediator of fibrosis. Various types of statins, doses, and delivery methods are being compared in large animal models to assess their relative efficacy in controlling fibrosis and enhancing regeneration in anticipation of clinical trials. A second project based on a successful AFIRM I program is working on strategies to block focal adhesion kinase (FAK), critical in inflammation and fibrosis, using an experimental small molecule developed by Pfizer. In addition, fibronectin (FN)-derived peptides are being evaluated for their ability to limit progression of burn injuries, a completely new approach to burn injury. Skin substitute and replacement approaches range from device development for full-thickness microscopic tissue column transfer, to the recent filing of a U.S. Food and Drug Administration (FDA) Investigational New Drug application (IND) to initiate a clinical trial for full-thickness burns and complex skin defects using the product, Stratagraft. These examples, along with the other projects described below, provide a comprehensive approach to enhancing tissue regeneration in the setting of combat wounds. As shown in **Table IV-1**, projects are grouped into three clinical challenge topic areas: skin replacement, optimization of the wound environment/promotion of skin regeneration, and scarless wound healing. Clinical trials are not included in Table IV-1, but are instead presented in a separate section of this chapter.

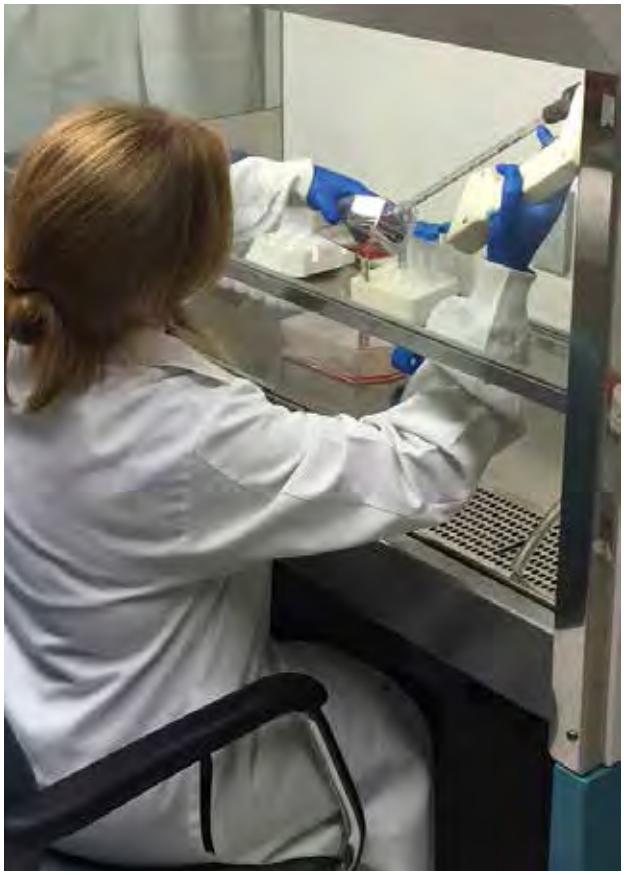


Table IV-1. Projects funded by RCCC, WFPC, and WRC by clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Skin Replacement	WFPC	4.2.5	In Situ Bio-printing of Skin for Battlefield Burn Injuries
		4.2.8	In Vitro Expanded Living Skin for Reparative Procedures
	WRC	SR-05	Novel Point-of-Care Cell Therapies for Full-thickness Wounds
		SR-07	Autologous Engineered Skin Substitutes with Pigment from Cultured Human Melanocytes
		SR-08	Skin Copying for Wound Repair
Optimization of the Wound Environment/ Promotion of Skin Regeneration	RCCC	4.6.1e	Pre-IND Studies for a Novel Cell Survival Peptide that Limits Burn Injury Progression
		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
	WRC	SR-03	Biomimetic Stem Cell Dressing for Skin Regeneration
		SR-04	Amniotic Fluid Derived Stem Cells for Enhanced Wound Healing
		SR-09	Localized Topical Metal Modulation to Inhibit Burn Progression
		SR-10	Novel Fibronectin Peptide Enhancers of Vascular Endothelial Cell Growth Factor to Promote Endothelial Cell Survival, Angiogenesis and Tissue Regeneration after Burns and Battle Injury
Scarless Wound Healing	RCCC	4.7.1	Adipose-Derived Therapies for Wound Healing, Tissue Repair, and Scar Management
	WRC	SR-01	Targeted Delivery of a Small Molecule Focal Adhesion Kinase Inhibitor for Scarless Wound Healing
		SR-02	Local Application of Statins to Reduce Scarring
		SR-11	Biomask for Skin Regeneration
		SR-12	Novel Polysaccharide Compound to Enhance Wound Healing and Decrease Scarring

Clinical Challenge Skin Replacement

The AFIRM researchers are focused on developing novel skin substitutes and skin replacement techniques to improve the outcomes of treatment of combat injuries. The ultimate objective for skin substitutes is to restore to injured skin the anatomy and physiology of normal, healthy, uninjured skin. There are currently no skin substitutes available which fully replace all of the structures or functions of uninjured skin, but researchers are continuing to advance the technology of facilitating wound repair through development of improved skin, skin substitutes, and skin regeneration therapies.

WFPC 4.2.5

In Situ Bio-printing of Skin for Battlefield Burn Injuries

Team Leader: *James J. Yoo, PhD (Wake Forest Institute for Regenerative Medicine [WFIRM])*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Burn Wound Repair*

Goals: The researchers' project goal is to develop a full-thickness biological skin that can be fabricated in situ using a portable bioprinter, and will result in a novel treatment for battlefield burns.

Accomplishments: The researchers have designed a portable skin printing system, and demonstrated the feasibility of in situ bioprinting for rapid care of skin wounds by showing that two different skin cell types could be directly delivered onto a wound in a mouse model as well as a porcine model. They have demonstrated that these skin cells remain viable and survive after printing. In addition, printed skin cells are able to form normal skin tissue and integrate with the surrounding skin, showing that full-thickness skin defects can be repaired with a bioprinter delivery system. The team scaled-up the bioprinting hardware and developed software for the bioprinter, in preparation for the large animal study, which they performed using a porcine model. Their results demonstrated that bioprinting autologous keratinocytes and dermal fibroblasts enhanced the healing rate of the burn wounds in the porcine model. This preclinical study suggests that the use of skin bioprinting is an alternative approach for rapid coverage of extensive skin wounds such as burn.

Future: The team completed this project.

WFPC 4.2.8

In Vitro Expanded Skin for Reparative Procedures

Team Leader: *Sang Jin Lee, PhD; James J. Yoo, MD, PhD; James H. Holmes, MD (Wake Forest)*

Team/Collaborating Partner Institution: *Wake Forest; North Carolina State University*

Target Clinical Application: *Autologous Skin Grafts for Burn Repair*

Goals: The ultimate goal of this project is to provide wounded Soldiers with large dimensions of autologous skin for reparative procedures. Specifically, the researchers will optimize expansion parameters for maximizing surface dimensions of skin, conduct validation studies of the expanded skin grafts in a preclinical burn animal model, and prepare materials for FDA approval for a clinical trial.

Accomplishments: The researchers demonstrated the clinical feasibility of using the expanded skin grafts in a pig full-thickness skin excisional model. They observed that cell/tissue viability was maintained and dermal structural integrity was preserved during the in vitro skin bioreactor. The expanded skin grafts showed high graft take rate, low wound contraction, and high integration of expanded skin onto wound site tissue. In addition, they submitted pre-IDE/IND documents to the FDA.

Future: The team will work to obtain approval for a clinical trial for the expansion of donor skin for the treatment of larger area burns.

WRC SR-05

Novel Point-of-Care Cell Therapies for Full-thickness Wounds

Team Leader: *Adam Katz, MD (University of Florida)*

Team/Collaborating Partner Institution: *University of Florida*

Target Clinical Application: *Therapy for Full-Thickness Wounds*

Goals: The goal of this project is to develop an autologous cell-based, point-of-care dermal replacement therapy that provides and/or facilitates clinical wound healing and/or scar results for full-thickness wounds similar to, or better than, a full-thickness skin graft or flap, using readily available adipose tissue as a cell source so as to minimize donor site morbidities.

Accomplishments: The researchers completed a number of major activities during the first year of AFIRM II, which include multiple isolations of adipose-derived cells (stromal vascular fraction [SVF] cells), dermal wound paste (DWP) formulation and culture, DWP analysis, testing of DWP conditioned media, design and approval of an initial in vivo study protocol, and initiation of this in vivo study. Their data demonstrated that DWP can be formulated with autologous point-of-care adipose derived cells in a reproducible fashion. They validated the use of a novel, single-use disposable device for cell isolation. The University of Florida team also showed that cells can be uniformly suspended within the paste in real time, and that the cells survive and proliferate within the paste over a period of 2 weeks. The formulation process was reproducibly safe with no evidence of bacterial contamination. Initial testing of paste-generated mediators demonstrated enhanced angiogenic signaling by paste formulations as evidenced by endothelial tube formation. Immunohistochemical analysis confirmed the presence of abundant endothelial cells within the paste.

Future: In the coming year, the University of Florida team will complete analytical studies (i.e. potency assays) related to the formulation and characterization of wound paste, and expand upon them with additional donors as needed. In addition, they will continue to complete the first of 3 proposed animal studies. This first animal study ("GFP tracking study"), using GFP donor murine cells in a syngeneic recipient, was initiated ahead of schedule, and data collection and analysis will be completed in the coming reporting period. Finally, the team plans to initiate the second of these animal studies, using Human adipose-derived stem cells (ASCs) wound paste (donor) in an immuno-compromised murine wound model with primary goals to demonstrate safety and efficacy ("human cell safety and efficacy study") earlier than planned.

WRC SR-07

Autologous Engineered Skin Substitutes with Pigment from Cultured Human Melanocytes

Team Leader: *Steven Boyce, PhD (University of Cincinnati)*

Team/Collaborating Partner Institution: *University of Cincinnati*

Target Clinical Application: *Skin Substitutes to Treat Burn Wounds*

Goals: The goal of this project is to demonstrate that a specific component of burn wound morbidity, uniformity of skin color, can be restored by incorporation and transplantation of autologous human melanocytes (hM) in autologous engineered skin substitutes (ESS).

Accomplishments: The researchers made substantial progress toward the goals of this project. They accomplished transplantation of isogeneic hM in the ESS format, and established the ability to cryopreserve hM. They transplanted ESS to athymic mice, and assessed for percentage of pigmented area grafted, density of hM/cm2 in healed skin, and pigment intensity in the healed skin. These parameters of skin pigmentation in ESS after grafting have been correlated with the same parameters in the original skin biopsy from which the ESS were generated. For these parameters, there were no statistical differences between the original tissue and the healed ESS.

Future: In the coming year, the team will continue preclinical characterizations using hM isolated from additional, unique donors to assure validity and reproducibility of the results needed to initiate clinical trials. In addition, they will prepare a Request for Designation and submit it to the FDA to determine the lead FDA Center (Biologics, Devices, Drugs) for regulation of clinical trials. After the regulatory designation is determined, they will prepare a regulatory protocol, and proposals for funding of a prospective clinical trial will be drafted for submission to qualified and interested agencies.

WRC SR-08

Skin Copying for Wound Repair

Team Leader: *Rox Anderson, MD (Massachusetts General Hospital)*

Team/Collaborating Partner Institution: *Massachusetts General Hospital*

Target Clinical Application: *Accelerated Wound Healing While Minimizing Donor Site Morbidity*

Goals: The purpose of this research is to copy the skin from a donor site onto the wounded site by transferring large numbers of full-thickness microscopic skin tissue columns (MTCs), with minimal to no donor site morbidity. MTCs also contain whole or partial hair follicles, sweat glands, vessels, lymphatics, fully pigmented epidermis, dermal and epithelial stem cells — all of the specialized elements that confer function to normal skin. The major goals of this project are to determine the MTC diameter limit for scar-free healing of donor sites in humans, increase the speed of skin-copying devices, to optimize skin-copy remodeling, and conduct a clinical trial for the application of MTCs to accelerate wound healing.

Accomplishments: The Anderson lab established and optimized the process of fabrication of harvesting-needles, and characterized the performance of fabricated needles, which is the key to determining the MTC diameter limit for scar-free healing in a clinical study that will follow the healing of skin sites grafted with harvesting-needles of different diameter size. The Institutional Review Board (IRB) approval for this study is pending and close to completion. The Anderson lab designed a bench-top tissue copier that features high speed and flexibility for harvesting MTCs, and implanting them in different matrix materials. The team also developed a non-contact, non-destructive, non-invasive, wide-field, point-and-shoot imaging method that takes advantage of the endogenous ultraviolet fluorescence of specific native molecules, and uses these molecules as optical biomarkers for cellular proliferation and dermal remodeling. It will be used to measure the fluorescence intensity associated with cellular proliferation, which is expected to increase where epithelialization occurs.

Future: The team will work towards determining the MTC diameter limit for scar-free healing in humans following IRB and Human Research Protection Office (HRPO) approval, by completing the clinical study. The researchers will also continue work toward increasing the speed of skin copying by: 1) testing and integration of hardware and software, 2) design review, 3) device testing and validation, and 4) device deployment. In addition, the team will continue the testing of commercially available matrix materials.

Clinical Challenge

Optimization of the Wound Environment/ Promotion of Skin Regeneration

Burn injuries can be extremely painful, debilitating, and complex to treat. AFIRM investigators are studying new ways to treat burn injuries to optimize the wound environment, promote skin regeneration, and prevent burn injury progression, the extension or deepening of burn injury over the first 72 hours after the inciting event. The prevention of burn injury progression will: 1) minimize tissue loss and the need for grafting, 2) shorten healing and hospitalization time, 3) lower rates of morbidity and mortality, and 4) decrease scarring and contracture.

RCCC 4.6.1e

Pre-IND Studies for a Novel Cell Survival Peptide that Limits Burn Injury Progression

Team Leader: *Richard Clark, MD (NeoMatrix Formulations, Inc.)*

Team/Collaborating Partner Institution: *NeoMatrix Formulations, Inc./Stony Brook University, New York*

Target Clinical Application: *Therapy to Prevent Burn Injury Progression*

Goals: The goal of this project is to develop a therapy to prevent burn injury progression, reduce inflammation, and induce healing in a porcine burn injury model by determining the optimal doses and treatment times for systemically administered 14-amino acid peptide P12 or P12 derivative. The research team will also develop a noninvasive monitoring system to predict burn injury progression.

Accomplishments: Under AFIRM I, the Clark lab discovered a number of FN domains that bind growth factors and enhance Recombinant Human Platelet Derived Growth Factor-BB activity on human dermal fibroblasts. Within these domains, a 14 amino acid peptide (P12) was most potent in vitro and limited burn injury progression in small and large animals. In ongoing work with P12, the research team has identified that P12 and a derivative, cP12, act as growth factor-enhancers, increase tissue cell survival and growth in culture, and limit burn injury progression in rat and porcine hot comb burn models and porcine burn vertical injury progression models. The team identified an optimal dose for cP12 to promote burn wound re-epithelialization (the required FDA primary outcome as per a Pre-IND meeting August 17, 2011). They validated cP12 blood assays in rat and dog blood at MicroConstants, Inc., and received final reports. Gene toxicity studies were completed at Covance and final reports were received. The team completed preliminary dosing and formal Good Laboratory Practices toxicokinetic studies at Charles River, Inc., and final reports are pending. The IND file organization was initiated by Target Health. The team also demonstrated that Forward Looking Infrared can monitor burn injury progression.

Future: The team will continue investigations on the mechanism of P12 bioactivity in both fibroblasts (supported by National Institutes of Health [NIH] R21 and future NIH grants) and endothelial cells (supported by AFIRM II). The researchers also plan to complete safety and efficacy studies with the porcine burn vertical injury progression model. The group will synthesize Good Manufacturing Practices (GMP) cP12 and GMP cP12 drug, and initiate the organization and writing of an IND for Phase I clinical trials.

RCCC 4.6.4

Polymeric, Antimicrobial, Absorbent Wound Dressing Providing Sustained Release of Iodine

Team Leader: *Carmine Iovine, PhD, Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Rutgers University*

Target Clinical Application: *Treatment of Burn Injury*

Goals: The goal of this project is to develop a treatment for infected traumatic wounds or thermal, heavily exuding burns.

Accomplishments: The team developed and patented a patient-conformable, highly absorbent sustained iodine releasing wound dressing based on the graft co-polymerization of starch with a polyester/urethane. The team coupled a pre-polymer polymerization process with a mold/cure/cut fabrication sequence and produced a uniform, well-defined product. Scale-up of a reproducible, controlled process at the laboratory scale has been completed. The team demonstrated that the in vitro sustained release characteristics were superior to commercially available silver and iodine-based devices. In addition, in vitro studies verified the broad anti-bacterial and anti-fungal action of the technology. Room temperature stability studies on the packaged device were executed to help define the shelf-life specifications for the final wound dressing product.

Future: No additional research is planned, as this project is now closed. The team will be seeking additional funding to complete the studies required for a 510(k) regulatory application.

RCCC 4.6.5

Topical P12 Therapy to Limit Burn injury Progression and Improve Healing

Team Leader: *Lauren Macri, PhD (Rutgers University), Richard Clark, MD (Stony Brook University)*

Team/Collaborating Partner Institution: *Rutgers University; Stony Brook University*

Target Clinical Application: *Therapy for Burn Injury*

Goals: The overall objective of this project is to deliver P12 or cP12 topically to a burn wound to inhibit burn progression, accelerate healing, and/or limit scar formation. The team will fabricate and characterize bio-erodible electrospun, Tyrosine-derived polycarbonates (TyrPC) fiber mats for the delivery of P12 or cP12 (P12- or cP12-loaded fiber mats) and recommend a suitable sterilization method for cP12-loaded fiber mats. TyrPC were chosen to make these mats, because they are readily processable, biocompatible, undergo hydrolytic degradation, and resorb benignly in a pre-determined period of time. The researchers will develop the porcine-excised hot comb burn model for horizontal progression as well as the porcine tangentially excised mid-dermal burn model for vertical progression, and evaluate the in vivo performance of peptide-loaded fiber mats in a porcine burn model.

Accomplishments: The team demonstrated linear P12 and cP12 release from ultrafast (<24 hours)- and fast (<4 days)-eroding TyrPC electrospun fiber mats. They determined that a suitable sterilization method for cP12-loaded fiber mats was electron-beam irradiation with 25 kGy. The team developed two animal models for the evaluation of topical therapies that may limit burn injury progression (the porcine-excised hot comb burn model for horizontal progression and the porcine tangentially excised mid-dermal burn model for vertical progression). In addition, they demonstrated in vivo safety of cP12-loaded fiber mats.

Future: This AFIRM project is closed. Additional funding has been secured under the Joint Warfighter Medical Research Program to focus future work on the 1) evaluation of cP12's stability in burn wound fluid; 2) delivery of cP12 (or its stable derivative) from gel matrix technologies, rather than from TyrPC, with hopes to eliminate the potential for polymer degradation product interference with cP12 bioavailability and bioactivity; and 3) demonstration of the efficacy of topical peptide on the inhibition of burn progression.

WRC SR-03

Biomimetic Stem Cell Dressing for Skin Regeneration

Team Leader: *Geoffrey Gurtner, MD (Stanford University)*

Team/Collaborating Partner Institution: *Stanford University*

Target Clinical Application: *Therapy for Accelerated Wound Healing and Skin Regeneration*

Goals: The overall goal of this project is to optimize the first-generation acute regenerative bandage. The bandage consists of a bone marrow-derived mesenchymal stem cell seeded pullulan-collagen hydrogel. The Gurtner lab will optimize the bandage by identifying the stem cell population(s) which are best able to promote accelerated and scarless wound healing, developing improved cell seeding and processing techniques, and testing the regenerative capacity of the optimized dressing in pre-clinical models.

Accomplishments: The team successfully identified a fetal wound gene transcription signature, which will be refined using an informatics-driven pathway analysis to identify additional candidates that may be implicated in the fetal regenerative environment. The lab considered multiple commercially available stem cells and identified four target cell lines suitable for the next phase of the project. The lab is in the final stages of collaboration review with these companies, and it anticipates acquiring these cells shortly. The group has reached out to clinical colleagues at Stanford who will obtain primarily isolated stem cell samples for comparison with the commercial cell lines.

Future: After acquiring all necessary stem cell lines (both commercially available and primarily isolated non-identifiable), the team will assay each stem cell population using microfluidic-based high-throughput single cell transcriptional analysis. The cell populations with profiles that most closely resemble the now-established fetal wound gene expression signature will be identified and isolated. Cell banks for these identified cell population(s) will be refined or established, depending on commercial or primary isolation. Following isolation of these stem cell populations, the researchers will optimize a pullulan-collagen biomimetic scaffold for cell delivery through cell-specific modifications in hydrogel porosity and three-dimensional (3D) composition. If the use of multiple stem cell populations is indicated, the effect of 3D spatial variance of stem cell seeding, or physical “stacking” of different stem cell seeded hydrogels will be tested in vitro for enhancement of cell differentiation capacity and cytokine expression using single-cell microfluidics, as well as traditional protein and RNA analyses. The lab will then compare the efficacy of immediate wound application of seeded hydrogels vs. delayed application of preserved hydrogels pre-seeded with stem cells. Additionally, wound healing efficacy will be compared between immediate-use and preserved pre-seeded hydrogels. Finally, various iterations of media and storage conditions before and after seeding will be tested for beneficial effects on long-term cell viability and maintenance of phenotype.

WRC SR-04

Amniotic Fluid Derived Stem Cells for Enhanced Wound Healing

Team Leader: *Shay Soker, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest University*

Target Clinical Application: *Therapy for Accelerated Wound Healing*

Goals: The overall goal of this project is to develop a therapy to improve wound healing by combining amniotic fluid stem (AFS) cells with an autologous mature skin cell population (keratinocytes) in treating full excisional skin wounds to accelerate the healing process. The specific goals of this project are to determine the combined effect of AFS cells and keratinocytes on full-thickness skin wound healing in pigs, and to develop current Good Tissue Practices- and current GMP-compliant processes to establish keratinocyte cultures for allogeneic cell therapy for Soldiers with burn wounds.

Accomplishments: The team tested the wound-healing properties of AFS cells by combining human AFS cells with a mature porcine skin cell population (keratinocytes) and treating full excisional skin wounds in a porcine model. Imaging, biopsies, and morphological assessment of the wounds were done to evaluate wound closure and re-epithelialization. In addition, the researches derived, expanded, characterized, and cryopreserved several AFS cell clones for future use in these wound healing studies. Their results showed that the treatment of full-thickness excisional wounds with AFS cells combined with autologous keratinocytes resulted in enhanced wound healing as evidenced by a shorter time to wound closure, less contracture of the wound, and faster re-epithelialization of the wound. In addition, the researchers compared results of wound healing using human AFS cells alone or in combination with autologous keratinocytes, as described above, with a study sponsored by a third party in which a biomaterial made from human amniotic membrane was used. The wound healing results in the pig were superior using the biomaterial; therefore, the researchers will be changing the project to include the use of the human amniotic membrane biomaterial instead of amniotic fluid-derived stem cells and keratinocytes, as originally planned.

Future: The team will examine the use of human amniotic membrane biomaterial incorporated into a gel, in place of the amnion-derived stem cells used in previous research, in a future study. The overall goal of the project remains the same, to develop a therapy to improve wound healing.

WRC SR-09

Localized Topical Metal Modulation to Inhibit Burn Progression

Team Leader: *Rajiv Bhushan, PhD (Livionex, Inc.)*

Team/Collaborating Partner Institution: *Livionex, Inc.*

Target Clinical Application: *Treatment to Prevent Burn Wound Progression*

Goals: In this AFIRM project, Livionex will demonstrate the in vivo effects of the topical use of its formulation on animal burn models and to complete IND/IDE enabling studies. The company plans to file an IND/IDE with the FDA to start clinical trials for the treatment of burn injuries. The goals of this project are to evaluate the efficacy of LF (Livionex formulation treatment lotion) in the prevention of dermal full-thickness burn in the porcine burn model, and to evaluate the effect of the time of initiating the treatment after burn injury on reducing the progression to full-thickness burns in porcine model. The team will meet with the FDA in a pre-IND meeting to obtain guidance on IND requirements.

Accomplishments: The research team has developed a formulation for use in therapy to inhibit burn progression. It is known that early down-regulation of inflammation via localized metal modulation soon after the occurrence of the injury may speed healing and prevent the systemic effects that often occur post burn injury. However, chelating agents that are used in reducing metals are charged molecules, and do not cross membrane boundaries. Livionex has overcome this barrier to using chelating agents, and has shown in vitro and in vivo that its methodology is very effective in down-regulating the inflammatory response. The team has prepared documentation for Institutional Animal Care and Use Committee and Animal Care and Use Review Office approval, trained lab personnel in the techniques to be employed, and made progress in improving/standardizing new methodologies to obtain additional data. The group has worked out programs for data interpretation and analysis.

Future: The team will complete the dose-response study of the efficacy of LF in pigs in the prevention of burn injury. The researchers will also start the study to establish the frequency of LF application that results in maximum efficacy against burn injury. Toward these objectives, the group will obtain the required approvals to conduct animal studies and train the research team in using the specimens of their rat model.

WRC SR-10

Novel Fibronectin Peptide Enhancers of Vascular Endothelial Cell Growth Factor to Promote Endothelial Cell Survival, Angiogenesis, and Tissue Regeneration after Burns and Battle Injury

Team Leader: *Richard Clark, MD (Stony Brook University)*

Team/Collaborating Partner Institution: *Stony Brook University*

Target Clinical Application: *Treatment to Prevent Burn Injury Progression*

Goals: The overall goal of this project is to develop and deliver FN-derived peptides that enhance growth factor activity that promotes endothelial cell survival and angiogenesis, thereby limiting burn injury progression, improving neovascularization, and hastening skin regeneration with minimal scarring.

Accomplishments: The team has made progress toward achieving the goal of using primary cell culture assays to screen for peptides which enhance pro-angiogenic activities of growth factors on human dermal microvascular cells (HDMEC). The researchers determined that the 14 amino acid FN peptide P12 is not the most potent peptide that enhances VEGF-driven HDMEC metabolism, and therefore they expanded the screen for optimal peptides to P12-parent domains in FNIII1. In addition, they performed an XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) metabolism assay under serum- and supplement-free conditions, and it was found to be insufficient for screening pro-angiogenic peptides. As a result, a more physiological reduced-serum proliferation assay was developed for this purpose.

Future: The team will continue screening for peptides that enhance pro-angiogenic activities of growth factors on HDMEC with proliferation assays recently developed. Since optimal pro-survival, pro-angiogenic FN peptides may enhance VEGF-driven activities other than proliferation, this screening will include assays for re-endothelialization, migration, and basement membrane invasion assays.

Clinical Challenge Scarless Wound Healing

The AFIRM researchers are focused on developing strategies to mitigate the scarring that results from fibroproliferative processes that occur during normal wound healing. Researchers aim to develop effective therapies to promote tissue regeneration while decreasing the inflammatory and fibrotic response. The complex interactions between various signaling molecules, extracellular matrix components, and cell populations complicate these efforts, and new approaches are being investigated to resolve these issues. Projects in this clinical challenge area specifically focus on development of an adipose-derived wound paste to mediate replacement of missing dermis, therapy to provide small molecule inhibition of a protein known to play a role in hypertrophic scarring, FAK, and development of therapy employing statins to reduce fibrosis in scar formation.

RCCC 4.7.1

Adipose-Derived Therapies for Wound Healing, Tissue Repair, and Scar Management

Team Leader: *Adam J. Katz, MD (University of Virginia [now University of Florida])*

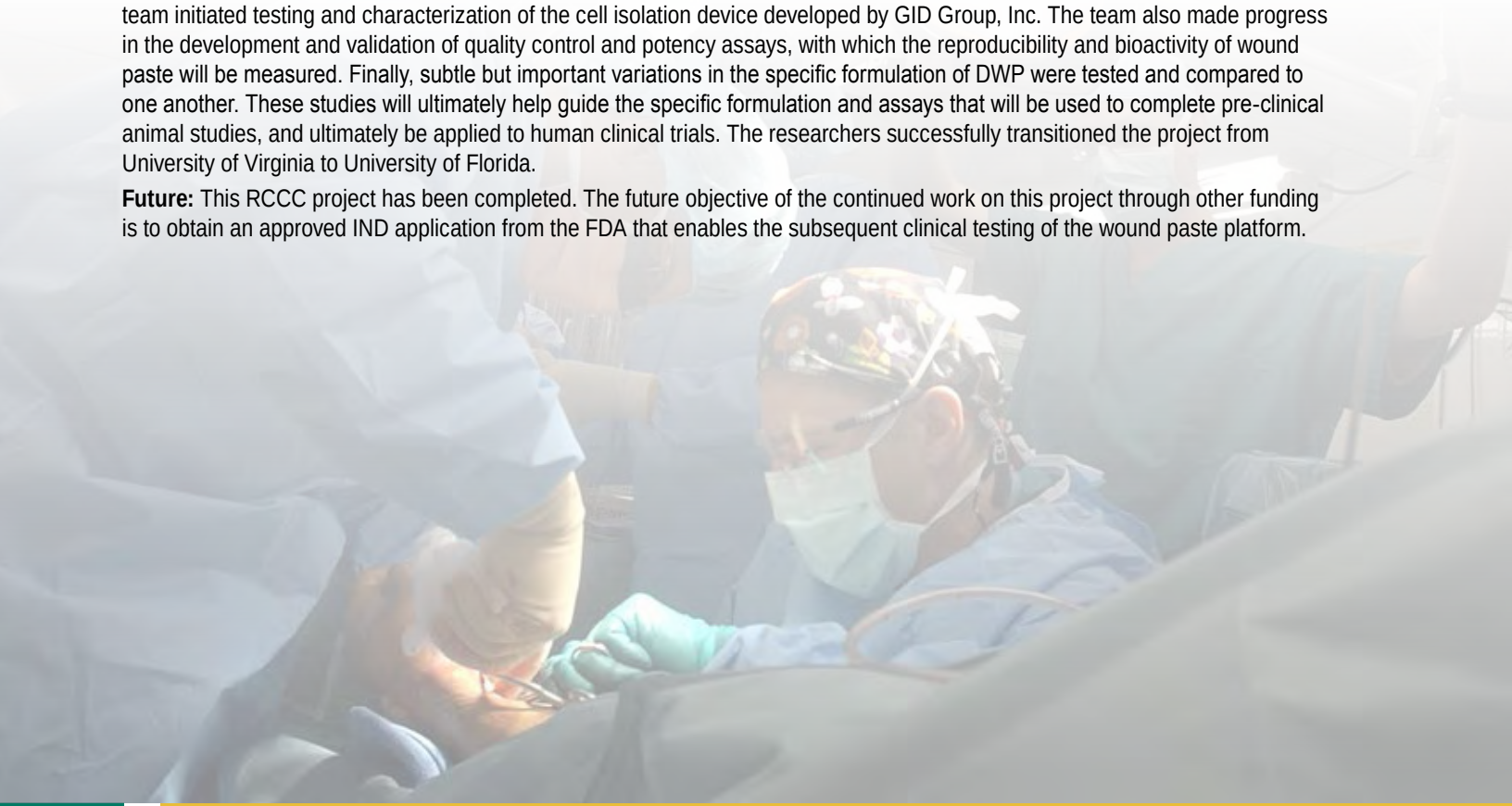
Team/Collaborating Partner Institution: *University of Virginia*

Target Clinical Application: *Therapy for Wound Healing*

Goals: The goal of this research team is to develop an autologous wound paste as a therapeutic platform that leverages the use of autologous, uncultured adipose-derived (SVF) cells obtained and prepared at the "point-of-care." The working hypothesis of the Katz team is that constructs composed of autologous adipose-derived SVF cells, human acellular dermal matrix, and glycosaminoglycan hydrogels can mediate the effective replacement of missing dermis in a more expedient timeframe and without the donor morbidity associated with currently available strategies.

Accomplishments: The team established a collection of formal standard operating procedures, guiding the Chemistry, Manufacturing, and Controls process for their DWP, which will also be used for future regulatory submissions. In addition, the team initiated testing and characterization of the cell isolation device developed by GID Group, Inc. The team also made progress in the development and validation of quality control and potency assays, with which the reproducibility and bioactivity of wound paste will be measured. Finally, subtle but important variations in the specific formulation of DWP were tested and compared to one another. These studies will ultimately help guide the specific formulation and assays that will be used to complete pre-clinical animal studies, and ultimately be applied to human clinical trials. The researchers successfully transitioned the project from University of Virginia to University of Florida.

Future: This RCCC project has been completed. The future objective of the continued work on this project through other funding is to obtain an approved IND application from the FDA that enables the subsequent clinical testing of the wound paste platform.



WRC SR-01

Targeted Delivery of a Small Molecule Focal Adhesion Kinase Inhibitor for Scarless Wound Healing

Team Leader: *Geoffrey Gurtner, MD (Stanford University)*

Team/Collaborating Partner Institution: *Stanford University*

Target Clinical Application: *Scarless Wound Healing*

Goals: The goal of this project is to develop a novel approach to scar reduction by performing small molecule inhibition of FAK, a protein that has been shown to play a critical mechanosensory role in hypertrophic scarring. Specific aims of the project include studies to optimize delivery of FAK inhibitors, demonstrate efficacy in murine wounds, and investigate the efficacy in porcine models of deep partial-thickness wounds.

Accomplishments: The team performed Western blot analysis to study the dose/response effects of FAK-inhibitor VS-6062 on FAK phosphorylation at tyrosine-397 in mouse skin fibroblasts. They performed immunofluoresence staining to study the dose effect of FAK inhibitor VS-6062 on the inhibition of cell proliferation in mouse skin fibroblasts. TUNEL staining was performed to study the dose effect of FAK-inhibitor VS-6062 on the apoptosis of mouse skin fibroblasts. The researchers also completed a number of tests to assess the basic physico-chemical characteristics (e.g., swelling ratios of FAK-1 imprinted and unloaded hydrogels and the in vitro release profile of FAK-1 from the hydrogel in water and 5% DMSO [95% water]) of the newly formulated drug releasing hydrogels.

Future: The researchers will perform in vitro experiments to define drug activity when suspended in a liquid solution or released from a pullulan-collagen bioscaffold. They will also determine in vivo drug release and activity using a murine excisional wound model.

WRC SR-02

Local Application of Statins to Reduce Scarring

Team Leader: *Thomas Mustoe, MD (Northwestern University)*

Team/Collaborating Partner Institution: *Northwestern University*

Target Clinical Application: *Scarless Wound Healing*

Goals: Statins are being investigated for scar reduction therapy as they have demonstrated anti-fibrotic properties in various models of fibrosis. The major goals of this project are to determine the optimal conditions (dosage, treatment time, and delivery method) for statin therapy to reduce hypertrophic scarring in the rabbit ear model. The team will also validate efficacy of statins and confirm the down-regulation of connective tissue growth factor (CTGF) in a pig ear scar model. Animal studies will ultimately support the transition to human investigator-initiated clinical trials.

Accomplishments: Previously, this team examined the effects of three different generic statins (simvastatin, lovastatin, and pravastatin) in their rabbit ear hypertrophic scar model and demonstrated that statins reduce hypertrophic scar formation by CTGF inhibition. Currently, they have proposed to utilize the rabbit ear model to address unanswered questions regarding statins, with implications for the method of local administration (local injection vs. topical application), type of vehicle used, the dosing frequency, and the potential effects of systemic administration. During this first year of the project, the team compared the efficacy of hydrophilic statins and hydrophobic statins.

Future: The team will continue ongoing efficacy experiments using the rabbit ear model to assess effective doses, delivery vehicle, and frequency. They will also initiate studies to validate efficacy of statins and confirm down-regulation of CTGF in the pig ear scar model.

WRC SR-11

Biomask for Skin Regeneration

Team Leader: *XingGuo Cheng, PhD (Southwest Research Institute)*

Team/Collaborating Partner Institution: *Southwest Research Institute; Wake Forest University; U.S. Army Institute of Surgical Research (USAISR)*

Target Clinical Application: *Scarless Wound Healing*

Goals: The overall goal of this research is to design and fabricate custom facial neodermis (biomask) with the correct topography of the face and thickness. The matrix is to be bi-layered with a spray-on siloxysilane covering. The custom made biomask will be fit to the injured face, to promote better healing and reduce scarring, in conjunction with stem cell therapy and wound vacuum-assisted closure. The major goals of this project over four years are to: 1) develop, fabricate, and evaluate a bi-layer custom facial neodermis, 2) evaluate custom facial bi-layer neodermis in vivo using a porcine model, 3) evaluate the biomask in vitro with incorporated adipose derived stem cells (ADSCs), and 4) evaluate the custom facial bi-layer neodermis product with and without ADSCs in vivo using a porcine model.

Accomplishments: The researchers and their collaborative teams have successfully fabricated the facemask using 3D imaging and printing. Both collagen and collagen/Integra-flowable wound matrix facemasks were successfully fabricated, and evaluations of permeability to water vapor were completed.

Future: The team will determine the oxygen permeability of the bi-layer constructs. This is a parameter that must be obtained for skin wound healing products.

WRC SR-12

Novel Polysaccharide Compound to Enhance Wound Healing and Decrease Scarring

Team Leader: *Peter Rubin, MD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh; WFIRM; USAISR*

Target Clinical Application: *Therapy to Enhance Wound Healing and Decrease Scarring*

Goals: The overall goal is to investigate a novel off-the-shelf polysaccharide compound (SYN01) (Synedgen, Inc., Claremont, CA) in a swine model of wound healing. SYN01 accelerates wound healing, reduces scarring and fibrosis, prevents infection, and disrupts biofilms for both combat burn wounds and complex open wounds.

Accomplishments: The team has isolated and banked multiple aliquots of ASCs from porcine groin tissue, and characterized the cell types of porcine ASCs. The group has tested and optimized conditions for labeling ASCs with PKH26 for post-operative tracking. The researchers are currently performing assays for toxicity in vitro and proliferation. They have obtained regulatory approval (Institutional Animal Care and Use Committee and Animal Care and Use Review Office) for porcine studies, and ordered two female pigs for testing the conditions of wound making, wound dressing, and optimization of SYN01 delivery and ASCs.

Future: The team will demonstrate that SYN01 will accelerate wound healing in a porcine excisional model, and it will work synergistically with negative pressure dressings and autologous adipose-derived stem cells. They are ready to perform and evaluate the first in vivo experiment to fine-tune the delivery options for their proposed treatments. The team will demonstrate that SYN01 will accelerate epithelialization and effective wound closure with wide meshed skin grafts in porcine burn wound models to prove that wounds can be covered with less donor skin. The researchers will assess the effectiveness of SYN01 in accelerating healing of infected wounds with biofilms, in both rodent and porcine models. The researchers will develop staged multimodality treatment regimen for infected de-gloving wounds and burn wounds using SYN01 and other interventions tested in AIMS 1-3. Ultimately, the team will initiate a limited Phase I/II clinical study to examine the safety and effectiveness of SYN01 applications in acute complex soft tissue wounds with infection.

Clinical Trials

AFIRM investigators are conducting clinical studies with a focus on burn wound healing, prevention of scarring after injury, and the use of skin substitutes in the treatment of wounds. The status of each clinical trial is summarized in **Table IV-2**, and additional details on these trials follow the table.

Table IV-2. AFIRM-funded Skin Regeneration projects with pending or active clinical trials.

Therapy/Product	Project Title	Consortium	Project Number	Trial Phase	Current Status
Autologous Fat Transfer for Scar Prevention and Remodeling (AFT-SPAR): A New Indication for an Existing Surgical Procedure	Clinical Trial: Autologous Fat Transfer for Scar Prevention and Remodeling (AFT-SPAR)	RCCC	4.7.3CT	I/II	Enrolling
Engineered Skin Substitute to Treat Burns	Clinical Trial: Expedited Availability of Autologous Engineered Human Skin for Treatment of Burned Soldiers – Lonza Walkersville, Inc.	RCCC	4.7.4CT	II	Pending
ReCell® Autologous Cell Harvesting Device	A Multicenter Comparative Study of the ReCell® Device and Autologous Split-Thickness Meshed Skin Graft in the Treatment of Acute Burn Injuries	WFPC	4.2.7a	Pivotal	Enrollment Complete
StrataGraft® Skin Tissue, Human Skin Substitute	Stratatech Technology for Burns	WFPC	4.2.9	I	Enrollment Complete
Ibis PLEX-ID Coupled PCR-ESI-Mass Spectrometry Assay	Detection of Pathogens in Burn Wounds	WFPC	4.2.10	Clinical Study	Enrollment Complete
StrataGraft Skin Tissue to Treat Complex Skin Defects	Clinical Evaluation of StrataGraft Skin Tissue as an Alternative to Autografting of Complex Skin Defects on the Face, Head, Neck, Hands, and Feet	WRC	SR-06	II	Pending

RCCC 4.7.3CT

Clinical Trial: Autologous Fat Transfer for Scar Prevention and Remodeling (AFT-SPAR)

Team Leader: *Adam J. Katz, MD (University of Florida)*

Team/Collaborating Partner Institution: *University of Florida; University of Virginia; (USAISR/Brooke Army Medical Center)*

Target Clinical Application: *Therapy for Scar Prevention and Management*

Goals: The goal of this clinical trial is to complete a Phase I/II clinical trial to evaluate the effectiveness of autologous fat transfer (AFT) for scar prevention and remodeling after injury. AFT is a single-stage procedure that involves the removal of adipose tissue from one site of a patient followed by the immediate and autologous transplantation/infiltration of this tissue into a different site of the same patient.

Accomplishments: The team has faced challenges to patient enrollment. However, the team is on track to complete enrollment for the delayed treatment cohort by the end of Calendar Year 2015.

Future: The study team has started to un-blind subjects in the late cohort group who have completed all follow-up evaluations, in order to begin data analysis.

RCCC 4.7.4CT

Clinical Trial – Expedited Availability of Autologous Engineered Human Skin for Treatment of Burned Soldiers - Lonza Walkersville, Inc. (LWI)

Team Leader: *David Smith, Head Therapeutic Cell Systems (LWI); Theresa D'Souza, PhD (LWI); Kerry Adamik, (LWI)*

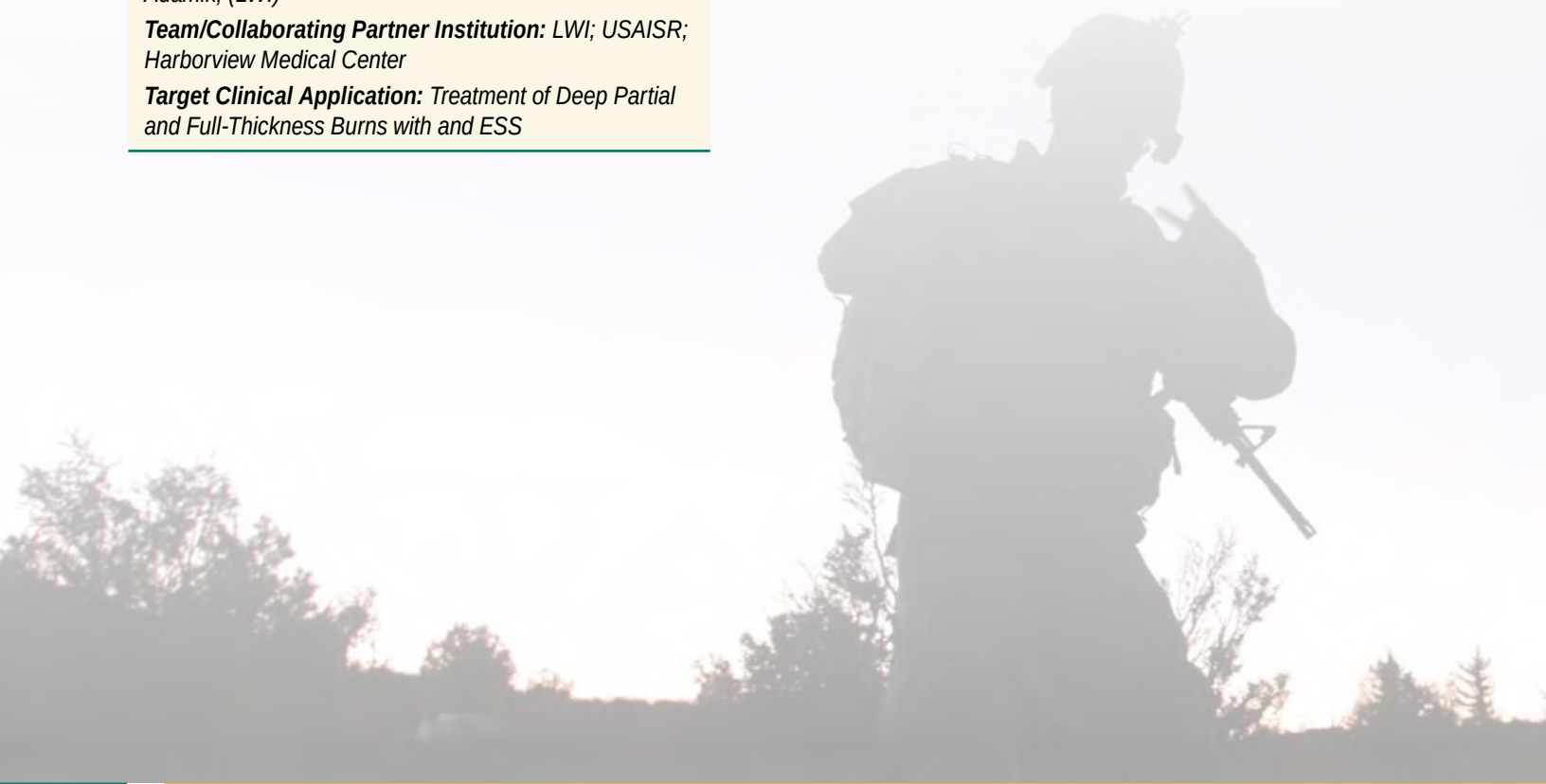
Team/Collaborating Partner Institution: *LWI; USAISR; Harborview Medical Center*

Target Clinical Application: *Treatment of Deep Partial and Full-Thickness Burns with and ESS*

Goals: The team will evaluate the safety and efficacy of their Autologous ESS-W compared to meshed, split-thickness autograft for treatment of deep partial- and full-thickness thermal burn wounds in adult patients in a Phase II clinical trial.

Accomplishments: LWI obtained IRB and HRPO approvals.

Future: LWI is planning to start the clinical effort in the beginning of 2016.



WFPC 4.2.7a

A Multicenter Comparative Study of the Recell® Device and Autologous Split-Thickness Meshed Skin Graft in the Treatment of Burn Injuries

Team Leader: *James Holmes IV, MD (Wake Forest)*

Team/Collaborating Partner Institution: *USAISR; Wake Forest School of Medicine; Royal Perth Hospital, Australia; Avita Medical Ltd.; Avita Medical Americas LLC; MedDRA Assistance, Inc.; BioStat International, Inc.*

Target Clinical Application: *Treatment of Second-degree Burn Injuries With an Autologous Skin Cell Suspension*

Goals: The project team has completed enrollment in this randomized, within-subject controlled study to compare the clinical performance of the Recell® device.

Accomplishments: Subject enrollment and treatment has been completed, and subjects continue to be followed until one year of follow-up is completed. Data from this clinical trial will be source-verified, analyzed, and published.

Future: The team will be starting a new cohort of 25 subjects, that will be recruited under this same IDE, but with a revised clinical study protocol. Data from the new cohort, together with the cohort studied under the AFIRM program, will substantiate Avita's Premarket Approval application.

WFPC 4.2.9

Stratatech Technology for Burns

Team Leader: *James Holmes IV, MD (Wake Forest)*

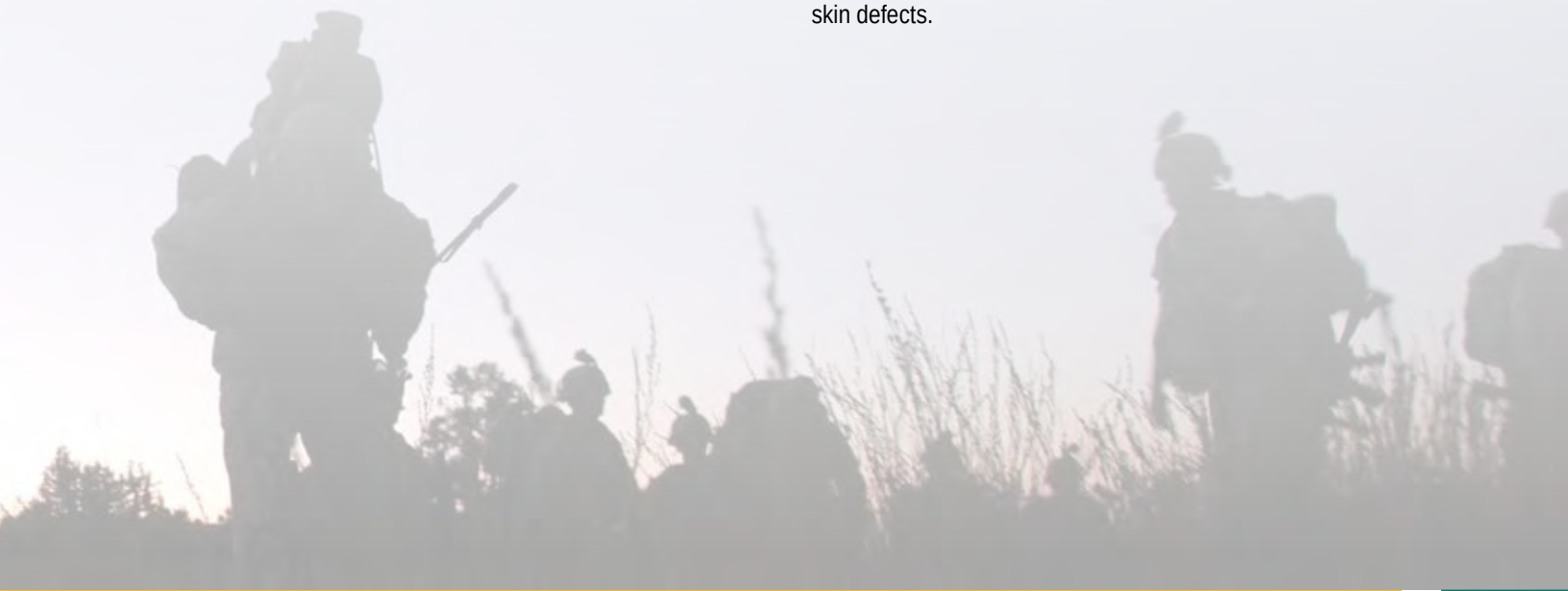
Team/Collaborating Partner Institution: *USAISR; University of Colorado Hospital; University of Wisconsin Hospital and Clinics; Arizona Burn Center; University of Texas Southwestern; Stratatech; ResearchPoint*

Target Clinical Application: *Wound Coverage and Healing of Deep Partial-Thickness Burns Using a Skin Substitute*

Goals: The team is conducting this clinical trial to evaluate the efficacy of StrataGraft® tissue in providing immediate wound coverage and accelerating healing of deep partial-thickness burns. StrataGraft® tissue is a readily available, viable, full-thickness, allogeneic human skin substitute that provides immediate wound coverage and secretion of growth factors, cytokines, and antimicrobial peptides to promote the healing of severe burns and other complex skin defects.

Accomplishments: Three cohorts of ten subjects each have been fully enrolled, with interim analysis between each cohort by the Wake Forest School of Medicine Institutional Data and Safety Monitoring Board.

Future: The team will use clinical data obtained from this study to inform the design of a Phase III study in patients with complex skin defects.



WFPC 4.2.10

Detection of Pathogens in Burn Wounds

Team Leader: *Sandeep Kathju, MD, PhD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh; Ohio State University*

Target Clinical Application: *Diagnosis of Burn Wound Infection*

Goals: The research team will work to complete this clinical trial and analyze the resulting data, in order to evaluate the utility of the Ibis PLEX-ID system in the diagnosis of burn wound infection.

Accomplishments: At the principal study site, University of Pittsburgh, the team has made substantial progress in recruiting for the ongoing study. At present, 140 patients have been enrolled in the trial. More than 150 samples have now been extracted and prepared for analysis, and have been subjected to the Ibis assay. The study team has analyzed results from the first 50 of these samples.

Future: The team will carry out the data analysis portion of the trial.

WRC SR-06

Clinical Evaluation of Stratagraft Skin Tissue as an Alternative to Autografting of Complex Skin Defects on the Face, Head, Neck, Hands, and Feet

Team Leader: *Lynn Allen-Hoffmann, PhD (Stratatech Corporation)*

Team/Collaborating Partner Institution: *Stratatech Corporation*

Target Clinical Application: *A Skin Substitute to Treat Complex Skin Defects in Cosmetically Sensitive Areas*

Goals: The primary focus of the clinical study is to evaluate the safety and efficacy of using StrataGraft® skin tissue as an alternative to autografting the full-thickness component of complex skin defects in cosmetically sensitive areas.

Accomplishments: The team is progressing with clinical trial preparations for planned initiation in 2016.

Future: Stratatech will work with each of the clinical sites to prepare regulatory submissions. After HRPO approval, patient enrollment may begin.

V: Genitourinary/Lower Abdomen Reconstruction

AFIRM OUR SCIENCE FOR THEIR HEALING

Background

A higher prevalence of dismounted patrols and the increased use of ground-based explosive weaponry by insurgents in the latter part of the recent U.S. armed conflicts resulted in a sharp increase in genitourinary, pelvic, and lower abdominal injuries. The mechanisms and constellation of these injuries was outlined in a report generated by the Dismounted Complex Blast Injury Task Force in 2011[1]. These devastating injuries impact elimination, sexual function, reproduction, endocrine function, and potentially, the overall psychological well-being of the injured Service member. The military medical community is in need of improved surgical reconstruction strategies, as well as the materials to treat these combat traumas and restore function.

[1] Dismounted Complex Blast Injury: Report of the Army Dismounted Complex Blast Injury Task Force, June 18, 2011. (*http://armymedicine.mil/Documents/DCBI-Task-Force-Report-Redacted-Final.pdf*)

V: Genitourinary/Lower Abdomen Reconstruction

The AFIRM's Genitourinary and Lower Abdominal Reconstruction Program addresses the majority of tissues and structures in the pelvic region, and focuses on repairing catastrophic tissue loss and restoring function. Although a new program area for the AFIRM, it capitalizes on the regenerative technologies and strategies established in other AFIRM program areas. The approaches being investigated include both engineered tissues and stem cell therapies, and the information gained may in turn contribute to the overall understanding of regeneration and repair for other tissue and organ systems.

Areas of Emphasis

The goal of the Genitourinary and Lower Abdomen Reconstruction Program is to develop and advance regenerative medicine-based technologies for the treatment of blast injuries to the genitourinary, pelvic, and anal regions. The current portfolio of projects focuses on reconstruction of abdominal wall (hernia repair), the anal canal/sphincter, bladder, testes, urethra, and penis. The researchers are investigating strategies that include engineered tissue scaffolds with cell seeding, three-dimensional (3D) printing of tissue constructs, promotion of vascular growth, and stem cells. Various small and large animal models are used to develop and test the strategies, and will be followed by human clinical trials. As shown in **Table V-1** below, the projects are focused on genitourinary and lower abdomen reconstruction. A summary of each project is provided. Clinical trials for some of the projects are planned during the next 3-5 years.

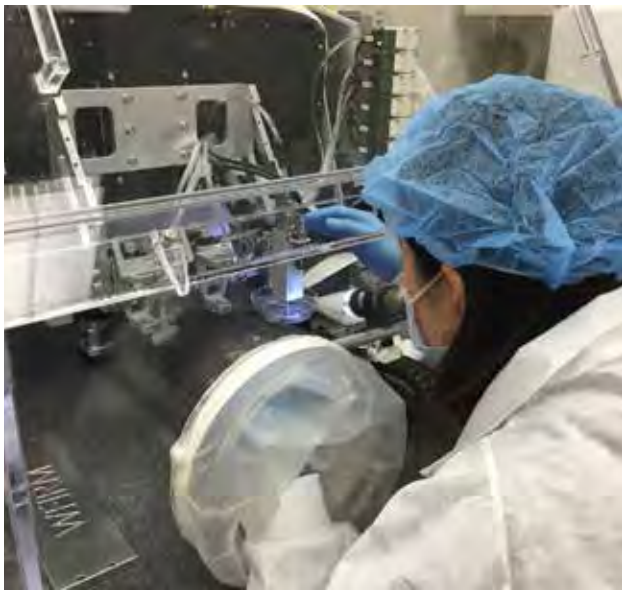


Table V-1. Projects funded by WRC by clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Genitourinary	WRC	GU-01	Engineered Bladder Tissue for Soldiers with Battlefield Injury
		GU-02	Urethral Tissue Repair Due to Battlefield Injury
		GU-03	Engineered Penile Tissue for the Repair of Battlefield Urologic Injuries
		GU-04	Engineered Testicular Tissue Organoids for Young Solders with Injury to the Testes
		GU-05	Restoration of Penile Tissue Function Using Stem Cells
Lower Abdomen	RCCC	4.4.3a	Functional Scaffold for Musculoskeletal Repair and Delivery of Therapeutic Agents
	WRC	GU-06	Engineering of Innervated Volumetric Skeletal Muscle Tissue for Accelerated Restoration of Pelvic Floor Muscle Function
		GU-07	Implantation of Bioengineered Innervated Terminal Gut as a Replacement Therapy for Injured Anus
		GU-08	Engineering Scaffold and Cell Therapy for Treatment of Fecal Incontinence Due to Defects in the Anal Sphincter
		GU-09	Medically Engineered Functional Anal Sphincters Using Composite Tissue Engineering and Novel Electrode (ME-FASTE)

Clinical Challenge Genitourinary

Work is underway to develop tissue constructs for the reconstruction of genitourinary structures. A common approach being used is to isolate or engineer bioscaffolds on which cells can be seeded and grown. The researchers are investigating bioprinting of testicular organoids, and they are also exploring low energy shock wave therapy to induce vascularization combined with stem cell injection to restore erectile function.

WRC: GU-01

Engineered Bladder Tissue for Soldiers with Battlefield Injury

Team Leader: *James Yoo, MD, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *Wake Forest Institute of Regenerative Medicine (WFIRM); Walter Reed National Military Medical Center*

Target Clinical Application: *Reconstruction of Bladder*

Goals: The goal of this project is to develop an engineered bladder tissue construct which incorporates a patient's own cells to restore functional bladder tissue. The effort will focus on modifying the established current Good Tissue Practices (cGTP)- and current Good Manufacturing Practices (cGMP)-compliant manufacturing processes and standard operating procedures (SOPs) for bladder damage by trauma in preparation for future clinical studies.

Accomplishments: During the past year, the team focused on modifying SOPs for developing engineered bladders to treat bladders damaged by trauma. Urothelial and bladder smooth muscle cells were successfully isolated, grown, and expanded in culture, and intracellular markers in the cultured cells were characterized using flow cytometry. The team is developing cGTP- and cGMP-compliant cell and tissue processing methods and is working to establish a manufacturing agreement for bladder tissue constructs. Further, the researchers completed the design of a canine model of trauma-induced small and fibrotic bladders.

Future: The team will further develop the canine fibrotic bladder model, and will continue to work on both the characterization and validation of SOPs for each procedure involved with bladder constructs.

WRC: GU-02

Urethral Tissue Repair Due to Battlefield Injury

Team Leader: *James Yoo, MD, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM; University of Washington*

Target Clinical Application: *Urethra Reconstruction*

Goals: The goal of the project is to develop tissue-engineered tubularized urethras that use the patient's own cells to restore functional urethral tissue. The team will develop and validate methods for cGTP- and cGMP-compliant cell and tissue processing for urethral implants, and will file an Investigational New Drug (IND) in preparation for future clinical trials.

Accomplishments: The team is developing cGTP- and cGMP-compliant cell and tissue processing methods for urethral implants. Urothelial and bladder smooth muscle cells were successfully isolated from human bladder tissue, and grown and expanded in culture. The team developed flow cytometry methods that were used to characterize intracellular markers in the cultured cells. Validation studies will use a rabbit model.

Future: The researchers will continue to work on the characterization and validation of SOPs for each procedure involved with urethral constructs. The team will prepare a pre-IND package in preparation for a pre-IND meeting with the U.S. Food and Drug Administration (FDA).

V: Genitourinary/Lower Abdomen Reconstruction

WRC: GU-03

Engineered Penile Tissue for the Repair of Battlefield Urologic Injuries

Team Leader: *John Jackson, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Reconstruction of Penis*

Goals: The goal of the project is to further develop a naturally derived acellular corporal tissue matrix that possesses the same architecture as native corpora in order to restore normal anatomical tissue configuration and erectile function. The effort will test the technology in a nonhuman primate model, and develop cGTP- and cGMP-compliant cell and tissue processing for the corporal implants. Results will be used to submit an IND package to the FDA, in preparation for a Phase I trial.

Accomplishments: Penile carvernosa endothelial and smooth muscle cells were successfully isolated and grown in culture, and the cultured cells were characterized using antibodies to cell type-specific markers for confirmation. The team prepared a decellularized scaffold by processing penile tissue from a donor monkey using a detergent solution and removing remaining DNA from the scaffold. The penile scaffold was then successfully reseeded with the cultured endothelial and smooth muscle cells, with both cell types observed to be present and spreading over the scaffold. Baseline sexual behavior observations and intracorporal pressures were begun as part of the animal study, with the initiation of surgical defects and implants expected in the next year. Additionally, the team submitted a review package to the U.S. Army Medical Research and Materiel Command Human Research Protection Office for the use of human cadaveric penile tissue requested in order to begin process development for human implants.

Future: The team will complete baseline sexual behavior observations and intracorporal pressures in non-human primates, and will create surgical defects for implantation of seeded penile scaffolds, Sexual behavior and intracorporal pressure will be monitored in the implanted animals. Human cadaveric penile tissue tissues will be used for developing cGTP- and cGMP-compliant procedures for tissue harvest, scaffold preparation, cell isolation, cell expansion, cell seeding, and implant handling.

WRC: GU-04

Engineered Testicular Tissue Organoids for Young Solders with Injury to the Testes

Team Leader: *Colin Bishop, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Reconstruction of Testes*

Goals: The goal of this project is to develop a 3D, bioprinted, functional testicular organoid that is capable of complete spermatogenesis and testosterone production in a regulated way. The project includes establishing and optimizing the bioprinting process and evaluating the constructs in a castrated mouse model.

Accomplishments: The team has isolated human Leydig, Sertoli, and spermatogonial stem cells (SSC) and extracellular matrix from commercially available human testes tissue. Cell culture lines for SSC, Leydig, and Sertoli cells were established and confirmed by the presence of cell type-specific markers. The team combined Leydig, Sertoli, and SSC cells in spheroid cultures and observed the formation of compact organoids. Confocal microscopy established that SSC appeared to self-organize to the center of the spheroid, with Sertoli and Leydig on the periphery, reminiscent of the structure of a human testis tubule.

Future: The team will establish and optimize printing/viability parameters for testis organoids, and evaluate bioprinting versus simple self-organization in spheroids. The team will assess viability and will use genomic, protein, and hormone assays to assess functionality of these constructs. Finally, the researchers will use an immunocompromised and castrated mouse model to evaluate functionality in vivo.

WRC: GU-05

Restoration of Penile Tissue Function Using Stem Cells

Team Leader: *John Jackson, PhD (Wake Forest University)*

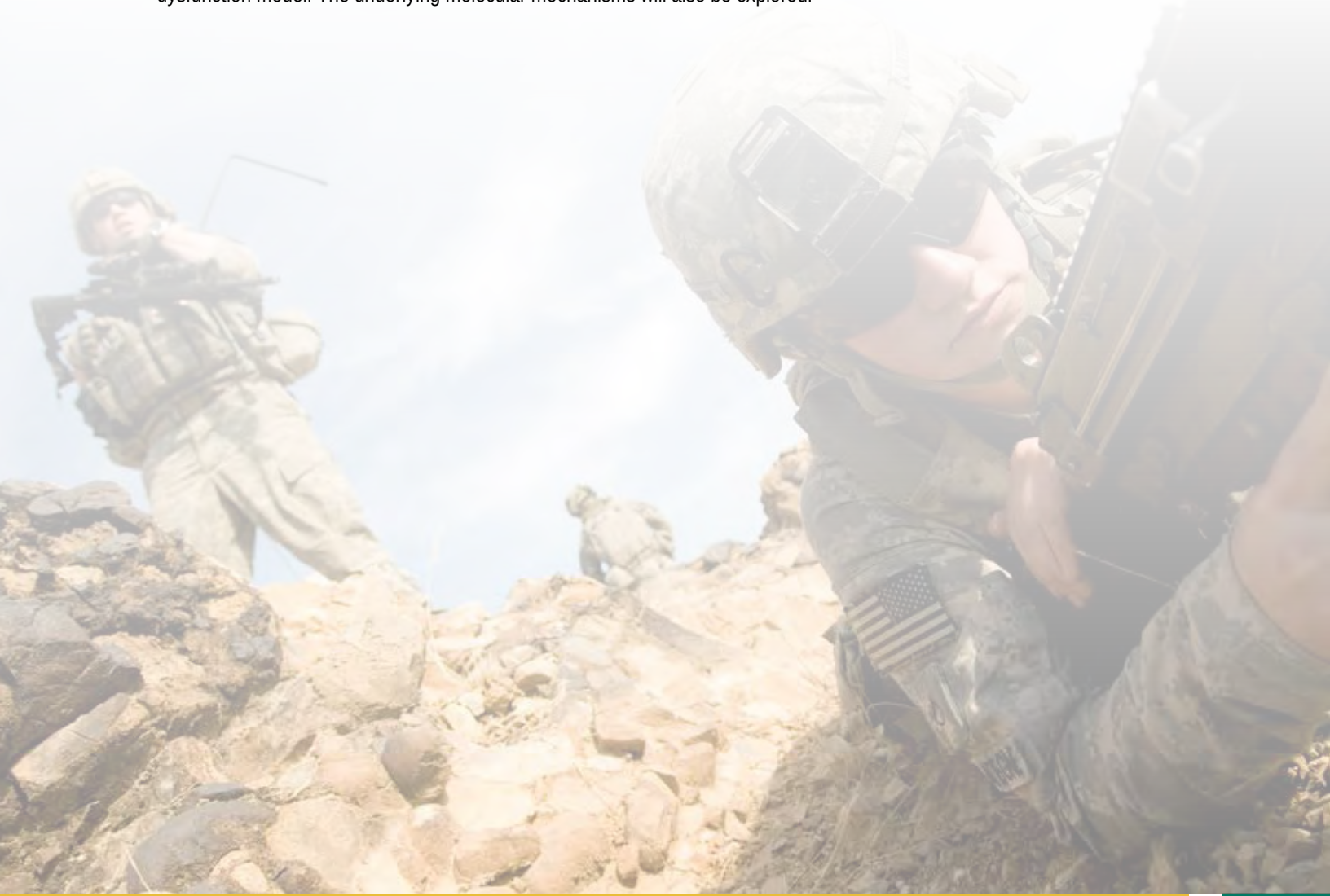
Team/Collaborating Partner Institution: *WFIRM; University of California, San Francisco*

Target Clinical Application: *Restoration of Erectile Function*

Goals: Low-energy shock wave therapy (LESWT) has been shown to improve function in a rat model of neurogenic erectile dysfunction (cavernosal nerve [CN] crush injury) as well as in diabetic men (neurologic and vascular injury). This project will evaluate the combination of LESWT and injection of exogenous stem cells in promoting angiogenesis to treat erectile dysfunction. The combination will be evaluated in a rat model with erectile dysfunction due to arterial and vascular insufficiency (ligation of internal iliac artery).

Accomplishments: The team has established rat models of erectile dysfunction involving internal pudendal bundle (IPB) ligation and cavernous nerve crush, alone or in combination, for use in their studies. Intracavernosal pressure monitoring was performed. Histological studies evaluated structure and the presence of neuronal nitric oxide synthase, tyrosine hydroxylase, von Willebrand factor, and smooth muscle actin. IPB ligation deteriorated the normal erectile function, damaged smooth muscle in the cavernous body, and degenerated the endothelium. The number of neuronal nitric oxide synthase-positive nerves decreased after either IPB ligation or CN crush. IPB ligation induced a reduction of tyrosine hydroxylase-positive nerves while CN crush led to an opposite trend. Rat model studies of LESWT were initiated. The team is characterizing human amniotic fluid-derived stem cells and human adipose-derived stem cells for use in the cellular therapy studies.

Future: The team will determine the effect of stem and progenitor cell therapy, in combination with LESWT, in their rat erectile dysfunction model. The underlying molecular mechanisms will also be explored.



Clinical Challenge Lower Abdomen

Work is underway to develop tissue constructs to reconstruct and restore function to the pelvic floor muscle system and anal sphincter, as well as repair hernia. Pelvic floor reconstruction requires large muscle constructs. Innervation is critical to the restoration of function. The primary approach involves engineering bioscaffolds on which cells can be seeded and grown into the various tissue types. Another approach combines tissue engineering to reconstruct the anal sphincter with microelectrodes incorporated to restore function. A technology previously developed and commercialized for rotator cuff repair is being translated for hernia repair.

RCCC 4.4.3a

Functional Scaffold for Musculoskeletal Repair and Delivery of Therapeutic Agents

Team Leader: *Kathleen Derwin, PhD (Cleveland Clinic)*

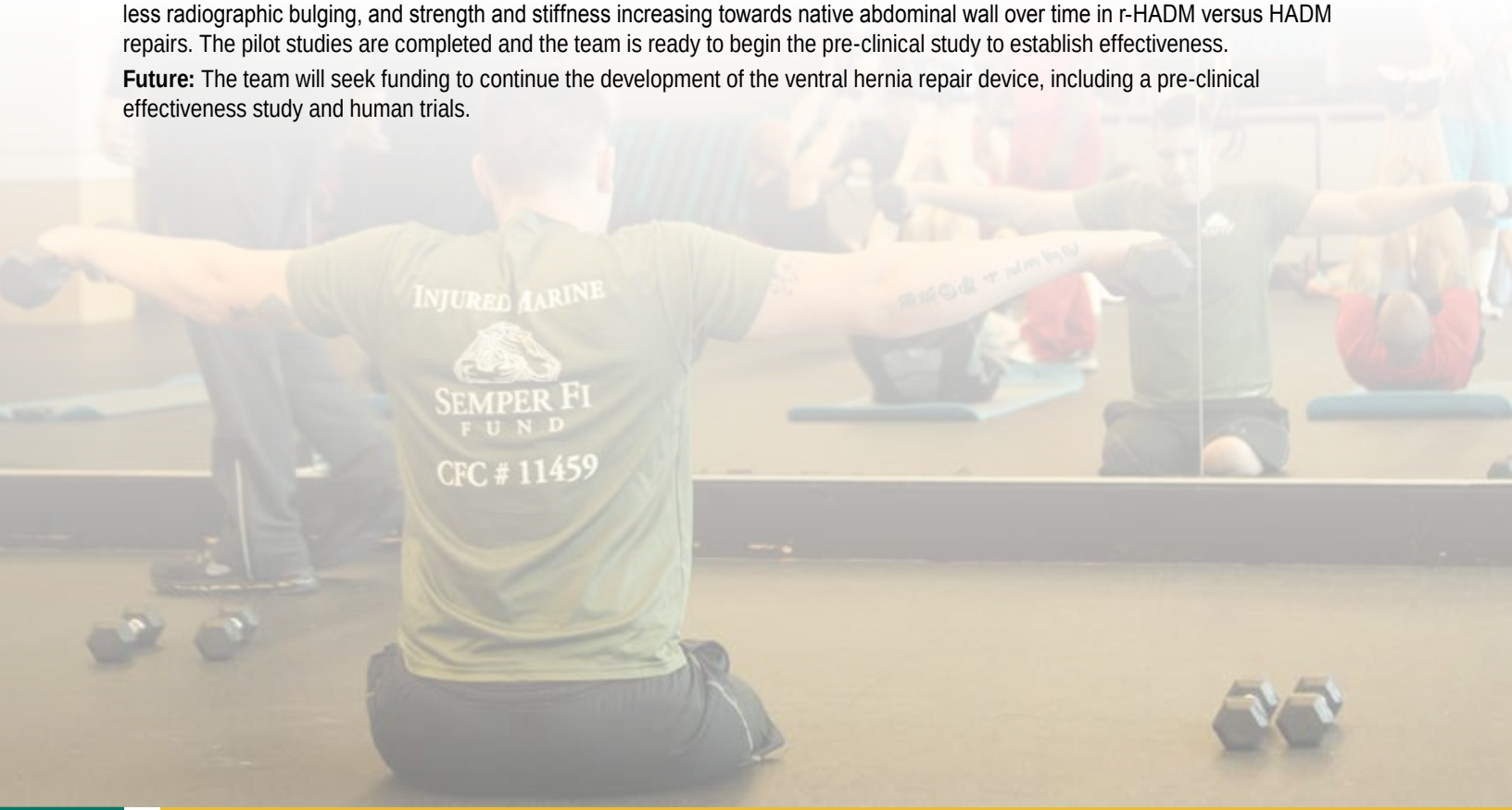
Team/Collaborating Partner Institution: *Cleveland Clinic; University Hospitals, Cleveland*

Target Clinical Application: *Repair of Hernia*

Goals: The goal of this project is to develop a ventral hernia repair device based on a reinforced extracellular matrix (ECM) technology. The effort involves developing prototypes, establishing a large animal (pig) ventral hernia model, and conducting pilot studies.

Accomplishments: The team previously developed and commercialized the reinforced ECM technology for rotator cuff repair. They completed the design of a reinforced human acellular dermis matrix (r HADM) device in which polymer fibers are stitched to the scaffold to provide targeted mechanical strength. A proof-of-concept experiment was performed in an in vitro enzyme digestion model to demonstrate that fiber reinforcement imparts mechanical durability to r-HADM during enzymatic degradation. The team also established a large animal (pig) ventral hernia repair model, and it developed computed tomography (CT) imaging methods for monitoring hernia repair bulging and hernia recurrence. They also established methods for retrieving tissue-device constructs and conducting biomechanical tests. Pilot studies were conducted in the porcine model which showed less radiographic bulging, and strength and stiffness increasing towards native abdominal wall over time in r-HADM versus HADM repairs. The pilot studies are completed and the team is ready to begin the pre-clinical study to establish effectiveness.

Future: The team will seek funding to continue the development of the ventral hernia repair device, including a pre-clinical effectiveness study and human trials.



WRC: GU-06

Engineering of Innervated Volumetric Skeletal Muscle Tissue for Accelerated Restoration of Pelvic Floor Muscle Function

Team Leader: *In Kap Ko, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Reconstruction of Pelvic Floor Muscle*

Goals: The goal of this effort is to further develop and optimize the fabrication of functional muscle constructs for restoring pelvic floor muscle anatomically and functionally. The effort will include optimizing fabrication of volumetric muscle constructs, assessing pre-patterning with acetylcholine receptor (AChR) as a means to increase innervation efficiency of constructs, and evaluating the approaches in an animal model of pelvic muscle injury.

Accomplishments: The team focused on optimizing the fabrication of volumetric muscle constructs using skeletal muscle cells. They successfully matured human muscle progenitor cells (hMPC) into myotube clusters, which was confirmed by staining for skeletal muscle markers. They investigated whether AChR clusters could be formed on the myotubes, as such clusters are a possible means to enhance neural integration. Human agrin treatment was found to be effective to fabricate AChR clusters on the hMPC-derived myotubes. The team next focused on increasing the thickness and organization of bioprinted muscle constructs. They successfully fabricated muscle constructs (3 layer, 0.1 cm thickness) using primary hMPC and human agrin treatment, which showed structural stability, cell viability, muscle differentiation, and innervating capability of the bioprinted construct. The team began working on fabrication methods for greater thickness constructs and encountered issues with structural stability, and oxygen and nutrient delivery to inner layers. A modified approach to the thrombin treatment portion of the process allowed fabrication of 0.3-0.6 cm thickness constructs with potentially viable cells in the inner layers. Assessment of the bioprinted constructs is ongoing for differentiation capability and structural integrity.

Future: The team will continue to establish and refine bioprinting methods for volumetric muscle constructs. Implantation studies will be initiated to test feasibility of their approach using an animal model of pelvic muscle injury.

WRC: GU-07

Implantation of Bioengineered Innervated Terminal Gut as a Replacement Therapy for Injured Anus

Team Leader: *Khalil Bitar, PhD (Wake Forest University)*

Team/Collaborating Partner Institution: *WFIRM*

Target Clinical Application: *Reconstruction of Anus*

Goals: The goal of this project is to develop a strategy for repairing injured anus that involves the use of autologous smooth muscle and neural progenitor cells from gut biopsies to bioengineer and implant intrinsically innervated internal anal sphincter. The effort will include pre-clinical large animal studies in order to move the technology towards clinical evaluations.

Accomplishments: The team is making progress validating the use of a rabbit model of partial sphincterectomy to serve as a model for injured anus and passive incontinence in humans. Fecal incontinence (FI) is induced by anterior hemi-circumferential sphincterectomy of the innervated anal sphincter (IAS). For the study, rabbits underwent surgeries that included intestinal biopsy for the purpose of isolating neural progenitor cells, partial sphincterectomy of the IAS to validate the injury model, and implantation of bioengineered intrinsically innervated IAS to determine if FI would be reversed. Results showed perineal soiling, altered stool form, and defecatory behavior similar to those seen in human FI. In addition, there is impairment in the Rectoanal Inhibitory Reflex and decreased anal resting pressures. Neural progenitor cells were successfully isolated from intestinal biopsies and then cultured, and IAS constructs were bioengineered. Implantation surgeries were also successful. The team is continuing to validate the model. Additionally, the researchers determined that it is feasible to isolate and co-culture neuroprogenitor cells from the appendix as an alternate cell source.

Future: The team will continue experiments to reach 6- and 12-month time points in the studies for validating the rabbit model. They also plan to bioengineer constructs using neurospheres from the appendix, and will compare their physiological responses to bioengineered tissue using neurospheres from the intestinal biopsies.

WRC: GU-08

Engineering Scaffold and Cell Therapy for Treatment of Fecal Incontinence Due to Defects in the Anal Sphincter

Team Leader: *Massarat Zutshi, MD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *Cleveland Clinic; Summa*

Target Clinical Application: *Reconstruction of Anal Sphincter*

Goals: The team is developing a scaffold with incorporated factors to chemoattract mesenchymal stem cells (MSC) to injured anal sphincter in order to promote new tissue formation. The approach is being studied in a pre-clinical rat model.

Accomplishments: The team has begun evaluating whether an SDF-1 and MCP-3 incorporated scaffold chemoattracts MSC, with formation of new tissue derived from the surrounding local tissues. The researchers developed scaffolds, isolated and expanded bone marrow-derived stem cells, and tested the functioning of the plasmid for the production of factors at a site of injury. The team created a standardized injury in the rat gluteus muscle to use as a model to study and optimize the combination of cells, scaffold, and plasmid for their anal sphincter repair strategy.

Future: The team will optimize the cell/scaffold/plasmid combinations, and use histologic evaluation to determine the effects on healing at different time points. The researchers will then proceed to anal sphincter studies.

WRC: GU-09

Medically Engineered Functional Anal Sphincters Using Composite Tissue Engineering and Novel Electrode (ME-FASTE)

Team Leader: *Stephen Feinberg, DDS, MS, PhD (University of Michigan)*

Team/Collaborating Partner Institution: *University of Michigan*

Target Clinical Application: *Reconstruction of Anal Sphincter*

Goals: This project seeks to develop a tissue-engineered/regenerative medicine approach in conjunction with the surgical techniques of muscle flap prefabrication and prelamination, and micro flexible electrodes to produce prefabricated, prevascularized, prelaminated composite soft tissue muscular flaps for functional reconstruction of the anal sphincter. Studies will use a rat model.

Accomplishments: The anal sphincter reconstruction includes: a) tissue engineering muco-cutaneous (M/C) constructs grown from the survivor's own biopsies, b) prelaminating the M/C cells constructs onto a flap of the survivor's skeletal muscle, c) surgically forming the prelaminated muscle flap into an anal sphincter with an internal muscosa lining and cutaneous layers externally surrounding the anal sphincter, and d) maintenance of optimal anal sphincter tone and relaxation with electrically conductive polymers, and multichannel, flexible electrodes and a stimulation system. The team has fabricated M/C constructs on an AlloDerm® scaffold by seeding human oral and skin keratinocytes on the same piece of AlloDerm® in separate compartments. The structural composition of the M/C construct was confirmed using routine histology and immunohistochemistry to identify epithelial markers that are differentially expressed in three distinct and separate anatomic areas of the anal replacement device: skin, mucosa, and functional zone. The team resolved the prior issues with the ability to manufacture large (3 cm x 5 cm) constructs. Optimal AlloDerm® thickness (14-20µm) and predictable contracture (15%-20%) were determined. Methodology was developed for, and pilot rat studies conducted for, anal sphincter surgical construction with concomitant muscle flap prelamination; AlloDerm® successfully maintained a sphincter and formation of biological incorporation of the acellular epidermal (AlloDerm®) layer. Muscle flaps prelaminated with AlloDerm® remained well vascularized and innervated with normal electromyogram (EMG) and potential for contractions. The team has determined that it should be feasible to activate a small, cylindrical construct of similar shape to an anal sphincter using currents below 100 microamps in both monopolar and bipolar configurations, and that electrode depth with respect to tissue surface had negligible effect on voltage distribution. Studies are ongoing to select the chronic stimulation regimens, as is a histological analysis of full-thickness dermal-epidermal constructs.

Future: The team plans to next develop human cell M/C constructs, define the stimulation parameters required to maintain active contraction of gracilis or latissimus dorsi muscle in rats, and graft M/C constructs into rats. The focus is to determine the long-term reliability of vascularization and innervation for implanted laminated M/C, 3D anal protective layers.

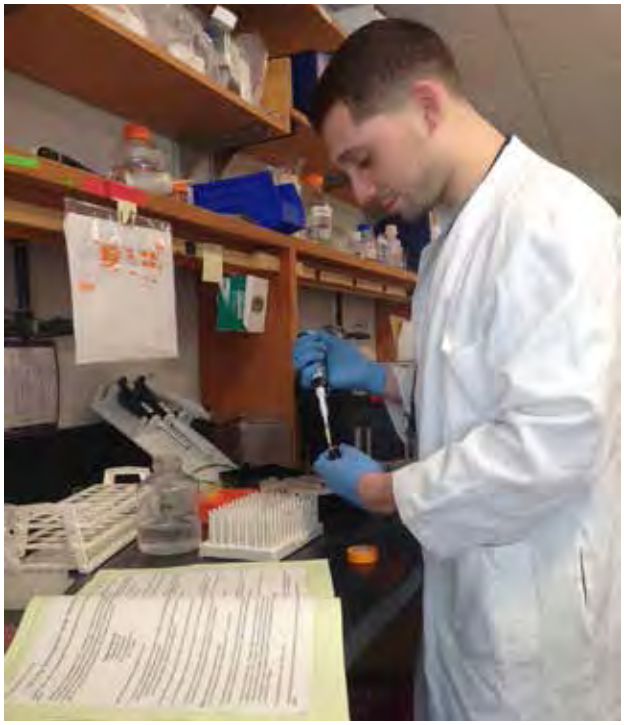
VI: Composite Tissue Allotransplantation and Immunomodulation

AFIRM OUR SCIENCE FOR THEIR HEALING

Background

Advances in military medicine have improved both the survival rate and treatment outcomes for severe combat trauma. Despite complex tissue reconstruction techniques, full restoration of function and appearance from significant extremity or craniomaxillofacial trauma may not be possible in all cases. Composite tissue allotransplantation (CTA) has emerged as a promising treatment modality in which tissue is transplanted from another individual. Numerous upper extremity and facial transplants have been performed in recent years, which have demonstrated success in restoring both appearance and function. However, stringent patient selection, constraints on donor selection, and the use of high-dose, multi-drug immunosuppression, which leaves the patient susceptible to a wide range of adverse effects, limit the widespread use of CTA.

VI: Composite Tissue Allotransplantation and Immunomodulation



The AFIRM's CTA and Immunomodulation Program is developing strategies which reduce the risks of the procedure, broaden the potential pools of candidates or donors, and reduce the adverse side effects of immunosuppression. Advances from the program are expected to improve long-term outcomes of hand and face transplantation for the wounded warrior.

Areas of Emphasis

The AFIRM CTA and Immunomodulation Program is divided into three areas: transplantation, immunomodulation, and graft preservation/surveillance. The current portfolio is primarily focused on immunomodulation strategies, with a few additional projects focused on graft preservation and surveillance. The researchers are developing new approaches to controlling the immune response during CTA, including inducing immune tolerance between the recipient and donor-grafted tissues, modulating the immune system, reducing inflammation, and decreasing the need for, and adverse effects of, long-term immunosuppression. The projects involve developing bone marrow- and stem cell-based immunomodulatory strategies, as well as immunosuppressive drug delivery approaches. The program is also focused on developing improved procedures and devices to better preserve the donor graft from the time it is removed from the donor until recipient blood flow is restored to the tissue. Additionally, the investigators are assessing ways to monitor the immune status of graft sites post-transplant. The program also is focused on understanding the mechanisms of, and strategies for controlling, the inflammation associated with ischemia-reperfusion injury (IRI) that can occur in some transplant cases. The research is conducted using in vitro, small, and large animal models, and the evaluation of some strategies has advanced to the human clinical trial stage. Insights gained from the CTA and Immunomodulation Program may have applicability for other types of transplants (kidney, liver, heart, etc.) as well as for other immunologically mediated conditions such as infection, inflammation, autoimmune disorders, and cancer.

As shown in **Table VI-1**, the current portfolio of AFIRM projects are grouped into two clinical challenge areas: immunosuppression and graft preservation/surveillance. A summary of each project is provided. Clinical trials are not included in Table VI-1 and are instead presented in a separate section of this chapter.

Table VI-1. Projects funded by RCCC, WFPC, and WRC by clinical challenge topic area.

Clinical Challenge	Consortium/ Institution	Project No.	Project Title
Immunomodulation	RCCC	4.3.1c	Composite Tissue Allograft Transplantation Without Lifelong Immunosuppression
		4.3.1e	Vascularized Composite Allograft Transplantation with Topical Immunosuppression
	WFPC	4.4.2	Hand Transplantation for Reconstruction of Disabling Upper Limb Battlefield Trauma – Translational Study
	WRC	CTA-01	Human Hematopoietic Cells Therapy for Transplantation Tolerance
		CTA-02	Towards a Preclinical Large Animal Tolerance Protocol for Vascularized Composite Allotransplantation in Swine
		CTA-03	Tolerance Induction to Vascularized Composite Allografts in a Pre-Clinical Large Animal
		CTA-04	Composite Tissue Allotransplantation without Life- Long Immunosuppression
		CTA-06	Post-transplantation Cyclophosphamide to Promote Immune Tolerance after Reconstructive Transplantation
		CTA-07	Translational Cell Based Immunomodulatory Therapies in Vascularized Composite Allotransplantation
		CTA-08	Tolerization of Vascularized Composite Allografts
		CTA-10	Vascularized Bone Marrow Regulates Alloresponses to Vascularized Composite Allografts
		CTA-11	Biomarker Guided Withdrawal of Immunosuppression in Recipients of Vascularized Composite Tissue Transplants
Graft Preservation and Surveillance	RCCC	4.3.1d	Portable Perfusion System to Increase Preservation Time of Isolated Limbs for Transplantation
	WRC	CTA-05	Non-Invasive Immune Monitoring to Improve Outcomes in Composite Tissue Transplantation
		CTA-12	Ischemia and Reperfusion Injury in CTA

Clinical Challenge Immunomodulation

Work is underway by AFIRM investigators to develop new approaches to vascularized composite allograft (VCA) transplants that include inducing immune tolerance between the recipient and donor-grafted tissues, modulating the immune system, and reducing inflammation, in order to achieve the ultimate goal of decreasing, or eliminating altogether, the need for long-term immunosuppression. The investigators are testing combinations of biological agents with bone marrow as a common element, and they are investigating a wound dressing embedded with drug-releasing nanospheres for administering immunosuppressive agents. Studies also include efforts to produce mixed chimeric hematopoietic stem cells that prevent rejection by allowing the recipient's immune system to view donor tissue as "self," not "other." Another project is investigating the functional mechanism of mesenchymal stem cells (MSCs) to stabilize the allograft vasculature during the ischemic conditions of the isolated tissue.

RCCC 4.3.1c

Composite Tissue Allograft Transplantation Without Lifelong Immunosuppression

Team Leader: *Maria Siemionow, MD, PhD (University of Illinois at Chicago)*

Team/Collaborating Partner Institution: *University of Illinois at Chicago; Tolera Therapeutics*

Target Clinical Application: *Immunomodulation for VCA*

Goals: The goal of this project is to develop enhanced standards for clinical modulation of the immune system to improve the safety and availability of VCAs. Investigators will examine whether human chimeric cells can be applied as a cellular therapy to eliminate the need for lifelong immunosuppression and investigate properties of cryopreserved human chimeric cells.

Accomplishments: During the course of this research study, the team demonstrated the feasibility of creating fused human chimeric cells ex vivo to test the fused cells as an immunomodulatory regimen. A population of fused human hematopoietic chimeric cells (HHCCs) was created from the cord blood of two unrelated donors. With the successful identification of fused chimeric cells, the team continued to conduct in vitro and preclinical studies of the chimeric cells towards the eventual aim of applying these cells as a novel cellular therapy approach for VCA transplantation. During the previous year, the research team studied many properties of the chimeric cells, including characterizing the genotype and phenotype before and after culturing, and discovered 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. Additionally, the team investigated the introduction of ex vivo fused human chimeric cells into an athymic nude rat model, which does not reject foreign biological material, and demonstrated that the human chimeric cells that were delivered into the femur were able to migrate into blood, bone marrow, and lymphoid organs. The introduced human chimeric cells were detectable in those tissues 24 hours after delivery and remained detectable in the rat up to 2 months later. With the results that human chimeric cells migrate from the site of injection to other bone components including natural stem cell niches, the team has demonstrated in the rat model the potential for the chimeric cells to become engrafted and survive in vivo.

Future: 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. To characterize the cryopreservation of the cells, the research team will investigate the stability, phenotypes, and genotype of cryopreserved chimeric cells both in vitro and in vivo. Following the 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.

RCCC 4.3.1e

Vascularized Composite Allograft Transplantation with Topical Immunosuppression

Team Leader: *David Sachs, MD, Curtis Cetrulo, Jr., MD (Massachusetts General Hospital), Joachim Kohn, PhD (Rutgers University)*

Team/Collaborating Partner Institution: *Massachusetts General Hospital; Rutgers University*

Target Clinical Application: *Immunomodulation for VCA*

Goals: The goal of this project is to investigate the topical delivery of two immunosuppressant drugs to reduce the need for high-dose systemic medicines that are associated with significant negative side effects.

Accomplishments: The research team formulated biodegradable drug delivery nanosphere-embedded dressings that provided sustained release of potent immunosuppressive agents. The drug-loaded nanospheres were stable over a range of temperatures, penetrated the dermis of cadaveric skin, and released drug over seven days from a gel. Investigators demonstrated the significant reduction in inflammation to skin grafts and wound beds when the drug-loaded nanospheres were applied to the recipient skin graft site or wound bed prior to graft placement in a baboon model. However, the topical nanosphere-delivery of single immunosuppressant agents did not prolong the survival of skin grafts or VCAs in animal models relative to the survival of skin grafts or VCAs treated with standard dressings.

Future: The RCCC project has been completed. In the future, the research team anticipates building on the findings of this project to develop an alternate formulation of the Tyrospheres to co-deliver different drugs that might be more effective in combination than alone in graft survival.

WFPC 4.4.2

Hand Transplantation for Reconstruction of Disabling Upper Limb Battlefield Trauma—Translational Study

Team Leader: *W.P. Andrew Lee, MD (Johns Hopkins University, School of Medicine)*

Team/Collaborating Partner Institution: *Johns Hopkins University; University of Pittsburgh*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to investigate whether the combination of bone marrow cell-based therapy and biologic agents such as CTLA4Ig can favor chimerism and promote tolerance induction in recipients of CTA transplants that could minimize or obviate the need for long-term immunosuppression.

Accomplishments: The research team completed studies that demonstrate the induction of immune tolerance in a mismatched swine hind limb transplant model using a regimen that included both donor bone marrow infusion and co-stimulatory blockade with the biological agent CTLA4Ig. The administration of CTLA4Ig effectively replaced whole body and thymic irradiation as a tolerance induction support therapy. In 5 of 8 animals receiving this regimen, the hind limb grafts survived indefinitely without immunosuppression. Investigators verified the systemic nature of this effect when a secondary skin graft to the recipient animals from the same donor failed to produce an immune response. When investigators removed the hind limb graft in this protocol, the induced tolerance was reversed, which indicated the essential role of vascularized bone marrow in the limb bone to elicit CTA tolerance.

Future: The research team continues to execute a clinical study of hand transplantation that is informed by the results of the bone marrow infusion procedure applied in this swine hind limb protocol. The clinical study will be completed with an aim to demonstrate the reduction of maintenance immunosuppression therapy, and transplant patients that are compliant with follow-up care are being observed while they are treated with low levels of a single immunosuppressive drug.

WRC CTA-01

Human Hematopoietic Cells Therapy for Transplantation Tolerance

Team Leader: *Maria Siemionow, MD, PhD (University of Illinois at Chicago)*

Team/Collaborating Partner Institution: *University of Illinois at Chicago*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to investigate properties of HHCCs and the immunomodulating potential of HHCCs to be further developed as supportive cellular therapies that can minimize long-term immunosuppression treatments in patients undergoing VCA transplantation procedures. The effort will develop an ex vivo procedure for creating fused donor-recipient (or "chimeric") human hematopoietic cells, characterize properties of the HHCCs to determine safety and feasibility of incorporating the HHCCs into a transplantation regimen, and assess the migration and maintenance of HHCCs in a rat model.

Accomplishments: During the first year of the study, the team established an ex vivo procedure to fuse the human hematopoietic cells from separate donors. The team developed a procedure to isolate mononuclear cells from bone marrow and enrich a hematopoietic stem cell population. They also successfully propagated the separate donor populations of hematopoietic cells in vitro, and labelled the populations of cells. The team also developed and evaluated the procedure to produce fused HHCCs, and they confirmed the efficacy and quality of producing chimeric fused human hematopoietic cells.

Future: The team will optimize procedures to propagate the fused HHCCs in vitro. Furthermore, the team will experimentally characterize the phenotype, viability, and genotype of the cultured HHCCs to confirm the composition of the HHCCs and produce preliminary data regarding the safety, stability, and feasibility for HHCCs to induce tolerance in VCA recipients.

WRC CTA-02

Towards a Preclinical Large Animal Tolerance Protocol for Vascularized Composite Allotransplantation in Swine

Team Leader: *David Sachs, MD (Massachusetts General Hospital)*

Team/Collaborating Partner Institution: *Massachusetts General Hospital*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to develop a supportive treatment protocol to induce tolerance for VCA transplantation. The effort will investigate a supportive therapy to generate a mixed chimerism phenotype and evaluate the induction of immunologic tolerance in a preclinical study of VCA transplantation. The outcomes of the study will be used to design a clinically relevant mixed chimerism protocol for inducing VCA tolerance.

Accomplishments: The team received local institutional and Animal Care and Use Review Office (ACURO) approvals to conduct the preclinical study protocols and performed the control VCA transplants in miniature swine. Flaps containing vascularized skin and underlying fascia from donor animals were harvested and transplanted in surgical defects in the recipient animals. These transplant procedures were performed on animals that were neither conditioned for immune tolerance nor immunosuppressed to establish the composition and dynamics of biological markers associated with allograft rejection. As expected in this control study, investigators observed a significant infiltration of recipient Langerhans cells that present antigen in skin biopsies from the donor site, indicative of pending rejection, within 2 days of the transplantation. The proportion of recipient Langerhans cell population in the graft tissue continued to increase, and gross features of graft rejection were observed by Day 7 or 8 after the surgery.

Future: The team has begun to examine biological markers and time to graft rejection in miniature swine that were conditioned with low-level total-body irradiation prior to the transplant procedure and administered previously harvested recipient peripheral blood mononuclear cells and immunosuppressant therapy. Further, investigators will complete the preclinical study with the third group of animals that will be treated with the conditioning protocol and administration of hematopoietic stem cells from the donor to generate mixed chimerism and modulate the rejection of the grafted tissue. After the completion of this VCA transplant animal study, the team will design a clinically relevant, mixed chimerism protocol for induction of VCA tolerance.

WRC CTA-03

Tolerance Induction to Vascularized Composite Allografts in a Pre-Clinical Large Animal

Team Leader: *David Mathes, MD (University of Washington)*

Team/Collaborating Partner Institution: *University of Colorado, Denver; University of Washington*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to design and test a non-myeloablative mixed chimerism protocol to tolerize a recipient to CTA without the need for long-term immunosuppression. The effort will examine CTA supportive therapy regimen including mobilized hematopoietic stem cells to establish mixed chimerism and induce immune tolerance. The effort will also elucidate the mechanism of action of immune tolerance and whether transient chimerism can induce tolerance for CTAs.

Accomplishments: The team received institutional and ACURO approval to initiate the animal treatment protocols. The team completed the planning and preparations to initiate the canine transplant procedures in the second year.

Future: The research team will perform 5 transplant procedures with supportive therapy of mobilized hematopoietic stem cells and non-myeloablative conditioning in the canine transplant model.

WRC CTA-04

Composite Tissue Allotransplantation without Life- Long Immunosuppression

Team Leader: *Maria Siemionow, MD, PhD (University of Illinois at Chicago)*

Team/Collaborating Partner Institution: *University of Illinois at Chicago*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to examine whether human chimeric cells can be applied as a cellular therapy to eliminate the need for lifelong immunosuppression and investigate properties of cryopreserved human chimeric cells. The effort will evaluate the safety and genotype stability of human umbilical cord blood-derived chimeric cells (HUCC) from two unrelated human umbilical cord blood donors, optimize HUCC cryopreservation techniques, and develop protocols to create and assess multichimeric cells for the potential to induce tolerance and enable organ donations from two or more unrelated donors.

Accomplishments: This project will build on the successful work by the laboratory to investigate the ex vivo fusion of human cord blood cells from two donors and the demonstration of the motility and longevity of human chimeric cells in the athymic nude rat model. During the first year of the project, the investigators received institutional and ACURO approval to initiate the animal protocols. The team also received approval to conduct the study using commercially purchased de-identified human cord blood cells. The team established procedures for histological, molecular, genotype, and phenotype analyses.

Future: During the upcoming year, the research team will test the safety and genotype stability of the human cord blood-derived chimeric cells after the cell fusion procedure utilizing umbilical cord blood from two unrelated human donors. The research team will also begin to investigate the protocol for cryopreservation of these cells, optimizing the protocol, and evaluating cells that had been cryopreserved for viability, proliferative properties, phenotype, and genotype stability.

WRC CTA-06

Post-transplantation Cyclophosphamide to Promote Immune Tolerance after Reconstructive Transplantation

Team Leader: *Gerald Brandacher, MD (Johns Hopkins University)*

Team/Collaborating Partner Institution: *Johns Hopkins University*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to evaluate a combined supportive therapy regimen to improve the outcomes following VCA transplantation to reconstruct tissue loss without the need for long-term, toxic immunosuppression. The effort will assess the combination of donor bone marrow and composite tissue transplantation with high-dose post-transplant cyclophosphamide in a mouse model to induce stable tolerance without myeloablative conditioning or long-term maintenance immunosuppression.

Accomplishments: The team received institutional approval and ACURO approval to initiate the animal protocols during the first year. The team successfully established a non-myeloablative conditioning regimen consisting of total body irradiation plus antibody-mediated T cell depletion on the day prior to transplantation followed by post-transplant cyclophosphamide administration. Results from this treatment protocol in a murine orthotopic hind limb transplant model demonstrated long-term graft survival without requiring maintenance immunosuppression. The team demonstrated that the supportive therapy regimen resulted in donor antigen-specific tolerance, which was confirmed by performing secondary full thickness skin grafts from donor-matched animals that survived, and from third party animals that were rejected.

Future: The research team will conduct in vitro testing and in vivo testing in this murine model to better understand the mechanisms of the observed tolerance phenomenon.

WRC CTA-07

Translational Cell Based Immunomodulatory Therapies in Vascularized Composite Allotransplantation

Team Leader: *Peter Rubin, MD (University of Pittsburgh)*

Team/Collaborating Partner Institution: *University of Pittsburgh; John Hopkins University*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to investigate the effectiveness of adipose-derived stem cell (ASC) and bone marrow mesenchymal stem cell (BM-MSC) treatments to minimize chronic drug-induced systemic immunosuppression following limb transplantation. The study is designed to demonstrate whether administering these supportive therapies in a VCA preclinical model will provide benefits alone or in combination to achieve allograft tolerance and reduce or eliminate the current requirement for drug-induced immunosuppression.

Accomplishments: The research team received an IRB exemption and HRPO concurrence to collect human bone marrow MSCs through the Center for Organ Recovery and Education at the University of Pittsburgh. Additionally, the investigators submitted animal study protocols for institutional and ACURO approval. The team refined procedures to harvest ASCs from Yorkshire pigs and to propagate the population of ASCs in culture. The team conducted initial in vitro studies of isolated ASCs and BM-MSCs to estimate the effectiveness of these stem cell populations to abrogate the expansion of growth-stimulated splenocytes. The team began to evaluate substituent groups of cells isolated from harvested bone marrow to identify cell populations that induce an immunosuppressive phenotype in an in vitro mixed lymphocyte reaction assay.

Future: The team will continue the in vitro characterization, improvement of isolation procedures, and the assessment of the immunosuppressive function of both ASCs and BM-MSCs. Concurrently, the team will be making preparations to perform in vivo experiments. In addition, the team will harvest fat, bone marrow, and blood from donors, and isolate MSCs from these tissues.

WRC CTA-08

Tolerization of Vascularized Composite Allografts

Team Leader: *Daniel Ceradini, MD (New York University)*

Team/Collaborating Partner Institution: *New York University*

Target Clinical Application: *Tolerance Induction for VCA*

Goals: The goal of this project is to develop novel approaches to facilitate tolerance of allogeneic tissue with decreased or no requirement for lifelong systemic immunosuppression to expand the clinical use of VCA transplantation. The effort will examine methods to modify VCAs between procurement and transplantation using immunosuppressive cells, and will involve evaluating conditions that prime MSCs for an immunomodulatory phenotype and populating allografts with immunomodulatory MSCs ex vivo to promote tolerance.

Accomplishments: During the first year of the project, the team received institutional approval to initiate the animal protocols. The research team demonstrated conditions under which MSCs could be primed in vitro for an immunosuppressive phenotype during culture expansion, maintain their proliferative capacity, and enhance functions critical for preventing acute rejection. The team's findings implicate the functional significance of MSCs to stabilize the allograft vasculature following the ischemic period between procurement and transplantation. Additionally, the team's results implicated two mechanisms, the response to alloantigen recognition and effector cell cytotoxicity, contributing to the functional inhibition of the acute rejection response by MSCs.

Future: During the upcoming year, the research team will focus on optimizing the MSC seeding efficiency in VCA flaps in vivo. Investigators will evaluate seeding efficiency, tissue distribution of seeded cells, and re-implantation success using both syngeneic and allogeneic models.

WRC CTA-10

Vascularized Bone Marrow Regulates Alloresponses to Vascularized Composite Allografts

Team Leader: *Rolf Barth, MD (University of Maryland)*

Team/Collaborating Partner Institution: *University of Maryland*

Target Clinical Application: *Immunomodulation for VCA*

Goals: The goal of this project is to investigate whether vascularized bone marrow transplanted as an allograft may provide the benefits of bone marrow transplantation without the associated morbidity of therapies required for bone marrow transplantation. The effort will examine the outcomes of vascularized bone marrow allograft in a well-established non-human primate (NHP) model of facial VCA and a clinical study of face transplantation with vascularized bone marrow.

Accomplishments: The team received approval from the institution and ACURO to initiate the animal protocols. In addition, investigators submitted documentation to the IRB for conducting the face transplantation that is still under review. Investigators ordered, received, and quarantined NHP animals, and the investigators screened the animals for mismatches to determine donor-recipient pairs. The team refined procedures to screen recipient peripheral blood for the presence of transplant donor cells, which is indicative of a surviving VCA transplant.

Future: The team will perform the facial VCA transplant procedures with the NHPs to evaluate the benefits of local versus distal vascularized bone marrow grafts. The team will monitor the recipients for health, transplant rejection, and chimerism. In addition, after HRPO approval to commence, the clinical team will recruit, list, and screen potential patients for the human clinical facial transplant procedure.

WRC CTA-11

Biomarker Guided Withdrawal of Immunosuppression in Recipients of Vascularized Composite Tissue Transplants

Team Leader: *Bohdan Pomahac, MD (Brigham & Women's Hospital)*

Team/Collaborating Partner Institution: *Brigham & Women's Hospital; Beth Israel Deaconess Medical Center*

Target Clinical Application: *Immunomodulation for VCA*

Goals: The goal of this project is to develop a feasible and safe short-term immunomodulatory support therapy regimen to minimize or possibly withdraw long-term immunosuppression treatments to CTA patients. The effort will involve a clinical study of CTA transplantation under a transient period of interleukin-2-mediated inhibition of immune response, during which the patients will be monitored to identify candidates suitable for immunosuppression withdrawal.

Accomplishments: The team obtained IND approval from the FDA to conduct the clinical study of interleukin-2 in the CTA recipients. The investigators submitted the clinical study protocol to the human research committee and received approval to proceed, and the subsequent submission of the study package to the HRPO is under review. The team has completed preparations to begin recruitment upon approval from the HRPO.

Future: After the HRPO approves the protocol, the team will commence the recruitment, enrollment, listing, and screening of 4-8 CTA candidates. The clinical study team will administer the tolerogenic protocol and transplantation procedures to these subjects and carry out an immune-monitoring protocol.

Clinical Challenge Graft Preservation and Surveillance

Work is under way to develop improved procedures and devices to better preserve isolated donor allograft transplant tissue between procuring the tissue from the donor and transplanting the tissue in the recipient. One project involves designing and evaluating an isolated limb perfusion device. Another effort is examining devices for non-invasive immune monitoring of the graft site after transplantation. Investigators are also working to elucidate the biological mechanisms of inflammation in IRI that occurs in some transplantation cases.

RCCC 4.3.1d

Portable Perfusion System to Increase Preservation Time of Isolated Limbs for Transplantation

Team Leader: *Bohdan Pomahac, MD (Brigham and Women's Hospital)*

Team/Collaborating Partner Institution: *Brigham and Women's Hospital*

Target Clinical Application: *Graft Preservation*

Goals: The goal of this project is to evaluate the feasibility that a perfusion medical device can attenuate the effects of IRI in amputated limbs for the preservation and replantation of an amputated limb.

Accomplishments: The team collaborated with Numia Medical to evaluate a functional prototype extracorporeal limb perfusion device. During the previous year, the team acquired the prototype device and tested perfusion parameters of the device. The team tested the perfusion device to make procedural improvements and demonstrated that the device can reliably perform a 12-hour perfusion of isolated limb at a defined arterial pressure and temperature. The team demonstrated the device could saturate the perfusion solution with oxygen. The team determined that the device could maintain some tissue viability without muscle fiber damage in the isolated limb.

Future: The project was completed. The research team aims to further investigate the functional prototype perfusion device with 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.

WRC CTA-05

Non-Invasive Immune Monitoring to Improve Outcomes in Composite Tissue Transplantation

Team Leader: *W.P. Andrew Lee, MD (Johns Hopkins University)*

Team/Collaborating Partner Institution: *Johns Hopkins University; Naval Medical Research Center*

Target Clinical Application: *Post-Transplant Monitoring of Grafts*

Goals: The goal of this project is to establish a non-invasive method for immune monitoring after VCA transplantation. The effort will evaluate the application of Raman Spectroscopy using real-time infrared digital cameras, visible reflectance spectroscopy, and near-infrared spectroscopy with a Bayesian classifier and molecular marker platform for effectiveness to monitor transplanted grafts and diagnose the rejection of grafts without the need for invasive tissue biopsies and advanced clinical signs of rejection.

Accomplishments: The research team received institutional and ACURO approvals to initiate the animal protocols, and a review of previously received IRB and HRPO approvals for human hand transplantations were deemed applicable to the human protocol aims of this study. During this year, the techniques for image protocols were optimized for visible reflectance using a 3CCD camera and the infrared imaging and functional near-infrared imaging digital cameras.

Future: The team will conduct the hind limb transplant procedure with donor-recipient mismatched animals. The team will use the light scatter imaging devices to monitor the progression of the tissue rejection process and correlate imaging data, physical characteristics, and biomarkers of skin rejection of the transplant to ascertain the diagnostic and mechanistic characteristics of tissue rejection.

WRC CTA-12

Ischemia and Reperfusion Injury in CTA

Team Leader: *Jerzy Kupiec-Weglinski, MD, PhD (University of California, Los Angeles)*

Team/Collaborating Partner Institution: *University of California, Los Angeles*

Target Clinical Application: *Graft Survival*

Goals: The goal of this project is to investigate the mechanisms that initiate and regulate innate immune-driven inflammation responses associated with IRI in a mouse hind leg transplantation model. The effort will develop a mouse orthotopic hind limb transplantation model in which the donor transplant has undergone extended cold preservation, investigate whether innate immunity markers in skin biopsies predict CTA outcomes, evaluate the mechanisms associated with IL-1R-mediated IRI-triggered CTA damage, and develop novel innate immune-based therapies against IRI in CTA small animal models.

Accomplishments: During the first year of the project, the investigators received institutional and ACURO approval to initiate the animal protocols. The surgical resident trained to achieve proficiency in the precise, microsurgical technique of mouse hind limb orthotopic transplantation.

Future: During the upcoming year, the team will perform orthotopic hind limb or full-thickness skin grafting microsurgical procedures on mice and collect a series of biopsies post-operatively. The research team will investigate whether innate immunity markers found in the skin biopsies predict CTA outcomes and whether such innate markers can be used as therapeutic targets.

Clinical Trials

AFIRM investigators are conducting clinical transplantation studies with a focus on testing strategies designed to improve long-term outcomes of allograft transplantation through modulating the immune response. The status of each clinical trial is summarized in Table VI-2, and additional details on these trials follow the table.

Table VI-2. AFIRM-funded CTA and Immunomodulation projects with pending or active clinical trials.

Therapy/Product	Project Title	Consortium	Project Number	Trial Phase	Current Status
Single-stage surgical reconstructive procedure for patients with severe cranial facial injuries	Clinical Trial: Composite Tissue Allograft Transplantation (Face)	RCCC	4.3.1aCT	I	Active
Therapeutic antibody to enhance tolerance to transplantation	Clinical Trial: Anti-TCR Monoclonal Antibody (TOL101) for Prophylaxis of Acute Organ Rejection in Patients Receiving Renal Transplantation	RCCC	4.3.1bCT	I/II	Closed
Stem cell supportive therapies to reduce long term use of immunosuppressive agents	Hand Transplantation for Reconstruction of Disabling Upper Limb Battlefield Trauma–Clinical Study	WFPC	4.4.2	I	Active
Stromal vascular fraction of white adipose tissue as support therapy for hand transplantation	Tolerization of Vascularized Composite Allografts with Adipose SVF Cells	WRC	CTA-09	I	Active

RCCC 4.3.1aCT

Clinical Trial: Composite Tissue Allograft Transplantation (Face)

Team Leader: *Maria Siemionow, MD, PhD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *Cleveland Clinic; U.S. Army Institute of Surgical Research*

Target Clinical Application: *Composite Tissue Allograft Face Transplantation*

Goals: This goal of this clinical study is to advance a single-stage surgical reconstructive procedure for patients with severe cranial facial injuries that spares the patient from multiple surgical procedures over many years.

Accomplishments: The team has continued to follow a previously conducted CTA face transplant patient for more than 4 years. The outcomes of that procedure demonstrate the safety and effectiveness of the single-stage treatment for persons with severe facial deficit and deformity. In objective tests following the original treatment, the patient has gained control of facial movements including a return of mastication, and the ability to speak clearly, smell, smile, frown, and kiss. Potential serious adverse events, such as infection and rejection that may occur with any transplant, have been successfully treated. The team has begun to recruit additional persons to qualify for a second facial transplantation procedure, and it has completed detailed evaluations on five individuals. One patient met the criteria and was approved to be listed for a face transplant procedure.

Future: The team will perform one CTA face transplant procedure and subsequent care for that patient, and list a second patient for face transplantation.

RCCC 4.3.1bCT

Clinical Trial: Anti-TCR Monoclonal Antibody (TOL101) for Prophylaxis of Acute Organ Rejection in Patients Receiving Renal Transplantation

Team Leader: *Maria Siemionow, MD, PhD (Cleveland Clinic)*

Team/Collaborating Partner Institution: *University of Colorado, Denver, Baylor University Medical Center, Cleveland Clinic, Medical University of South Carolina, St. Barnabas Medical Center, University of Michigan, University of Utah, University of Kentucky, University of Virginia*

Target Clinical Application: *Tolerance Induction for Organ Transplantation*

Goals: The goal of this clinical study is to test the safety and tolerability of the therapeutic antibody TOL-101 as a conditioning agent administered prior to transplantation to enhance patient tolerance of the transplant organ.

Accomplishments: The team originally proposed the study to be executed as a two-part (Parts A and B) Phase I/II clinical study designed to assess safety and tolerability of the TOL-101 investigative drug in conjunction with a standard immunosuppressive regimen for renal transplantation. During the Phase I/Phase II Part A clinical study, investigators enrolled 36 patients who were treated with standard immunosuppressive regimen or the standard immunosuppressive regimen with the TOL-101 agent. Following the renal transplantation surgeries, patients were monitored for safety and potential drug-related adverse events. Tissue samples from 60 biopsies collected from these patients were submitted to the Cleveland Clinic Pathology Core for analysis. Investigators identified 27 serious adverse events, of which only one serious adverse event, a case of pneumonia, was potentially related to the investigative drug. The team submitted a clinical study report on the safety of TOL-101 to the FDA. The FDA approved the elimination of Part B of the Phase I/II clinical trial, enabling investigators to begin planning a Phase III clinical trial.

Future: The RCCC clinical study has been completed. The objectives of the Phase I/II clinical trial (Part A), which leveraged Department of Defense funds with funds from the manufacturer, Tolera Therapeutics, were exceeded. The FDA approved the elimination of the originally proposed Part B of the Phase I/II clinical trial and approved plans to design an adequately powered Phase III study for further development of the therapeutic antibody.

WFPC 4.4.2

Hand Transplantation for Reconstruction of Disabling Upper Limb Battlefield Trauma—Clinical Study

Team Leader: *W.P. Andrew Lee, MD (Johns Hopkins University, School of Medicine)*

Team/Collaborating Partner Institution: *Johns Hopkins University; University of Pittsburgh*

Target Clinical Application: *Immunomodulation for VCA*

Goals: The goal of this clinical study is to develop a post-transplantation surgery immunomodulatory protocol combining donor bone marrow stem cell (BMSC) and tacrolimus therapies designed to reduce dosing or frequency of maintenance immunosuppression in patients.

Accomplishments: The team designed the clinical investigation to assess the immunomodulatory potential of transient donor BMSC infusion therapy to boost the depletion of donor white blood cells combined with long-term, tapered dosing of the immunosuppressive agent, tacrolimus. Among the 6 hand transplant patients treated with the combination BMSC and tacrolimus regimen, the outcomes demonstrate the immunomodulatory regimen to be safe, effective, and well tolerated as long as the patient was in compliance with the regimen and physician's recommendations. The team has monitored patients and determined that the clinical progress, and immunologic and functional outcomes were on track in all subjects compliant with medication and physical therapy.

Future: The clinical team will continue to monitor the long-term outcomes of the patients. In addition, the team recently screened 6 civilian upper extremity amputee patients, of which 4 met the qualifications for transplantation.

WRC CTA-09

Tolerization of Vascularized Composite Allografts with Adipose SVF Cells

Team Leader: *Joseph Kutz, MD (Jewish Hospital)*

Team/Collaborating Partner Institution: *Christine M. Kleinert Institute; Cardiovascular Innovation Institute; Jewish Hospital*

Target Clinical Application: *Immunomodulation for VCA*

Goals: This goal of this clinical study is to investigate the effectiveness of stromal vascular fractions (SVF) of white adipose tissue to promote healing and modulate immune responses to treat episodes of graft rejection in hand transplants.

Accomplishments: During the first year of the project, the team formally established the clinical collaboration between Wake Forest, Jewish Hospital, the Christine M. Kleinert Institute, the Cardiovascular Innovation Institute, and the University of Louisville. The clinical protocol from previously conducted hand transplantation procedures was amended to include the study of the SVF cells, and the institutional approval was received. Eight (8) potential patients were screened against inclusion and exclusion criteria, and classified as medically acceptable to undergo the procedure. They will continue to undergo social support, psychological, and financial screening.

Future: Potential patients will continue to be evaluated for qualification, with an aim of enrolling sufficient numbers of patients to perform 1-2 hand transplant procedures each year of the study, and to provide SVF therapy to patients who experience graft rejection. For each transplant, the team will monitor progress after the procedure and will apply the SVF treatment protocol for any episodes of graft rejection as defined by skin biopsy.

VII: AFIRM Statistics

AFIRM OUR SCIENCE FOR THEIR HEALING

Introduction

The AFIRM is a large, multifaceted complex biomedical research and development (R&D) 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 R&D to find medical solutions to treat our Service members with severe combat wounds. AFIRM investigators and product developers represent over 70 research universities and hospitals, and 22 commercial partners at the core institutions and collaborating organizations.

VII: AFIRM Statistics

While previous chapters of this report demonstrate the depth of the AFIRM's individual research projects, here the research consortium as a whole is displayed 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 presenting aggregated program data. This chapter also provides tangible, scientific outcomes attributable to AFIRM-supported research: inventions disclosed, patent applications filed, research or review articles published, and conference presentations and posters presented. The AFIRM data shown in this chapter cover the 6 years of the program, with particular emphasis placed on the AFIRM in Program Year 6 (PY6).

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 350 Rutgers–Cleveland Clinic Consortium (RCCC) and Wake Forest–Pittsburgh Consortium (WFPC) researchers contributed to the AFIRM research activities in PY6.[1] The distribution of these scientists and students is illustrated in **Figure VII-1a**. For example, 108 faculty members from the RCCC and the WFPC contributed to the AFIRM in PY6. In addition, 48 postdoctoral associates and fellows, and 118 scientific and technical staff contributed to the AFIRM studies in PY6.[2] This same period of time includes the first year of the Warrior Restoration Consortium (WRC), and 340 research faculty, scientists, fellows/trainees, and students contributed to the WRC, many of whom were active in the RCCC or WFPC of the first AFIRM program. The distribution of the WRC scientific and technical staff is shown in **Figure VII-1b**.

Another highlight of the AFIRM is the substantial recruitment and training of the next generation of scientists to advance regenerative medicine

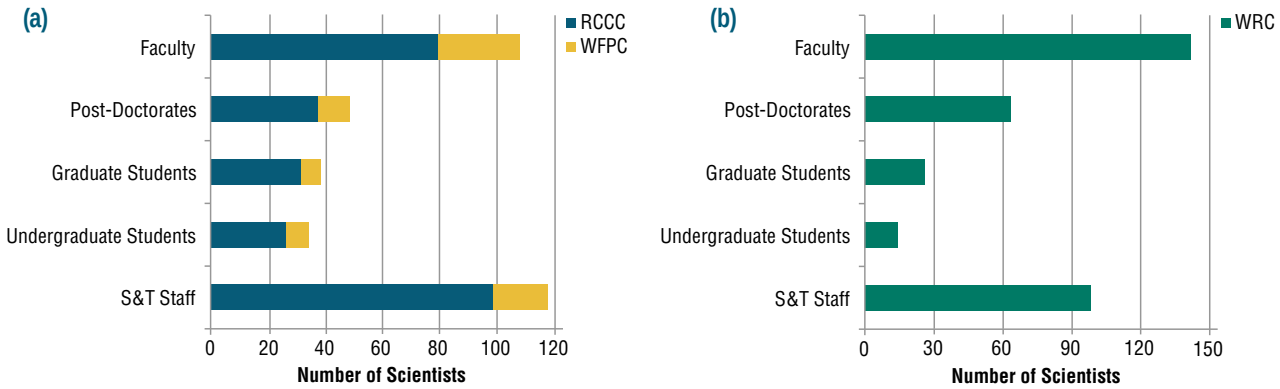


Figure VII-1. Distribution of scientists and students contributing to the (a) WFPC and RCCC of the original AFIRM program during PY6, and (b) the first year of the WRC in PY6.[3]

[1] For the purpose of this annual report, PY6 is defined as the period from June 1, 2013, to September 14, 2014, that contains the mostly overlapping sixth year of the first AFIRM program (June 1, 2013, to May 31, 2014) and first year of the WRC program (September 15, 2013, to September 14, 2014). 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; 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 PY6.

R&D into the future. More than 60 undergraduate and graduate students received valuable scientific training through WFPC- and RCCC-sponsored research projects in PY6 (Figure VII-1a), and more than 50 students received training during the first year of the WRC in PY6 (Figure VII-1b).

The numbers of AFIRM-supported graduate students who completed their degree requirements each year of the program are shown in **Figure VII-2**. During PY1, 3 graduate degree recipients were supported through the AFIRM. In each of the following 5 years, between 12 and 24 graduate degree recipients had received training support through the AFIRM. For the 6-year span of the program, a total of 85 graduate students supported through the AFIRM completed their degree requirements (64 received doctoral degrees and 21 received Master's degrees).

The AFIRM program was organized to permit an evolution of the technical and scientific focus. The program was originally organized into five research focus areas: Limb and Digit Salvage, Craniofacial Reconstruction, Burn Repair, Scarless Wound Healing, and Compartment Syndrome; however, the emphasis of the research shifted over time.[4] With the WRC, the research focus areas have been reconfigured as Craniomaxillofacial Regeneration, Composite Tissue Allotransplantation and Immunomodulation, Extremity Regeneration, Genitourinary/Lower Abdomen Reconstruction, and Skin Regeneration.

Figure VII-3 depicts the proportion of all core personnel who worked on the different program areas during the sixth year of the WFPC and RCCC (VII-3a) and the first program year of the WRC (VII-3b).[5]

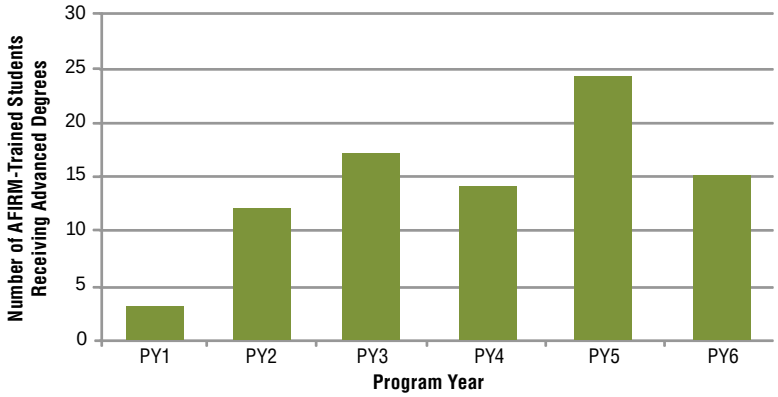


Figure VII-2. Number of graduate degrees awarded to students who received training through the AFIRM.

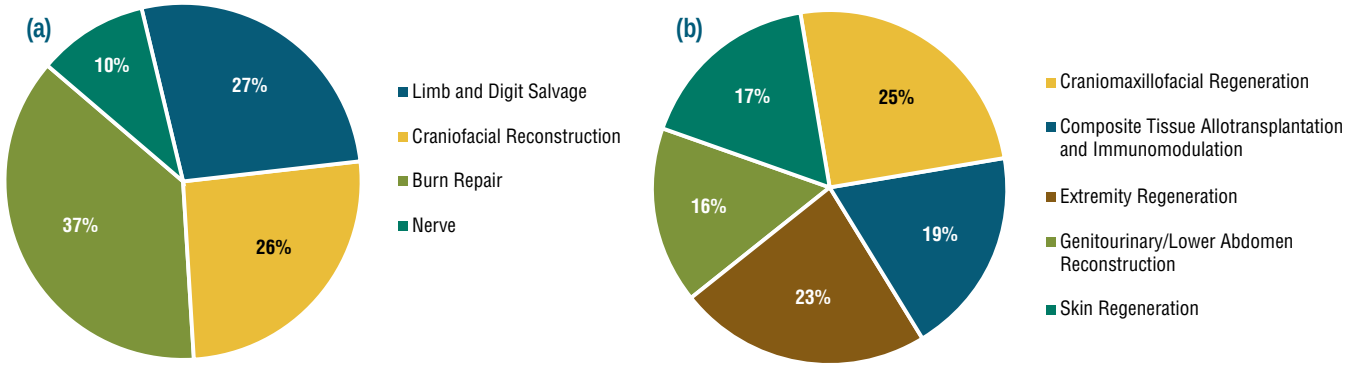


Figure VII-3. Percentage of personnel conducting research in the AFIRM program across the research main focus areas of (a) the WFPC and RCCC of the original AFIRM program, and (b) the WRC in PY6.

[4] As the AFIRM program has matured, the overarching research focus areas have been redefined by the consortia to more accurately describe the focus of the research. To compare the consortia across different years, Figure VII-3a displays the percentage of personnel conducting research according to the main focus areas active during PY6.

[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.

Honors and Achievements

The AFIRM program's faculty members are highly accomplished in their respective scientific fields. From June 2013 through September 2014, 26 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 and awards from private foundations; recognition of excellence at conferences; and faculty teaching awards. The distribution of honors received is shown according to the type of conferring organization (**Figure VII-4**).[6] The complete lists of honors and awards received by the AFIRM faculty during PY6 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 initiation of 60 newly awarded grants, contracts, or subcontracts in PY6.[7]

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 program year 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 program year. 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, RCCC, and WRC investigators in PY6 was 89 (**Figure VII-5**).[8] In addition, 57 peer-reviewed manuscripts were published in PY6 by WFPC, RCCC, and WRC investigators. The complete lists of AFIRM researchers' publication and presentation citations from PY6 are shown in **Appendix B**.

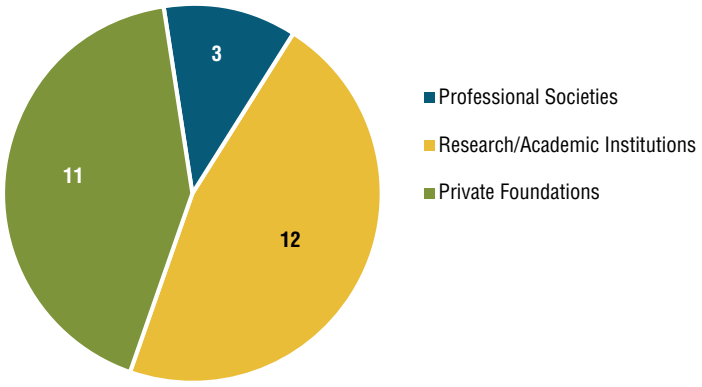


Figure VII-4. Distribution of honors and awards to AFIRM faculty by type of conferring organization in PY6.

[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 new grants or contracts includes those reported with funding start dates within PY6 and those that did not report funding dates.

[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.

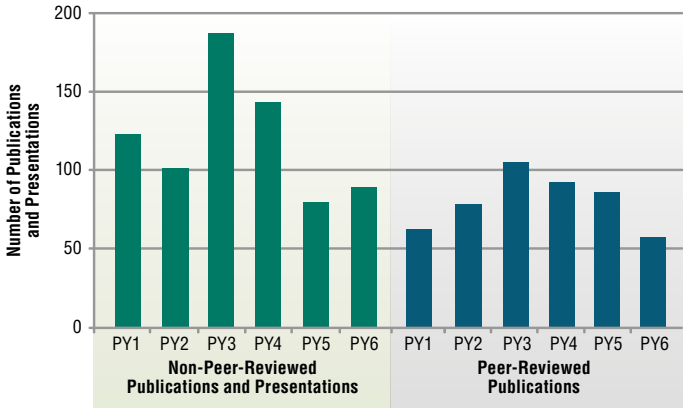


Figure VII-5 AFIRM-sponsored research findings disseminated to the scientific community through presentations and publications.

Inventions, Patent Applications, and Patents

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

During the first 6 years of the program, more than 110 invention disclosures were reported by AFIRM investigators[10], of which more than 90 patent applications were filed with Government patent offices. Based on annual reports, the USPTO has granted 14 patents to these applications. The distributions of invention disclosures, patent applications, and granted patents are shown in **Figure VII-6**.

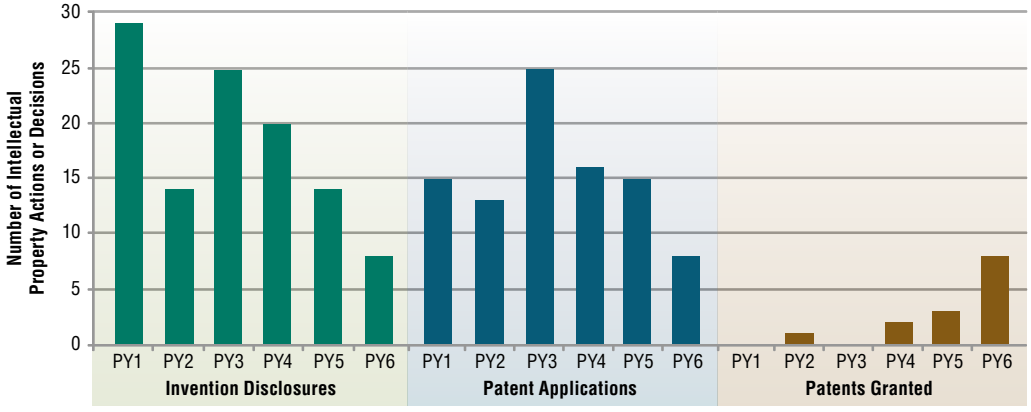


Figure VII-6. AFIRM-attributable invention disclosures, Government patent applications filed, and patents granted.

[9] Definitions of AFIRM-attributable inventions, patent applications, and patents were developed to standardize the self-reported intellectual property, and these 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."

Developmental Accomplishments and Milestones

Preclinical Models and Clinical Studies

In PY6, AFIRM researchers completed the development and/or validation of 11 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.

For investigators to conduct human clinical studies, they must first receive 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 6 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 PY6, sponsors affiliated with the AFIRM submitted 4 IDE applications and 6 IND applications.[11] Two (2) IDE applications and 4 IND applications were approved. Other sponsors have initiated pre-IND and pre-IDE meetings with the FDA, and have begun to prepare IND or IDE applications.

Through PY6, AFIRM investigators advanced numerous products through clinical study planning, approval, and execution stages. Six (6) clinical trials were open to patient enrollment in PY6, including 1 diagnostic protocol, 3 interventional device protocols, and 1 each of Phase I and Phase I/II protocols.[12] An additional 8 studies, including 5 Phase I and Phase I/II studies, had been submitted to IRBs or human research protection offices. One (1) diagnostic study was also open to enrollment in PY6. **Figure VII-7** 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 PY6, nearly 30 commercial organizations were involved in the AFIRM program in a variety of capacities, of which 16 are AFIRM members or are collaborating with the AFIRM, and the other partners provided materials or services under contracts or agreements.[13]

[11] Prior to PY6, IND and IDE applications submitted to the FDA for medical devices and therapeutics under investigation by the AFIRM were not reported.

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

[13] 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.

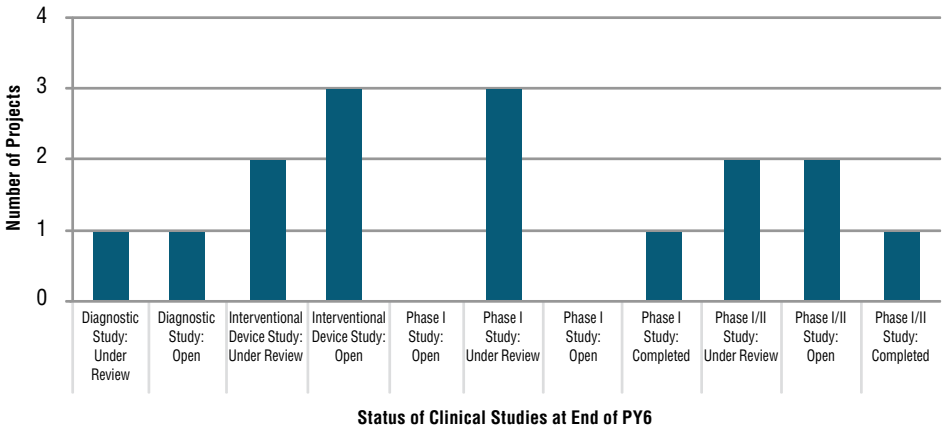
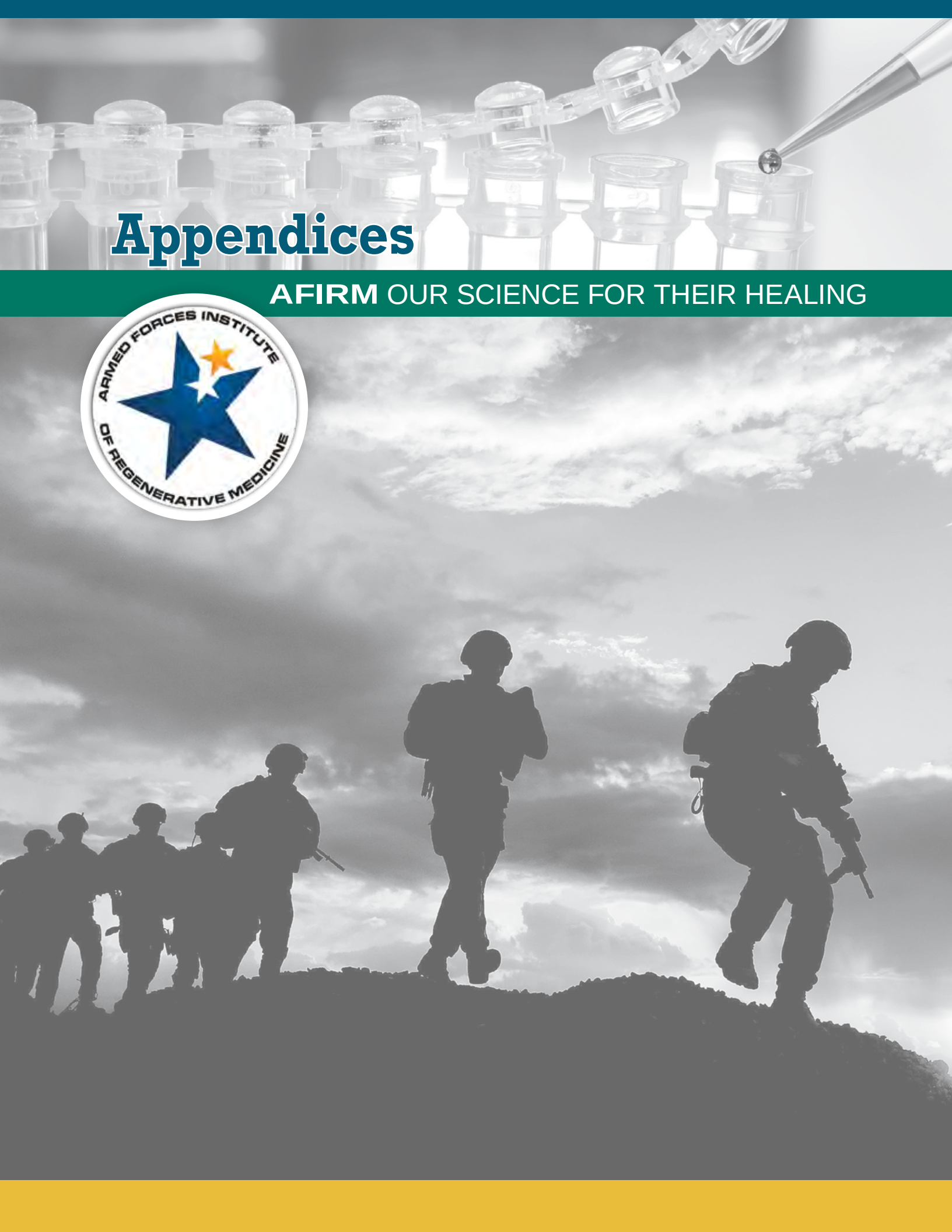


Figure VII-7. A snapshot of the clinical protocol stages of AFIRM products at the end of PY6.



Appendices

AFIRM OUR SCIENCE FOR THEIR HEALING

ARMED FORCES INSTITUTE OF REGENERATIVE MEDICINE

Appendix A: Honors and Awards to AFIRM Faculty

During the reporting period from June 2013 through September 2014, 26 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

Kohn, J (Rutgers University): Research Award for Meritorious Leadership and Contributions to New Jersey's Life Sciences Community Healthcare Institute of New Jersey

Langer, R (Massachusetts Institute of Technology): Julio Palmaz Award, Biomed SA

Langer, R (Massachusetts Institute of Technology): Breakthrough Prize in Life Sciences, Breakthrough Prize

Langer, R (Massachusetts Institute of Technology): Biotechnology Heritage Award Chemical, Heritage Foundation

Langer, R (Massachusetts Institute of Technology): International Prize in Nanotechnology, RUSNANOPRIZE

Langer, R (Massachusetts Institute of Technology): Kyoto Prize in Advanced Technology, Inamori Foundation

Langer, R (Massachusetts Institute of Technology): Queen Elizabeth Prize for Engineering, Queen Elizabeth Prize Foundation

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

Langer, R (Massachusetts Institute of Technology): Mack Memorial Award, Ohio State University

Langer, R (Massachusetts Institute of Technology): Honorary Degree, University of Western Ontario

Langer, R (Massachusetts Institute of Technology): ETH Zurich Chemical Engineering Medal, ETH Zurich

Sachs, D (Massachusetts General Hospital): Medawar Prize, The Transplantation Society

Siemionow, M (Cleveland Clinic Foundation): Casimir Funk Natural Sciences Award, Polish Institute of Arts and Sciences of America

Siemionow, M (Cleveland Clinic Foundation): Editors' Pick – "Effectiveness of Topical Immunosuppressants in Prevention and Treatment of Rejection in Face Allotransplantation," Transplantation, 95(10), Transplantation (Journal)

Siemionow, M (Cleveland Clinic Foundation): Great Immigrants, Carnegie Corporation of New York

Siemionow, M (Cleveland Clinic Foundation): Honorary Membership, Association of Polish Surgeons

Siemionow, M (Cleveland Clinic Foundation): Ireneusz Wierzejewski Medal of Recognition for Contributions to Polish Orthopaedics, Polish Association of Orthopaedics and Traumatology

Siemionow, M (Cleveland Clinic Foundation): Postgraduate Poster Award (3rd place) for "Human Di-Chimeric Cells: A New Approach for Tolerance Inducing Protocols in Transplantation," Senior Author, Case Western Reserve University's National Center for Regenerative Medicine 4th Annual Retreat

Siemionow, M (Cleveland Clinic Foundation): 2013 Innovator Award for Epineural Sheath Technology for Fat Volume Maintenance, Cleveland Clinic Innovations

Siemionow, M (Cleveland Clinic Foundation): Honoris Causa Doctorate, Poznan University of Medical Sciences

Sundback, C (Massachusetts General Hospital): Poster of Distinction: MGH Scientific Advisory Committee

Wake Forest–Pittsburgh Consortium

Ibrahim, Z (Johns Hopkins University School of Medicine): Uniformed Services University of Health Sciences Research Day, USUHS

Lee, WPA (Johns Hopkins University School of Medicine): JK Hardesty Award, Plastic Surgery Research Council

Lee, WPA (Johns Hopkins University School of Medicine): Best Research Poster, Department of Surgery Research Day

Marra, K (University of Pittsburgh): Best Research Advisor Award, University of Pittsburgh

Mikos, AG (Rice University): Distinguished Engineering Alumnus Reward, Perdue University

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 2013 through September 2014, corresponding to the overlapping period of Program Year 6 of the first AFIRM consortia and the first program year of the WRC. 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.

Arya D, Chang S, DiMuzio P, Carpenter J, Tulenko TN. Sphingosine-1-phosphate promotes the differentiation of adipose-derived stem cells into endothelial nitric oxide synthase (eNOS) expressing endothelial-like cells. *J Biomed Sci*. 2014 Jun 4; 21:55. doi: 10.1186/1423-0127-21-55. PMID: 24898615.
Bergfeld W, Klimczak A, Stratton JS, Siemionow MZ. A 4-year pathology review of the near total face transplant. *Am J Transplant*. Epub 2013 Aug 6. DOI: 10.1111/ajt.12379.
Bichara DA, Pomerantseva I, Zhao X, Zhou L, Kulig KM, Tseng A, Kimura AM, Johnson MA, Vacanti JP, Randolph MA, Sundback CA. Successful creation of tissue-engineered autologous auricular cartilage in an immunocompetent large animal model. *Tissue Eng Part A*. 2014 Jan; 20(1-2):303-12. doi: 10.1089/ten.TEA.2013.0150. Epub 2013 Oct 4. PMID: 23980800.
Bushman J, Mishra B, Ezra M, Ghul S, Schulze C, Chaudhury S, Ripoll D, Wallqvist A, Kohn J, Schachner M Loers G. Tegaserod mimics the neurostimulatory glycan polysialic acid and promotes nervous system repair. *Neuropharm*. 2014 Apr; 79:456-66. doi: 10.1016/j.neuropharm.2013.09.014. Epub 2013 Sep 22. PMID: 24067923.
Bushman J, Vaughan A, Sheihet L, Zhang Z, Costache M, Kohn J. Functionalized nanospheres for targeted delivery of paclitaxel. *J Control Release*. 2013 Nov 10; 171(3):315-21. doi: 10.1016/j.jconrel.2013.06.017. Epub 2013 Jun 20. PMID: 23792807.
Cervantes T, Bassett E, Tseng A, Kimura A, Roscioli N, Randolph MA, Vacanti JP, Hadlock TA, Gupta R, Pomerantseva I, Sundback CA. Design of composite scaffold and 3D shape analysis of tissue engineered ear. *J R Soc Interface*. 2013 Oct 6; 10(87):20130413. doi: 10.1098/rsif.2013.0413. Epub 2013 Jul 31. PMID: 23904585.
Cirillo V, Clements BA, Guarino V, Bushman J, Kohn J, Ambrosio L. A comparison of the performance of mono- and bi-component electrospun conduits in a rat sciatic model. *Biomaterials*. 2014; Oct; 35(32): 8970-82. doi: 10.1016/j.biomaterials.2014.07.010. Epub 2014 Jul 29. PMID: 25085857.
Coffman KL, Siemionow MZ. Ethics of facial transplantation revisited. *Curr Opin Organ Transplant*. Apr; 19(2):181-7 Review (2014).
Ezra M, Bushman J, Shreiber D, Schachner M, Kohn J. Enhanced femoral nerve regeneration after tubulization with a tyrosine-derived polycarbonate terpolymer: Effects of protein adsorption and independence of conduit porosity. *Tissue Eng Part A*. 2014 Feb; 20(3-4):518-28. doi: 10.1089/ten.TEA.2013.0092. Epub 2013 Nov 12. PMID: 24011026.
Felgueiras HP, Sommerfeld SD, Murthy NS, Kohn J, Migonney V. Poly(NaSS) functionalization modulates the conformation of fibronectin and collagen Type I to enhance osteoblastic cell attachment onto Ti6Al4V. *Langmuir*. 2014 Aug 12; 30(31):9477-83. doi: 10.1021/la501862f. Epub 2014 Aug 1. PMID: 25054428.
Gibran NS, Wiechman S, Meyer W, Edelman L, Fauerbach J, Gibbons L, Holavanahalli R, Hunt C, Keller K, Kirk E, Laird J, Lewis G, Moses S, Sproul J, Wilkinson G, Wolf S, Young A, Yovino S, Mosier MJ, Cancio LC, Amani H, Blayney C, Cullinane J, Haith L, Jeng JC, Kardos P, Kramer G, Lawless MB, Serio-Melvin ML, Miller S, Moran K, Novakovic R, Potenza B, Rinewalt A, Schultz J, Smith H, Dylewski M, Wibbenmeyer L, Bessey PQ, Carter J, Gamelli R, Goodwin C, Graves T, Hollowed K, Holmes J 4th, Noordenbas J, Nordlund M, Savetamal A, Simpson P, Traber D, Traber L, Nedelec B, Donelan M, Baryza MJ, Bhavsar D, Blome-Eberwein S, Carrougher GJ, Hickerson W, Joe V, Jordan M, Kowalske K, Murray D, Murray VK, Parry I, Peck M, Reilly D, Schneider JC, Ware L, Singer AJ, Boyce ST, Ahrenholz DH, Chang P, Clark RA, Fey R, Fidler P, Garner W, Greenhalgh D, Honari S, Jones L, Kagan R, Kirby J, Leggett J, Meyer N, Reigart C, Richey K, Rosenberg L, Weber J, Wiggins B. Summary of the 2012 ABA Burn Quality Consensus conference. *J Burn Care Res*. 2013 Jul-Aug; 34(4):361-85. doi: 10.1097/BCR.0b013e31828cb249. PMID: 23835626.
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Appendix B

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

Lewitus DY, Rios F, Rojas R, Kohn J. Molecular design and evaluation of biodegradable polymers using a statistical approach. *J Mater Sci Mater Med*. 2013 Nov; 24(11):2529-35. doi: 10.1007/s10856-013-5008-0. Epub 2013 Jul 26. PMID: 23888354.
Lewitus DY, Smith KL, Landers J, Neimark AV, Kohn J. Bioactive agarose carbon-nanotube composites are capable of manipulating brain-implant interface. *J Appl Polym Sci*. 2014 Jul 15; 131(14). doi: 10.1002/app.40297. PMID: 25382868.
Lukaszuk M, Kwiecien G, Madajka M, Uygur HS, Drews D, Siemionow M. Repair of the peripheral nerve gap with epineural sheath conduit to prevent muscle denervation atrophy in the diabetic rat model. *Polish J Surg*. 85(7): 387-294, 2013 Jul 1; doi: 10.2478/pjs-2013-0059.
Ozturk C, Uygur S, Ozturk CN, Lukaszuk M, Siemionow M. Feasibility of using external jugular vein and its branches as y- and x-shaped vein grafts for bridging of arterial defects and providing additional arterial sources for free flap applications in rat model. *J Reconstr Microsurg*. 2014 Jul; 30(6):371-4. doi: 10.1055/s-0033- 1361930. Epub 2014 Feb 17.
Sommerfeld SD, Zhang Z, Costache MC, Vega SL, Kohn J. Enzymatic surface erosion of high tensile strength polycarbonates based on natural phenols. *Biomacromol*. 2014 Mar 10; 15(3):830-6. doi: 10.1021/bm4016539. Epub 2014 Feb 3. PMID: 24432806.
Zhu J, Lin F, Brown DA, Clark RA. A fibronectin peptide redirects PDGF-BB/PDGFR complexes to macropinocytosis-like internalization and augments PDGF-BB survival signals. *J Invest Dermatol*. Epub 2013 Nov 7. doi: 10.1038/jid.2013.463.

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

Hwang CM, Lee BK, Green D, Jeong SY, Khang G, Jackson JD, Atala A, Lee SJ, Yoo JJ. Auricular reconstruction using tissue engineered alloplastic implants for improved clinical outcomes. *Plast Reconstr Surg*. 2014 Mar; 133(3):360e-369e. doi: 10.1097/01.prs.0000438460.68098.4b. PMID: 24572881.
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Table B-1c. Peer-Reviewed Publications: Warrior Restoration Consortium.

Allen AB, Gazit Z, Su S, Stevens HY, Guldberg RE. In vivo bioluminescent tracking of mesenchymal stem cells within large hydrogel constructs. *Tissue Eng Part C Methods*. 2014 Oct; 20(10):806-16. doi: 10.1089/ten.TEC.2013.0587. [Epub 2014 Apr 3.] PMID: 24576050.

Boyce ST, Zimmerman RL, Supp DM. Tumorigenicity testing in athymic mice of cultured human melanocytes for transplantation in engineered skin substitutes. *Cell Transplant*. Epub 2014 Jul 23. PMID: 25199067.

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Woodall JD, Schultz BD, Sosin M, Barth RN. Large animal models for vascularized composite allotransplantation. *Curr Transpl Rep*. 2014 Jun 19; (1), 190-6. Epub 2014 Mar 7. DOI 10.1007/s40472-014-0026-5.

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Appendix B

Tables B-2a, **B-2b**, and **B-2c** display 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 2013 through September 2014. 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 2013 through September 2014 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.

Bobkiewicz A, Kwiecien G, Uygur HS, Madajka M, Siemionow M. Human Epineural Sheath Conduit as an Allograft Alternative for Peripheral Nerve Repair: Anatomy and Feasibility Study in Sheep Model (e Poster), ASPN Annual Meeting, Kauai, Hawaii, January 10-12, 2014.
Bobkiewicz A, Uygur H, Kwiecien G, Madajka M, Siemionow M. Epidural Sheath Jacket as a New Surgical Technique for Neuroma Prevention in the Rat Sciatic Nerve Model: A Preliminary Report (Oral Presentation), PSRC 59th Annual Meeting, New York, New York, March 6-9, 2014.
Bobkiewicz A, Uygur HS, Kwiecien G, Madajka M, Siemionow M. Epineural Sheath Jacket as a New Surgical Technique for Neuroma Prevention in the Rat Sciatic Nerve Model: A Preliminary Report (e Poster), AAHS Annual Meeting, Kauai, Hawaii, January 8-11, 2014.
Cwykiel J, Askar M, Siemionow M. Development of a New Immunomodulatory Supportive Therapy for Transplantation Protocols: Ex Vivo Engineered Human Chimeric Cells. A Preliminary Study (Oral Presentation). Scientific Free Paper Session: Basic, Clinical, Experimental, 11th Meeting of the International Hand and Composite Transplantation Society, Wroclaw, Poland, August 30, 2013.
Cwykiel J, Kwiecien G, Askar M, Siemionow M. Migratory Pathways of Human Ex-vivo Created Chimeric Cells in the Athymic Nude Rat Model: A Preliminary Report (Oral Presentation). ASRM Annual Meeting, Kauai, Hawaii, U.S.A, January 11-14, 2014.
Cwykiel J, Kwiencien G, Askar M, Siemionow M. The Potential of Ex-Vivo Created Human Chimeric Cells for Migration and Engraftment in the Nude Rate Model (Poster). World Transplantation Congress, San Francisco, California, June 26-31, 2014.
Cwykiel J, Szopinski J, Ozturk C, Siemionow M. Low Temperature Volume Replacement Fluids Increases Inflammation in the Rat Cremaster Muscle Microcirculation Model: A Preliminary Study, Cleveland Clinic Foundation's 32nd Annual Research Day, October 10, 2013.
Kwiecien G, Cwykiel J, Madajka M, Bobkiewicz A, Uygur S, Siemionow M. Donor-Recipient Chimeric Cell Tranplantation as a Novel Rescue Therapy for Acute Radiation Syndrome: A Preliminary Report (Oral Presentation). PSRC 59th Annual Meeting, New York, New York, March 6-9, 2014.
Kwiecien G, Cwykiel J, Madajka M, Bobkiewicz A, Uygur S, Siemionow M. Donor-Recipient Chimeric Cell Transplantation as a Novel Rescue Therapy for Acute Radiation Syndrome: A Preliminary Report (Oral Presentation). American Association of Plastic Surgeons 93rd Annual Meeting, Miami, Florida, April 5-8, 2014.
Kwiecien G, Lukaszuk M, Uygur HS, Madajka M, Siemionow M. Epineural Sheath Conduit Supported with Bone Marrow Stromal Cells as an Alternative to Nerve Autograft Repair in Diabetic Conditions (Poster). 7th Biennial Meeting: World Society for Reconstructive Microsurgery, Chicago, Illinois, July 12-14, 2013.
Kwiecien G, Uygur HS, Bobkiewicz A, Madajka M, Caplan A, Siemionow M. Nerve Gap Repair with Human Epineural Sheath Conduit Supported with Human Mesenchymal Stem Cells (Oral Presentation). ASPN Annual Meeting, Kauai, Hawaii, January 10-12, 2014.
Kwiecien GJ, Cwykiel J, Madajka M, Bobkiewicz A, Uygur S, Siemionow MZ. Abstract 50: Donor-Recipient Chimeric Cell Transplantation as a Novel Rescue Therapy for Acute Radiation Syndrome: A Preliminary Report. *Plast Reconstr Surg.* April; 133(4 Suppl):1013-4 (2014).
Madajka M, Ozturk C, Szopinski J, Uygur HS, Kwiecien G, Bobkiewicz A, Siemionow V, Siemionow M. Long Nerve Defects Repair with Epineural Sheath Conduit – Large Animal Model (Poster). ASRM Annual Meeting, Kauai, Hawaii, January 11-14, 2014.
Madajka M, Ozturk C, Uygur HS, Lukaszuk M, Kwiecien G, Borkiewicz A, Szopinski J, Siemionow V, Siemionow M. Nerve Regeneration Through an Epineural Sheath Conduit – Restoration of Long Nerve Defects (Oral Presentation). Scientific Free Paper Session: Basic, Clinical, Experimental, 11th Meeting of the International Hand and Composite Transplantation Society, Wroclaw, Poland, August 30, 2013.
Madajka M, Ozturk C, Uygur HS, Lukaszuk M, Siemionow M. Characteristics of the Human Epineural Sheath Properties for Enhancement of Nerve Regeneration – A Preliminary Report (Oral Presentation), ASPN Annual Meeting, Kauai, Hawaii, January 10-12, 2014.
Ozturk C, Szopinski J, Madajka M, Mendiola A, Siemionow M. Using Naïve Epineural Sheath as a Nerve Conduit and Protective Patch in Peripheral Nerve Reconstruction (Oral Presentation), Free Paper Session: Nerve and Brachial Plexus. 7th Biennial Meeting: World Society for Reconstructive Microsurgery, Chicago, Illinois, July 13, 2013.
Siemionow M, Kwiencien G, Madajka M, Uygur S, Bobkiewicz A, Caplan A. Functional Results of Peripheral Nerve Repair with Human Epineural Sheath Conduit Supported with Human Mesenchymal Stem Cells (Poster Presentation). World Transplantation Congress, San Francisco, California, June 26-31, 2014.

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

Siemionow M. Cellular Therapies in Face Transplantations (Oral Presentation). 6th Congress of Modern Oncology, Poznan, Poland, March 27-29, 2014.
Uygur HS, Kwiecien G, Bobkiewicz A, Madajka M, Siemionow M. Epineural Sheath Tube Prevents Fat Graft Absorption – A Preliminary Report (e-Poster). ASPN Annual Meeting, Kauai, Hawaii, January 10-12, 2014.
Uygur HS, Ozturk C, Bozkurt M, Kwiecien G, Madajka M, Siemionow M. A New Vascularized Cervical Lymph Node Transplantation Model: An Anatomic Study in Rat (Oral Presentation). ASRM Annual Meeting, Kauai, Hawaii, January 11-14, 2014.
Uygur HS, Ozturk C, Kwiecien G, Djohan R, Siemionow M. Sheep Hemi-facial Allotransplant Model: An Anatomic Study in Cadavers (Poster). 7th Biennial Meeting: World Society for Reconstructive Microsurgery, Chicago, Illinois, July 12-14, 2013.
Uygur HS, Siemionow M. Prevention of Fat Graft Absorption by Epineural Sheath Tube – A Preliminary Report (Oral Presentation). 11th Annual Meeting of the International Federation for Adipose Therapeutics and Science, New York, New York, November 22-24, 2013.
Uygur S, Kwiecien G, Bobkiewicz A, Madajka M, Siemionow M. Application of Epineural Sheath for Fat Graft Volume Maintenance (Oral Presentation). American Association of Plastic Surgeons 93rd Annual Meeting, Miami, Florida, April 5-8, 2014.
Uygur S, Ozturk C, Kwiecien G, Djohan R, Siemionow M. Sheep Hemifacial and Auricular Transplantation Models: An Anatomic Study. Ann Plast Surg, April; 72(4):469-74 (2014).
Uygur, HS, Ozturk C, Kwiecien G, Djohan R, Siemionow M. A New Large Animal Hemifacial Transplantation Model: An Anatomic Cadaver Study in Sheep (Poster). ASRM Annual Meeting, Kauai, Hawaii, January 11-14, 2014.

Table B-2b. Presentations and Non-Peer-Reviewed Publications: Wake Forest–Pittsburgh Consortium.

Ibrahim Z, Leto Barone AA, Wu L, Sarhane KA, Furtmueller G, Shores JT, Cooney DS, Sacks JM, Lee WPA, Brandacher G. Immune Tolerance Across a Full MHC Barrier in a Swine Hind Limb Transplantation Model Using a Combined Costimulatory Blockade and Donor Bone Marrow Cell Infusion. *J Am Coll Surg*. 2013 (Oct); Vol.217, No. 3S:92-93.
Leto Barone AA, Ibrahim Z, Khalifian S, Sarhane KA, Furtmüller G, Fryer M, Goldberg L, Wimmers E, Wu L, Alrakan M, Shores J, Bonawitz SC, Sacks JM, Gordan CR, Raimondi G, Cooney DS, Lee WPA, Brandacher G. The Role of Donor Antigen Persistance in Maintaining Immune Tolerance to a Vascularized Composite Allograft. *Plast Reconstr Surg*. 2014(Mar); Suppl 3, Vol. 133, 21.
Leto Barone AA, Ibrahim Z, Sarhane KA, Furtmueller G, Wang Y, Wang YL, Mill DT, Fryer M, Khalifian S, Alrakan M, Shores J, Bonawitz SC, Gordon C, Sacks JM, Raimondi G, Cooney DS, Sun Z, Lee WPA, Brandacher G. Either Abatecpt or Belatacept in Combination With Donor Bone Marrow Infusion Induces Immune Tolerance Across a Full MHC Barrier in a Translational Large Animal Vascularized Composite Allotransplantation Model. *Transplantation*. 2014(Jul); Suppl 1, Vol. 98.
Prim P, Kang HW, Lee YS, Wysk R, Jackson JD, Atala A, et al. Bioreactor Assessment for Soft Tissue Expansion And Growth. 15th Annual Conference of the North Carolina Tissue Engineering and Regenerative Medicine Society, October 28, 2013, Old Salem Convention Center, Winston-Salem, North Carolina.
Sivak WN, Bliley JM, Marra KG. Polymeric Biomaterials for Peripheral Nerve Regeneration: Fabrication and Use of a Polycaprolactone Guide. In Walker JM (Ed.), Methods in Molecular Biology: Axon Growth and Regeneration: Methods and Protocols. New York: Springer (2014, 1162:139-48).

Table B-2c. Presentations and Non-Peer-Reviewed Publications: Warrior Restoration Consortium.

Allen-Hoffmann L. (Plenary Presentation). The Symposium on Advanced Wound Care, April 2014.
Anderson RR. Emerging Procedural Therapies. 5th Annual Project C.A.R.E. Multidisciplinary Training Summit. Naval Medical Center, San Diego, California, December 8, 2013.
Anderson RR. Surgery on the Microscale — Lasers, Micrografting, and Skin Copying for Wound Care. San Antonio Wound Healing Seminar, Southwest Research Institute, San Antonio, Texas, May 22, 2014.
Azari K. An Update on the UCLA VCA Program. (Oral Presentation) Eleventh Meeting of the International Hand and Composite Tissue Allotransplantation Society, Wroclaw, Poland, August 29-31, 2013.
Barth R, Klassen D, Bojovic B, Woodall J, Schultz B, Brazio P, Drachenberg C, Shaffer C, Kelly N, Rodriguez E, Bartlett S. Immunologic and Clinical Outcomes 20 Months After Full Face Transplant. (Poster Presentation) World Transplant Congress, San Francisco, California, July 2014. *Am J Transplant 2014*; 14(S3): 412.
Barth RN. Basic, Translational and Clinical Research in VCA: What are the Goals? Sunrise Symposium, Mechanisms of Allograft Injury in VCA. World Transplant Congress, San Francisco, California, July 2014.
Boyce ST. Next Generation Grafts and Matrices. Wound Healing Society and Symposium on Advanced Wound Care, Orlando, Florida, April 2014.
Boyce ST. Tissue Engineering and Regenerative Medicine to Reduce Scar. 7th World Congress on Pediatric Burns, Boston, Massachusetts, August 2014.
Chang JB, Soares MA, Massie JP, Duckworth A, Rao N, Kim C, Mehta K, Hua A, Rabbani P, Saadeh PB, Ceradini DJ. Primed Mesenchymal Stem Cells Prevent Endothelial Activation and Improve Allograft Perfusion Following Transplantation. Plastic Surgery Research Council 59th Annual Meeting, March 2014.
Chang JB, Soares MA, Massie JP, Kim C, Duckworth A, Rabbani P, Saadeh PB, Ceradini DJ. Ex Vivo Mesenchymal Stem Cell Therapy Promotes Allograft Tolerization via Indoleamine-2,3-Dioxygenase and Protects Vascular Networks from Ischemia-Reperfusion Injury. 3rd Annual Helen L. and Martin S. Kimmel Center for Stem Cell Biology Retreat, New York, New York, February 2014.
Chang JB, Soares MA, Massie JP, Kim C, Duckworth A, Rabbani P, Saadeh PB, Ceradini DJ. Ex Vivo Mesenchymal Stem Cell Therapy Promotes Allograft Tolerization Via Indoleamine-2,3-Dioxygenase and Protects Vascular Networks from Ischemiareperfusion Injury. Center for Skeletal and Craniofacial Biology, New York, New York, May 2014.
Cheng A, et al. Early Macrophage Phenotype in Healing and Non-healing Segmental Bone Defects. The 60th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Cheng XG. Collagen-based Biomask as a Promising Skin Graft for Facial Burn. (Oral Presentation) Trauma Care Breakout Session at Military Health System Research Symposium (MHSRS), Fort Lauderdale, Florida, August 2014.
Christ G. Development of a TEMR Technology Platform for Treatment of Volumetric Muscle Loss Injuries. (Invited Talk) WFIRM Regenerative Medicine Essentials Course, Winston-Salem, North Carolina, July 23, 2014.
Christ G. Muscle Progenitor Cells for Tissue Engineered Muscle Repair (TEMR) of Volumetric Muscle Loss (VML) Injuries: Cleft Lip as "First-in-Man" Target Indication. (Invited Talk) Fetal Therapy and Maternal-Fetal Tolerance: Overcoming Barriers to Successful Fetal Stem Cell Transplantation, San Francisco, California, April 17-18, 2014.
Christ G. Muscle Tissue Engineering and Muscle Regeneration for the Treatment of Traumatic Injuries. (Invited Talk) Regenerative Rehabilitation Symposium, San Francisco, California, April 10-11, 2014.
Christ G. Tissue Engineering for Muscle Repair. (Invited Talk) Advances in Tissue Engineering Short Course, Rice University, Houston, Texas, August 15, 2014.
Christ G. Translational Studies of Muscle Repair and Regeneration. (Invited Talk) University of Virginia Department of Biomedical Engineering, Charlottesville, Virginia, January 24, 2014.
Dosier C et al. Delivery Time of Stem Cell Loaded Hydrogels Affects New Bone Formation in a Critically Sized Femoral Segmental Defect. The 60th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Dunn M. A Tissue Engineered Load Sharing Scaffold for Meniscal Regeneration. 2014 Annual Meeting of the American Academy of Orthopaedic Surgeons, New Orleans, Louisiana, March 11-15, 2014.
Dunn M. An In Vitro Characterization of Meniscal Root Attachment Mechanics in a Large Animal Model. Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Dunn M. Improved Fixation of Tissue-Engineered Meniscus Implant Using Interference Screws. Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Dunn M. Stiffer Polymer Fiber May Reduce Extrusion and Decrease Peak Contact Stresses Associated with a Fiber-Reinforced Collagen Sponge for Meniscus Replacement. Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Dunn MG. An Academic Perspective of Translational Research. Gordon Research Conference on Musculoskeletal Biology and Bioengineering, August 3, 2014.
Elisseeff J. Presentation at American Association of Anatomists, San Diego, California, April 27, 2014
Elisseeff J. Presentation at Materials Research Society Meeting, San Francisco, California, April 24, 2014
Elisseeff J. Translating Biomaterials in Regenerative Medicine. 7th Annual Translational Regenerative Medicine Wound Care Conference, Ohio State University, March 13, 2014.
Garza L. MHSRS, Fort Lauderdale, Florida, August 2014.
Gregory K. Treatment of Extremity Compartment Syndrome Using Autologous Bone Marrow Mononuclear Cells in Swine. (Poster) MHSRS, Fort Lauderdale, Florida, August 2014.

Table B-2c. Presentations and Non-Peer-Reviewed Publications: Warrior Restoration Consortium (cont.).

Katz A. Fat Transfer for Scar Prevention and Remodeling: A Phase I/II Randomized Blinded Clinical Trial. (Presentation) 2014 IFATS Asia International Conference, Shanghai, China, July 19-20, 2014.
Krishnan L, et al. Alginate-based rhBMP2 Hybrid Delivery System Is an Effective alternate to Vital Bone Autografts in the Healing of Critically Sized Bone Defects. The 60th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Kupiec-Weglinski. Ischemia-Reperfusion Injury in VCA vs. SOT. (Plenary Oral Presentation) Eleventh Meeting of the International Hand and Composite Tissue Allotransplantation Society, Wroclaw, Poland, August 29-31, 2013.
Liao H-T, Lauren K, Marra K, Rubin P. Platelet-rich Plasma Promotes Fat Graft Survival via Stemness and Angiogenesis on Adipose-derived Stem Cells. Plastic Surgery Research Council, New York, New York, March 7-9, 2014.
Marra K. MHSRS, Fort Lauderdale, Florida, August 2014.
Mikos AG. Biomaterials for Tissue Engineering. 13th Congress of the Japanese Society for Regenerative Medicine, Kyoto, Japan, March 4-6, 2014.
Mikos AG. Biomaterials for Tissue Engineering. University of British Columbia Department of Chemical and Biological Engineering, Vancouver, Canada, 2014.
Mikos AG. Biomaterials for Tissue Engineering. University of Houston Department of Chemical and Biomolecular Engineering, Houston, Texas, 2014.
Mikos AG. Biomaterials for Tissue Engineering. University of Texas Medical School at Houston Department of Pediatrics, Houston, Texas, 2014.
Mikos AG. Synthetic Scaffolds for Tissue Engineering. Advances in Tissue Engineering, Houston, Texas, August 2014.
MT A Li et al. Amniotic Membrane as a Treatment Strategy in a Novel Volumetric Muscle Loss Model in the Rat Biceps Femoris. The 60th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Priddy LB et al. An Alginate-Based Delivery System Localizes Bone Regeneration in High-Dose BMP-2 Delivery. The 60th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, March 15-18, 2014.
Rubin P. MHSRS, Fort Lauderdale, Florida, August 2014.
Rubin P. Plastic Surgery Research Council, New York, New York, March 7-9, 2014.
Schultz B, Woodall J, Brazio P, Klassen D, Shaffer C, Rodriguez E, Bojovic B, Bartlett S, Barth R. Transient Detection of Microchimerism Using Quantitative RT-PCR for Insertion/Deletion Polymorphysms After Full Face Transplant. (Poster Presentation) World Transplant Congress, San Francisco, California, July 2014. *Am J Transplant 2014*; 14(S3): 411.
Shupe T. American Society of Cell and Gene Therapy Annual Meeting, May 21, 2014.
Shupe T. MHSRS, Fort Lauderdale, Florida, August 2014.
Shupe T. Society for Biomaterials Annual Meeting, April 2014.
Spicer PP, Young S, Kasper FK, Athanasiou KA, Mikos AG, Wong MEK. Tissue Engineering in Oral and Maxillofacial Surgery. In Lanza RP, Langer R, and Vacanti JP (eds), Principles of Tissue Engineering. Fourth Edition, 2014 (November 29, 2013), 1487-1506.
Talley AD, Kalpakci KA, Zienkiewicz KJ, Wenke JC, Guelcher SA. Space Maintenance and New Bone Formation with Polyurethane Biocomposites in a Canine Saddle Defect Model. (Oral Presentation) Society for Biomaterials Annual Meeting, Denver, Colorado, April 16-19, 2014.
Talley AD, Kalpakci KA, Zienkiewicz KJ, Wenke JC, Guelcher SA. Space Maintenance and New Bone Formation with Settable Bone Grafts in a Canine Mandibular Ridge Saddle Defect Model. (Oral Presentation) Society for Injectable Osteoarticular Biomaterials Annual Meeting, Nantes, France, May 5-8, 2014.
Tam J, Wang Y, Fisher J, Farinelli W, Franco W, Purschke M, Sakamoto F, Doukas A, Anderson RR. In Vivo Reconstitution of Full-Thickness Skin Features from Microscopic Skin Tissue Columns. Tissue Engineering and Regenerative Medicine International Society – Americas, November 10-13, 2013.
Tam J. Tissue Copying for Wound Repair. 7th World Congress on Pediatric Burns, Shriners Burns Hospital for Children, Boston, Massachusetts, September 1, 2014.
Tatara AM, Shah SR, Puperi DS, Watson BM, Grande-Allen KJ, Wong ME, Mikos AG, Kasper FK. Mitigation of Biofilm Formation with an Elastomeric Barrier Membrane System. 2014 Annual Meeting & Exposition – Society for Biomaterials, Denver, Colorado.
Wang H. Translational Peripheral Nerve Research: What Tools Do We Have? (Invited speaker) 12th International Microsurgical Symposium, Botucatu, Brazil, September 12-13, 2014.
Windebank AJ. Regeneration in the Nervous System. (Invited Keynote speaker) Neurorehabilitation Summit, Rochester, Minnesota, May 19, 2014.
Windebank AJ. Regenerative Medicine Approaches to Repair and Protection of the Nervous System (Mary Murphy Endowed Lecture in Biology). Clark University, Dubuque, Iowa, March 18, 2014.
Windebank AJ. Regenerative Medicine Approaches to Repair and Protection of the Nervous System (Neurology Visiting Professor). University of Puerto Rico - Medical Sciences Campus, January 17, 2014.
Wong ME. Tissue Engineering in Oral and Maxillofacial Surgery. Advances in Tissue Engineering, Houston, Texas, 2014.
Wong ME. Tissue Engineering Principles in the Management of Atrophic Maxillary Defects. Academy of Osseointegration 2014 Summit: Current Best Evidence for the Management of the Edentulous Maxilla, Oak Brook Hills, Illinois, 2014.

Appendix C: Patents, 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 2013 – September 2014 for PY6).
- 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 Patent Full-text and Image Database (http://patft.uspto.gov/), or the USPTO Patent Application Full Text and Image Database (http://appft.uspto.gov/). The database queries were used to (1) identify patents and patent applications filed for self-reported inventions, and (2) identify and validate filing dates for patents and patent applications.

Patents:

Research advances achieved during the AFIRM program resulted in 8 patents granted by the USPTO to AFIRM investigators during this program year:

- Biohybrid Elastomeric Scaffolds and Methods of Use Thereof (#8,535,719). Freytes DO, Gilbert TW, Guan J, Stankus J, Wagner WR (University of Pittsburgh).
- Bioresorbable Tissue Engineered Fibrocartilage Replacement with Three-Dimensional Matrix of Fibers (#8,623,085). Balint EA, Dunn MG (University of Medicine and Dentistry of New Jersey).
- Bone Substitute Compositions, Methods of Preparations and Clinical Applications (#8,557,235). Kumta PN, Sfeir CS, Roy A (University of Pittsburgh).
- Inflammation-Regulating Compositions and Methods (#8,529,897). Washburn NR, Bencherif SA, Sun LT (Carnegie Mellon University).
- Isocyanate Manufacture (#8,552,217). Bhattacharyya S, Guelcher SA, Gopal D, Burello M (Vanderbilt University).
- Matricryptic ECM Peptides for Tissue Reconstruction (#8,716,438). Agrawal V, Tottey S, Johnson SA (University of Pittsburgh).
- Methods and Compositions Related to Targeting Wounds, Regenerating Tissue and Tumors (#8,470,780). Ruoslahti E, Järvinen T (University of California, Santa Barbara).
- Surgical Device for Skin Therapy or Testing (#8,765,468). Boyce ST (University of Cincinnati).

Appendix C

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.

- Bone Substitute Compositions, Methods of Preparations and Clinical Applications (20140004161). Kumta PN, Sfeir CS, Roy A (University of Pittsburgh).
- Bone Substitute Nanocomposites and Methods of Synthesis Using Multiphosphorylated Peptides (20150045304). Beniash E, Sfeir CS (University of Pittsburgh).
- Cryopreservation of Viable Human Skin Substitutes (20140271583). Allen-Hoffmann BL, Pirnstill JC, Gratz KR, Comer AR (Stratech Corporation).
- In-vivo Bioreactor System and Method for Tissue Engineering (20140024112). Galiano RD, Mustoe TA, Chavez-Munoz CI (Northwestern University).
- Polypeptides and Methods of Use. (20150087597). (Stony Brook University).
- Polyurethane Composite for Wound Healing and Methods Thereof (20130295081 A1). Hafeman A, Davidson J, Nanney LM, Adolph E (Vanderbilt University).
- Reducing Cutaneous Scar Formation and Treating Skin Conditions (20150065431). Xu W, Hong SJ, Galiano RD, Mustoe TA (Northwestern University).
- Surgical Device for Skin Therapy or Testing (20140287020 A1). Boyce ST (University of Cincinnati).

Invention Disclosures:

Invention disclosures are not publicly reposed in standard databases; therefore, the AFIRM consortium reports are the only information source for inventions disclosed. The provided information did not indicate a date when the inventions were filed with the institutional technology licensing office, and most records did not indicate a case reference number assigned to the invention. Due to these limitations, all invention disclosures without a date or reference number were assumed, but not validated, to have been filed from June 2013 through September 2014. Also, self-reported patent applications that only listed an invention disclosure number, but not a patent application filing number or serial number, were considered invention disclosures and not patent applications. Seven (7) invention disclosures were made by AFIRM faculty during this period, of which 5 are among the patent applications listed above. Two (2) additional invention disclosures include:

- Composite Guide. Marra K (University of Pittsburgh).
- Cryosyringe Designed for Sterile Liquid Nitrogen Freezing, Quick Thawing and Injection of Cellular Slurries. Garza LA, Kang S, Meyerle JH (Johns Hopkins University).

Appendix D: Description of 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
AAT ..... acellular adipose tissue
AC ..... articular cartilage
AChR ..... acetylcholine receptor
ACS ..... absorbable collagen sponge
ACURO ..... Animal Care and Use Review Office
ADSC ..... adipose derived stem cell
AFIRM ..... Armed Forces Institute of Regenerative Medicine
AFS ..... amniotic fluid stem
AFT ..... autologous fat transfer
AFT-SPAR ..... Autologous Fat Transfer for Scar Prevention and Remodeling
ah-SC ..... autologous human Schwann cell
APC ..... antigen presenting cell
ASC ..... adipose-derived stem cell
b-rhBMP-2 ..... biotinylated recombinant human bone morphogenetic protein-2
βTCP ..... beta-TCP
BMA ..... bone marrow aspirate
BM-MNC ..... bone marrow mononuclear cell
BMSC ..... bone marrow stem cell
BM-MSC ..... bone marrow mesenchymal stem cell
BMP-2 ..... bone morphogenetic protein-2
BOD ..... Board of Directors
CaP ..... calcium phosphate
CBER ... Center for Biologics Evaluation and Research
CCTD ..... chronic caprine tibial defect
CDER ..... Center for Drug Evaluation and Research
CDRH ..... Center for Diagnostics and Radiological Health
CF ..... Craniomaxillofacial Regeneration
CFMD ..... canine femoral multidefect
cGMP ..... current Good Manufacturing Practices
cGTP ..... current Good Tissue Practices
CN ..... cavernosal nerve
CPC ..... calcium phosphate cement
CRMRP ..... Clinical and Rehabilitative Medicine Research Program
CS ..... compartment syndrome
CT ..... computed tomography
CTA ..... Composite Tissue Allotransplantation and Immunomodulation
CTGF ..... connective tissue growth factor
CTP-O ..... osteogenic connective tissue progenitors
DBM ..... demineralized bone matrix
DECM ..... dermal ECM
DoD ..... Department of Defense
DS ..... density separation
DT ..... desaminotyrosyl tyrosine
DTE ..... desaminotyrosyl tyrosine ethyl ester
DWP ..... dermal wound paste
EC ..... endothelial cell
ECM ..... extracellular matrix
EMG ..... electromyogram
eNOS ..... endothelial nitric oxide synthase
EPC ..... endothelial progenitor cell
ER ..... Extremity Regeneration
ESS ..... engineered skin substitutes
EVPOME ..... Ex Vivo Produced Oral Mucosa Equivalent
FAK ..... focal adhesion kinase
FDA ..... U.S. Food and Drug Administration
FI ..... fecal incontinence
FN ..... fibronectin
GDNF ..... glial cell line-derived neurotrophic factor
GMP ..... Good Manufacturing Practices
GU ..... Genitourinary/Lower Abdomen Reconstruction
HCT/P ..... Human Cells, Tissues, and Cellular and Tissue-Based Products
HDMEC ..... human dermal microvascular cells
hDSC ..... human dental stem cell
HHCC ..... human hematopoietic chimeric cell
hM ..... human melanocytes
hMPC ..... human muscle progenitor cell
HRPO ..... Human Research Protection Office
HUCC ..... human umbilical cord blood-derived chimeric cells
IAS ..... innervated anal sphincter
IIPT ..... Integrating Integrated Project Team
IDE ..... Investigational Device Exemption
IND ..... Investigational New Drug

Appendix E

IPB ..... internal pudendal bundle
IRB..... Institutional Review Board
IRI..... ischemia-reperfusion injury
LD ..... latissimus dorsi
LESWT..... low-energy shock wave therapy
LV..... low-viscosity
LV-MG..... LV/MasterGraft®
LWI..... Lonza Walkersville, Inc.
M..... million
M/C..... muco-cutaneous
MCA ..... mineralized cancellous allograft
ME-FASTE ..... Medically Engineered Functional Anal Sphincters Using Composite Tissue Engineering and Novel Electrode
MIT ..... Massachusetts Institute of Technology
MRI ..... magnetic resonance imaging
MS..... magnetic separation
MSC..... mesenchymal stem cell
MTC ..... microscopic skin tissue column
MuNTIS ..... Muscle–Nerve Tissue Integration System
NC..... neural cell
NGT..... nerve guide tube
NHP..... non-human primate
NIH ..... National Institutes of Health
OBEI ..... Oregon Biomedical Engineering Institute
OHSU ..... Oregon Health & Sciences University
OSMEA ..... organic stretchable microelectrode array
PBSM..... porous bone space maintainer
PCL..... poly(caprolactone)
PCLF..... PCL fumarate
PDO..... polydioxanone
PES..... poly(ether sulfone)
PEUU ..... polyester-urethane urea
PLA..... polylactic acid
PLGA..... poly(lactic-co-glycolic acid)
PMMA ..... porous poly(methyl methacrylate)
PMO ..... Project Management Office
POG..... particulate oxygen generator
PPy/PCTC ..... PolyPyrrole/PolyCaprolactone-block-polyTetrahydrofuran-block-polyCaprolactone
PRP ..... platelet-rich plasma
PY6..... Program Year 6
R&D..... Research and Development
RCCC ..... Rutgers–Cleveland Clinic Consortium
r HADM..... reinforced human acellular dermis matrix
rhBMP-2..... recombinant human bone morphogenetic protein-2
SEM ..... scanning electron microscopy
SIS..... small intestinal submucosa
SMC..... smooth muscle cell
SOP..... standard operating procedures
SPO..... sodium percarbonate
SR..... selective retention
SRG..... Skin Regeneration
SSC..... spermatogonial stem cells
SVF ..... stromal vascular fraction
TA ..... tibialis anterior
TCP..... tri-calcium phosphate
TEMR..... Tissue Engineered Muscle Repair
TEBV ..... tissue-engineered blood vessel
TENG ..... tissue engineered nerve graft
TIRM..... Tissue Injury and Regenerative Medicine
TyrPC ..... tyrosine-derived polycarbonate
UBM ..... urinary bladder matrix
USAISR ..... U.S. Army Institute of Surgical Research
USAMRMC ..... U.S. Army Medical Research and Materiel Command
UMDNJ..... University of Medicine and Dentistry of New Jersey
USPTO ..... U.S. Patent and Trademark Office
UTHSC ..... University of Texas Health Science Center at Houston
VA ..... Department of Veterans Affairs
VCA..... vascularized composite allograft
VEGF..... vascular endothelial growth factor
VML ..... volumetric muscle loss
WFIRM..... Wake Forest Institute for Regenerative Medicine
WFPC..... Wake Forest–Pittsburgh Consortium
WIPO ..... World Intellectual Property Organization
WRC..... Warrior Restoration Consortium

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