Regenerative Medicine
Challenges to be Addressed by the Rutgers-led AFIRM Team

1: Vascularity in engineered tissue
2: New ways to create better biomaterials
3: Growing tissues with engineered scaffolds
4: Fat transfer to reduce scarring and improve wound healing
5: Preventing the progression of burn injury
6: Facial reconstruction
7: Tissue transplantation
8: Limb salvage
1: Vascularity in engineered tissue

- In our body, no living cell can be more than 200 micron away from a blood vessel. Our body is composed of a dense grid of tiny blood vessels that bring oxygen and nutrients to every living cell. One of the challenges of engineering tissue has been the need to include a microvascular bed within the tissue. This challenge has so far not been met.

- The laboratory of Professor Joseph Vacanti at Massachusetts General Hospital is developing a bioengineering approach to the creation of functional microvascular beds using microfabrication technology.

- The images below were contributed by Professor J. Vacanti. Image (A) is an example of a functional, engineered microvascular bed, designed using computational biology methods. Image (B) shows a confluent layer of aortic endothelial cells growing in these engineered blood vessels. The cells formed a natural lining similar to that found in living blood vessels.
2: New ways to create better biomaterials

- Our dream to regenerate tissues and to heal large wounds depends in part on the availability of unique “biomaterials” that can be used to support the initial attachment and growth of cells - ultimately leading to newly formed tissues such as nerves, blood vessels, skin, bone, or muscle.
- To accelerate the development of these critically needed biomaterials, the laboratory of Professor Joachim Kohn at Rutgers University develops advanced methods for the discovery of new biomaterials.
- Referred to as the “Combinatorial-Computational Approach”, Kohn’s methodology has been used by several companies to bring new medical products to the clinic.
- AFIRM’s research and development programs require a wide range of improved biomaterials, some of which will be developed by Kohn.
- A specific example, illustrating the power of the Combinatorial-Computational Approach is the development of a revolutionary, coronary stent for which Kohn designed the stent material to meet the requirements of this particular application.

The image above has been provided by REVA Medical Inc. (San Diego) showing the current prototype of a fully degradable coronary stent made of a tyrosine-derived polycarbonate developed in the laboratory of Professor Kohn at Rutgers University. This stent is currently in clinical trials in Germany and Brazil.
Use of microprinted slides to study stem cell differentiation

The laboratory of Professor Robert Langer at MIT develops high-throughput assays to evaluate the interactions between cells and biomaterials. Langer’s work complements the Combinatorial-Computational Approach of Kohn. The collaboration between these two leading biomaterials laboratories is a unique strength of AFIRM.

The image on the left was contributed by Professor Langer, showing three representative microspots from a test slide featuring 1728 different biomaterials. Each spot was seeded with the same mesenchymal (adult) stem cells. Lineage-specific staining was then used to show that the originally identical stem cells had differentiated into three different cell types (each having a unique color) depending on the specific spot they were seeded on.

This is a milestone achievement which will be used in AFIRM’s studies aimed at creating clinically useful tissue scaffolds such as conduits for nerve regeneration.

*Alliance for Regenerative Medicine Participating Institutions:* Rutgers, the State University of New Jersey ♦ Cleveland Clinic ♦ Carnegie Mellon University ♦ Case Western Reserve University ♦ Dartmouth-Hitchcock Medical Center ♦ Massachusetts General Hospital/ Harvard Medical School ♦ Massachusetts Institute of Technology ♦ Mayo Clinic ♦ Northwestern University ♦ Stony Brook University ♦ University of Cincinnati ♦ University of Medicine and Dentistry of New Jersey ♦ University of Pennsylvania ♦ University of Virginia ♦ Vanderbilt University
3: Growing tissues with engineered scaffolds

- Significant research has enabled scientists to create very sophisticated tissue scaffolds - polymeric implants that allow cells to grow and form functional tissue in predetermined shapes. Within the Rutgers-led AFIRM team, several laboratories collaborate in the development of new tissue scaffolds for bone, nerve, and muscle regeneration. Tissue scaffolds can also be used in creating new body parts such as ears or noses.

- The illustration below is a composite image, showing on the left a scaffold from the laboratory of Professor Linda Griffith (MIT) based on a 3-D printing technique licensed to Therics Inc. In the middle is a micrograph showing the interior structure of a new scaffold design from the laboratory of Professor Kohn at Rutgers. The image on the right is a magnification of a small section of the middle image, illustrating the delicate scaffold structure comprised of larger “macropores” and much smaller “micropores”. The combination of macro and micropores within the same scaffold has been shown by Kohn and his colleagues to improve the rapid regeneration of bone in a preclinical animal model.
Use of a tissue scaffold to create a new nose or ear

➢ Above, a polymer scaffold before and after seeding with chondrocytes for regeneration of a nose. Note the place for the nostrils at the bottom of the polymer scaffold. On the right is an image showing an engineered human ear replacement in tissue culture. Images contributed by the laboratory of Professor Langer at MIT.

➢ These artificially grown body parts are part of AFIRM’s goal to regenerate the facial features of seriously injured service members such as the person shown on the right (image reproduced with permission from US Army MRMC). First clinical trials using this technology could start in about 2 years.
4: Fat transfer to reduce scarring and to improve wound healing

- Adipose tissue (fat) contains biologically active cells which can excrete growth factors and cytokines leading to improved healing and the remodeling of scar tissue. The promise of autologous fat transfer is that a simple procedure (which can be performed at the same time as other surgery is done) will improve the healing of wounds as well as reduce the amount of scarring caused by the injury.

- Autologous fat transfer is one of the new surgical procedures that will be explored by AFIRM to evaluate its potential benefits for our wounded service members.

- This is illustrated on the next page. These images were contributed by the laboratory of Dr. Adam Katz (University of Virginia)
Fat transfer therapy for wound healing, tissue repair and scar management

Adipose tissue-derived therapies for wound healing, tissue repair and scar management. Adipose (fat) tissue can be harvested using minimally-invasive suction methods (A). Stem cells are then isolated and expanded in a culture dish (B). The fat-derived stem cells can be formed into discrete modular units (C) and/or seeded onto biocompatible scaffolds (C). The formulated therapies can be delivered by a variety of methods (spray, injection or implantation) to open wounds (D) with the intent to assist and enhance wound healing.

*Alliance for Regenerative Medicine Participating Institutions: ♦Rutgers, the State University of New Jersey ♦Cleveland Clinic ♦Carnegie Mellon University ♦Case Western Reserve University ♦Dartmouth Hitchcock Medical Center ♦Massachusetts General Hospital/Harvard Medical School ♦Massachusetts Institute of Technology ♦Mayo Clinic ♦Northwestern University ♦Stony Brook University ♦University of Cincinnati ♦University of Medicine and Dentistry of New Jersey ♦University of Pennsylvania ♦University of Virginia ♦Vanderbilt University
5: Preventing the progression of burn injury

- Once a person has been seriously burnt, the initial burn injury tends to worsen for several days after the injury. This process is called “injury progression”.

- AFIRM will explore innovative ways to limit the progression of burn injuries in the days following the injury. By administering certain commonly used food additives either before war fighters go on dangerous missions, or as soon as possible after the injury has occurred, it may be possible to diminish the damage caused by burns. This would be a very significant benefit to those service members who are injured by explosive devices. But, this therapy could also be of great value to civilian burn victims.

- The following images were contributed by Adam J. Singer, MD, Professor and Vice Chairman for Research, Department of Emergency Medicine, Stony Brook University and Medical Center

The two images above show what started as identical burn injuries. The burn in the left image was left untreated. The originally separated rectangular burn areas increased in size over time, typical of burn injury progression. In the right image, an initially identical burn was treated with a common food additive. The burn areas remained clearly separated and the beginning of new hair growth (starting to cover the dark burn eschar) is a sign of the cutaneous viability of the treated wound areas.
6: Facial reconstruction

- Unfortunately, major battlefield injuries to the face are common. Even if the victim survives, the destruction of a person’s face causes more serious, long-lasting psychological problems than any other injury.

- AFIRM will therefore study several new approaches to help those service members who sustain major injuries to the face.

- For the repair of irregularly shaped bone defects, an injectable bone scaffold would be ideal. Such a polymer scaffold has been developed by a team of scientists from Carnegie Mellon University (CMU) and Vanderbilt University.

- To repair a large bone defect in the face, Professor Kohn’s lab will provide newly designed bone regeneration scaffolds that are able to guide bone growth into the polymer scaffold.

- The promise of this technology has been established in preclinical studies, but the new bone regeneration scaffold has never been used in humans. AFIRM will explore this technology, and if it is found to be of benefit to patients, AFIRM will develop this new tissue scaffold technology for the benefit of both civilian and military patients.

*Alliance for Regenerative Medicine Participating Institutions:  ❄️Rutgers, the State University of New Jersey ❄️Cleveland Clinic ❄️Carnegie Mellon University ❄️Case Western Reserve University ❄️Dartmouth Hitchcock Medical Center ❄️Massachusetts General Hospital/ Harvard Medical School ❄️Massachusetts Institute of Technology ❄️Mayo Clinic ❄️Northwestern University ❄️Stony Brook University ❄️University of Cincinnati ❄️University of Medicine and Dentistry of New Jersey ❄️University of Pennsylvania ❄️University of Virginia ❄️Vanderbilt University*
7. Tissue Transplantation

- In severe facial injuries, reconstruction may no longer be an option. For the most severely injured patients, AFIRM will explore the use of tissue transplants for reconstruction of face and limbs.
- Key to the success of the procedure is a new method, developed by AFIRM researcher Maria Z. Siemionow, MD at the Cleveland Clinic, to induce “immune tolerance” in the transplant recipient. Earlier transplant procedures required massive immunosuppression (with its related side effects). Dr. Siemionow has developed a method to reduce or even eliminate the need for long-term treatment of patients with aggressive immune suppression drugs.
- This revolutionary procedure has been tested in animal studies with results extending out 2-3 years. The individual components of this therapy all have been tested for safety in humans, and AFIRM will explore the safety and efficacy of the complete procedure in humans. If successful, severely injured patients needing transplantation of face and limb tissues may have a chance to enroll in clinical trials in the near future.

Face transplants without immunosuppression were demonstrated using white (“Norway”) and dark (“Sprague-Dawley”) rats. The white rat was the recipient of the transplant. The black rat was the organ (e.g., face) donor. Two years after the procedure, the recipient rat shows no signs of rejecting the face tissue transplanted from the donor. The recipient rat is fully immune-competent, meaning that it is capable of fighting off normal diseases and infections. Most transplant recipients today receive immunosuppression drugs, which compromises the immune system and can lead to other chronic conditions.

Images contributed by Dr. Maria Z. Siemionow, Cleveland Clinic

*Alliance for Regenerative Medicine Participating Institutions:◆ Rutgers, the State University of New Jersey ◆ Cleveland Clinic ◆ Carnegie Mellon University ◆ Case Western Reserve University ◆ Dartmouth Hitchcock Medical Center ◆ Massachusetts General Hospital/Harvard Medical School ◆ Massachusetts Institute of Technology ◆ Mayo Clinic ◆ Northwestern University ◆ Stony Brook University ◆ University of Cincinnati ◆ University of Medicine and Dentistry of New Jersey ◆ University of Pennsylvania ◆ University of Virginia ◆ Vanderbilt University
The injuries to arms and legs following blast injuries and severe civilian trauma often result in the loss of large regions of tissue in the middle portion of the limb, disrupting the healing and use of the hand or foot.

Despite many advances in reconstructive surgery, current methods to reconstruct these tissues are inadequate in many settings. Presently, when preservation, repair or regeneration of these ‘bridging’ tissues cannot be reliably achieved, an amputation of the arm or lower leg above the injured area becomes the best and only option. The AFIRM is dedicated to developing new regenerative medicine therapies for helping save and rebuild injured limbs.

The AFIRM Limb Salvage Program will focus on using new technologies in regenerative medicine and tissue engineering to provide surgeons with advanced tools and new options for repair and regeneration of these critical bridging tissues. The goal is to allow victims of severe military or civilian trauma to recover from their injuries while reducing the need for limb amputation.
Rapid processing of autogenous adult stem cells for bone regeneration

Repairing missing or fractured bone is often the first step in successful limb salvage. The human body produces stem cells capable of building new bone on a continual basis. The AFIRM program in Limb Salvage, led by George F. Muschler, M.D., of the Cleveland Clinic, will concentrate on developing improved methods for harvesting and concentrating a patient’s own stem cells to repair fractured or missing bone and creating new biomaterials to help those cells grow successfully in an injured patient.

Bone Marrow or Fat Cells Collected With a Needle During Surgery

Rapid Concentration and Selection of Stem Cells in the Operating Room

Regenerate of Missing Bone

Restore Missing Stem Cells to the Injury Site

Images provided by George Muschler, MD, and Maciej Zborowski, PhD, Cleveland Clinic

*Alliance for Regenerative Medicine Participating Institutions: Rutgers, the State University of New Jersey • Cleveland Clinic • Carnegie Mellon University • Case Western Reserve University • Dartmouth Hitchcock Medical Center • Massachusetts General Hospital/Harvard Medical School • Massachusetts Institute of Technology • Mayo Clinic • Northwestern University • Stony Brook University • University of Cincinnati • University of Medicine and Dentistry of New Jersey • University of Pennsylvania • University of Virginia • Vanderbilt University*
Bridging of nerve gaps

- A second critical need in limb salvage is the regeneration of functional nerve. In the absence of motor and sensory nerves connecting the hand or leg to the brain, the salvaged limb may provide no benefit to the patient.
- The Rutgers-led AFIRM team has developed a major research effort, involving the laboratories of Drs. Langer (MIT), Yaszemski (Mayo Clinic), Siemionow (Cleveland Clinic) to address the regeneration of nerve as a critical component of the limb salvage program.
- The images below illustrate our approach to the challenge of bridging a major nerve gap.

Images by M, Yaszemski, MD, PhD, Mayo Clinic, D. Anderson, PhD, MIT, and R.Langer, PhD, MIT

*Alliance for Regenerative Medicine Participating Institutions: * Rutgers, the State University of New Jersey ◆ Cleveland Clinic ◆ Carnegie Mellon University ◆ Case Western Reserve University ◆ Dartmouth Hitchcock Medical Center ◆ Massachusetts General Hospital/Harvard Medical School ◆ Massachusetts Institute of Technology ◆ Mayo Clinic ◆ Northwestern University ◆ Stony Brook University ◆ University of Cincinnati ◆ University of Medicine and Dentistry of New Jersey ◆ University of Pennsylvania ◆ University of Virginia ◆ Vanderbilt University