White Paper
“Liquid Bandage and Tissue Regeneration”
April 7, 2010

Problem To Be Studied

This project will be divided into two arms, which will run concurrently. Project arm 1 will develop and test the liquid bandage, and project arm 2 will develop and test the tissue regeneration technology.

Arm 1: Liquid Bandage

This project arm will develop hemostatic devices which contain recombinant human clotting factors (fibrinogen, thrombin, and Factor XIII) which are produced in the milk of transgenic mammals. Some of these hemostatic devices will be in liquid form, hence the term “liquid bandage.” All components of the planned hemostatic devices will be completely absorbable by the body. The recombinant human clotting factors form a firm, durable clot that will stop exsanguinating bleeding in a soldier on the battlefield. The recombinant hemostatic devices of project arm 1 will have several advantages over existing blood clotting products, including less cost, greater efficacy, increased safety, and more potential applications. In the event of blast injuries, penetrating wounds to organs, or in the operating room, the recombinant hemostatic devices would be applied directly to a wound and immediately stop the bleeding. Preliminary research indicates that these recombinant hemostatic devices can be stored at room temperature for at least one year. Concurrent with the work in project arm 1, technology will be developed to regenerate tissue (arm 2), especially skin, in conjunction with the utilization of the recombinant hemostatic devices.

Arm 2 - Tissue Regeneration

Despite decades of research, there is no permanent, practical and economical dermal replacement therapy. Recent technologies involving adult stem cells, nanomaterials, and clotting components have progressed to the point that a major breakthrough in dermal regeneration is now possible. This project will utilize those developments to create a technique of skin replacement using a combination of adult stem cells, clotting components, growth factors, extracellular matrix products, and nanomaterials. These components will be integrated into an absorbable scaffolding material similar to that used in some of the hemostatic devices in project arm 1. Such an integrated product would be applied to an open wound, and would function as an epidermal/dermal (skin) replacement. The collaborative effort required for this project arm would include experts in the fields of stem cell biology, recombinant protein technology, nanomaterial engineering, wound healing, regenerative medicine, and surgery. Such a team already has been assembled.

Significance and/or Uniqueness of the Proposed Effort

There has been a tremendous need for a treatments of life-threatening hemorrhage which can be applied in the battlefield and/or hospital setting, especially by someone with minimal medical training. Immediate access to life-saving medical care (e.g., blood transfusion and surgery) often is not possible when a soldier or civilian patient has a life-threatening injury with severe bleeding. This research addresses this problem with the development of therapies for severe bleeding that can be used on the battlefield or in the hospital with minimal to no training or preparation. The therapies contain recombinant clotting factors which are applied as a sealant (with an aerosol propulsion system) or embedded in a biodegradable nanoengineered bandage. These proteins do not come from human sources, so transmission of viral illnesses (such as hepatitis or HIV) will not be an issue. Hemostatic products based on plasma-derived clotting factors (fibrinogen and thrombin) are commercially-available, but none of these products contain Factor XIII (fibrin stabilizing factor). This latter factor is vital for clot strength and stability.
Preliminary data collected by the investigators of this proposal in a porcine model of arterial injury and solid organ injury have demonstrated the feasibility and efficacy of several iterations of these recombinant hemostatic devices. For example, The hemostatic bandage, which consists of recombinant fibrinogen, thrombin, and Factor XIII embedded in a PLA (polylactide) matrix, was able to stop hemorrhage from a 5 mm femoral arteriotomy in swine after 3 minutes of finger pressure, while the animals maintained a mean arterial pressure of >120 mm Hg. In addition, the aerosol-driven sealant (which mixes solubilized fibrinogen, thrombin, and Factor XIII in an applicator tip as the therapy is administered) was able to control hemorrhage from grade V hepatic lacerations and hepatic lobectomies, also in swine. The abundant availability of recombinant Factor XIII (Cross-linking Factor) is the stand-out characteristic of these recombinant hemostatic devices. As far as is known, the investigators are the only group in the world which has access to this recombinant factor in such great amounts. The addition of Factor XIII to fibrinogen-based clotting devices greatly enhances their efficacy, secondary to the cross-linking effect of Factor XIII on polymerized fibrin (nascent clot).

Conventional treatment of full-thickness skin loss involves the application of autologous split-thickness skin grafts, in which epidermis and dermis are harvested from the patient and transplanted to the site of injury. This grafting technique provides a complete epidermal component for the wound, but it creates both donor-site morbidity and is inadequate as a dermal substitute. Current commercially available dermal scaffolds have offered limited progress in the treatment of deep dermal wounds. Implantation of a biodegradable dermal scaffold that is antimicrobial and supportive of native cellular ingrowth, yet also function as an immediate skin replacement, would be of immense value.

**Potential Military Relevance**

Hemostasis, wound healing, and regenerative medicine have become research priorities of the Department of Defense and the Department of Veterans Affairs. For example, more than 29,000 U.S. troops have been wounded in Iraq. For those that have lost their lives, uncontrolled hemorrhage is the leading cause of preventable combat-related deaths. Most of these deaths occur in the field before the injured can be transported to a treatment facility. The immediate treatment of the injured soldier centers on three factors: stopping blood loss, starting treatment and avoiding infection. Current methods for controlling blood loss for battlefield injuries involve application of a cloth bandage. While traditional bandages help to facilitate clotting, they must be changed frequently, which often results in more bleeding. This project will eliminate the need for applying or changing cloth bandages by developing a more effective liquid bandage to stop the bleeding and initiate tissue regeneration. Economical, effective, and safe blood clotting formulations will have great utility in the control of trauma-related hemorrhage in military situations. The liquid formulation proposed in this project could easily be carried by soldiers and stored at base hospitals or triage areas. This product will form a quicker and stronger clot, which will result in less blood loss and more lives saved. This project’s tissue regeneration component will be particularly beneficial to treatment of military personnel with major burns or skin loss from blast trauma. The development of an effective, relatively simple, and economical technique of dermal replacement would have a profound effect in the field of regenerative medicine, and would be highly valued by the DoD, Veterans Health Administration, and other federal institutions.

**Conclusions**

The work of this proposal will develop recombinant hemostatic devices for use on the battlefield for trauma-related hemorrhages, which are the leading cause of preventable combat-related deaths. These hemostatic devices will contain recombinant human clotting factors produced in the milk of transgenic mammals. These hemostatic devices will be completely absorbable by the body, and will be less expensive, more effective, safer, and have more potential applications than existing alternatives. These hemostatic devices also will be
transportable and easily stored. The work of this proposal also will develop a technique to replace skin with an engineered dermal/epidermal construct, which will be particularly beneficial in the treatment of military personnel with severe burns or skin loss secondary to blast trauma.

 Brief Curriculum Vitae for PI & Key Personnel

Name: Mark A. Carlson, MD  
Role: Principle Investigator  
Position/Title: Associate Professor, Department of Surgery, University of Nebraska Medical Center, Omaha, NE  
Education: BA, College of Wooster, OH, 1984; MD, Case Western Reserve University, 1989  
Statement: Dr. Carlson, the PI of the project, is a researcher in wound healing and a general surgeon. He has experience in multiple in vivo and in vitro healing models.

Name: Iraklis I. Pipinos, MD  
Role: Co-Investigator  
Position/Title: Associate Professor, Department of Surgery, University of Nebraska Medical Center, Omaha, NE  
Education: MD, University of Crete, Greece, 1992; PhD, University of Crete, Greece, 2004  
Statement: Dr. Pipinos, a co-investigator, is a researcher in vascular biology and a vascular surgeon. He has experience in multiple in vivo and in vitro vascular biology models.

Name: Jason M. Johanning, MD  
Role: Co-Investigator  
Position/Title: Associate Professor, Department of Surgery, University of Nebraska Medical Center, Omaha, NE  
Education: BA, Northwestern University, 1990; MD, University of Kansas, 1994  
Statement: Dr. Johanning, a co-investigator, is a researcher in vascular medicine and a vascular surgeon. He has experience in multiple in vivo and in vitro vascular medicine models.

Name: William H. Velander, PhD  
Role: Co-Investigator  
Position/Title: Chair, Department of Chemical & Biomolecular Engineering, University of Nebraska-Lincoln  
Statement: Dr. Velander, a co-investigator, has expertise in hematology, hemostasis, and recombinant protein technology.

Name: Gustavo Larsen, PhD  
Role: Co-Investigator  
Position/Title: LNKChemsolutions LLC, Principal; Professor and Associate Chair, Dept of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln  
Education: BS, University of Mar del Plata, Argentina, 1985; PhD, Yale University, 1992  
Statement: Dr. Larsen, a co-investigator, has expertise in nanomaterials and chemical biology.

Name: Wilson H. Burgess, PhD  
Role: Co-Investigator  
Position/Title: Chief Scientific Officer, Propulsion Technologies  
Education: BA, University of Virginia, Charlottesville, 1976; PhD, University of Virginia, Charlottesville, 1981.  
Statement: Dr. Burgess, a co-investigator, has expertise in growth factor biology, signal transduction, and protein chemistry.
List of Relevant Publications


