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## **Small Business Evaluation and Entrepreneur's (SBEE) Program**

*A collaboration between the NU Nanoscale Science and Engineering Center NSEC and  
Kellogg Graduate School of Management*

*Directors: Professors Barry Merkin and Chad A. Mirkin*

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### **PROPOSAL**

The purpose of the new Small Business Evaluation and Entrepreneurs (SBEE) Program is to facilitate the commercialization of new technological developments, by providing scientists with the tools to successfully launch new businesses. Through this program scientists propose ideas for starting businesses to a team of Kellogg students. Students select proposals and provide research and write business plans for presentation to investors. Your completed proposal will be forwarded for review to Kellogg students for inclusion in the SBEE Program. Please be advised that limited time and resources make acceptance of all projects submitted impossible. However, projects will be solicited throughout the year.

Date: **November 9, 2006**

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**Title of Project: Blood Clotting on Nanostructures**

#### **Summary:**

*(Describe the underlying technological development that you feel has potential for commercialization, suggested length 1 page)*

**(see page 2)**

#### **Potential Applications:**

*(Describe the potential applications for this new technology and target markets, suggested length ½ -1 page)*

**(see page 3)**

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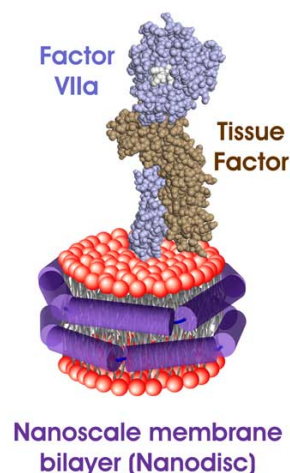
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## Summary: Blood Clotting on Nanostructures

The underlying technological development is the production of novel, nanoscale protein-membrane complexes that stabilize the body's natural activator of the blood clotting cascade in a highly active state. These nanostructures will be employed as high-efficiency hemostatic agents to control excessive bleeding in surgery, trauma, battlefield injuries, and in patients with compromised hemostasis.

**Tissue Factor**—Tissue factor (TF) is the protein responsible for triggering blood clotting in normal hemostasis [1]. It does this by binding coagulation factor VIIa (a plasma serine protease) with high affinity. TF is *by far* the most potent known initiating agent of the blood clotting cascade, and is therefore potentially attractive as the active principle for manufacturing high-efficiency, topical hemostatic agents to stop excessive bleeding. Currently available clinical hemostatic agents do not activate the blood clotting cascade via TF, however. Instead, they activate clotting through artificial means (typically via the less efficient contact pathway of blood clotting). The difficulty in using TF in topical hemostatic agents is because it is an integral membrane protein whose activity requires it to be embedded in an appropriate phospholipid membrane. Membrane proteins are notoriously difficult to handle, formulate, immobilize and stabilize using conventional technology.



**Nanodiscs**—The Sligar laboratory at the University of Illinois at Urbana-Champaign has developed a supramolecular composite particle, termed Nanodisc, which consists of a phospholipid bilayer ringed by a Membrane Scaffold Protein (MSP). Self-assembly of Nanodiscs is reproducible and highly efficient, yielding monodisperse preparations. Nanodisc technology has great potential in overcoming many of the difficulties posed by more traditional methods of handling and delivering integral membrane proteins such as TF [2].

**Tissue Factor-Nanodiscs (TF-Nanodiscs) as Hemostatic Agents**—We have developed methods for efficiently incorporating TF into the stabilized nanoscale membrane within Nanodiscs (TF-Nanodiscs). We have also demonstrated that TF-Nanodiscs can efficiently assemble the activator complex of the blood clotting cascade, and that TF-Nanodiscs exhibit potent blood clotting activity *in vitro*. We are currently initiating *in vivo* testing in animals of the efficacy of TF-Nanodiscs in controlling surgical bleeding. TF-Nanodiscs have many advantages over conventional liposome-based technologies, including much smaller size and greater stability. Both TF and MSP can be produced with high yield using efficient bacterial expression systems, making the production of TF-Nanodiscs cost-effective. The MSP moiety of Nanodiscs can be readily derivatized, allowing permanent attachment of TF-Nanodiscs to solid surfaces such as collagen sponges or wound dressings. TF-Nanodiscs immobilized onto such solid supports represent a stable, efficient hemostatic agent capable of accelerating blood clotting when applied topically.

Our laboratory has also recently discovered that inorganic polyphosphate is a natural modulator of blood clotting and fibrinolysis [3]. Polyphosphate can be used in conjunction with hemostatic agents such as immobilized TF-Nanodiscs to enhance their function.

### References

1. Morrissey JH and Mutch NJ. Tissue factor structure and function. In: *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (Fifth Edition), RW Colman, VJ Marder, AW Clowes, JN George and Goldhaber SZ, eds. (Lippincott Williams & Wilkins, Philadelphia), pp 91-106, 2006.
2. Service RF. Sushi-like discs give inside view of elusive membrane proteins. *Science* **304**:674, 2004.
3. Smith SA, Mutch NJ, Baskar D, Rohloff P, Docampo R, and Morrissey JH. Polyphosphate modulates blood coagulation and fibrinolysis. *Proc Natl Acad Sci USA* **103**:903-908, 2006.

## Potential Applications

Life-threatening bleeding can complicate surgery or trauma, and uncontrolled hemorrhage remains one of the major causes of death due to battlefield injuries. Life-threatening bleeding is also an important medical problem in patients with compromised hemostatic systems. Vulnerable patients include those with congenital and acquired hemophilia, von Willebrand disease, thrombocytopenia, liver failure, patients undergoing anticoagulant therapy, and patients with other hereditary and acquired bleeding tendencies. Existing treatments to control bleeding suffer from a variety of drawbacks, some of which are described below. There is, therefore, a large unmet need for improved hemostatic agents to treat life-threatening bleeding.

**Potential Products and Markets**—The products to be developed include: (1) Hemostatic agents to be used during surgery; and (2) Hemostatic dressings to treat traumatic bleeding, to be used by first responders. Hemostatic agents useful to treat surgical bleeding would include TF-Nanodiscs immobilized onto substances such as collagen sponges or collagen granules that would be highly biocompatible and could be left in place during and after surgery. The target market would be surgeons. Hemostatic dressings to treat traumatic bleeding by first responders would include TF-Nanodiscs immobilized onto wound dressings that could be applied topically to sites of bleeding. Target markets include the military, first responders to injury/trauma, and possibly the general public.

**Drawbacks with Other Hemostatic Agents**—Very high dose recombinant human factor VIIa (administered by intravenous injection) has been developed for use as a pharmaceutical agent, marketed under the brand names NovoSeven® and NiaStase®. NovoSeven is FDA approved for treating bleeding episodes in hemophilic patients who have intractable inhibitors, and who therefore fail to respond to factor VIII replacement therapy. It is also increasingly being used off-label as a more general hemostatic agent, and in fact the US military currently uses NovoSeven to treat bleeding from battlefield injuries when other hemostatic agents fail. A drawback to treating bleeding episodes with recombinant factor VIIa is that the cost of treating a *single* bleeding episode with NovoSeven can easily exceed \$50,000.

Other existing alternative hemostatic agents, including topical hemostatic agents, are generally much weaker activators of blood clotting compared to the enzymatic cascade involving TF, and have limited efficacy in controlling severe bleeding. Collagen and oxidized cellulose are commonly used to help control bleeding in surgery, acting as hemostatic agents via promoting platelet activation and adhesion. While platelets can help propagate the clotting cascade, they have little ability to initiate it. For this reason, topical hemostatic agents like collagen may also include thrombin. Thrombin is the last enzyme in the clotting cascade, contributing to hemostasis both by accelerating platelet activation and by clotting fibrinogen in hemorrhaging blood. As the last enzyme in the cascade, thrombin must be used at concentrations that are orders of magnitude higher than would be necessary for TF, the initiator of the cascade. Fibrin sealants ("fibrin glue") are also used to control surgical and traumatic bleeding. They consist of thrombin and fibrinogen introduced together into a wound site (sometimes also with fibronectin, factor XIII and/or calcium chloride), whereupon the thrombin clots the fibrinogen *in situ*.

Newer topical hemostatic agents approved for treating bleeding in human patients include those made from chitin (or chitosan) and mineral-based agents such as QuikClot® (which contains zeolite). The mechanism of action of this class of agents is not well understood, but may include activation of the contact pathway of clotting. Contact of QuikClot material with body fluids results in a highly exothermic reaction that can cause severe burns. Several studies of existing topical hemostatic agents have concluded that, while some have limited efficacy for treating bleeding in severe trauma (including battlefield wounds), none is ideal and there is considerable room for improvement. In addition, topical hemostatic agents still require the application of considerable local pressure on wounds for relatively long periods of time to achieve hemostasis.