

# National Textile Center

## FY 2003 (Year 12) Project Proposal

### Project No.

F03-MD15.

Competency: Fabrication

### BIO-ACTIVE BANDAGES

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#### Project Team:

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#### Objective:

Embedding functionally active genetically engineered mammalian cells in textile fibers can have a wide range of application in medical science from screening to drug delivery. The overall objective of this project is to develop a fabric that will have functionally viable genetically engineered mammalian cells embedded in it. The first goal of the project is to develop an appropriate biomaterial that can be used as a substrate for growing the genetically engineered cells as well as lends itself amenable to fabrication as a textile product like a bandage or a fabric. The second goal is to store the transfected cells embedded in the fabric in a stable state for extended time periods at ambient temperatures. This will be accomplished by desiccation of the cells that will keep them in a state of suspended animation without compromising their viability. This work will form the basis of developing a bio-active bandage that will have genetically modified fibroblasts (skin cells) that can secrete growth factors that are of paramount importance to the treatment of burn patients. In addition to developing a clinically relevant textile product, the project will develop a mechanistic understanding of cell-fabric interaction and transport of biomolecules across the fabric that will potentially form the basis of future drug delivery products that are non invasive, minimally toxic and cost effective.

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#### Relevance to NTC Mission:

The development of bio-active fabrics began under NTC grant M00-D03. This technology uses bacterial and mammalian cells to perform useful functions in textile products. Work under the previous grant established bio-active fabric designs that sustained cellular life and function over time. The next step in bringing bio-active fabrics to market is to create a working product whose performance cannot be matched by any other means. The bio-active bandage is such a product. Using cutting edge advances in genetic engineering, cellular preservation along with state of the art microfabrication technology, we are in a position to create a product that is far superior to anything currently on the market. Due to U.S. dominance in these fields, products like bioactive bandages represent an area of textile manufacturing in which U.S. companies have a huge advantage. The success of this project will establish a new textile product line for many types of topical drug delivery. In addition, textile products that incorporate dried mammalian cells have powerful applications in tissue engineering and bio-artificial organs where the ability to construct 3-D living structures while the cells are in a dry state offers huge advantages. These products will not only have significant and lucrative medical applications; but they will also represent the first commercial application of the bio-active fabric concept. The knowledge gained from this work will continue to expand the possibilities for using cellular micro-machines to create functional clothing in the future.

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#### State of the Art:

*Bio-active Fabric:* Previous work by Prof. Alex Fowler (funded by NTC grant M00-D03) has formed the basis of bio-active fabric design. These fabrics use living mammalian or bacterial cells to generate or consume chemicals in textile products. Among the long term goals for this effort are the creation of fabrics that are self-cleaning or that can regenerate chemical coatings using their own imbedded bio-reactors. This is the only project to our knowledge that focuses on putting cells to work in a textile product, rather than on eliminating them. A number of different designs were developed and tested. One of the most powerful designs proved to be a fabric based on dried

mammalian cells. Mammalian cells can perform complex functions and can be genetically engineered to secrete chemicals useful to humans; but the nutritional and environmental conditions required to maintain healthy cultures of mammalian cells are quite restrictive. In order to make mammalian cells useful in a textile product, it is necessary to put the cells into some form of stasis until they are needed, so that they do not prematurely exhaust their nutritional source, and so they are not damaged by the environmental conditions to which ordinary textiles are subject. *Preservation of Mammalian Cells:* Traditionally mammalian cells have been preserved in the frozen state; but this requires them to be maintained at liquid nitrogen temperatures. Our laboratory has recently demonstrated the ability to preserve and recover mammalian cells stored in a dried state. Successful preservation of mammalian cells by desiccation requires the addition of sugar to the intracellular environment prior to drying. Sugars do not normally penetrate the cellular membrane unless actively transported by the cell; but we are able to load sugars into the cell using an engineered pore forming protein based on the toxin produced by staphylococcus aureus. This engineered pore can be opened or closed by the addition or removal of zinc from the extracellular solution. This pore allows us to load controlled amount of intracellular sugar. Desiccation using intracellular sugar has helped stabilize cellular membranes (Chen et al., 2001). More importantly we have recently shown that we can dry fibroblasts (cell that make up the dermal layer of the skin) and recover 80% of them after rehydration. We have also shown that fibroblasts genetically transfected to produce Keratinocyte Growth Factor (KGF) recover their ability to produce and secrete KGF following drying and rehydration. We are one of only three labs in the world capable of desiccating cells and recovering them. This expertise coupled with our ongoing textile research, this puts us in a unique position to create fabrics based on dried mammalian cells.

*Wound Healing:* There has been substantial work using growth factors in the field of wound healing. Numerous studies have shown that the application of human growth factors to a wound can greatly increase healing rates, and can stimulate the healing of certain non-healing wounds such as diabetic ulcers (Ulubayram et al., 2001). Bandages incorporating growth factors have been developed; but because the growth factors are so hard to produce and maintain, these bandages are extremely expensive (on the order of \$1,000 per bandage). They are in use, therefore, only by the military. By incorporating mammalian cells capable of producing growth factors such as KGF into a bandage, we can create a bandage that would greatly accelerate wound healing. Since the problems associated with growth factor purification and storage are eliminated, bio-active bandages that produce growth factor would be far less expensive than currently available growth factor bandages. A bio-active bandage that produces growth factor in-situ, therefore, represents a product whose performance cannot be matched by other means and will provide a clear example of the power of the bio-active fabric concept.

Chen, T., Acker, JP, Eroglu, A., Cheley, S., Bayley, H., Fowler, A and Toner, M., 2001, "Beneficial effect of intracellular trehalose on the membrane integrity of dried mammalian cells," *Cryobiology*, v. 43, pp. 168-181.

Ulubayram, K, Nur Cakar, A., Korkusuz, P., Ertan, C. and Hasirci, N., 2001, "EGF containing gelatin-based wound dressings," *Biomaterials*, v. 22, pp. 1345-1356.

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#### **Approach:**

Optimization of cellular substrate for mammalian cell survival and growth factor production will be our first goal. This involves material selection and surface topography fabrication. Preliminary work (M00-D03) has shown that 3T3 fibroblasts that are genetically transfected to produce KGF can grow and function on a variety of substrates that are suitable for drying and incorporation into a poly-laminate fabric. Among the most promising substrates are cotton fabric, microfabricated polydimethylsiloxane (PDMS) and Biobrane (a commercially available skin substitute composed of a knitted nylon fabric bonded to an ultrathin silicone layer). However, optimization of the substrate geometry and the appropriate cellular density for controlled release and transport of KGF into the skin is required. Microfabrication of the cellular substrate may have significant impact on the ability of the cells to withstand drying and rehydration as well as on their level of KGF production. We will use photolithography techniques already well established in our lab to microfabricate PDMS molds. PDMS layers manufactured in this way can produce microenvironment at the cellular level. Different PDMS topographies will be created to control cellular density and distribution. The overall design of the bio-active bandage will mimic existing products like an ordinary band-aid and will be constructed using a poly-laminate fabric. The outer layer will be a water impermeable layer to which adhesive can be applied. The intermediate layers of the laminate will consist of traditional absorbant material that will be selected based on the specific application and a substrate material housing the mammalian cell colony. The layer in contact with the skin will be water permeable and hydrophilic to allow for absorption of blood and other excreted liquids by the bandage, and to allow for passage of growth factors or other drugs into the wound. Different combination of materials and manufacturing processes will be tested for the outer and inner fabric layers that will ensure a robust product that allows for easy transport of KGF.

The next goal will be the preservation of the bio-active bandage with the cells embedded in them. The effect of surface geometry on the drying and rehydration kinetics will be studied using gravimetric analysis. Various techniques including FTIR (Fourier Transform Infrared Spectroscopy), pyranine fluorescence imaging and FRAP (Fluorescence Recovery After Photobleaching) will be used to quantify the physico-chemical environment of the cells during and after desiccation in order to mechanistically understand and therefore optimize desiccation. Cells grown on the substrates will be loaded with 0.2 M trehalose using the H5 pore forming protein that enables cell permeabilization. They will then be vacuum dried at 37°C to about 8% moisture. Cellular viability post rehydration will be tested using clonogenic assays and production of KGF will provide functional viability. Once the optimal desiccation protocol has been designed, substrates containing dried mammalian cells will then be incorporated into poly-laminate fabric designs. The completed fabric will be vacuum sealed in mylar to prevent changes in cellular moisture during storage. Successful introduction of the bio-active bandage into the commercial market will rely on the ability to reliably store the bandages so that bio-activity is maintained. Excessive drying can lead to cell death, and insufficient drying leads to an unstable system whose cells deteriorate and die over time. We will perform detailed studies of the effects of storage duration, storage temperature and moisture content before and after storage on the survival and function of the fibroblasts, as well as on the overall KGF production of the bandage. Intracellular sugar concentration, packaging materials and storage conditions will also be optimized to promote long term stability of the system.

A critical optimization required for the eventual applicability of the bandages is the adequate delivery of the growth factor over a reasonable period of time. A time course study of the bio-active bandage (post rehydration) will be performed to assess its applicability in drug delivery. In order to accomplish this objective, water loss from the cellular matrix has to be minimized while maximizing the transport of the growth factors. The moisture loss of the bandage system will be measured gravimetrically over time. Different approaches will be taken to maximize the moisture holding capacity of the bandage while allowing the rapid transport of KGF. KGF production as a function of temperature, moisture content and time will be monitored. The most successful fabric designs will be further tested *in-vivo* by application to the skin of nude mice. Temperature of the cell colonies will be measured as well as moisture contents and KGF production while the bandage is adhered to the mouse. These studies will test how much drug can be delivered by a bio-active bandage under realistic conditions.

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**This Year's Goal:**

Our goal for this year is to design and begin testing a bio-active bandage that incorporates genetically transfected 3T3 fibroblasts in the dry state. The bandage will be tested based on KGF production following rehydration of the bandage system immediately after drying. Material selection for the cellular substrate, inner and outer layer material will be completed in preparation for studies on long-term storage of the bandages.

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**Outreach to Industry:**

The Bio-active fabric project M00-D03 has already generated tremendous industry interest from companies all over the world. Among the companies that have contacted our group regarding possible licensing, collaboration or product design are Adidas, Nike, Unilever, Proctor and Gamble, Natural Fibers Corp, EFE Co. Ltd., Sageous and Popular. In addition we continue to receive international media attention. We have performed more than 30 interviews for the international press, including articles for Business Week, the New York Times and Fox News. This attention and industry interest will clearly carry over into the Bio-active bandage project.

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**New Resources Required:**

A new FRAP system will be required at UMD. Micofabrication facilities are already available at Harvard Medical School. Cell culture facilities are available at UMD and HMS. Textile manufacturing facilities are available at UMD. Most of the expense will go toward support of grad students, post docs and chemicals.

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