National Textile Center
FY 2003 (Year 12) Continuing Project Proposal

Project No.
M02-CL04

Competency: Materials

Biomimetic Manufacturing of Fibers: Materials Development

Project Team:

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Objective:

The ultimate vision of this on-going research program is to produce precisely specified polymeric materials having properties engineered to provide specific solutions to complex applications. We have met most of our foundational objectives. The objective of the present proposal is to actualize this vision by completing development of our biomimetic production methods. Through these methods, designed proteins produced via the molecular biology and transgenic plant protocols we have now developed can be processed into products using a technology similar to solution processing. By the end of the three years of this project, we will have fibrous and film-like materials composed of designed protein polymer structures**.**

Progress Statement:

The prototype bioreactor for protein production in recombinant yeast has been constructed and is operating. Currently, we are scaling up the production to enable pilot spinning experiments to be carried out on purified recombinant protein. The flow purification, concentration and ordering modules have been engineered, and will be placed inline with the bioreactor to complete the production and spinning system. A “super-expressor” for yeast has been identified and isolated, and will be incorporated into the bioreactor when appropriate.

Transgenic plants harboring the spidroin 1-collagen copolymer gene have been identified and RNA expression verified. Expression of the protein appears to be minimal but may simply represent inefficiency in the detection system. Optimization of isolation of the copolymer protein and characterization are underway. Second generation transformation vectors that will improve detection have also been designed and constructed. These will be introduced into tobacco in the coming weeks.

Experiments to increase our understanding of the natural spinning systems through research on native dragline silk, and the *Nephila clavipes* spinning apparatus are currently in progress. Our spider farm currently houses 12 mature female *N. clavipes*. In our process modeling efforts, we have successfully modeled the flow through a semi-hyperbolic channel with a long L/D ratio, simulating the spider duct.

Our outreach efforts are detailed below:

1. M. Ellison, A. Abbott, et al., Biomimetics: An Holistic Approach to Advanced Fibrous Materials. Invited keynote presentation (Ellison) at AUTEX 2002, July 2002, Bruges, Belgium.

2. M. Ellison, A. Abbott, et al., Biomimetic Advanced Materials Manufacturing. Lecture series (Ellison) at Dong Hua University, Shanghai, Oct. 2002.

3. F. Teule, S. Abbott, M. Ellison, and A. Abbott. 2001. Production of novel fiber polymers in plants, Critical reviews in Biomedical Engineering. In press

4. F. Teule, S. Jung, J. Wood, W.R. Marcotte, M.S. Ellison, and A.G. Abbott. 2002. Biomimetic Manufacturing of Fibers. In, "Design and Nature," C.A. Brebbia, L. Sucharov, and P. Pascolo, eds. WIT press, pp 379-390.

Next Year's Goals:
The second year will see a completion of the bioreactor-based spinning system, and further improvements in the expression of our synthetic genes in plants and other organisms. We will add a concentration gradient to our model of the flow through hyperbolic channel, and conduct additional molecular modeling work. Additional property characterization of native silk will be done, using Raman spectroscopy in concert with mechanical deformation. Electrical properties of native silk will be measured via TSC and DMTSC, and thermal properties by DSC and DMTA.

The third year will culminate in a Biomimetic product that has been characterized and compared with natural products.

Approach:

To a large extent, our approach will be to continue the work discussed above, focusing on end-use production. There remain a few items to explore as regards the molecular biology work. One fundamental problem is extra-cellular secretion of the proteins. We found that the bi-component copolymer gene is well expressed and secreted into the culture media greatly facilitating protein purification, while the single component gene protein product, although produced in high quantities, does not get secreted. We are thus investigating ways to improve the secretion of the homopolymer proteins based on the data collected from the copolymer studies. Solution of this problem is of fundamental importance to commercial scale production of these types of structural proteins, however, we are additionally developing methods for large-scale protein purification from cellular extracts. In this regard, we are implementing temperature and pH regimes that appear to be a workable alternative to secretion for purification of these engineered proteins in the cases where the proteins are not secreted from the cell.

Finalizing the development of our in situ purification technique, and developing more broad applications, is one of the main thrusts of the proposed work. As mentioned above, the histidine tag is used to tie one end of the protein, allowing the other to remain free to orient in the flow. With coiling precluded by our method, concentrations sufficient for inducing the lyotropic LC phase transition can be obtained. In this manner, we will be able to affect the requisite order in the protein to form materials with desired properties. As we enhance our understanding of the function of self-assembly in the ordered aggregation of protein materials, and study the structure of the inner surface of the spider duct (SEM/AFM and Raman microprobe) for any potential assembly templating functions, we will be able to improve our forming systems.

Constructing models of the structure and predicting (at some level) the physical properties of our proteins will be critical to moving toward our ultimate goal. The infrastructure (hardware and software) is mostly in place; the primary need will be the support of students.

Finally, protein production at reasonable levels is of paramount importance to the objective of this program. We have developed a continuously growing bioreactor for microbial production of the engineered proteins. This system is integrated directly into a spinning system such that proteins produced in the bioreactor can be directly concentrated and spun. Additionally, the system is flexible and allows introduction of proteins produced in other systems, such as plants to be fed in line to the spinning system at the appropriate stages for purification, concentration, and spinning. The bioreactor system is complete, running and proteins are being purified for spinning. We expect to complete the proof of concept for this system this year as proposed and within the renewal period, produce fibers for study. For the studies proposed here, we are initially expressing the desired proteins in yeast and are continuing to explore transgenic plant protein production. All expression constructs contain a *myc* epitope that can be used for analysis of presence and relative levels of recombinant protein.

Outreach to Industry:
There are several companies in diverse industries eager for useable results in this area. These include agricultural companies (seed producers, for example), medical companies interested in bioactive wound dressing and prosthesis materials, and high-performance materials (fibers and other forms) companies. By keeping our high profile, and through proactive outreach efforts, we will be able to engage several companies in each of these areas.

New Resources Required:
This project is strongly dependent on people and expendable supplies. There are no new major equipment resources needed.