Development of Bio-Active Fabrics

Goal: To develop fabrics that contain micro-fabricated bio-environments and biologically activated fibers. These fabrics will have genetically engineered bacteria or mammalian cells incorporated into them, that will enable them to generate and replenish chemical coatings and chemically active components.

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Abstract

Biotechnology is revolutionizing in the way problems are solved in almost all areas of life. Bacteria are used to remediate oil and chemical spills; genetically engineered plants resist pests and weed killers; and bioreactors are used to produce drugs and enzymes to treat cancer and unclog drains. The scope and usefulness of bio-engineered products are constantly increasing. Clothing is an ideal medium in which to implant mobile bio-environments. Niche applications for bio-active fabrics exist in the medical and defense industries - e.g. drug producing bandages or protective clothing with highly sensitive cellular sensors - but bio-fabrics may form the basis of a whole new line of commercial products as well: fabrics that literally eat odors with genetically engineered bacteria, self cleaning fabrics and fabrics that continually regenerate water and dust repellents. Our project aims at developing new technologies for the incorporation of biologically

active fibers and micro-environments with the goal of producing durable fabrics at reasonable cost. We have genetically engineered bacteria that allow for the non-invasive quantification of bacterial viability and function in fibers and fabrics. We are developing techniques for introducing and culturing these bacteria in select hollow fibers. We also intend to explore the use of poly-laminate fabrics and fabricated micro-environments for the creation of bio-active fabrics. The success of this effort will open an entirely new area of textile production in which the rapidly increasing number of genetic engineering tools can be used to improve fabric design and performance.

Introduction

The tools of genetic engineering enable people to design and create cell based machines to perform useful tasks for mankind. The number and effectiveness of these living devices is rapidly expanding. Recent examples include bacteria that are engineered to remediate chemical and oil spills, bacterial bioreactors for the generation of drugs and chemicals, and engineered plants that resists pests and disease. The number and type of these cell based machines that will be developed in the future is unknown. Only the most basic and obvious cell based devices have yet to be realized; but it is becoming increasingly clear that these genetically engineered machines represent a new paradigm in micro-fabrication. Bacterial and mammalian cells are incredibly efficient and compact machines that can now be designed and modified to perform the functions of our choice. We are just beginning to take advantage of these biological microdevices.

Clothing is an obvious habitat for biological micro-machines. Clothing is specifically designed to provide a comfortable environment for living cells. Clothing materials are generally bio-friendly (non-toxic to cells); and sources of heat, moisture and even nutrients for cellular micro-devices are all readily available from the human body. There are many obvious

applications that a clothing based bioreactor might be able to accomplish. The control of odors in clothes and shoes could be accomplished by secretion of deodorizers of by the bacterial digestion of odor producing proteins. Water repellent coatings on jackets or shirts could be continually replenished by imbedded bacteria, or self-cleaning clothes could be envisioned in which oil and protein digesting bacteria act continuously. Limiting our thinking. however, to these obvious applications is to assume that little or no progress will be made in the design of cell based machines. These example applications represent the use of simple cellular devices that can be easily designed now. The goal of this project is to prepare ourselves not only for the inclusion of the simple cell based devices of today; but for the unknown yet potentially awesome cell based devices of the future.

Our vision is to create fabric based bio-reactors in which colonies of mammalian cells or bacteria can live and function for extended periods of time. We want to learn which types of fabric based bioreactors are best for promoting growth and function of the cells. We want to develop methods for making the cells and their environments more tolerant of cold, variations in humidity and washing. We want to characterize the working life of a clothing based bioreactor using current cell strains in specially designed fabrics; and identify ways of extending that working life through improvements in the cellular environment or in the cells themselves. We ultimately hope to be in position to incorporate any cell based machine into a bio-active fabric that can promote the cells' growth and function over long periods of time.

Research Plan

There are three bio-active fabric designs that we intend to explore initially. The first will be based on hollow fibers. We intend to explore a number of natural hollow fibers and certain

man-made fibers as possible micro-environments for bacterial bio-reactors. Milkweed fibers and cotton fibers that have been treated so that their hollow core is re-opened will be studied first. These natural fibers are biofriendly, having no toxic effects on the cells. Hollow fibers will be filled with bacteria in agar growth medium. Typically bacteria grow very rapidly until they have consumed all of the available nutrients in the particular environment. After the nutrients are consumed bacteria go into either a dormant or spore phase in which they no longer function. For our project we need to determine how long the bacteria remain active and functional in the hollow fiber environment. The agar gel naturally inhibits bacterial growth by spatially confining the cells. The cells will be further constrained by the fiber walls. Under these conditions it is unknown how long the bacteria will remain functional, or what percentage will continue to function. By changing the consistency of the agar gel we can control the growth kinetics of the bacteria and thus their functional life span. Clearly the fiber diameter and the ability of the bacteria to migrate through and around the fiber walls will also effect the growth kinetics. We are in the process of assessing the functional life span of e. coli bacteria in natural (cotton and milkweed) hollow fibers. We will quantify the effect of fiber diameter, gel consistency and nutrient level on the length and amount of function in the bacteria.

In order to quantify bacterial function it is necessary to have a bacteria whose function can be readily assessed. In our proposal we identified as one of our first year's aims to create a GFP (green fluorescent protein) producing strain of e. coli. We have accomplished this goal already and are in the process of quantifying the relationship between measured fluorescence and bacterial viability. GFP is a fluorescent protein that is produced by the bacteria. If the bacterium is dead or dormant the GFP will not be produced and the cell will not be fluorescent. When the cell is functional the cell will produce GFP. The GFP emits green light when it is excited by ultraviolet

radiation. As part of this project we have established a research microscopy lab that includes a Nikon E-600 fluorescent microscope capable of exciting and capturing the fluorescent signal of GFP. The fluorescent signal is captured using a digital SPOT camera. This high quality digital camera is capable of quantitatively assessing fluorescent intensity. By measuring the intensity of green light emitted by fibers containing GFP producing e. coli we can quantitatively evaluate the level of bacterial function over time.

We have also identified and acquired an e. coli strain that produces bioluminescent luciferase. Bioluminescence is what makes fire-flies and plankton glow. Unlike fluorescent proteins, bioluminescence does not require excitation with ultra-violet radiation in order to emit a signal. The bacteria emit light as long as they are functioning, and the strength of the emitted light is a measure of their functional level. We intend to use both GFP and luciferase producing bacteria to quantify function. The advantage of GFP is that the fluorescent signal is very strong and thus will provide sensitive assay. The luciferase signal is relatively weak; but the ability to observe the signal without UV excitation could be advantageous for monitoring function in-situ rather than under the microscope.

Once we have assessed the functioning life span of bacteria in hollow fibers we will begin to explore ways of extending the life span. We will initially explore changing the formulation of the agar growth medium. We will change its consistency and nutrient content to try and improve the growth and functioning characteristics of the bio-active fibers. We will genetically modify the bacteria to be lysine deficient. Lysine is an essential amino acid that bacteria (and other organisms) produce. Using genetic engineering we can render them incapable of producing lysine and thus make their growth impossible unless lysine is supplied to them externally. We can then formulate growth media with controlled amounts of lysine to control the bacterial growth. Still

using lysine deficient bacteria we will explore the use of micro-fabricated time release capsules to release lysine over time. We can study the effect of controlled bacterial growth on the amount of time we can sustain bacterial life in a particular fiber, and on the level of bacterial function that we can maintain over that time.

Once we have developed understanding and control of bacterial growth and function in individual hollow fibers we will incorporate bio-active hollow fibers into fabrics. Within the fabric the fiber will exist in a particular micro-environment in which the humidity, temperature and airflow will all be affected by the overall construction of the fabric. We will expose the fabrics to variations in humidity, temperature and mechanical loading and examine their effect on the performance of the bio-active fibers.

Other fabric designs will be studied subsequent to the hollow fiber based fabric. Poly-laminate fabrics will be explored. The construction of bio-active poly-laminate fabrics is fairly straightforward. A variety of gas permeable shell fabrics will be used to contain bacterial layers. Experience in the incorporation of micro-electronics into fabrics indicates that poly-laminate construction may be fairly fragile. On the other hand the poly-laminate design would allow for massive numbers of bacteria to be incorporated into a single fabric which may lead to a very high level of function.

A third bio-active fabric will be based on engineered micro-environments. Using micro-fabrication technology we will create cellular microenvironments capable of sustaining cell life and function. We propose to construct environments out of polydimethylsiloxane (PDMS), a bio-friendly and easily manipulated material that we have successfully used to manipulate the environment of cells in the bio-artificial liver. PDMS cellular micro-environments can be incorporated into yarns and non-woven fabrics to create bio-active fabrics.

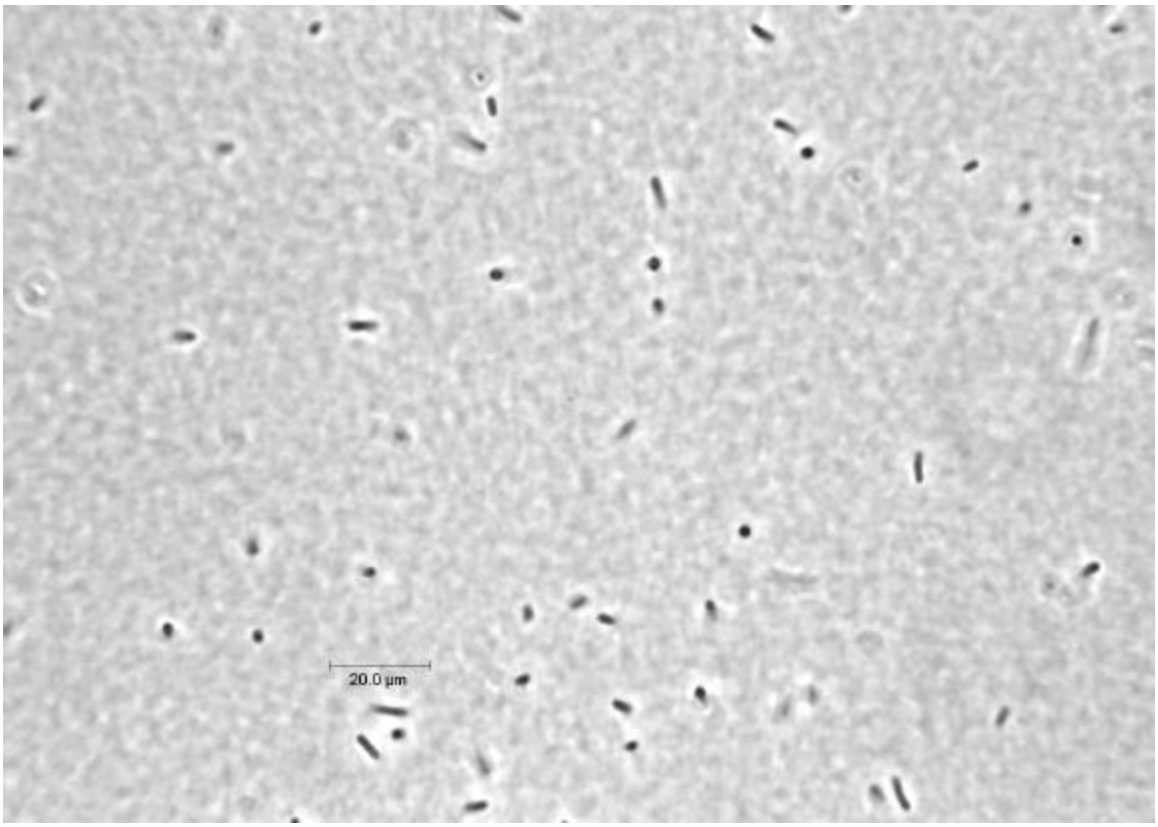
Progress to Date

Nikon E-600 Research Microscope with SPOT RT Digital Camera. Part of the new Bioengineering Laboratory at UMass Dartmouth where bacteria will be grown and incorporated into fibers.

Our first efforts have been aimed at establishing a bioengineering laboratory at UMass Dartmouth and at training our mechanical engineering research personnel in the growth and observation of bacteria. The bioengineering laboratory currently consists of an autoclave for sterilizing instruments and preparing bacterial growth medium, a shaking incubator for the growth of bacteria in broth and gel, a weighing station for preparation of media and a research microscope. The microscope is capable of phase, DIC and fluorescence microscopy. The attached SPOT camera allows one to digitally capture images and quantify fluorescent intensity. The quantification of light intensity is critical to our studies of bacterial function using GFP and Luciferase. All of this equipment is now operational.

Bacterial Incubator at UMass Dartmouth. The device is capable of incubating bacteria in gel medium or broth due to its oscillating shelves.

Our mechanical engineering research assistant, Mr. Wenjian Wang, spent his early summer with our collaborators at Harvard Medical School. He learned basic techniques for bacterial culture. Transferring this technology to UMass he has now grown and observed e. coli bacteria in our bioengineering laboratory.



E. coli bacteria cultured in the UMass Dartmouth Bioengineering Laboratory (1000x).

Our Harvard Medical School collaborators have engineered and isolated two strains of bacteria that we will use to quantify bacterial function in hollow fibers and bio-active fabrics. The first strain of bacteria is bioluminescent because we have introduced the plasmid pCGLS1 which encodes the lux operon. The lux operon is a cluster of genes, (luxCDABE) isolated from the nematode symbioant bacterium Photorhabdus luminescens. In addition to the enzyme responsible for bioluminescence (luciferase), this operon also encodes the biosynthetic enzymes

for the proper substrate, thus addition of exogenous substrate is not necessary to generate the bioluminescent signal and the bacteria can be quantitated and viewed in real time.

The second strain of bacteria that we are using is fluorescent because we have introduced the plasmid, pGFP-5 that expresses a gene encoding the green fluorescent protein (GFP). The GFP gene was originally isolated from the jelly fish Aequorea victoria. The GFP protein as well as any bacteria that contain it fluoresces bright green upon exposure to UV or blue light. We have isolated clones of both of these bacterial strains expressing each of these reporter genes and grown up large quantities of these bacteria. Presently, we are working out the optimal conditions for the adsorption of these genetically modified bacteria to our materials and fabrics as well as the non-invasive and real time methods to monitor bacterial cell number and metabolic activity.

We have identified three types of hollow fibers that we will study and compare to one another: cotton that has been treated to re-open natural hollow core, kapok which has a large natural pore, and hollofill - a commercially available hollow fiber. We are about to begin the development of methods for insertion of growth media and bacteria into these fiber types.

Publications and Presentations

While we have not generated any publications yet, we have already presented work on this project at the Intelligent Textiles 2000 conference held in Providence Rhode Island. The talk was presented by Prof. Fowler and was entitled *"Development of Bio-Active Fabrics."* Contacts made at the conference included researchers from MIT who had attempted to incorporate micro-electronics into fabrics and the head of research and development for Adidas who was very interested in pursuing this technology for the creation of odor free sneakers. We have arranged to contact Adidas after making developing our first bio-functional fabrics.

Project Web Site: *http://www.mne.umassd.edu/faculty/alexbio.html*