LOADING OF GENETICALLY ENGINEERED BACTERIA INTO HOLLOW MILKWEED FIBERS

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ABSTRACT

The genetically engineered bacteria can already be designed and cultured according to clients' demands. However, the lack of cost-reasonable functional carriers of bacteria limits their commercial mass application. Clothing is an ideal medium in which to implant mobile bio-environments. Our project aims at developing new technologies for the incorporation of biologically active fibers and microenvironments with the goal of producing durable fabrics at reasonable cost. Ultimately these fabrics may form the basis for new lines of commercial products such fabrics that literally eat odors with genetically engineered bacteria, or fabrics that generate drugs for use in bandages. Our immediate objectives are to establish basic fabric designs that can sustain bacterial life and function for long periods of time.

We have developed the ability to grow GFP-producing E.coli and assess their viability and function based on fluorescent intensity. We have set up a set of techniques for loading E.Coli into select hollow fibers. We have tested the functional period of GFP E.coli in milkweed under several typical conditions. Our image analysis code can measure and compare loading effects between hollow fibers, and report numbers of functional GFP bacteria in a microscope field. A mechanical unloading technique based on union of centrifugal separation and enumeration of CFU (colony forming units) is under development to quantify the long term functional levels of bacteria within the fibers.

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. 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 micro-devices. 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 also some obvious initial 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 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. 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 cell-based devices of the future. Our vision is to create fabric-based bioreactors 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 bioactive fabric that can promote the cells' growth and function over long periods of time.

METERIALS AND METHODS

Natural hollow fibers

Milkweed fibers and cotton fibers that have been treated so that their hollow core is re-opened have been explored as possible micro-environments for bacterial bio-reactors. These natural fibers are bio-

friendly, having no toxic effects on the cells. We have developed a vacuum pressure impregnation system for loading the bacteria into hollow fibers. The fibers are placed in a beaker within an evacuated chamber. Culture broth is allowed to enter the beaker while the vacuum is maintained so that the fibers are immersed. Release of the vacuum causes atmospheric pressure to force the fluid into the hollow core.

Typically bacteria grow very rapidly until they have consumed all of the available nutrients in the particular environment. By changing the nutrient level we can control the growth kinetics of the bacteria and thus their functional life span. The fiber diameter and the ability of the bacteria to migrate through and around the fiber walls will also affect the growth kinetics. We have qualitatively assessed the functional life span of E.eoli bacteria in milkweed hollow fibers under a variety of conditions. We have found that some bacteria remain alive and functional for up to two weeks in a simple milkweed fiber. We are developing techniques to quantify function of the bacteria over time.

E.Coli strain with pGFP-5 plasmid

In order to quantify bacterial function it is necessary to have a bacteria whose function can be readily assessed. We secured a GFP (green fluorescent protein) producing strain of E. coli. 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. Using this bacteria we can determine whether the bacteria is alive and retains its genetically engineered function over time.

Fluorescent microscope

We used a Nikon E-600 fluorescent scope 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. Our initial studies were aimed at merely establishing whether any bacteria remained alive and functional in the milkweed as a function of time. We assessed this by looking at the maximum number of functional bacteria that could be found within a single 1000X microscopy field as a function of time and storage condition.

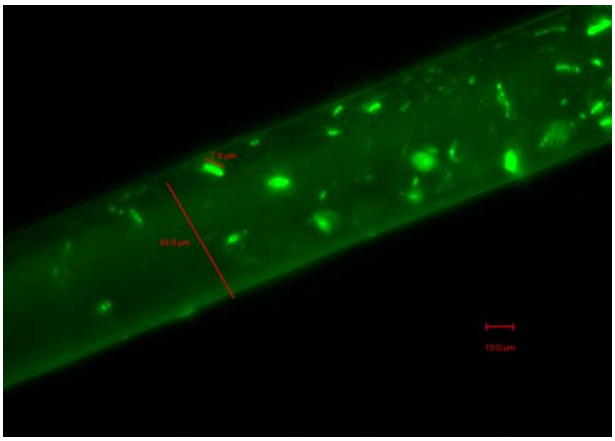

Figure 1. Genetically engineered E. coli in a milkweed fiber three days after loading.

Digital image analysis

In order to save material and time consume in the quantification process, we developed a digital image analysis code that can measure and compare loading effect between hollow fibers, and report numbers of functional GFP bacteria in a microscope field. Its main principle is a method called region growing used in the field of image processing. This is a sound technique for studying individual fibers, but remained impractical for the study of many fibers at the same time.

Centrifugal unloading and CFU enumeration

Quantification of long term function in bacteria loaded into large numbers of milkweed fibers is being accomplished using centrifugal separation and enumeration of CFU. The centrifugal separation is accomplished by spinning the 2 cm. long fibers at 35,000 RPM. This results in an acceleration of up to 1.47 m/s^2 for the E. Coli within the milkweed. Our vacuum loading procedure was repeated 13 times into hollow milkweed fibers. We observed an average loading effect of 0.0708 milliliters of bacteria containing solution per milligram of milkweed fiber. A negative control was performed 10 times without loading. The average loading was 0.00221 milliliters per mg of fiber. More than 30 times more bacteria were present in fibers that were vacuum loaded, than were present in fibers merely immersed in identical broth.

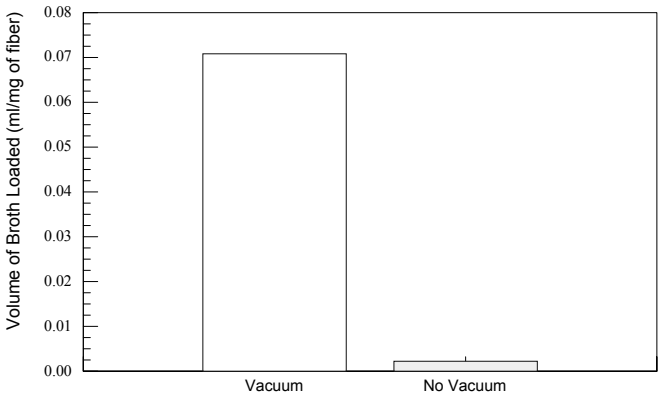

Figure 2. Effect of vacuum loading on bacterial impregnation of milkweed fibers.

RESULTS AND DISCUSSION

Vacuum loading of genetically engineered bacteria into milkweed fibers has been shown to be effective using both digital microscopy and CFU assessment following centrifugal unloading.

Long term function of bacteria in the milkweed environment is a function of nutrient level, temperature and environment humidity. Studies to quantify these effects using centrifugal unloading and CFU analysis are underway.

ACKNOWLEDGEMENTS

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