DESCRIPTION OF THE RESEARCH PROJECT FOR THE 2019 SUMMER RET SITE

Project: Bioengineering Therapies for Peripheral Nerve Repair

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Project Summary

This research topic is inspired by the Armed Forces Institute of Regenerative Medicine (AFIRM) Extremity Injury Treatment research program. The focus of this program area aims to deliver products that may decrease the need for amputation following severe trauma, enabling wounded warriors to restore form and function to damaged arms, hands, legs, and feet. This is linked to the NSF **big idea** of

Growing Convergence Research, where merging of ideas, approaches, tools, and technologies from interdisciplinary sciences can be implemented to stimulate discovery. This is applied in this project by utilizing novel usages of cells (biology), biomaterials (chemistry), and scaffolds (engineering) to create bioengineering procedures to restore a level of function in traumatic nerve injuries. The central **research challenge** or objective is to utilize functionalized biomaterials and cells to direct and align regeneration of nerve across traumatic peripheral nerve injury gaps that otherwise won't regenerate either spontaneously or through surgical intervention.

In addition to warriors in the battlefield, peripheral nerve injuries impact an additional 1.4 million Americans each year with of 20 million people currently living with the effects of traumatic nerve injury [1]. In severe peripheral nerve injuries, nerve pathways, connections, and the extracellular matrix surrounding the nerve that lead to the sensory or motor targets are disrupted [2]. If the injury gap is approximately 2 centimeters or larger, functional recovery is extremely limited [3, 4]. This deeply impacts quality of life due to the loss of connections between the central nervous system and the bodies extremities.

A key reason for poor functional recoveries is that neurons lack the proper guidance, alignment, and signaling from the damaged extracellular matrix to allow for targeted growth across injured tissue [5, 6]. The extracellular matrix is vital to tissue regeneration by acting as a "bioactive" scaffold for cells that can instruct cell behavior and function. This project will address the urgent need to promote functional recoveries in traumatic nerve injury through the use of bioactive polymers such as the proposed engineered biomaterials combined with an extracellular matrix. Our lab currently utilizes a model for using cells to assemble a natural, extracellular matrix that mimics the extracellular matrix formed in the body and will be used in this project (Figure 1A) [7, 8]. The extracellular matrix will then be utilized by engineering micropatterned polymers (Figure 1B) to possess the proper physical and chemical signals to interact with cells and become a "bioactive polymer" with an extracellular matrix coating. This is accomplished by allowing and directing cells to align on the polymer, secrete and assemble the extracellular matrix, and achieve an aligned, bioactive extracellular matrix combined with biomaterials.

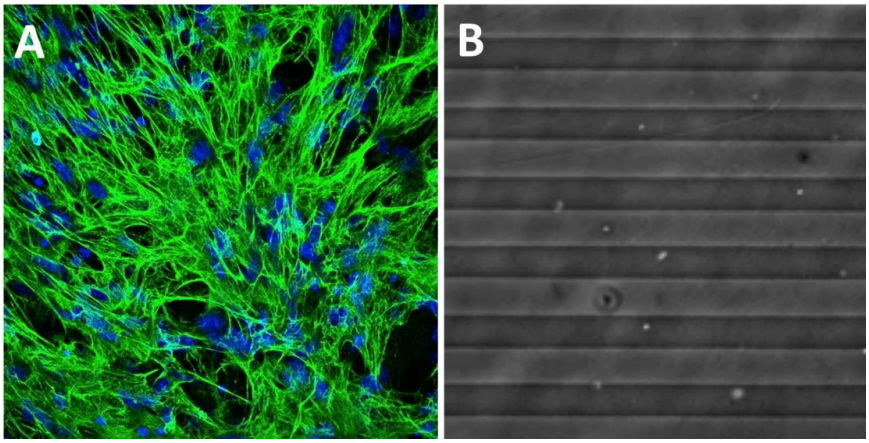

Figure 1: (A) Fibroblast cells secrete and assemble an extracellular matrix in culture. This image is stained for a major extracellular matrix protein fibronectin (green) and the cell nucleus with DAPI (blue). Structure of the extracellular matrix is fibrillar across the surface with cells interspersed throughout. (B) Polymers can be utilized to micropattern directionality on which cells will adhere and align themselves. A simple, chemical interface is applied to polymers in certain orientations to allow for preferential cell adhesion on lines.

This research will answer the **guiding question**: Can we direct alignment of cells and the deposition and alignment of extracellular matrix by cells through the use of engineered biomaterials? To answer this question, the following tasks are proposed to be undertaken.

- Synthesis of micropatterned biomaterials
- Culture fibroblast cells on patterns to assess and quantify alignment
- Assess and quantify extracellular matrix deposition and alignment following cell culture
- Recellularization of extracellular matrix to analyze neurite and glial cell alignment

Training Provided

Teachers will first learn about the concepts of bioengineering, particularly as it relates to the challenges and goals facing current biomedical issues. They will then be trained in three overarching areas: biomaterial synthesis, cell culture, and immunofluorescent microscopy. These skills will be utilized to synthesize biomaterials for which they will perform cell culture experiments on in order to gain fundamental knowledge about the interactions between cells and biomaterials. Following cell culture on biomaterials, fluorescent microscopy skills will be utilized in order to analyze and quantify the results of the cell-biomaterial interactions for designing and targeting future experiments and goals. In particular, training will be provided to decipher and analyze cellular images gained through immunofluorescent microscopy.

Research Facilities

The research will be conducted primarily in the Harris laboratory at the University of Cincinnati. This is a 653 ft^2 wet laboratory space and 194 ft^2 microscopy laboratory space designed and set up for bioengineering research. Additional laboratory space for cell culture is shared with the Department of Biomedical Engineering and includes biosafety hoods, cell culture incubators, centrifuge, and microscopes. The RET participants research will be experimental in nature and be performed using the variety of equipment housed in the lab to carry out bioengineering research including the ability to synthesize biomaterials, perform cell culture, and analyze cell and protein response.

Ideas for Classroom Implementation

Throughout the project, teachers will be introduced to and gain knowledge into the emerging field of bioengineering, and in turn be able to expose students to the basic concepts of bioengineering. Teachers will be able to conceptualize and communicate to students the timeline and setup from basic, fundamental research, engineering solutions to particular medical problems, and finally to the final clinical trials involved in promoting a new drug or therapy.

Teachers will be able to integrate the following concepts to the classrooms:

- **Middle school (Grades 6-8):** What is basic and translation research? Where are each of these types of research performed? What is the link between a medical problem, basic research, and translational research? What is needed to ultimately target and approve medical therapies?
- **High School (Grades 9-12):** How do we use cell culture to model phenomena or disease in the body? How do we we quantify and analyze certain populations of cells to gain insight into cellular processes. What role does basic research play in directing how we approach animal model and human trial studies?

- **College (Grades 13-14):** Advanced knowledge of the extracellular matrix and how the extracellular matrix interacts with cells. More intensive nerve biology and tissue biology training including the repair process undergone following injury. This can also include current strategies for regeneration of nerve and the drawbacks with a project designed towards creating and promoting a new therapy for a medical issue.

In addition, specific biomaterials can be created using a 1-step process in the classroom by first mixing a base and curing agent and then allowing 24 hours to solidify. Materials can be provided to allow students of all ages to mold a biomaterial in differing configuration and stiffness in order to model a tissue replacement (Figure 2). These can also provide a basis to understanding how cells interact with biomaterials and what the culture surfaces can entail and how they appear.

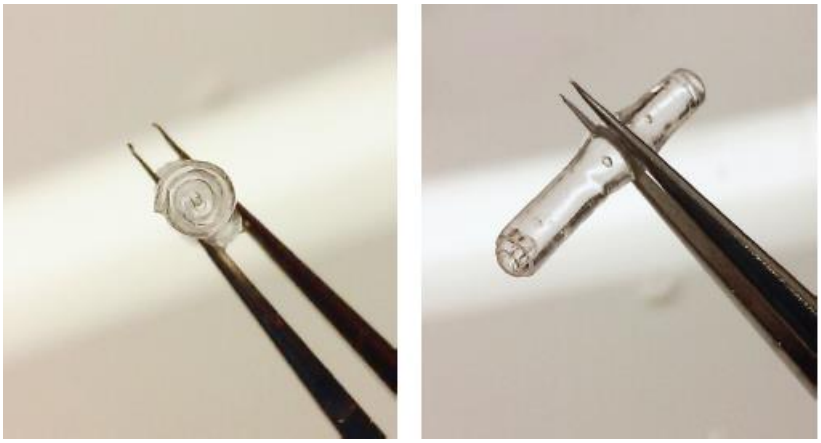

Figure 2: Cross-sectional and longitudinal profiles of a polymer rolled up into a spiral scaffold. These scaffolds are cylindrical structures that can be manipulated with tweezers or your hands.

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