DESCRIPTION OF THE RESEARCH PROJECT FOR THE 2020 SUMMER RET SITE

Project 1: Tissue Engineering Approach for Diabetic Chronic Wound Healing

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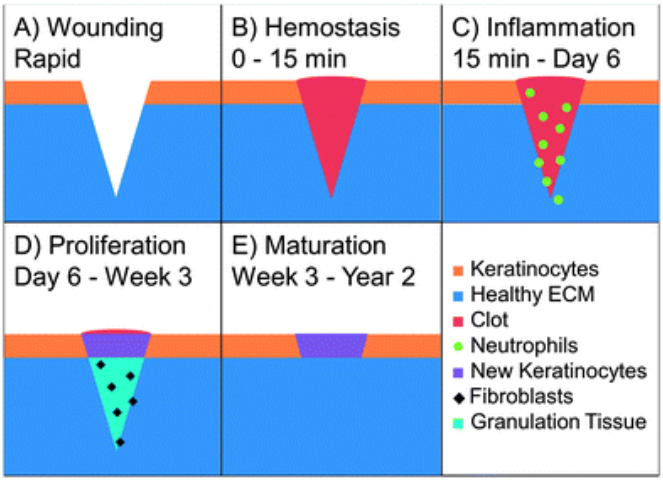
Background and Significance

The ***big idea*** driving this project is using a tissue engineering approach for diabetic chronic wound healing. Chronic wounds, also known as chronic skin ulcers, affect more than 6.5 million people in the United States alone.[1] Chronic wounds are a burden on both patients and the healthcare system. The slower healing process of chronic wounds as compared to acute wounds leads to painful and often unsuccessful recoveries. In many instances, the sole clinical solution is to amputate the affected limb to prevent further morbidity of the patient. Furthermore, scarring of wounds after current treatment necessitates needed improvements in aesthetics of the wound

post-healing. The treatments and care for chronic wounds can also cost developed countries up to approximately 2-3% of their healthcare budgets over time.[2]

The key factor distinguishing chronic wounds from acute wounds is the healing process is arrested at a certain stage of recovery, most commonly the inflammation stage. A key component of inflammation is an increased level of matrix metalloproteinases (MMPs) that degrade the extracellular matrix and growth factors. This, in turn keeps fibroblasts from being prominent in the wound regeneration process as is necessary for optimal healing.[1]

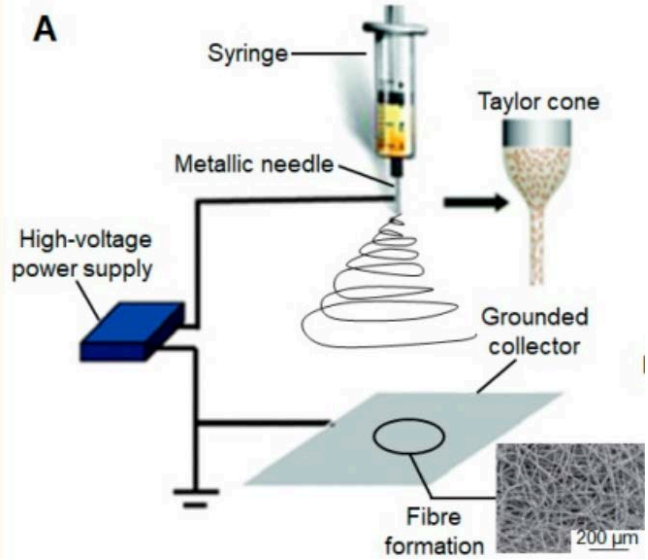


The five stages of wound healing[1]

One of the numerous types of chronic wounds are diabetic ulcers, which usually occur on the legs and feet. Diabetes specifically exacerbates the slow healing process in chronic wounds due to factors such as neuropathy, poor circulation, blood sugar levels, inflammation, hindered immune system function, and the increased chance for infection.[1] The current treatment for these diabetic ulcers is negative pressure wound therapy, which has been shown to decrease wound size and healing times.[3] However, a major drawback to this method is effectiveness is greatly reduced in larger wounds.[4] Other treatment options include moisture-modulating dressings, which are not optimal for patient comfort, and hyperbaric oxygen therapy, which lacks long-term therapeutic success.[1, 3]

Research Challenge and Guiding Questions

The electrospinning process has the potential to create 3-D nanofiber scaffolds that allow for a faster and more long-lasting wound healing process for chronic wounds, particularly diabetic ulcers. Electrospun scaffolds, made from a myriad of different biocompatible polymers, can be further functionalized both chemically and physically to enhance and promote the wound healing process. The qualities that are vital in a scaffold for regeneration are high porosity, moisture-retention, high surface-to-volume ratio, antimicrobial activity, and growth factors or proteins to enhance cell proliferation and migration.[1]



The electrospinning process[5]

The ***research challenge*** is to compare two well-researched polymers in the field of electrospinning for wound healing: synthetic polymer poly(lactic-co-glycolic) acid (PLGA) and natural polymer chitosan (derived from chitin). Previous studies have successfully created nanofiber scaffolds using these polymers and have grown fibroblast cells on them, indicating that they are not toxic at a cellular level. Both PLGA and chitosan are bio-degradable, though they differ in degradation rates. PLGA can be tailored to degrade in user defined rates depending on composition, whereas chitosan degrades relatively quickly. PLGA also has more inherent mechanical strength than chitosan, but as a natural polymer, chitosan has been shown to have higher levels of antibacterial properties when compared to PLGA.[6] In this project, each of these polymers will be combined with one natural additive at a time, yarrow and goldenrod. Both of these additives are known to have anti-inflammatory properties.[7, 8] Epidermal growth factor (EGF) will also be added to nanofiber mats, as it has been shown to significantly improve wound healing activity in similar studies.[1] The ***guiding questions*** for the project are: 1) what impact will addition of natural additives, yarrow and goldenrod have on the performance of each of these polymers, and 2) what impact will the addition of EGF have on the performance? It is hypothesized that wound closure will occur the fastest and most thoroughly on the natural polymer with a natural, anti-inflammatory additive.

Research Plan

The broad-view progression of this research project is as follows:

- Create polymer solutions for each PLGA and chitosan condition that contain EGF, EGF + yarrow, EGF + goldenrod, and that do not contain EGF.
- Separately electrospin each of these polymers to form aligned nanofiber mats of the same thickness.

    - Use the co-axial electrospinning apparatus, to have the EGF + polymer +/- additive solution in the core (for extended-period release) and the polymer +/- additive solution in the shell.
- Further functionalize the nanofiber mats by chemically attaching EGF to the surface of the nanofibers for burst release.
- Seed fibroblast cells onto the electrospun mats and allow for growth for a week.
- Cut each of the six types of electrospun mats into equally-sized pieces and inflict identical “wounds” into the cell surface (same depth, width, and shape).
- Observe the speed of wound closure and the rate of proliferation of fibroblasts (and later keratinocytes) across the six set-ups.

Expected Outcomes

In the case that the hypothesis is supported in this study, by faster migration of fibroblasts into the wound and a higher rate of proliferation of fibroblasts in the chitosan + additive mats, this will indicate the importance of possessing anti-bacterial activity, cell-fiber attachment points, and anti-inflammatory additives in wound closure. Even lacking definitive results, the study will be an important indicator of the qualities that are most important in a treatment option for the closure of chronic wounds such as diabetic lower-limb ulcers.

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: electrospinning of the scaffolds, cell culture, and immunofluorescent microscopy. These skills will be utilized to synthesize tissue engineered scaffolds 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 scaffolds, 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 Esfandiari 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 and immunolabeling of samples will be performed in the Harris Laboratory which 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 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 biomaterial and how they interact with cells. More intensive wound biology and immune response training including the repair process will be gained. This can also include current strategies for regeneration of skin and the drawbacks with a project designed towards creating and promoting a new therapy for a medical issue.

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