Bioinstructive Scaffolds Through a Multiscale Approach Mimicking Tissue Mechanics and Regulating Differentiation

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Statement of Purpose: Regenerative engineering is structured around three main components: stem cells, biochemical factors such as growth factors or cytokines, and biomaterial scaffolds. The scaffold architecture presented to cells has been demonstrated to play a critical role in cellular responses, i.e. proliferation and differentiation, ultimately establishing the "cell niche" and affecting tissue regeneration, reparation, or replacement. While significant progress has been made in the regenerative engineering field, questions persist.

The areas of scaffold design and fabrication need significant improvement. This project presents a prime achievement within the soft tissue engineering field: a novel nanofiber scaffold structure with tailorable mechanical properties based on a "Chinese-fingertrap" design. The scaffolds are fabricated from an innovative electrospinning technique. Furthermore, this project analyzes the effects of fiber diameter in tissue engineering constructs and the relationship to stem cell differentiation through intracellular signaling. Cell signaling pathways are investigated to determine the target proteins of interest, specifically for the application of tendon tissue engineering. The results of this project have a broad impact and promising project potential with the opportunity to span across various tissue engineering applications, including vascular engineering, nerve regeneration, and ligament and tendon engineering.

Methods: Scaffold synthesis was modified from a basic electrospinning method. Solution components and electrospinning conditions are shown in **Table I**.

Table I: Electrospinning conditions for scaffold synthesis

Polymer Solution	10% PCL, 10% gelatin 2,2,2-Trifluoethanol (TFE) each, 0.003% glacial acetic acid
Voltage, Flow Rate	16 – 18 kV, 1.5 mL/hr
Target Distance	26 cm
Humidity	40% - 60%
Temperature	21°C - 24°C

To create a tube-shaped scaffold with bi-axially aligned fibers, the polymer solution was spun with an innovative electrospinning set-up: Fibers were targeted to a dual directional oscillating rod. The nanofiber alignment was controlled with magnets flanking the target area, which created fibers aligned parallel to the rod, while the rotational speed could be changed to control fiber alignment and ultimately mechanical properties of the scaffold. Samples were evaluated with environmental scanning electron microscopy (ESEM) to confirm nanofiber alignment. To investigate optimal conditions for the specific application of tenogenic differentiation, a gradient of fiber diameters versus growth factor concentration (GDF-5) has been designed. At timepoints of 2 hours and 24 hours, a quantitative antibody array will detect changes in cell lysates for up to 43 different kinases. A Chemi-IR Detection Kit will convert the chemiluminescent signals to signals for near-infrared fluorescent Western blot detection. MAPK, focal adhesion and cell:cell signaling will be highlighted in the analysis.

Results: The project innovation includes the Chinese finger trap concept, shown in **Figure 1A,** which utilizes an overlapping criss-cross fiber design to improve substrate retention and mechanical properties. **Figure 1B** illustrates the device set-up for scaffold production.

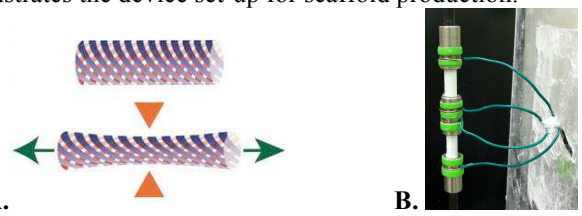

Figure 1: **A.** Chinese fingertrap scaffold design (top), under tension (bottom) **B.** Novel electrospinning set-up

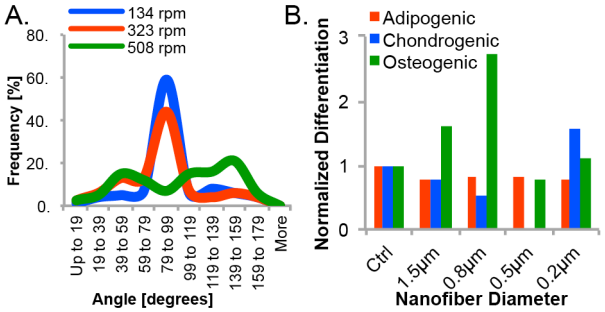

Figure 2: A. Line graph demonstrates fiber alignment relative to speed **B.** Fiber diameter effects on phenotype

The line graph comparison (**Figure 2A**), generated from ESEM scaffold images and ImageJ, illustrates varying fiber arrangement based on rotational speed of the electrospinning device. The steep single blue peak displays alignment in one direction while the green line demonstrates bi-axial alignment with two generalized peaks; red presents intermediate alignment. **Figure 2B** establishes that cells actively recognize changes in their microenvironment and respond to variability in scaffold nanostructure leading to differentiation.

Conclusions: These results provide promising potential for the proposed scaffold design to be used as bioinstructive geometry. By harnessing fiber architecture and diameter to align cells in an organized manner and replace or augment growth factors via cell contact inhibition and signaling pathways, this scaffold is an encouraging approach for tissue engineering applications. Additional research will be performed specifically for the tendon tissue engineering application to elucidate the cellular mechanisms to describe the signaling pathways and protein functions, enhance the tenogenic lineage, and improve tendon scaffold mechanics to reach physiological ranges.