

# Nanostructured Scaffolds for Tissue Engineering Applications

Nathaniel Hoglebe

Chemical Engineering, University of Dayton

**NNIN REU Site: Nano Research Facility, Washington University in St. Louis, St. Louis, MO**

**NNIN REU Principal Investigator(s): Dr. Younan Xia, Biomedical Engineering, Washington University in St. Louis**

**NNIN REU Mentor(s): Dr. Jingwei Xie, Biomedical Engineering, Washington University in St. Louis**

**Contact: hogrebnj@notes.udayton.edu, xia@biomed.wustl.edu, xiej@seas.wustl.edu**

## Abstract:

Polymeric nanofiber scaffolds with both random and aligned fiber orientations were fabricated through the process of electrospinning. A linear gradient of the bone mineral hydroxyapatite was successfully deposited onto the surface, and the bone mesenchymal stem cells of rats were then cultured onto the scaffolds. Preliminary results illustrate that a higher cell density was found on areas of low mineral content.

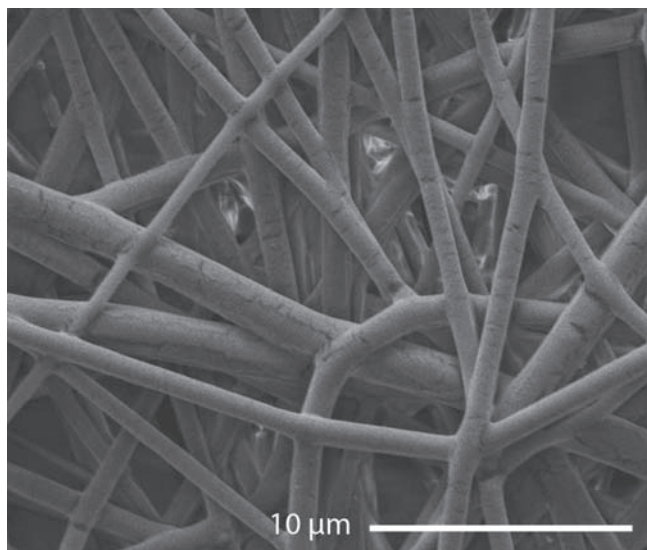


Figure 1: Scanning electron microscope (SEM) image of an unmineralized scaffold with random nanofiber orientation.

## Introduction:

One of the current challenges to successful implementation of tissue regeneration techniques into mainstream modern medical practice is the lack of the essential extracellular structure that promotes proper cell attachment, migration, proliferation, and differentiation. Nanofibers fabricated through the process of electrospinning the synthetic biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) offer a promising prospect for this necessary scaffold in repairing damaged tissue because of their high porosity and large surface area [1].

In particular, when coated with a gradient of the bone mineral hydroxyapatite, these nanofibers can potentially mimic the tendon-to-bone attachment site, which is a place of much localized stress due to its non-uniform tissue composition as it changes from soft tendon to hard bone. The objectives of this project, therefore, were to fabricate PLGA nanofiber scaffolds with both random and aligned orientations of the

fibers, deposit hydroxyapatite onto the surface of the scaffolds in a gradient-like fashion, culture bone mesenchymal stem cells of rats onto these scaffolds, and finally characterize the cell activity in response to both varying concentrations of hydroxyapatite and orientations of the nanofibers in an effort to determine if these biomineralized scaffolds will be useful in the regeneration of damaged tendon-to-bone attachment sites.

## Experimental Procedure:

Electrospinning, the process by which a solution of dissolved polymer is turned into nanofibers, was used to fabricate the scaffolds. An electric current was applied to the tip of a syringe in order to induce a charge on the outgoing droplet, creating an electric field that stretched the dissolved PLGA into thin nanofibers as the solvent evaporated. The fibers were deposited on a uniform piece of aluminum foil to obtain a random orientation but were collected in the empty space between two pieces of metal to achieve a parallel formation of the nanofibers.

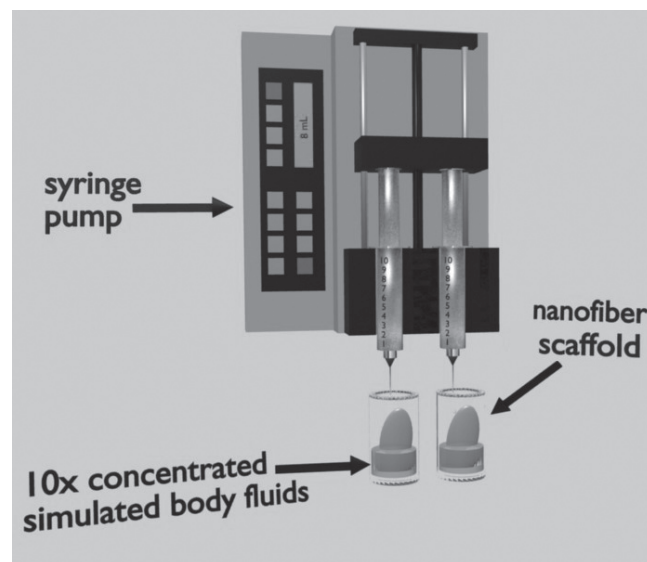


Figure 2: Schematic of the biomineralization process.

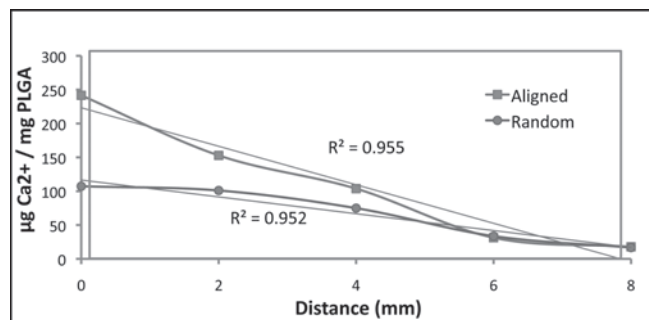


Figure 3: Graph of the mineral gradient.

Once the nanofibers were fabricated, the scaffolds were mounted onto copper wire frames and placed into glass vials. A gradient of hydroxyapatite was created by dripping 8 mL of a solution of ten times concentrated simulated body fluids and sodium bicarbonate over the course of an hour into the vials via a syringe pump. Since deposition is directly related to immersion time, the bottom of the scaffold obtained a higher concentration of mineral than the top, resulting in a linear gradient.

Once dried, the nanofiber scaffolds were mounted onto glass slides and prepared for cell culture. The bone mesenchymal stem cells (BMSC) of rats were grown onto the scaffolds using standard cell culture techniques using both proliferating and differentiating media. The scaffolds were monitored throughout the growth period for their attachment, proliferation, and differentiation characteristics in response to varying mineral content and nanofiber orientation.

### Results and Conclusions:

The calcium ion concentration was measured with both inductively coupled plasma mass spectroscopy (ICP-MS) and energy dispersive x-ray spectroscopy (EDX) to determine hydroxyapatite content. The results indicate a relatively linear deposition pattern corresponding to immersion time. After seven days of cell culture on the scaffolds with gradations in mineral content, preliminary results suggest that cell proliferation was greater in areas of lower mineral content.

This outcome can possibly be attributed to the fact that the scaffolds retain a higher porosity in regions of lower mineral content, and thus there are more sites to which the cells can attach. This result was surprising, however, because of its apparent contradiction with previous work, but it could be due to variations in the cell culture process, such as the use of different cell types [2]. Rat bone mesenchymal stem cells, for example, could possibly retain a fibroblast phenotype and thus tend to attach on a site with lower mineral content.

Alkaline phosphatase staining was used to determine the differentiation characteristics of the bone mesenchymal stem cells in differentiating media. Fluorescence micrographs indicate that there was a higher concentration of osteoblasts in areas of low mineral content, which can be attributed to the fact that there was an overall higher cell concentration in these regions.

The result of immunohistochemistry tests indicate that bone mesenchymal stem cells secreted type I collagen on both the high and low mineral content regions, while they secreted very little type II collagen anywhere on the scaffolds. This outcome indicates the development of cells with characteristics of tendon and bone cells. If type II collagen secretion was desired to mimic the fibrocartilage found between tendon and bone, alterations in the cell culture process could be performed.

### Future Work:

The preliminary results indicate that nanofiber scaffolds coated with a non-uniform layer of hydroxyapatite hold much promise for the regeneration of the tendon-to-bone interface due to their ability to yield a gradient of cell proliferation and behavior, which is critical to the structural integrity of the attachment site. Further research includes current in vivo experimentation of rat rotator cuffs that will attempt to determine the full extent of nanofiber scaffold utility in modern regenerative medicine.

### Acknowledgments:

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### References:

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- [2] Li, X., et al. Nanofiber scaffolds with gradations in mineral content for mimicking the tendon-to-bone insertion site. *Nano Letters* 2009; 9:2763-2768.

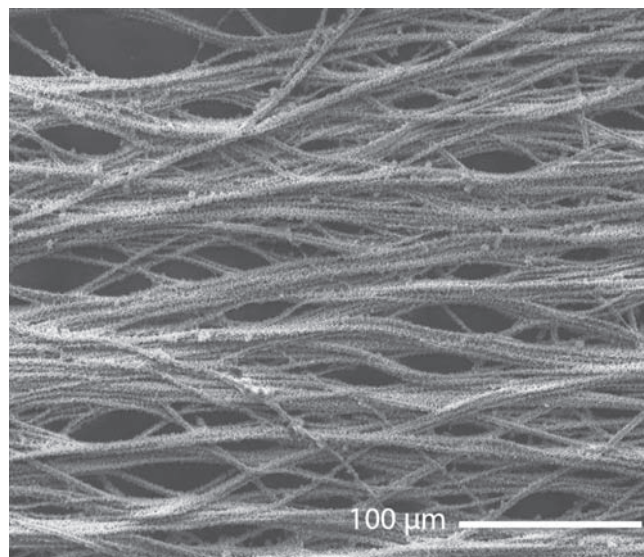


Figure 4: SEM of aligned nanofibers coated with mineral.