Fabrication and characterization of a graded co-electrospun mesh to mimic the bone-ligament interface

Satyavrata Samavedi, Aaron S Goldstein, Abby W Morgan.
Department of Chemical Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Statement of Purpose: A major concern with current anterior cruciate ligament (ACL) implants is their inability to integrate from the ligament through the bone-ligament interface into the bone. The interface is characterized by a gradual gradient in mechanical, functional and biochemical properties from the bone to the ligament and presents a challenge to mimic. Electrospinning is a useful tool to fabricate polymeric scaffolds and co-electrospinning has the added advantage of being able to produce graded scaffolds by using two different polymers that have properties similar to the bone and the ligament. In this study, we have demonstrated the ability to co-electrospin and characterize graded meshes that represent the bone-ligament interface. Polycaprolactone (PCL) and silk were the polymers chosen to represent the bone and ligament ends respectively. Meshes were mineralized to help promote selective growth of osteoblasts.

Methods: Solutions of PCL and silk were doped with fluorescent dyes DiI and DiO and co-electrospun from two syringes present on opposite ends of a rotating grounded target. The syringes were off-set along the length of the target to produce a gradient of either polymer. Post spinning, the meshes were treated with 5× simulated body fluid (SBF) to achieve mineralization atop the existing mechanical gradient. The samples were observed under a scanning electron microscope (SEM) to confirm presence of fibers and mineral. Resultant images were imported into ImagePro Plus software for analysis of fiber diameter. To determine the nature of gradient present, the dyes were extracted from the meshes and fluorescence read under a plate reader. A plot was generated to indicate the variation in concentration of either dye along the length of the mesh. Alizarin Red staining was performed to determine presence of mineral on the surface of the meshes. X-ray diffraction (XRD) and thermogravimetric analysis (TGA) were performed to determine mineral composition and content in the meshes.

Results: SEM analysis (Fig. 1) of co-electrospun PCL-PCL meshes (as proof of concept), doped with dyes DiI and DiO, revealed fibers with almost negligible bead formation. The average fiber diameter was found to be 0.42 ± 0.17 microns.

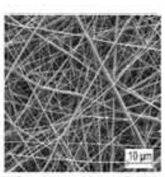

Figure 1. SEM image of mesh revealing presence of nanofibers

The fibers imaged on glass cover slips (Fig. 2) revealed that while PCL fibers doped with DiI were present at one end and those doped with DiO present at the other, cover slips that were sampled from the middle revealed the presence of a mixture of the two types of fibers.

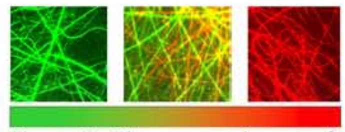

Figure 2. Fluorescent images from three spots along the length of mesh, indicating presence of gradient

The plot (Fig. 3) generated from dye extraction showed that while the concentration of DiI decreases in one direction, that of DiO decreases in the opposite direction. Alizarin Red staining confirmed the presence of mineral deposits on meshes treated with 5× SBF (Fig. 4). XRD studies indicated presence of calcium in the mineralized meshes. These studies were then repeated with graded PCL-silk meshes.

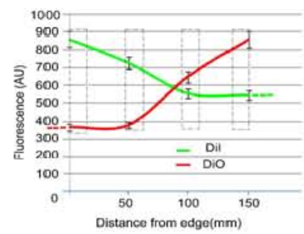

Figure 3. Plot depicting variation of dye concentration

Figure 4. Control and Alizarin Red stained meshes

Conclusions: Co-electrospinning was applied to fabricate graded electrospun meshes, consisting of two different polymers. The presence of a gradient was proven using fluorescent dyes. Mineralization was achieved on the fibers to improve osteoconductivity for bone tissue growth. Future studies would involve tensile testing of the graded meshes. The ability of the graded template to help form gradients in osteoblast and fibroblast proliferation will also be investigated.

References:

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