Aligned Nanofiber/Hydrogel Composite Scaffolds for Peripheral Nerve Regeneration

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Statement of Purpose: Tissue engineering grafts are needed for the use of regenerating aligned soft tissues including nerve, spinal cord, tendon, and muscle. Utilizing aligned nanofibers in tissue engineering grafts enhances the alignment and elongation of resident cells. However, aligned nanofibers are generally utilized as dense 2D nanofiber films only, which is inadequate for 3D soft tissue regeneration. Specifically, 2D films typically feature dense fiber packing that blocks cell infiltration. Here, continuous aligned nanofibers are embedded in a cell degradable gelatin hydrogel. The gelatin matrix stabilizes delicate nanofibers at low packing densities that do not limit cell penetration into the hydrogel matrix. The fabrication process utilizes a dip coating procedure [1] to make thin aligned nanofiber/hydrogel films that can be assembled into thick 3D structures using a unique layer-by-layer assembly process that leverages the thermo-reversible and permanent UV crosslinking properties of methacrylated gelatin (GelMe) hydrogel composite films.

Methods: An 18 wt% polycaprolactone (PCL) solution was electrospun utilizing a custom automated track collector unique to our lab [2]. Highly aligned fibers were collected on a rack and then mounted on thin plastic frames to stabilize the fibers. Fiber arrays were then dip coated in gelatin or GelMe solution resulting in hydrogel/fiber composite films. Several processing parameters were tested to determine their role on film thickness. To create multi-layer 3D composites, hydrogel/fiber composite films were dipped at 37°C (above gelation temp), and placed on previous layers that had been allowed to cool (below gelation temp). UV light was projected on the stack for 30 seconds after the addition of each layer to partially crosslink.

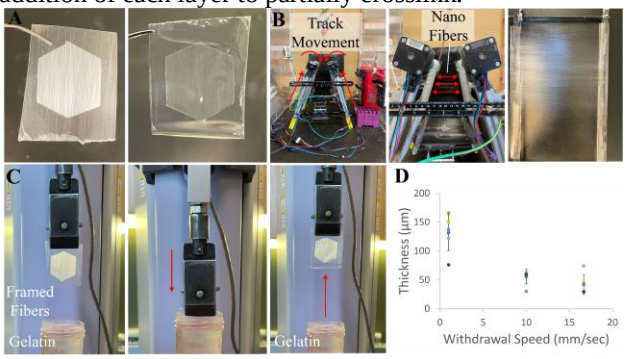

Figure 1: *(A) Framed PCL Nanofibers with and without gelatin coating. (B) Electrospinning to create the fibers (C) Gelatin Dip Technique to coat fibers. (D) Thickness vs. Withdrawal Speed Graph (n=6).*

Results: Local melting at layer interfaces during assembly encourages layer-layer integration for cohesive 3D structures. Tunable spacing between aligned fibers in the composites is controlled through 2D fiber array density and layer thickness. 2D composite film thickness ranged from 41 µm to 136 µm by varying conditions including dip temperature, withdrawal speed, solution concentration, and fiber spacing. Two millimeter thick 3D blocks were constructed with 25 layers. Segments cut from the block were wrapped in a PCL nanofiber conduit to create a 2 mm diameter implantable graft. These grafts were used to replace a 1 cm long gap in a rat sciatic nerve model. Tissue integrated well with the graft and new tissue growth into the grafts was observed. Segments similar to these 3D composite grafts are also suitable for an in vitro infiltration model that is currently being evaluated to test the effect of various graft parameters on cell infiltration into 3D aligned nanofiber/hydrogel composite grafts. Ongoing work is being conducted to optimize graft parameters and to include biomolecule gradients to enhance peripheral nerve regeneration.

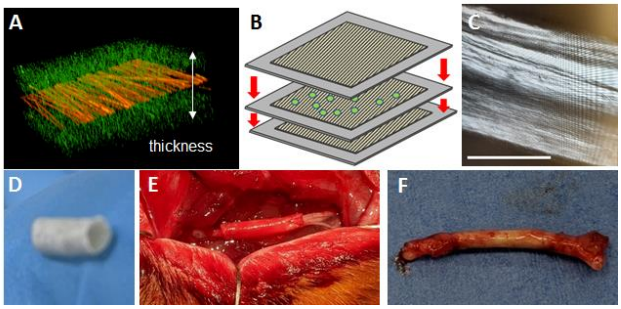

Figure 2: *(A) Gelatin hydrogel (green)/ aligned PCL nanofiber (red) composite film. (B) Layer-by-layer assembly of cohesive 3D structure. (C) Brightfield microscopy reveals aligned fibers in 3D composite (scale bar = 1 mm). (D) Composite grafts wrapped in a conduit were (E) implanted in a rat sciatic nerve gap model and (F) retrieved after 1, 2, 3, or 4 weeks.*

References:

1.) Beachley V, Wen X. Mater Sci Eng C Mater Biol Appl. 2009 Oct 15;29(8)
2.) Beachley V, Katsanevakis E, Zhang N, Wen X. Adv Healthc Mater. 2013 Feb;2(2):343-51.

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