Electrospun gelatin scaffolds with biological and architectural cues direct cell proliferation and alignment

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Statement of Purpose: Electrospinning is a process in which electrostatic force is utilized to stretch a charged polymeric solution towards a collector plate with opposite charge. We have developed an electrospinning process, which utilizes an array of electrodes to fabricate polymeric nanofibers of various dimensions into three-dimensional highly organized architectures. Biomedical applications for electrospun nanofiber scaffolds include, but are not limited to, preparing tissue engineering scaffolds, bioactive coatings, and wound healing dressings. Our goal is to develop electrospun scaffolds embedded with several angiogenic cues (e.g. growth factors, cells) that can be used in the area of therapeutic angiogenesis. In this study, bFGF was loaded onto electrospun scaffolds with variable fiber architecture and endothelial cell proliferation was determined as a function of growth factor concentration and scaffold morphology.

Methods: Scaffolds were prepared via electrospinning of 10% gelatin B solution in HFIP (1,1,1,3,3,3 Hexafluoro-2-propanol) at 15kV, 10cm needle-to-collector distance (N-t-C), and 4mL/hr feed rate from an 18GA blunt needle. Scaffolds were then crosslinked in 25% glutaraldehyde solution vapor for 1 hour at room temperature after which they were washed in a 0.1mM solution of glycine overnight. On seeding day, scaffolds were first washed in 70% alcohol for 1 hour and then rinsed with unsupplemented media three times for 15 minutes each time to remove any residues. Scaffolds were then inserted into 24 well plates, one per well, and supplemented with 10µL of bFGF solution at desired concentration. Therefore, loading was achieved via physical absorption of the bFGF solution into the gelatin scaffolds. A 10µL aliquot of 10,000 endothelial cells (HUVEC) was then deposited onto the center of each scaffold. Cells were allowed to attach for 30 minutes prior to adding 2mL of unsupplemented media per well. All groups were incubated for 24 hours at 37º C and 5% CO_2. The cells were stained with Calcein AM and cell attachment and proliferation was quantified using fluorescent microscopy.

Cytoskeletal arrangement of the attached cells was also assessed as a function of fiber orientation of the electrospun scaffolds. Briefly, 10,000 cells were loaded onto gelatin B electrospun scaffolds and allowed to grow in 2mL of supplemented endothelial cell medium for 5 days under 5% CO_2 and 37°C. On day 5, cells were stained with Alexa Fluor 488 phalloidin and DAPI, according to the manufacturer's protocol (Invitrogen Corporation by Life Technologies Colsberg, CA), to target and visualize both F-actin and cell nucleus using fluorescent microscopy.

Results: bFGF was loaded onto electrospun gelatin scaffolds at variable concentrations, and induced cell proliferation at concentrations higher than 25 ng/ml in unsupplemented medium.

Cytoskeletal assessment as a function of scaffold architecture (i.e. fiber alignment) showed a pronounced effect in F-actin arrangement of the seeded cells between aligned, random, and bare wells. Cells seeded onto randomly aligned scaffolds remained relatively rounded and their F-actin filaments had a random orientation without any extensions. On the other hand, cells seeded onto scaffolds with aligned fiber orientation had their F-actin filaments aligned along the fibers of the scaffold.

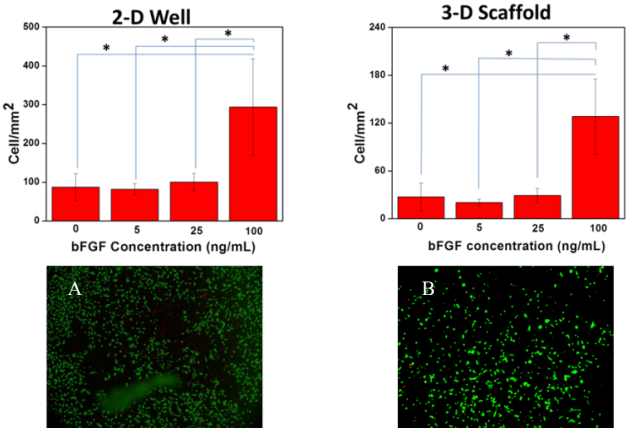

Figure 1: HUVEC proliferation on culture plates (2-D) and electrospun gelatin scaffolds with random fiber orientation as a function of bFGF loading concentrations. Fluorescent images (4x magnification) of cells growing on 2-D wells (A) and electrospun scaffolds with random fiber orientation (B). * Indicates $p<0.05$.

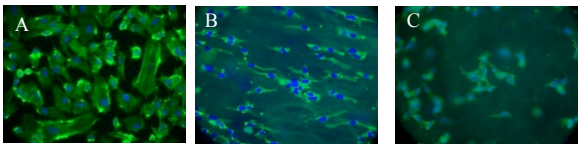

Figure 2: Cytoskeleton arrangement of HUVECs on electrospun gelatin scaffolds. (A) Random fiber orientation, B) Aligned fiber orientation, C) Bare well. (40X)

Conclusions: bFGF loaded scaffolds induced cell proliferation as a function of growth factor loading concentration. In addition, the fiber orientation of the electrospun fibers directed cell proliferation and alignment.

Acknowledgments: This work was supported by a grant to FMA by the National Institutes of Health (NIBIB-1R21EB012136-01)