Localized delivery of bFGF from elctrospun scaffolds for guided angiogenesis

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Statement of Purpose: Therapeutic angiogenesis strategies to treat peripheral artery disease and critical limb ischemia include cytokine delivery (i.e. VEGF, PDGF-BB, FGF-2) stem cell transplantation, gene therapy, and tissue engineering strategies that combine cytokine/gene therapy with stem cell administration. Even though a number of delivery methods have been developed to control the release kinetics and localization of cytokines to promote vascularization, directionality and organization of the newly formed vasculature has yet to be achieved (1,2). The objective of this study is to demonstrate that guided angiogenesis is possible by controlling the spatial and temporal delivery of basic fibroblast growth factor from three-dimensional electrospun scaffolds.

Methods: Synthesis of electrospun gelatin scaffolds loaded with bFGF: A 10 wt% gelatin B solution was loaded in our electrospinning apparatus. The feed rate, needle-to-collector distance and electrode polarity was adjusted to achieve patterned nanofibers with specific properties (i.e. fiber orientation). The resulting scaffolds were further crosslinked with glutaraldehyde for 24 hrs. Subsequently, the scaffolds were washed, sterilized and loaded with bFGF via absorption. Three-dimensional angiogenesis assay: Briefly, cytodex beads were coated with HUVEC of passages 2-4 at a concentration of 2×10^6cells / 2500 beads and left overnight in a 37 ºC/ 5% CO_2 incubator. The coated beads were then embedded into fibrin gels and allowed to crosslink for 20 min at 37 ºC. Subsequently, growth factor releasing constructs were placed on top of the fibrin gels and supplemented with medium. Microscopic evaluation was done at days 3, 6 and 9, where vessel length and number of sprouts per bead were determined as a function of bFGF concentration and scaffold morphology (random or aligned fibers).

Results: Capillary formation (i.e. sprouts length and number of sprouts per bead) was a function of bFGF loading concentration for both types of electrospun scaffolds with aligned or random fiber orientation. The overall length of sprouts at day 9 ranged from 220.28µm ± 77.86 to 261.18 µm ± 70.98 (Fig. 1) and the average number of sprouts per bead ranged from 10.94± 1.04 to 3.25±0.93.

Conclusions: Electrospun gelatins scaffolds with variable fiber orientation were loaded with bFGF and the biological activity of the releasing growth factor was evaluated in a 3-D in vitro angiogenesis assay. The bFGF loading concentration affected both number of sprouts per bead as well as length of sprout over a period of 9 days.

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References: (1) Silva E, et al. J.Throm. Haem. 2007;5(3):590-598. (2) Chen RR, et al. Pharm.Res. 2007;24(2):258-264.

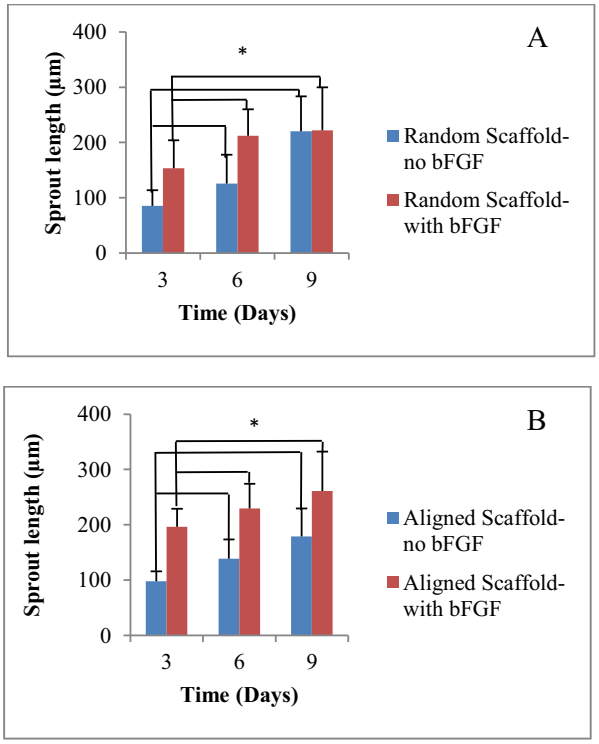

Figure 1: Average length of HUVEC sprouting with respect to bFGF (100 ng/scaffold) release from electrospun scaffolds with random (A) or aligned (B) fiber orientation. Statistical significance between groups with $p < 0.05$.

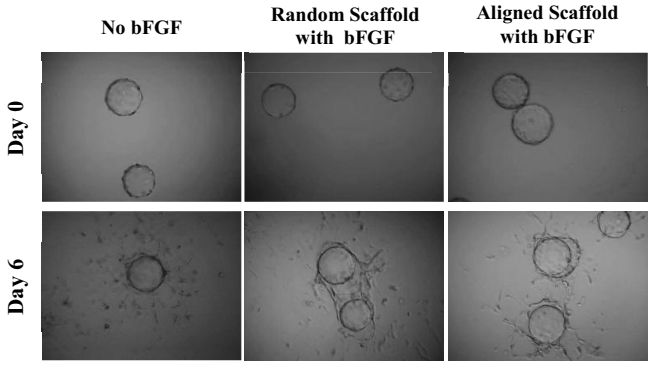

Figure 2: Light microscopic images of cell sprouting in a fibrin gel matrix in response to bFGF release from elecrospun scaffolds with variable fiber orientation. Scaffolds were loaded with 100 ng of bFGF per scaffold.