In Vitro Evaluation of Biomimetic Nanofiber-Based Scaffolds for Rotator Cuff Repair

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Statement of Purpose: Surgical repair of rotator cuff tears and chronic degeneration of cuff tendons represent significant challenges. The lack of functional solutions has motivated the development of alternative grafts for augmenting rotator cuff repair [1-4]. Our objective is to design a biomimetic augmentation graft for rotator cuff repair. Nanofiber-based scaffolds are of interest since the average fiber diameter is similar to that of native collagen. Additionally, scaffold functionality can be enhanced by introducing structural anisotropy to the matrix design [5].

The objective of this study is to characterize the structural properties of the nanofiber-based scaffolds and to evaluate the effects of matrix fiber alignment on the response of human rotator cuff fibroblasts (RCFs). Specifically, cell attachment and matrix deposition on aligned and unaligned poly(lactide-*co*-glycolide) (PLGA) nanofiber meshes will be determined. It is hypothesized that cell adhesion and matrix deposition will be directed by nanofiber alignment and organization.

Methods: *Scaffold Fabrication* – Aligned and unaligned nanofibrous scaffolds of PLGA 85:15 were formed via electrospinning [6]. Briefly, a 30 vol% solution of PLGA in DMF and ethanol was electrospun at 1.0 mL/hr with an applied voltage of 8-10 kV. A rotating collecting target was utilized to form the aligned fiber mesh.

Scaffold Characterization– The effects of mesh alignment on scaffold porosity, pore diameter (Mercury porosimetry, n=2), tensile properties (40 mm gauge length, 10 mm/min, n=6), and permeability (n=6) were determined. The effects of media incubation (37°C, 12 hrs) on glass transition temperature (T*g*) of the electrospun mesh were determined with differential scanning calorimetry (n=3).

Cells and Cell Culture – Human RCFs were derived from explant cultures of tissues obtained following rotator cuff surgery (female, aged 65-70 years). The cells were maintained in DMEM plus 10% serum, 1% non-essential amino acids, and 1% antibiotics. The nanofiber scaffolds were UV sterilized and seeded with RCFs ($3.14x10^4$ cells/cm^2). Cell and fiber alignment, as well as matrix elaboration were determined at 1, 7, and 14 days.

End-Point Analyses – Cell attachment was examined by fluorescence confocal microscopy (n=3) and scanning electron microscopy (SEM, n=3). Fiber alignment was visualized with confocal reflection microscopy (n=3) [7]. Alignment was quantified using custom software [8]. The mean vector length (MVL; $0 \le r \le 1$, 0=random, 1=aligned), mean angle (MA; $-90° \le \theta \le 90°$, 0°=horizontal orientation) and angular deviation (AD; 0°=aligned, 40.5°=random). Matrix deposition (collagen types I and III) was evaluated by immunohistochemistry.

Results: *Effects of Fiber Alignment on Scaffold Properties* – The average pore diameter, porosity and permeability of the aligned and unaligned meshes were not significantly different (Table 1). However, the aligned matrix measured a significantly higher stiffness when compared to the unaligned mesh ($p<0.05$). Incubation in DMEM at 37°C significantly lowered the T*g* of the electrospun mesh. Interestingly, the as-fabricated nanofiber mesh exhibited higher T*g* when compared to PLGA polymer ($p<0.05$, Table 2).

Table 1	Average Pore Diameter (μm)	Porosity (%)	Permeability (m^4/N·s)	Stiffness (N/mm)
Aligned	3.7±0.9	79.5±2.9	(7.87±2.47)E-12	25.5±2.8
Unaligned	5.2±0.9	79.5±1.4	(57.22±6.28)E-13	7.2±1.6

Table 2	Aligned (T*g*,°C)	Unaligned (T*g*,°C)	Pellets (T*g*,°C)
As-fabricated	54.1±1.3	52.1±0.98	49.15±0.09
Experimental	40.7±1.2	38.5±0.32	46.3±0.10

Table 3		MA ± AD (°)		MVL	
A Fibers	A Cells	2.12 ± 16.2	2.02 ± 19.1	0.84	0.78
UA Fibers	UA Cells	-14.8 ± 37.9	-28.6 ± 36.6	0.12	0.57

Cell Alignment and Matrix Deposition – Fibroblast morphology on the aligned mesh was elongated and the cells attached in the direction of the long axis of the fiber (Figs. 1, 2). In contrast, cells on the unaligned mesh were randomly distributed (Fig. 1). Quantitative analysis revealed that the alignment and orientation indices (MA, AD, MVL) of the cells were similar to those of the underlying mesh fibers (Table 3). Moreover, the deposition of collagen types I and III was also consistent with the original fiber organization in the mesh (Fig. 1).

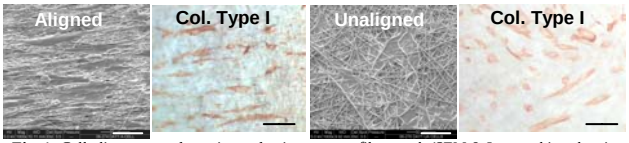

Fig. 1: *Cell alignment and matrix production on nanofiber mesh (SEM & Immunohistochemistry).*

Discussion: The structural anisotropy of the aligned and isotropy of the unaligned mesh guided human rotator cuff fibroblast attachment and matrix deposition. Controlled cell response on the biomimetic nanofiber scaffold resulted in a more physiologically relevant matrix for rotator cuff repair. Matrix fiber orientation thus has a profound effect on fibroblast response and is a critical factor for consideration in the design of functional scaffolds for rotator cuff repair.

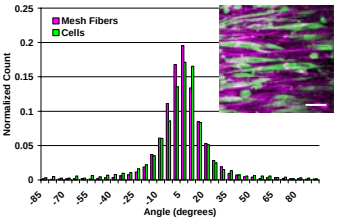

Fig. 2: *Cell and nanofiber orientation at day 14 for aligned mesh. (60x, scale bar=50μm).*

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References: 1) Dejardin *et al.*, 2001; 2) Thomopoulos *et al.*, 2002; 3) Funakoshi *et al.*, 2006; 4) Iannotti *et al*., 2006; 5) Costa *et al*., 2003; 6) Reneker and Chun, 1996; 7) Brightman *et al.*, 2000; 8) Karlon *et al.*, 1998.