Effect of Scaffold Fiber Diameter on Human Tendon Fibroblast Response

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Introduction: Rotator cuff repair is a common shoulder procedure with 75,000 surgeries performed each year[1]. Injuries to the rotator cuff occur mostly (76%) at insertion[2], which at best heals with a disorganized scar tissue. Decreased collagen fibril diameter has been reported to be associated with scar tissue[3], and since fibril diameter is directly related to the mechanical strength of the tendon, a healing tissue with smaller fibril diameter thus gives rise to inferior biomechanical properties[4]. Consequently, our approach to functional tendon-bone integration focuses on controlling the diameter of fibrous scaffolds in order to enhance mechanical properties and optimize cell response on the scaffold. The **objective of this study** is to evaluate the response of human rotator cuff fibroblasts (hRCFs) on aligned polylactide-co-glycolide (PLGA) scaffolds with different fiber diameters. **It is hypothesized** that cell proliferation, matrix synthesis and gene expression will depend on fiber diameter. It is expected that the results of this study will improve our understanding of cell-material interactions critical for functional rotator cuff repair.

Methods: *Scaffold Fabrication*: Aligned scaffolds with varying fiber diameters were formed by electrospinning[5] of PLGA (85:15, Lakeshore) in N,N-dimethylformamide with 30%, 46%, 62% and 70% polymer weight/solvent volume ratio. Fiber diameter was measured using SEM and Image-J (n=3). Solution viscosity was determined by oscillatory shear tests (n=4, Advanced Rheometer), and mechanical properties were measured at 5mm/s using an Instron system (n=6). *Cell Culture*: The hRCFs (aged 50, male) were seeded on the scaffolds ($3x10^4$ cells/cm^2), and cultured in fully supplemented media with ascorbic acid for four weeks. *End-Point Analyses (1, 3, 7, 14, 28 days)*: Total DNA (n=6) was quantified by the Picogreen Assay. Glycosaminoglycan (GAG) and collagen synthesis (n=6) were assessed by DMMB and Sircol assays, respectively. Integrin (β1, αv, and α5) expression (n=3) was analyzed by RT-PCR.

Results: As expected, fiber diameter increased significantly with increasing solution viscosity (Fig. 1A&B) at all PLGA concentrations, with a less prominent effect observed at lower concentrations. Tensile strength of scaffolds with a smaller fiber diameter (0.67±0.09μm, nanofiber) was significantly lower than that of 3.30±0.31μm (microfiber) (Fig. 1C). In this study, we examined cell response on scaffolds with fiber diameters of 0.67±0.09μm and 3.30±0.31μm. It was observed that total cell number on the scaffolds was independent of fiber diameter and did not change significantly over time (Fig. 2A). However, significantly higher amount of both GAG (Fig. 2B) and collagen (Fig. 2C) were synthesized at Day 28 by fibroblasts cultured on scaffolds with a smaller fiber diameter. Additionally at Day 3, β1 integrin expression was down-regulated on the nanofiber scaffolds (Fig. 2D, $p<0.05$).

Discussion/Conclusions: In this study, fibroblast proliferation was found to be independent of fiber diameter, and this result is in agreement with those reported for NIH 3T3 mouse fibroblasts grown on aligned PLGA meshes (0.14-3.6μm)[6]. It is interesting to note that scaffolds with the smaller fiber diameter (0.67μm) supported significantly higher deposition of both GAG and collagen. The nanofiber group examined here exhibits a larger fiber diameter than that of the scar tissue found at the healing tendon site (46±11nm)[3], thus it is possible that unlike the microfiber group, the submicron fiber diameter scaffold more closely approximates the native tendon matrix which consists of fibrils ranging from 20 to over 280 nm[7]. It was also observed that fibroblasts on microfiber meshes (3.3μm) expressed a higher level of β1. Since the production of β1 and αv are associated with healing tendons and ligaments[8,9], the cells are possibly detecting the diameter difference between the meshes and native tissue, and mounting a repair response. Similar levels of α5 expression indicate that cells attach to both scaffolds equally[10]. Overall, the results of this study demonstrate that fiber diameter is an effective modulator of tendon fibroblast response and the functional significance of these observed changes will be investigated in our future studies.

References: [1] Vitale MA. J Shoulder Elbow Surg. 2007; [2] Itoi E. J Orthop Res. 1995; [3] Alaseirlis DA. Connect Tissue Res. 2005; [4] Dressler MR. J Orthop Res. 2002; [5] Moffat KL. Tissue Eng Part A 2009; [6] Bashur CA. Biomaterials 2006; [7] Silver FH. J Long-Term Effect Med Impl. 1992; [8] Harwood FL. Connect Tissue Res. 1998; [9]Schreck PJ. J Orthop Res. 1995; [10] Kaabeche K. J Cell Sci. 2005.

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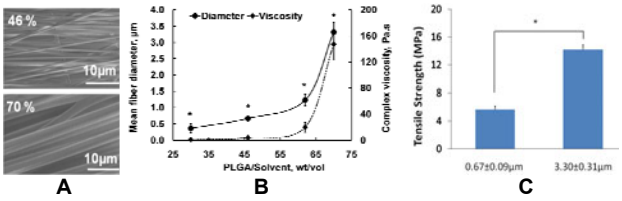

Figure 1. A) SEM of 46&70% PLGA fibers, B) Effect of concentration on fiber diameter and viscosity, C) Effect of fiber diameter on tensile strength. * indicates significance at $p<0.05$.

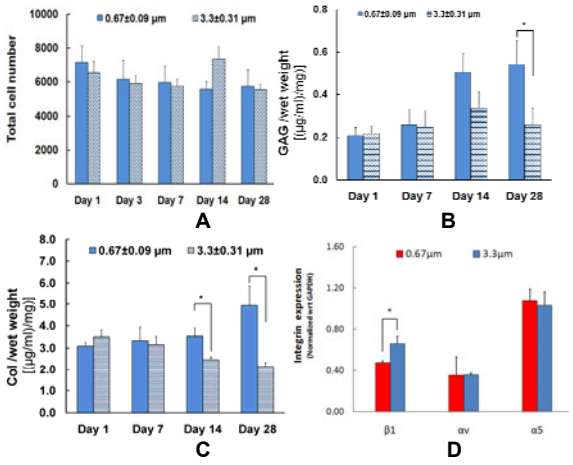

Figure 2. A) Proliferation of hRCFs, B) GAG, C) collagen synthesis, and D) integrin expression at Day 3. * indicates significance at $p<0.05$.