# Scaffold Fiber Diameter and Alignment Co-Regulate Tendon Fibroblast Response

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**Statement of Purpose:** The nano- and microstructure of tendons contribute to overall tissue integrity[1]. Structural characteristics of biomaterial scaffolds, namely fiber diameter and organization may serve as critical templating parameters for directing cell-mediated healing[2-5]. Cells respond to their environment through actin stress fibers and focal adhesions which activate downstream signaling pathways[6]. These signaling processes regulate cell functions including, adhesion, migration, morphology, proliferation, gene expression and differentiation. Thus the interactions between cells and their local environment will be important in tissue homeostasis and during wound repair. The **objective of this study** is to assess the impact of fiber diameter of unaligned nanofiber scaffolds as well as fiber orientation on cell response. It is **hypothesized** that both fiber diameter and organization can be used to guide tendon fibroblast adhesion, which will in turn influence cell proliferation, matrix deposition, and phenotypic expression. The elucidation of cell-biomaterial interactions is anticipated to provide critical insight towards optimization of scaffold design and inducing desired cell responses.

**Methods:** *Scaffold Fabrication:* Unaligned scaffolds were produced by electrospinning solutions of PLGA dissolved in DMF and acetone onto a stationary plate. Fiber diameter was modulated by varying the solvent ratio, polymer weight% (32, 43, 50), flow rate, needle gauge and distance to the collector. Aligned nanofibers were collected on a rotating mandrel. SEM was used to measure fiber diameter[2]. *Cells & Cell Culture:* Human rotator cuff fibroblasts (hRC-FB) were derived from explant culture and seeded on nanofibers (p3-5, 30,000 cells/cm$^2$), with monolayer as control. *Cell Response:* cell viability (n=3) (Live/Dead) and alignment (n=3)[7] were deteremined. DNA (n=5, PicoGreen) and collagen (n=5, hydroxyproline) were quantified and qRT-PCR (n=5) was performed. Active-Rac1 was quantified using an ELISA assay. Immunohistochemistry for actin and paxillin and matrix histology were performed. *Statistical Analyses:* A two-way ANOVA was performed and the Tukey-Kramer test was used for all pair-wise comparison at $p<0.05$.

**Results:** *Effects of Diameter*: Alignment analysis (MA=0, MVL=1, CSD=0) indicates that initially, micron fibers promote the greatest degree of cell alignment, albeit by Day 28 no significant differences was noted between groups (Fig.2). At day 1, a higher ($p<0.05$) RhoA expression was detected on 390nm fibers compared to all other groups, while Rac1 was upregulated on the 390nm vs. 740nm group. Active Rac1 measured via ELISA was greater on nanofiber compared to microfiber scaffolds. Higher cell number ($p<0.05$) and more intense matrix staining (H&E) were seen on nanofiber versus microfiber. *Effects of Alignment*: Adhesion differences were apparent between the aligned and unaligned (740nm) groups within 1 hour post seeding, with cells forming focal adhesions and polarized along the long axis of fibers (Fig.2). These difference persisted over time ($p<0.05$, Fig. 2). At day 1, while no difference was evident in RhoA and Rac1 expression due to alignment, Cdc42 was upregulated on aligned scaffolds. Active Rac1 was lower on aligned compared to the unaligned group ($p<0.05$).

**Discussion & Conclusions:** The results suggest that on the unaligned scaffolds fiber diameter regulates cell response, with the greatest cell alignment seen on microfibers, while cell growth and biosynthesis were the highest on the nanofibers. The upregulation of Rho GTPases on nanofiber compared to microfiber, indicates great cell mobility, as Rac1, RhoA and Cdc42 have been reported to work in a cooperative manner to promote cell movement, an important aspect of the wound healing process[8]. These observations suggest that the unaligned nanofiber scaffolds may resemble matrix in a state of injury thus stimulating cells migration and biosynthesis as part of the tissue repair process.

**References:** (Beck, *et al.* 2005, Erisken, *et al.* 2013, Bashur *et al.*, 2006, Moffat *et al.*, 2009, Subramony *et al.*, 2013, Giancotti *et al.*, 1997, Costa *et al.*, 2003, Nobes & Hall, 1999). **Acknowledgements:** NIH/NIAMS (AR055280, AR056459) Drs. M. Sheetz and T. Iskratsch (Columbia), Dr. J. Brown (PSU)

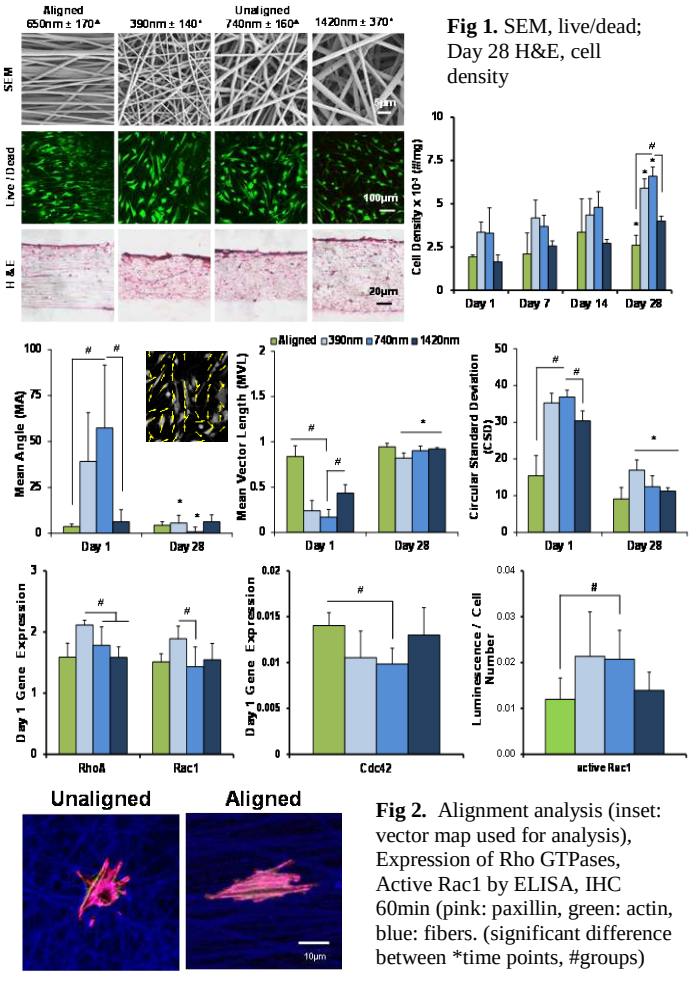


**Fig 1.** SEM, live/dead; Day 28 H&E, cell density

**Fig 2.** Alignment analysis (inset: vector map used for analysis), Expression of Rho GTPases, Active Rac1 by ELISA, IHC 60min (pink: paxillin, green: actin, blue: fibers. (significant difference between *time points, #groups)