Combined Treatment of a Tendon Gap with a Biomimetic Electrospun Scaffold, Stromal Cells and GDF5

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Statement of Purpose: The natural ability of tendons and ligaments to heal is sub-optimal with a high risk of failure in tension even with appropriate post-surgical therapy. Large tendon gap defects often must be reconstructed and augmented with grafts. Autografts and allografts are extensively used; however both pose potential risks. Regenerative strategies to repair tendon injury could overcome shortcomings and modulate a regenerate tendon that is biomechanically, biochemically and histologically similar to the native tissue.

We have previously shown that poly (DL-lactide-co-glycolide) (PLAGA) polymers fabricated into non-woven fibers (Ø=800μm) that dimensionally mimic the tendon collagen bundles can promote proliferation and support infiltration of primary adipose- derived stromal cells (ADSCs), and simulate ADSCs to differentiate into a tenocyte-like phenotype. Scaffold mechanical properties are comparable to human flexor tendon. We have characterized the healing response in a rat Achilles tendon gap defect model that simulates a lacerated tendon and is bridged with an electrospun, non-woven scaffold delivering either ADCSs or surface immobilized Growth/Differentiation Factor-5 protein (GDF5). This combinatorial approach modulates the regenerative process to repair and remodel, and achieve functional tendon sooner. This work was performed at UVA.

Methods: Briefly, a 21% (w/v) PLAGA 65:35 in THF:DMF (3:1) was delivered at 3mL/hr using a blunt 18G needle. A positive DC current of 25kV applied to the needle initiated electrospinning, and fibers were deposited onto a grounded mandrel (2.5mm Ø) rotating at 100rpm at an air gap of 30cm.

GDF5 was immobilized by covalent amide bonds to PLAGA. Non-woven scaffolds were treated with 2mM KOH for 30mins, and functionalized with GDF5 by EDC/NHS chemistry for 4hr at 25°C. Completely lacerated F344 rat Achilles tendon was not repaired or augmented with a tubular scaffold (approved by IACUC). In the augmented groups, the scaffold was seeded with ADSCs overnight or immobilized with GDF5, and tissue retrieved at 4 and 8 weeks post-surgery.

In vitro, the mechanical properties and cellular response of ADSCs seeded on GDF5 composite scaffold was analyzed. *In vivo*, the repaired tendon gap defect was characterized using real-time PCR to measure gene expression of tendon phenotype and ECM markers. Tendons were tested in tension to determine mechanical properties, and were analyzed histologically.

Results: (*In Vitro*) Bioactivity of the immobilized GDF5 protein was verified by cell proliferation and gene expression assay. 100ng/mL GDF5 has been previously reported to significantly up-regulate tendon phenotype and ECM markers in *in vitro* culture. Cell number increased, and gene expression of neo-tendon (scleraxis) and mature tendon (tenomodulin) phenotype marker in ADSCs seeded on GDF5 composite scaffold was equivalent to treatment with 100ng/mL GDF5 supplemented media. The mechanical properties of the scaffold following GDF5 protein immobilization are comparable to the scaffold alone.

(*In Vivo*) Type I collagen (col 1) is the most abundant tendon ECM component, and responsible for its tensile properties. The non-woven biomimetic scaffold bridged the tendon gap defect and delivery of ADSCs or immobilized GDF5 enabled an earlier increase in col1α1 gene expression beginning at 4 weeks. Tenomodulin (Tnmd) is a marker of the mature tendon phenotype, and gene expression was increased (4 and 8 weeks) in the ADSCs and GDF5 immobilized groups, suggesting earlier maturation of cells within the regenerate tissue (**Fig 1A**).

The scaffold stabilizes the repair tissue, and is critical to prevent rupture during the reparative phase. The new tendon tissue was stronger and tensile modulus increased significantly when the gap defect was repaired with a non-woven graft seeded with ADSCs (8 weeks, **Fig. 1B**).

At 8 weeks, the scaffold was completely replaced with new tendon tissue. Immobilization of GDF5 yielded a regenerate tendon having high collagen concentration (**Fig. 1C**), and a composition similar to native tendon identified by a higher proportion of large diameter collagen fibrils suggesting faster tissue remodeling and functional recovery.

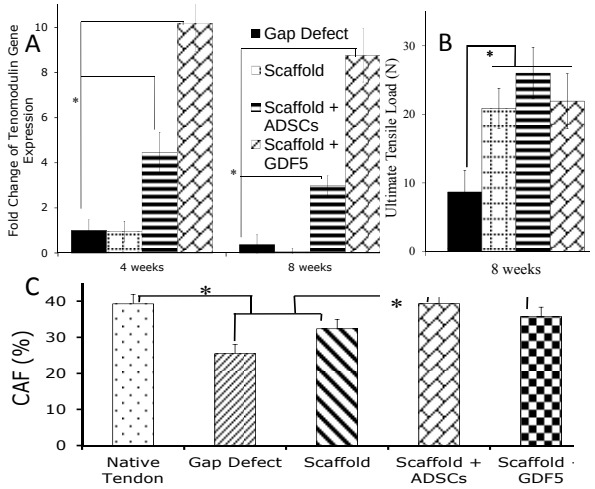

Figure 1. (A) Tnmd gene expression, (B) Ultimate load, and (C) Collagen concentration of the regenerate tissue.

Conclusions: GDF5 tethered onto the fiber surface is biologically active and equivalent to 100ng/mL GDF5 concentration. Complete regeneration of a large tendon gap defect is reported evidenced by early recovery of tendon function and tissue composition. Reconstruction of large tendon defect with stromal cells or immobilized GDF5 resulted in robust ECM synthesis and early tissue maturation. Regenerate tendon is similar to native tendon tissue.