Extracellular Matrix Mimicking Tubular Scaffolds: Accelerated Achilles Tendon Gap Defect Regeneration

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Statement of Purpose: Improving the functional outcome of lacerated tendons with a combination of surgical intervention and controlled therapy is the focus of the current tendon repair paradigm. The repair is a slow process with variable outcomes and complications that lead to failure to regain full functionality. Current approaches use either an autograft or a freeze-dried allograft to augment and reestablish a gap defect. Biodegradable polymers are an attractive alternative to overcome both the shortage of autografts and the immunogenicity of allografts. The scaffold designed aims to dimensionally mimic the collagen bundle size evident in native tendon architecture. The poly (DL-lactide-*co*-glycolide) (PLGA) scaffold fabricated by electrospinning technique has high porosity and large pore size which promotes adhesion, proliferation and infiltration of adipose derived stromal cells (ADSCs). They have customizable mechanical properties and can be fabricated into various shapes and sizes. Tubular PLGA scaffolds bridge Achilles tendon gap defect and accelerate neotendinogenesis with improved mechanical strength and organization as compared to controls.

Methods: PLGA 65:35 was electrospun into a sheet or a tubular scaffold composed of fibers. Scaffolds which mimic the collagen bundle size of native tendon are selected and further characterized. Stromal cells isolated from rat inguinal fat pad are maintained in culture up to three passages and seeded onto the selected scaffold to characterize proliferation. Concentration kinetics of GDF5 protein to modulate gene expression of ECM components and markers of tendon phenotype in ADSCs was evaluated by real time – PCR. For *in vivo* studies, tubular scaffolds were used to bridge an Achilles tendon gap defect in Fischer 344 rats (approved by IACUC). Repair at 4 and 8 weeks post-surgery was evaluated using histological techniques, gene expression and mechanical testing.

Results: PLGA 65:35 scaffolds are composed of fibers having diameter 500 – 1100nm. They are 48% porous with a mean pore diameter of 13.3µm. Confocal microscopy shows ADSCs adhered to the electrospun scaffolds with extending processes, and significantly higher cell numbers were quantified on the scaffolds over time. Gene expression of ECM and tendon phenotype markers was significantly upregulated in ADSCs on treatment with GDF5 protein. 100ng/mL GDF5 protein concentration was most potent with increased collagen Type I and scleraxis (neotendon marker) gene expression. The tensile stress of the scaffold is 80MPa. Tubular scaffolds used to bridge the tendon gap defect (Fig. 1) were present at 4 weeks post-surgery with cells growing around and into the lumen of the scaffold. At 8 weeks, the polymer had almost completely resorbed and small remnants were evident in a few of the animals. H&E staining at 8 weeks, showed significant cellular alignment in the repair tissue (Fig. 2A) and Masson's Trichrome (Fig. 2B) showed intense collagen staining and alignment suggesting that augmentation with a nanofiber PLGA scaffold can promote cellular organization and extracellular matrix deposition. Gene expression analysis of the regenerate tendon showed that augmentation with the tubular scaffold leads to an increase in the expression of the genes for collagen Type I and of the neotendon marker scleraxis (Fig. 2C). Tensile testing at 8 weeks showed increased modulus and strength in repairs bridged with the tubular scaffold. At 8 weeks, the regenerate tissue in the augmented tendon had strength comparable to the fabricated scaffold in vitro.

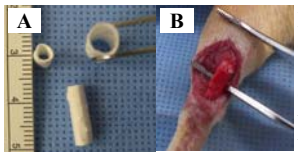

Fig. 1 (A) PLGA scaffold composed of nonwoven electrospun fibers can be fabricated into tubes of different diameters. (B) Achilles tendon gap defect augmented using tubular electrospun scaffolds.

Conclusions: The electrospun tubular PLGA 65:35 scaffold composed of fibers which mimic the collagen bundle size promotes the healing of a critical sized Achilles tendon defect. Our data shows that the regenerate tendon tissue is mechanically superior to the controls. Additionally, the scaffold is completely cellularized, replaced with neotendinous tissue containing extracellular matrix with organized collagen bundles. The degradation rate and mechanical properties of the tubular scaffold can be tailored for optimum healing with the potential for development toward clinical uses. Future studies will investigate electrospun tubular scaffolds that are tailored to deliver stromal cells and growth factors locally to modulate and enhance repair and to improve tendon function.

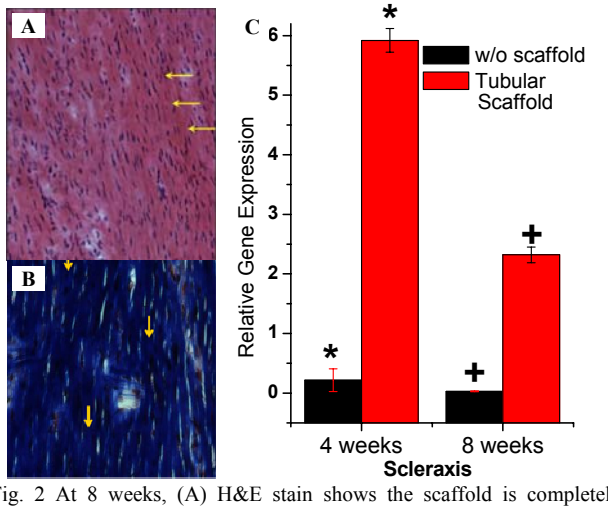

Fig. 2 At 8 weeks, (A) H&E stain shows the scaffold is completely degraded and replaced by tendinous tissue with extensive cell alignment, (B) Masson's Trichrome stain shows dense collagen bundles in repair tissue bridged with tubular scaffold, (C) Scleraxis gene expression. At 4 and 8 weeks, Scx expression increased significantly in Achilles tendon repairs with the tubular scaffold. (n=8, $p<0.05$).