Development, Characterization and Cell-Seeding of a Novel Biocompatible Scaffold for Tendon and Ligament Reconstruction

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Statement of Purpose: An ideal tendon scaffold would be: 1) naturally-derived from allogeneic material, 2) decellularized to decrease inflammatory potential and host immune response, 3) biocompatible, 4)characterized by sufficient porosity/micro-architecture to allow seeding/infiltration of the patient's own cells prior to/after implantation, and 5) distinguished by sufficient biomechanical integrity to withstand rehabilitation until complete remodeling has occurred. The tissue-engineered tendon scaffold has the potential to significantly improve the treatment of tendon and ligament injuries, especially those associated with tumor, trauma, and congenital deficiencies where autograft or allograft tissue might not be available in sufficient quantity for reconstruction.[1] We hypothesize that tendon can be engineered to enhance host cell infiltration and remodeling. The goal of this study was to employ a simple process that combines decellularization and chemical oxidation to decellularize and modify the dense architecture of the tendon in order to expose the underlying substructure. We expect that this treatment will result in a more porous graft that retains biomechanical properties after modification, is biocompatible *in vitro*, and possesses negligible inflammatory potential *in vivo*. We also expect cells to readily infiltrate the graft *in vitro and in vivo*.

Methods: Flexor digitorum profundus (FDP) tendons were harvested from the long digit of 56 day old Leghorn chickens. Tendons were trypsinized, then decellularized and oxidized using an aqueous solution containing 2% Triton X-100 and 1.5% peracetic acid for 4 hours. The tissue was then rinsed several times with distilled, deionized water. DNA content was determined pre- and post-decellularization/oxidation. Cellularity and micro-architecture were assessed using light microscopy and scanning electron microscopy, respectively. *In vitro* biocompatibility was assessed using the MTS™ (Promega, Madison, Wisconsin) and Neutral Red assays.[2] For mechanical testing, fresh-frozen FDP tendons and decellularized and oxidized FDP tendon scaffolds were cut into "dogbones" with a custom punch and placed on an uniaxial load frame for biomechanical analysis. Cell seeding was performed *in vitro* and assessed microscopically. Cell infiltration *in vivo* was also qualitatively assessed after subcutaneous implantation in a murine model.

Results/Discussion: Histology: Abundant cellular material, specifically nuclear material, was evident after hematoxylin and eosin (H&E) staining of fresh-frozen leghorn flexor digitorum profundus tendon prior to decellularization. The micro-architecture via SEM was also extremely dense, with little porosity. After oxidation and decellularization, no nuclear material was evident. In addition, scanning electron microscopy revealed significant differences in the micro-architecture following oxidative treatment. DNA Content: DNA content after treatment (0.0701 μg DNA / mg tissue dry weight, n=8) was decreased, compared to that of untreated tendons (0.3077 μg DNA / mg tissue dry weight, n=16), $p<0.001$. *In Vitro* Biocompatibility: Mitochondrial activity determined using the MTS™ for the FDP tendon scaffold (1.23 +/- .155, n=10) differed significantly from the positive control (1.654 +/- .179, n=10), ($p<0.001$). However, cell viability determined by the Neutral Red assay for the FDP tendon scaffold (.204 +/- 0.044, n=10) was not significantly different from the positive control (.231 +/- 0.104, n=10), ($p=0.455$). Mechanical testing: The elastic modulus was reduced in the tendon scaffolds (2.68 MPa) to 77% of that observed for fresh-frozen tendons (3.50 MPa). The FDP tendon scaffolds possessed a maximal stress (MPa) at break that was 74% (33.04 MPa) of that observed for fresh-frozen tendons (44.53 MPa). These differences were not statistically significant, $p>0.05$. *In Vitro* Cell Seeding and *In Vivo* Host Cell Infiltration: Cell penetration into regions normally occupied by cells in native tissue, i.e. the interfascicular regions of the tendon was observed. Host cell infiltration by fibroblast-like cells was also observed as early as 3 days after implantation subcutaneously in an immune competent mouse. A qualitative increase in host cell infiltration was observed at 21 days. No evidence of a gross inflammatory reaction or capsule formation surrounding the implanted scaffold was observed at the time of explantation, either grossly or microscopically.

Conclusions: FDP tendon can be processed to generate a tissue-engineered scaffold that: 1) has significantly decreased DNA content quantitatively, as well as decreased/absent cellular material histologically, 2) is biocompatible in vitro 3) has significant alterations in the micro-architecture, specifically porosity, which might improve cell-seeding and targeted host-cell infiltration and ultimately, graft incorporation and remodeling, 4) retains approximately 75% of the biomechanical properties of fresh-frozen FDP tendon, 5) is successfully seeded with cells *in vitro*, and 6) is readily infiltrated by fibroblast-like host cells *in vivo* as early as 3 days.

References: 1. Chang J. J Hand Surg. 2006;31A:349-3582 2. Piente J-L. J Biomed Mater Res 2001;55:33-39.