INTRODUCTION:
Types I and II collagens are extremely important building blocks in growth and development as well as orthopaedic tissue repair. To date, the field has sought to understand transcriptional and post-translational modifications to the collagen molecule during development. Less is known about the genetic and molecular basis behind tendon and insertion healing and its relationship to repair biomechanics. In particular, investigators still do not fully understand the patterns of collagen I and II gene expression leading to protein synthesis and repair stiffness in healing tendon tissues. In this study, we created a defined injury in the patellar tendon middesstance in a specialized doubly transgenic murine model to monitor changes in both Col 1 and Col 2 expression. We hypothesized that injury would create local, intense and rapid changes in Col 1 expression with no changes in Col 2 expression. We also hypothesized that these changes would precede increases in biomechanical stiffness and strength.

METHODS:
Subjects: The mouse containing the transgene pOBCol3.6GFPtpz with a 3.6 Kb fragment of the rat col 1a1 promoter enhancer sequence and GFP-T was acquired courtesy of David Rowe, U. Conn Health Center. GFP-T expression in these transgenic mice is evident in skin and tendon as well as osseous tissues [1]. Plasmid pcoll2-ECFP was derived by replacing the β-gal gene with ECFP (Clontech, Palo Alto, CA) in the expression gene containing the mouse type II collagen promoter and enhancer [2] (provided by W. Horton, Northeastern Ohio College of Medicine). This pcoll2-ECFP construct was injected into mice blastocysts. Mice transgenic for either pOBCol3.6GFPtpz or pcoll2-ECFP were then bred to produce doubly transgenic animals. No apparent phenotypic differences were observed between the doubly and non-transgenic mice. A total of forty-two 10 week old subjects were assigned for surgery. Twelve of these were evaluated for gene expression at 1, 2, and 4 weeks (n=4 each) and another 18 for biomechanics at 4 and 6 weeks (n=9 each). Twelve others served as non-operative controls.

Surgical Procedure: The study was approved the institution’s IACUC. Each animal was initially anesthetized with 5% isoflurane and maintained under a 3% flow during surgery. Both hind limbs were exposed under loupe magnification and a central-third punch defect was created in one tendon as previously described [3]. The contralateral limb was used as a sham operation. All animals were allowed to ambulate and feed ad lib after surgery.

Histologic Studies: Each mouse was euthanized under standard protocol, and both knees were harvested to evaluate gene expression. Knee joints were fixed with 4% paraformaldehyde overnight, and then allowed to decalify with a 5M EDTA/PBS solution for 7 days. Each was then soaked in 30% sucrose followed by OCT and then frozen on dry ice. Limbs were then sectioned in the sagittal plane (25μm thick) to visualize the tendon injury site and adjacent tissues. Sections were examined with a Zeiss inverted fluorescent microscope with filters for GFP-T (Col 1) and ECFP (Col 2) fluorescence. Additional filters permitted detection of any autofluorescence.

Biomechanical Evaluation: The patella was held in place with a custom-designed cone-shaped fixture (design courtesy of Dr. Soslowsky at U Penn). Each specimen was immersed in 37°C saline bath, preloaded to 0.02 N, preconditioned for 50 cycles at 1% strain, and then failed in tension at 0.1%/s. Resulting force-displacement curves permitted us to compute stiffness in the linear region and maximum force. The effects of time and injury were assessed statistically using student’s paired t-test (p<0.05).

RESULTS:
Histology: Collagen 1 gene expression (col 1) is at baseline at 1 week post-surgery compared to sham and non-operative controls and then increases by two weeks before declining at 4 weeks (Fig 1A-D) showing injury vs. sham). At one week, Col 1 is not expressed in an abundant nature in the swollen tendon. At 2 weeks, the Col 1 expression became intense and localized to the tissues immediately surrounding the repair site. At 4 weeks, there was less Col 1 gene expression than at 2 weeks, and healing tendon fibrils were found in an organized fashion.

Collagen 2 was not present in the repair process of the healing central third patellar tendon injury.

Biomechanics: Repair stiffness and maximum force were 32% and 40% of sham control values at 4 weeks post injury (p<0.05) (Fig. 2). Six weeks post-surgery, stiffness and maximum force had increased but were still significantly less than corresponding sham values (68% and 52% of sham, respectively; p<0.05; Fig. 2).

REFERENCES:

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