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Murine patellar tendon biomechanical properties and regional strain patterns during natural tendon-to-bone healing after acute injury

Steven D. Gilday [a,b,*], E. Chris Casstevens [c], Keith Kenter [c], Jason T. Shearn [a], David L. Butler [a]

[a] Biomedical Engineering Program, University of Cincinnati, Cincinnati, OH, United States
[b] Medical Scientist Training Program, University of Cincinnati, Cincinnati, OH, United States
[c] Department of Orthopaedic Surgery, University of Cincinnati, Cincinnati, OH, United States

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ABSTRACT

Tendon-to-bone healing following acute injury is generally poor and often fails to restore normal tendon biomechanical properties. In recent years, the murine patellar tendon (PT) has become an important model system for studying tendon healing and repair due to its genetic tractability and accessible location within the knee. However, the mechanical properties of native murine PT, specifically the regional differences in tissue strains during loading, and the biomechanical outcomes of natural PT-to-bone healing have not been well characterized. Thus, in this study, we analyzed the global biomechanical properties and regional strain patterns of both normal and naturally healing murine PT at three time points (2, 5, and 8 weeks) following acute surgical rupture of the tibial enthesis. Normal murine PT exhibited distinct regional variations in tissue strain, with the insertion region experiencing approximately 2.5 times greater strain than the midsubstance at failure (10.80 $\pm$ 2.52% vs. 4.11 $\pm$ 1.40%; mean $\pm$ SEM). Injured tendons showed reduced structural (ultimate load and linear stiffness) and material (ultimate stress and linear modulus) properties compared to both normal and contralateral sham-operated tendons at all healing time points. Injured tendons also displayed increased local strain in the insertion region compared to contralateral shams at both physiologic and failure load levels. 93.3% of injured tendons failed at the tibial insertion, compared to only 60% and 66.7% of normal and sham tendons, respectively. These results indicate that 8 weeks of natural tendon-to-bone healing does not restore normal biomechanical function to the murine PT following injury.

1. Introduction

Tendon injuries occur in a diverse patient population and commonly result in pain, disability, and significant healthcare costs (United States Bone and Joint Initiative, 2011). Frequently injured tendons include the Achilles, patellar, and quadriceps tendons of the lower extremity, the biceps and rotator cuff tendons of the upper extremity, and the flexor and extensor tendons of the hands (Clayton and Court-Brown, 2008). Treatment of such injuries routinely requires reattachment of a ruptured tendon to its bony insertion, but this presents a challenge due to the extreme difference in material properties between soft and hard tissue. To cope with this material mismatch, the uninjured tendon-to-bone insertion site, also known as the enthesis, exhibits a gradual transition between the compliant tendon and the much stiffer bone via a fibrocartilaginous transition region (Benjamin et al., 2002). Gradations in matrix composition, collagen alignment, cell phenotype, and mineralization (Genin et al., 2009; Thomopoulos et al., 2003; Thomopoulos et al., 2006) help facilitate optimal force transmission while also dissipating potentially damaging interfacial stress concentrations between these mechanically dissimilar materials (Liu et al., 2011; Shaw and Benjamin, 2007). Unfortunately, once disrupted, the insertion site does not regenerate its complex natural architecture and is instead replaced by scar tissue, resulting in a mechanically inferior interface that is susceptible to further injury (Galatz et al., 2006; Kinneberg et al., 2011; Newsham-West et al., 2007; Rodeo et al., 1993).

Functional tissue engineering (FTE), an evolving discipline which emphasizes the restoration of normal mechanical function in damaged load-bearing tissues, has been proposed as a promising alternative to traditional tendon repair strategies (Butler et al., 2000; Guilak et al., 2003). Fundamental to the FTE paradigm is the need to measure the biomechanical properties of normal and naturally healing tissues under physiologic as well as failure loads in order to establish quantitative benchmarks against which tissue-engineered repairs can be compared (Butler et al., 2000). Working towards this goal, our research group determined that in vivo patellar tendon (PT) forces in the rabbit (Juncosa et al., 2003) and goat (Korvick et al., 1996) reached 21% and 40% of normal PT failure force, respectively, during simulated activities of daily living (ADLs). Then, using these

* Corresponding author at: Biomedical Engineering Program, 827 Engineering Research Center, 2901 Woodside Drive, Cincinnati, OH 45221, United States. Tel.: +1 513 253 9351; fax: +1 513 556 4162.
E-mail addresses: gildays@mail.uc.edu, sdgilday@gmail.com (S.D. Gilday).

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physiologic force thresholds as mechanical benchmarks, we evaluated the relative success of various tissue-engineered tendon repairs in a full-length rabbit PT defect model (Butler et al., 2008). Although this FTE approach did yield improved mechanical outcomes compared to natural healing alone, the vast majority of our tissue-engineered PT repairs still fail prematurely at the distal insertion, indicating a need for better strategies to stimulate tendon-to-bone healing.

More recently, our attempts to regenerate functional tendon–bone interfaces have necessitated moving from large animal models such as the rabbit to the more genetically tractable mouse. The availability of transgenic and knockout mice has permitted detailed studies of PT enthesis development (Liu et al., 2012; Liu et al., 2013; Sugimoto et al., 2013) and PT natural healing (Dyment et al., 2012; Dyment et al., 2013; Scott et al., 2011). Lower costs and higher throughputs also make murine models an attractive option for screening the efficacy of novel therapeutic treatments for tendon-to-bone healing before scaling up to more clinically relevant model systems. However, applying the FTE paradigm to PT repair in the mouse has proven difficult because the peak in vivo forces in the murine PT are unknown and the mechanical properties of native murine PT, specifically the regional (insertion site vs. midsubstance) differences in tissue strains during loading, have not been adequately described. Furthermore, the biomechanical outcomes of natural tendon-to-bone healing after murine PT enthesis injury have not been well characterized.

Thus, the objective of this study was to analyze the global biomechanical properties and regional strain patterns of (1) *normal* murine PT and (2) *naturally healing* murine PT at three time points (2, 5, and 8 weeks) following acute surgical rupture of the tibial enthesis. We hypothesized that normal murine PT would exhibit regional variations in tissue strain, with the more compliant insertion region experiencing larger strain than the stiffer midsubstance. We also hypothesized that at all time points following enthesis injury, healing tendons would exhibit reduced global biomechanical properties and increased strain in the insertion region compared to contralateral shams, resulting in failure initiation at the insertion site.

2. Materials and methods

2.1. Experimental design

Patellar tendon dimensions, structural and material properties, regional strain patterns, and failure locations were assessed at three different post-injury time points (2, 5, and 8 weeks) in a cohort of 30 twenty-week-old (20.3 ± 0.5 weeks; mean ± SD) male CD-1 wild-type mice. Twenty-week-old mice were chosen for this study because they are skeletally mature adults whose patellar tendons are large enough to allow for the creation of standardized, repeatable surgical injuries and the biomechanical testing of normal and healing tissues in vitro. The study time points were carefully selected in order to capture both the proliferative and remodeling phases of tendon healing and to keep consistent with our group's previous work on natural healing of murine PT (Dyment et al., 2012). Following surgical injury, naturally healing tendons ($n=10$ per time point) were directly compared with contralateral shams ($n=10$ per time point). Inter-animal comparisons were also made using a separate group of normal, unoperated patellar tendons ($n=10$) from healthy twenty-week-old male CD-1 mice.

2.2. Murine patellar tendon injury model

All murine surgeries were performed by one coauthor (ECC) and were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Mice were anesthetized with 4% isofluorane, subcutaneously injected with 1 mg/kg buprenorphine, and both hind limbs were shaved and aseptically prepped. Using surgical loupes (2.5 ×), small (0.5–1 cm) longitudinal skin incisions were made to expose the PT in each limb. An acute surgical injury was then created in the left PT while the contralateral PT was subjected to a sham procedure.

2.2.1. Surgical injury (Fig. 1A)

Using a previously described surgical technique (Dyment et al., 2012), two full-length longitudinal incisions were created in the left PT in order to isolate the central-third portion of the tendon from adjacent medial and lateral struts. The central-third of the PT was then transected at its distal insertion into the tibia. Any remaining soft tissue at the insertion site was removed with microsurgical scissors and the enthesis was further disrupted by using a small jigsaw blade to create a shallow bony defect. Care was taken not to damage the intact struts. The tendon's proximal patellar insertion was also left intact. The transected central-third was laid back in its normal anatomic position between the struts with the distal end in close proximity to its original insertion site on the tibia, but no attempt was made to physically reattach the tendon tissue to the bone.

2.2.2. Contralateral sham (Fig. 1B)

The central-third of the right PT was isolated from the struts as described above but was not transected at the distal insertion. Thus, sham-operated tendons retained a structurally intact tendon–bone interface at both the tibial and patellar ends.

In both injured and sham limbs, skin incisions were closed with 5–0 prolene suture. Mice were allowed full range of motion and unlimited cage activity immediately following surgery. No gait alterations or behavioral changes were noted as a result of the surgical procedure. At the designated post-surgical time point (2, 5, or 8 weeks), mice were euthanized by carbon dioxide asphyxiation and frozen at −20 °C to await biomechanical testing.

2.3. Biomechanical testing and analysis

On the day of testing, murine hind limbs were thawed and dissected to expose the PT. After noting gross morphological appearance, the central-third of each PT

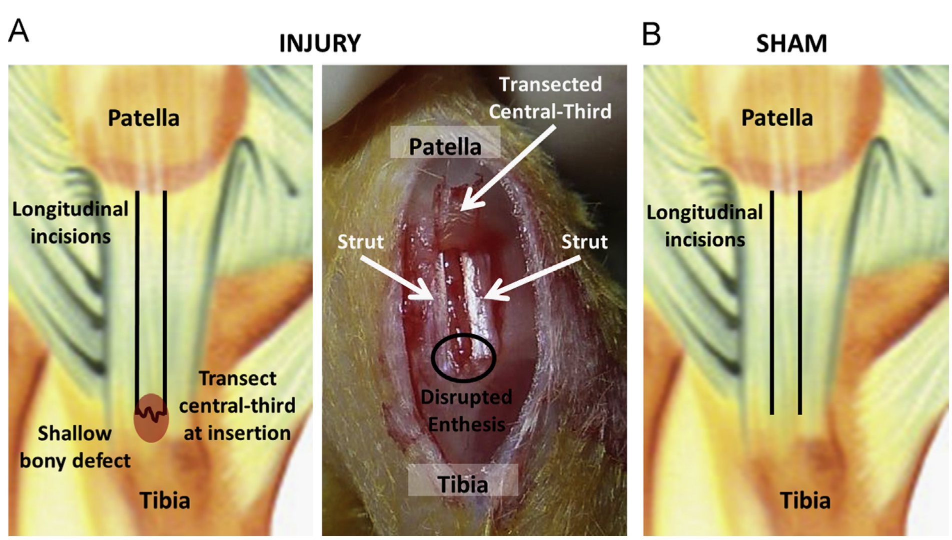

Fig. 1. Murine patellar tendons were subjected to either an acute surgical injury or a contralateral sham procedure. To create the surgical injury (A), two full-length longitudinal incisions were made to isolate the central third of the tendon, which was then transected at the tibial insertion. A shallow bony defect was created in the tibia and the transected central-third was laid back in its normal anatomic position between the medial and lateral struts to facilitate tendon-to-bone healing. For the contralateral sham procedure (B), longitudinal incisions were made to isolate the central third of the tendon, but the tibial insertion was left intact.

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