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Short communication

## Quantitative comparison of three rat models of Achilles tendon injury: A multidisciplinary approach



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## ABSTRACT

The Achilles tendon, while the strongest and largest tendon in the body, is frequently injured. Inconclusive evidence exists regarding treatment strategies for both complete tears and partial tears. Well-characterized animal models of tendon injury are important for understanding physiological processes of tendon repair and testing potential therapeutics. Utilizing three distinct models of rat Achilles tendon injury, the objective of this study was to define and compare the effects and relative impact on tendon properties and ankle function of both tear severity (complete tear versus partial tear, both with post-operative immobilization) and immobilization after partial tear (partial tear with versus without immobilization). We hypothesized that a complete tear would cause inferior post-injury properties compared to a partial tear, and that immediate loading after partial tear would improve post-injury properties compared to immobilization. All models were reproducible and had distinct effects on measured parameters. Injury severity drastically influenced tendon healing, with complete tear causing decreased ankle mobility and tendon mechanics compared to partial tears. One week of plantarflexion immobilization had a strong effect on animals receiving a partial tear. Tendons with partial tears and immobilization failed early during fatigue cycling three weeks post-injury. Partial tear without immobilization had no effect on ankle range of motion through dorsiflexion at any time point compared to the pre-surgery value, while partial tear with immobilization demonstrated diminished function at all post-injury time points. All three models of Achilles injury could be useful for tendon healing investigations, chosen based on the prospective applications of a potential therapeutic.

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### 1. Introduction

The Achilles tendon, the strongest and largest tendon in the body, is frequently injured. After surgical repair, patients risk re-rupture and can have long-term deficits in function, with the rate of return to pre-injury activity as low as 16% (Barfod et al., 2014). Current recommendations from the American Academy of Orthopedic Surgeons regarding surgical repair vs. non-surgical repair are primarily based on patient preference and/or surgical risk factors, and evidence for the use of casts for either operative or non-operative patients is inconclusive (Chiodo et al., 2010). Some clinical evidence supports the use of early weight-bearing and mobilization (Kangas et al., 2003; Maffulli et al., 2003). We

have recently published several studies supporting early return to activity using a rat model of complete Achilles rupture (Freedman et al., 2016; Freedman et al., 2017A; Freedman et al., 2017B). Little clinical data exists regarding best practices for treating partial tears, but treatment is typically non-operative (Filardo et al., 2010; Hartgerink et al., 2001; Hsu et al., 2017).

Animal models of tendon injury are essential for understanding physiological processes of tendon repair and for testing the effects of potential therapeutics (Hast et al., 2014). We have adapted and utilized three rat models of Achilles tendon injury; first, a complete, full-thickness, full-width tear with surgical repair and post-operative immobilization (Freedman et al., 2016); second, a full-thickness, partial-width tear without surgical repair and with post-operative immobilization; and third, a full-thickness, partial-width tear without surgical repair or post-operative immobilization (Beason et al., 2012; Boorman-Padgett et al., 2018). The full-thickness model encompasses several aspects of a surgically repaired full-thickness Achilles tear, including blunt transection

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(using the back of a scalpel blade) rather than sharp to better replicate the “brush-like” tendon end pathology (Barfred, 1973), as well as clinically relevant suture repair and use of plantarflexion immobilization (McCoy and Haddad, 2010; Kadakia et al., 2017). Clinical partial rupture has been defined as “visible disruption of collagen fibers within the tendon” (Allenmark, 1992) which is achieved in the partial tear model presented here.

However, comparisons of the effects of these injury models on tendon mechanics, ankle joint function, tendon histological properties, and heterotopic ossification have not previously been made. The objective of this study was to quantitatively define and compare the effects and relative impact on tendon properties and ankle function of the three Achilles tendon injury models. We hypothesized that animals receiving a complete tear would have inferior mechanical properties and ankle function compared to those receiving a partial tear, and that immediate loading after a partial tear would improve post-operative mechanical properties and ankle function compared to immobilized tendons. Through this study, we will comprehensively characterize these three Achilles tendon models for future use in the field.

## 2. Methods

### 2.1. Study design & surgical procedures

These studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (400–450 g, approximately 4–5 months;  $n = 144$ ) were used; this age corresponds with slow to no skeletal growth, most closely correlated with young adult human age range. Animals were divided into 3 groups; the first group ( $n = 48$ ; complete tear + immobilization, CT + IM) underwent full-thickness, blunt transection and modified Kessler repair of the right Achilles tendon (Fig. S1A) followed by one week of limb immobilization (Freedman et al., 2016, Fig. S1C). The second group ( $n = 48$ ; partial tear + immobilization, PT + IM) underwent full-thickness, partial-width transection of the right Achilles tendon (1.5 mm biopsy punch in central tendon which removes ~60% of tendon width, Fig. S1B) without repair followed by one week of limb immobilization (Boorman-Padgett et al., 2018). The third group ( $n = 48$ ; partial tear only, PT) underwent full-thickness, partial-width transection of the right Achilles tendon without repair. Animals were sacrificed at post-operative week 1, 3, or 6 ( $n = 16$ /group/time point). Time points were selected to represent timing of early, intermediate, and late rehabilitation strategies, as both temporal improvements and structural changes occur throughout this time period after a complete tear and repair (Freedman et al., 2017A). Study design is diagrammed in Supplemental Fig. 2.

### 2.2. In vivo functional evaluation

Animals in 6 week groups underwent longitudinal in vivo ambulatory and passive ankle joint mechanics assessments (Freedman et al., 2016). **Ankle range of motion and stiffness:** Passive ankle joint range of motion (ROM) and stiffness through plantarflexion and dorsiflexion were quantified using a custom torque cell and orientation device (Peltz et al., 2009) on anesthetized animals ( $n = 16$ /group) with LabView. Functional ankle joint properties (toe and linear ankle stiffness; ankle ROM) were evaluated in a blinded fashion using customized MATLAB software (Freedman et al., 2016; Fryhofer et al., 2016, Peltz et al., 2009). Data were collected pre-op for all groups, at 2, 3, and 6 weeks for CT + IM and PT + IM groups, and at 2, 4, and 6 weeks for PT group. Data were normalized to the average pre-op value for each group. **Quantitative ambulatory assessment:** Patterns of hindlimb ambulation were

quantified using an instrumented walkway, consisting of two load/torque cells and a dorsal view camera to provide non-invasive measures of limb function as described (Caro et al., 2014; Sarver et al., 2010). Data were analyzed to calculate ground reaction forces (lateral, braking, propulsion, vertical) and spatiotemporal parameters (step width, stride length, velocity) of the injured limb. Data was collected pre-op for all groups, at 3 and 6 weeks for CT + IM and PT + IM groups, and at 2, 4, and 6 weeks for PT group. Animals were weighed at the time of each assessment, and ground reaction forces were normalized to percent animal body-weight at that time. Data were then normalized to average pre-op values for each group.

### 2.3. Mechanical testing & $\mu$ CT

Animals assigned to mechanical testing ( $n = 10$ /group/time point) were frozen at  $-20\text{ }^{\circ}\text{C}$  and thawed for dissection prior to tendon cross-sectional area measurement using a custom laser device. Mechanical testing was performed as described (Freedman et al., 2016; Fryhofer et al., 2016) using load-controlled fatigue testing including preconditioning, stress relaxation, dynamic frequency sweeps, a ramp to 35 N, and fatigue cycling until failure. The 5–35 N cyclic loading protocol corresponds with 10–70% of the ultimate failure load of an uninjured rat tendon, chose to recreate the theorized range of in vivo loading endured by the Achilles tendon during hopping (Fukashiro et al., 1995) as well as previous cyclic loading studies (Wren et al., 2003). The ramp to 35 N is considered the initial part of the cycling protocol and is used to calculate quasistatic tendon properties. Images were acquired during testing to track strain optically (Freedman et al., 2014). Fatigue parameters were calculated at 5, 50, and 95% of fatigue life (defined by corresponding percentage of cycles to failures) to describe the three phases of fatigue behavior. If tendons did not withstand the initial ramp to begin fatigue testing, fatigue parameters were not calculated. Post-test, tendons were scanned using  $\mu$ CT (Scanco Medical  $\mu$ CT 35) at 55 kVp with a 21  $\mu\text{m}$  resolution to assess for presence of heterotopic ossification (HO). Three-dimensional standard microstructural analysis was performed to determine the geometric trabecular bone volume/total volume (BV/TV) fraction as well as bone mineral density (BMD). While a portion of the calcaneus was scanned for tissue orientation, it was not included in the quantification; only heterotopic calcifications in the tendon were measured.

### 2.4. Histology

Achilles tendons were harvested at animal sacrifice ( $n = 6$ /group/time point), processed with paraffin, sectioned at 7  $\mu\text{m}$ , stained with hematoxylin & eosin, and analyzed as described (Thomopoulos et al., 2003; Tucker et al., 2017) including semi-quantitative, blinded grading for cell density and nuclear shape as a proxy for overall cell shape, and quantitative polarized light microscopy for collagen alignment in the injury region ( $n = 2$ –3 ROI per sample which were individually assessed and then averaged) (Gimbel et al., 2004). Images were graded between 1 and 3 for nuclear shape (1 = more spindle shaped and 3 = more rounded shape) and between 1 and 4 for cellularity (1 = fewer cells and 4 = higher number of cells). A grading scale was created within the set of all images from all models at each time point, and at least two images per region were assessed per specimen. To define the grading scale, images were blinded and sorted from least to most number of cells (for cellularity) and most spindle shaped to roundest (for nuclear shape). The ranked images were divided into three or four equal groups (Grades 1–3 or 1–4), and the median image from each group was assigned as a “standard” for the corresponding grades for each time point.

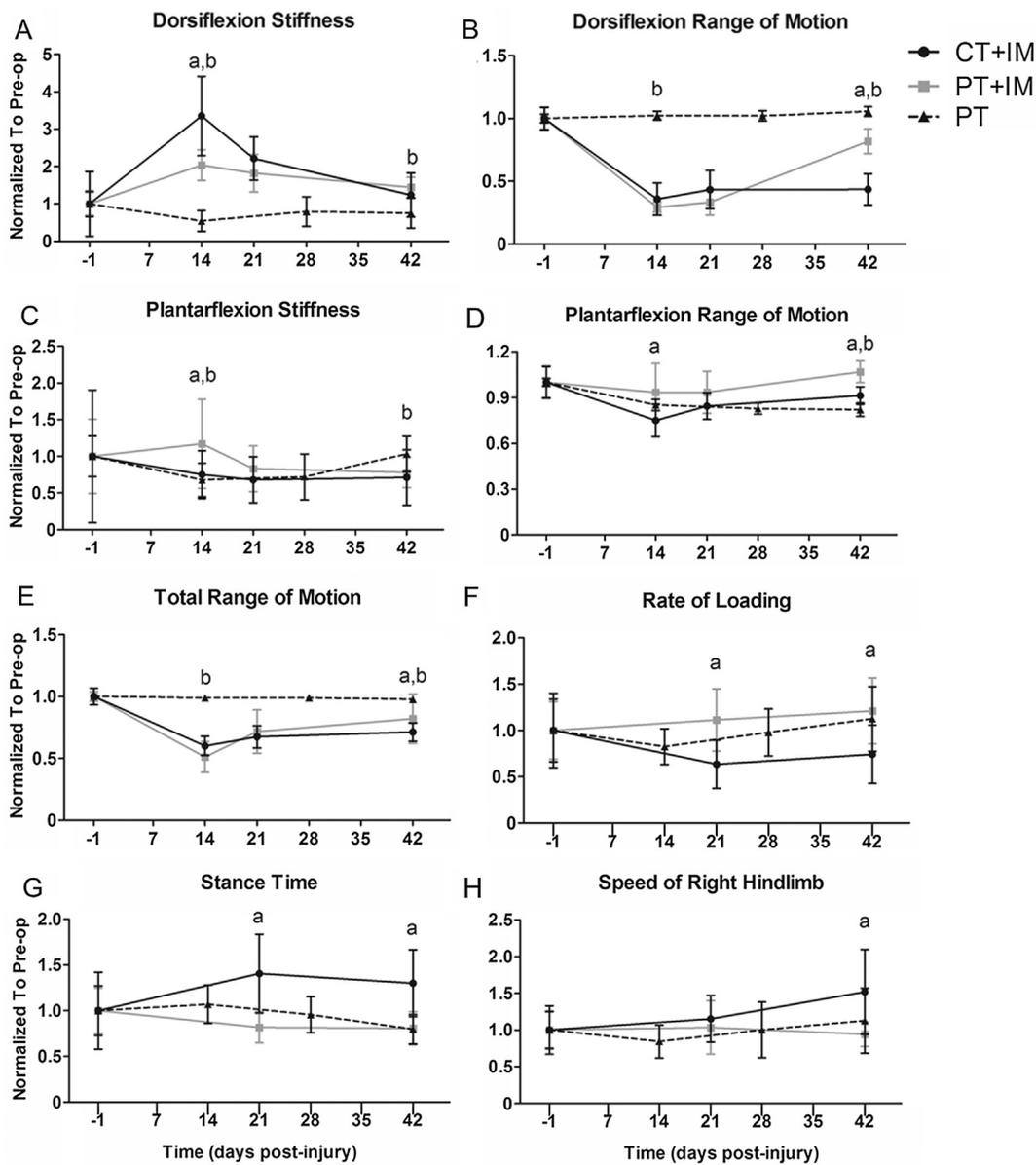
2.5. Statistics

Statistical comparisons were made between the CT + IM and PT + IM groups and between the PT + IM and PT groups, based on our hypotheses. Comparisons for functional assessments, mechanics, collagen fiber organization, and  $\mu$ CT metrics were made using two-way ANOVAs with Bonferroni post-hoc tests for planned comparisons with GraphPad. Mechanical properties were also assessed with respect to changes over time within each model. Individual one-way ANOVAs for each model were run for cross-sectional area, stress relaxation, modulus, stiffness, cycles to failure, and for dynamic modulus at each tested frequency, with post-hoc Bonferroni tests with corrections for multiple comparisons. Semi-quantitative histological comparisons were made using Kruskal-Wallis tests at each time point.

3. Results

3.1. Functional assessments

Ankle joint stiffness and range of motion (ROM) through dorsiflexion were significantly altered in CT + IM and PT + IM groups relative to their own pre-surgery value (Fig. 1A, B). Ankles from complete tendon tears were stiffer than both partial tear groups at 14 days post-injury, but by 6 weeks, were only stiffer than the PT group (Fig. 1A). In contrast, CT + IM and PT + IM groups had similarly diminished dorsiflexion ROM (~60% decrease) at 14 days (Fig. 1B). PT + IM joints regained significantly more ROM by 6 weeks, while CT + IM joints did not recover (Fig. 1B). Few differences existed in plantarflexion parameters (Fig. 1C, D), so total ROM reflects changes in dorsiflexion motion (Fig. 1E). CT + IM ani-



**Fig. 1.** Functional Assessments. Passive joint testing demonstrated that post-injury (A) dorsiflexion stiffness was increased and (B) dorsiflexion ROM was decreased for CT + IM group compared to PT + IM as well as PT + IM compared to PT only. PT + IM demonstrated (C) increased plantarflexion stiffness and (D) increased range of motion compared to both other models. (E) Total range of motion reflected changes seen in dorsiflexion. Ambulatory analysis showed that (F) rate of loading and (G) stance time were decreased in the CT + IM group, as well as (H) increased speed. All data is normalized to pre-injury values. Data is presented as mean  $\pm$  SD at each time point. CT, complete tear; PT, partial tear; IM, immobilization. (a) denotes significant difference between CT + IM and PT + IM ( $p < 0.025$ ); (b) denotes significant difference between PT + IM and PT ( $p < 0.025$ ).

mals also had significantly slower rate of loading (Fig. 1F) and longer stance time (Fig. 1G) during ambulation than PT + IM, even though overall speed was increased at 6 weeks (Fig. 1H).

### 3.2. Mechanical properties

At 3 and 6 weeks post-injury, cross-sectional area was larger for CT + IM tendons compared to PT + IM (Fig. 2A). Stress relaxation was increased in PT compared to PT + IM at 1 week, but decreased at 3 and 6 weeks (Fig. 2B). Dynamic frequency sweeps demonstrated increased dynamic modulus in PT tendons compared to PT + IM at 3 weeks, and in PT + IM tendons compared to CT + IM at 6 weeks (Fig. 2C). These differences were not frequency-dependent. Similarly, PT + IM modulus was also significantly greater than CT + IM at 1 and 6 weeks, and was significantly lower than PT at 3 weeks (Fig. 2D). Similar differences were seen in stiffness at 1 and 3 weeks, but no differences were seen by 6 weeks post-injury (Fig. 2E). PT tendons withstood significantly more fatigue cycles before failing than PT + IM tendons at 3 and 6 weeks (Fig. 2F), and only PT tendons were able to produce a reliable fatigue response at 3 weeks (Fig. 2F, G). Tissue modulus (Fig. 2G) and both secant and tangent stiffness (data not shown) measured during fatigue testing were greater in PT + IM tendons than in CT + IM at 6 weeks, but there were no differences between PT and PT + IM groups in these metrics at this time point (Fig. 2G).

When assessing properties over time within each model, CT + IM tendons significantly increased in cross-sectional area over time (Fig. S1A), while differences in PT + IM CSA were only seen between 1 W and 3 W. However, no properties improved from 3 weeks to 6 weeks in the CT + IM group, although tendons were able to undergo fatigue cycling at 6 weeks (Fig. 2G). Interestingly, several parameters of tendon mechanical properties decreased in the PT + IM group from one week (no loading, animals were euthanized with their casts on) to three weeks (after two weeks of post-immobilization cage activity). Tendon cross-sectional area (Fig. S3A) increased, but material properties including modulus (Fig. S3C) and dynamic modulus (Fig. S4B) significantly decreased. Conversely, tendons receiving partial tear injuries that were not immobilized did not see a significant change in cross-sectional area

over time, and viscoelastic and material properties were also unchanged over time (Figs. S3 and S4).

### 3.3. Histological observations

No differences were determined in cell number (cellularity) (Fig. 3A), nuclear shape (Fig. 3B), or collagen organization (Fig. 3C). Collagen organization at the injury site at 1 week was not able to be quantified. Representative histological images from the injury site at 3 weeks post-injury are shown in Fig. 3D.

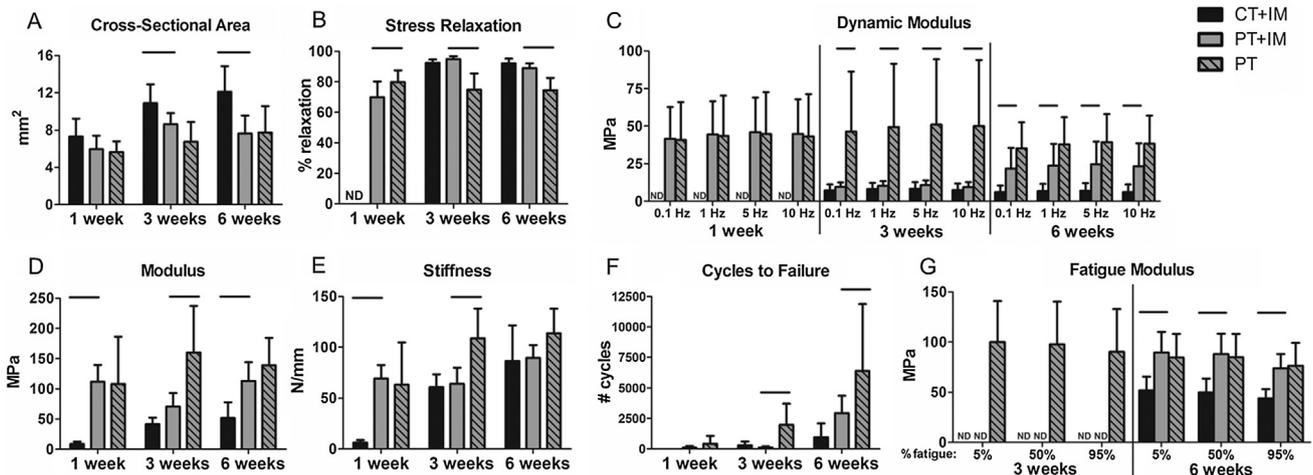
### 3.4. $\mu$ CT

The presence of heterotopic ossification was observed in almost all samples in all groups at all time points (4–6 positive for calcification out of  $n = 6$ /group/time point for all groups; no apparent differences between models). Bone volume was significantly higher in CT + IM tendons than PT + IM tendons at six weeks (Fig. 4A); however, this mineralized tissue had decreased tissue mineral density (Fig. 4B). Representative 3D renderings created from scans of tendon-calcaneal complexes at 6 weeks post-injury are shown in Fig. 4C.

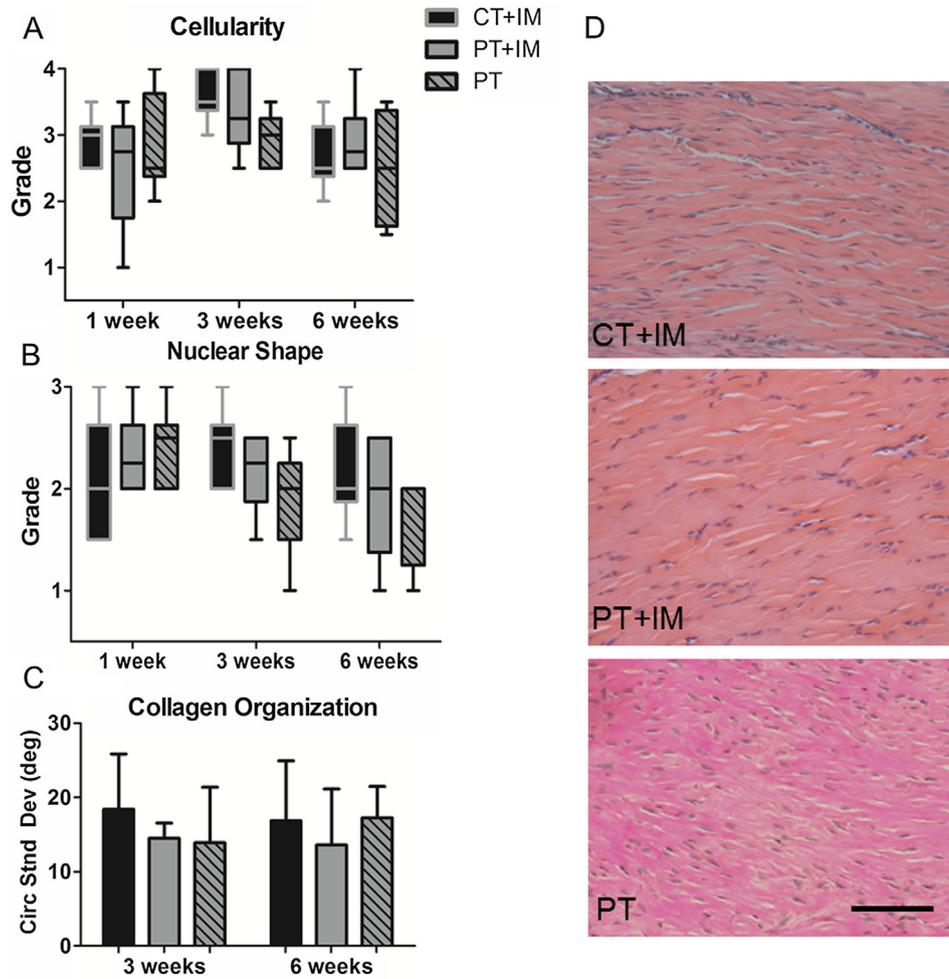
## 4. Discussion

This study investigated differences in ankle function, tendon mechanics, and HO in three different models of Achilles injury. All models were reproducible and had distinct effects on measured parameters.

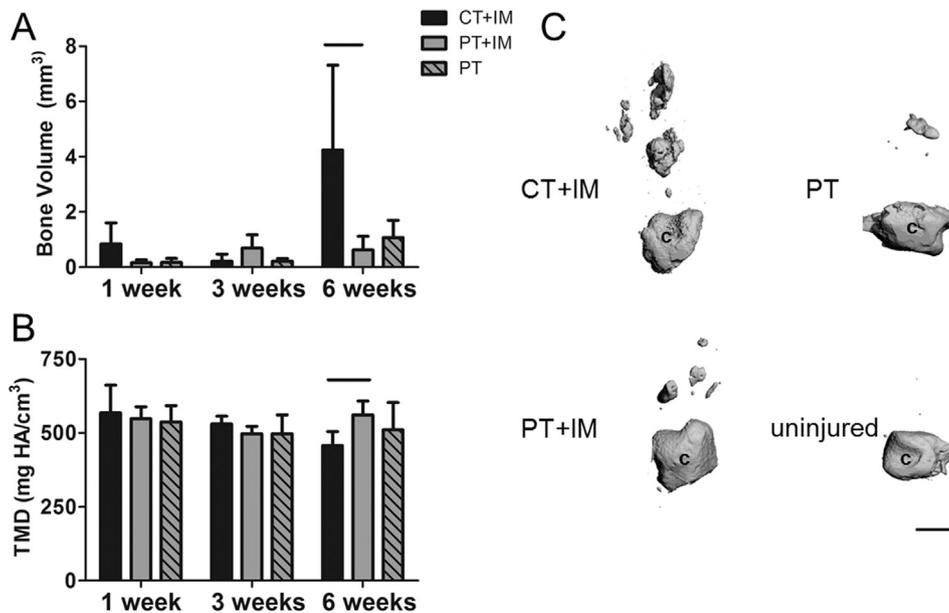
Injury severity (CT vs PT) had a drastic influence on tendon healing, with complete tears causing diminished ankle mobility (Fig. 1A–D) and decreased tendon mechanics (Fig. 2) throughout post-injury time points compared to partial tears, which appeared to return function more closely to baseline values by six weeks post-injury (Fig. 1). Changes in loading rate and stance time of the injured limb (Fig. 1F, G) indicate that CT animals are altering ambulation patterns more severely, which may be due to loss of function or increased pain (Caro et al., 2014). CT tendons were larger in cross-sectional area (Fig. 2A) and contained more HO than PT



**Fig. 2.** Mechanical Properties. Injury model affects (A) tendon cross-sectional area at 3 and 6 weeks (uninjured Achilles cross-sectional area:  $4.1 \pm 0.4 \text{ mm}^2$ ). Immobilization affects (B) stress relaxation at all time points (uninjured Achilles stress relaxation:  $71 \pm 7\%$ ). Both injury mode and immobilization alter (C) dynamic modulus (uninjured Achilles dynamic modulus:  $85.9 \pm 29.5 \text{ MPa}$  at 0.1 Hz,  $92.5 \pm 30.9 \text{ MPa}$  at 1 Hz,  $96.3 \pm 31.8 \text{ MPa}$  at 5 Hz, and  $95.0 \pm 32.3 \text{ MPa}$  at 10 Hz), (D) tissue modulus (uninjured Achilles tissue modulus:  $295 \pm 59 \text{ MPa}$ ), and (F) tendon stiffness (uninjured Achilles tendon stiffness:  $133 \pm 26 \text{ N/mm}$ ). Immediate load bearing improves (F) cycles to failure at 3 and 6 weeks (uninjured Achilles tendon CTF:  $3941 \pm 1618$  cycles); and complete tear (CT) decreases (E) fatigue modulus at 6 weeks (uninjured Achilles tendon fatigue modulus:  $173 \pm 22 \text{ MPa}$  at 5% fatigue life,  $181 \pm 26 \text{ MPa}$  at 50% fatigue life,  $161 \pm 17 \text{ MPa}$  at 95% fatigue life). Data is presented as mean  $\pm$  SD. CT, complete tear; PT, partial tear; IM, immobilization; ND, data was not able to be collected. Solid lines indicate significant differences ( $p < 0.025$ ).



**Fig. 3.** Histological Properties. No differences were identified in (A) cell density (cellularity), (B) nuclear shape, or (C) collagen organization between models (uninjured Achilles tendon collagen organization:  $5.5 \pm 1.6$  deg). (D) Representative images of  $200\times$  magnification H&E stained sections of the injury site three weeks post-injury. Data in A and B is presented as median  $\pm$  IQR with minimum and maximum whiskers. Data in C is presented as mean  $\pm$  SD. Scale bar in D:  $100 \mu\text{m}$ . CT, complete tear; PT, partial tear; IM, immobilization.



**Fig. 4.**  $\mu\text{CT}$  Properties. CT + IM showed (A) increased heterotopic bone volume but (B) decreased tissue mineral density at 6 weeks post-injury. (C) Representative 3D renderings of  $\mu\text{CT}$  reconstructions of calcaneus-Achilles complexes six weeks post-injury. The calcaneus was not included in the quantitative  $\mu\text{CT}$  analyses. Data is presented as mean  $\pm$  SD. Scale bar in C:  $2 \text{mm}$ . CT, complete tear; PT, partial tear; IM, immobilization; c, calcaneus. Solid lines indicate significant differences ( $p < 0.025$ ).

tendons (Fig. 4A). Heterotopic ossification has been associated with tendon thickening in clinical assessments (Yu et al., 1994). However, differences in mineral density between groups (Fig. 4B) suggest that the mechanisms of HO development or maturation may vary between models (Isaacson et al., 2011). Notably, cross-sectional area for all groups at 6 weeks was still at least 50% larger compared to uninjured Achilles tendon area, potentially at least partially due to HO formation in all models. Although increased cross-sectional area can be associated with a degenerative tendon state (Weinstabl et al., 1991), it is also a sign of increased matrix due to exercise (Rooney et al., 2015) and is noted after injury as a result of increased matrix and scar tissue production (Frölke et al. (1998)). Unfortunately, there is no consistent correlation between tendon thickness and clinical outcomes after injury.

Heterotopic ossification is characterized by the ectopic formation of lamellar bone within soft tissue and is known to occur after localized trauma, including surgical operations (Amar et al., 2015). Rat Achilles tenotomy has previously been used as a model of HO formation to investigate potential therapeutics (Zhang et al., 2016). Although the mechanism through which HO initiates is not fully understood, it has been hypothesized that it is related to the post-traumatic release of bone morphogenetic protein 2 which in turn mediates increases in inflammatory markers, immune cell recruitment, local tissue remodeling, and vascularization (Amar et al., 2015). The full-width injury model described in this paper involves blunt transection of the tendon followed by a complex suturing technique to best re-approximate the tendon ends. Compared to the simple sharp biopsy punch injury in the partial-width injury, this full-width injury requires significantly more handling of the tendon with forceps as well as multiple suture passes. Therefore, we hypothesize that the increase in HO in CT + IM tendons is likely due to increased trauma to the tendon tissue during the injury procedure. Analogously, the clinical presence of heterotopic ossification in the Achilles tendon is associated with trauma or surgery. However, overall incidence of HO after Achilles injury is unknown, and perhaps underreported, as it can be asymptomatic (Richards et al., 2008).

Our second tested variable, one week of plantarflexion immobilization, had a strong effect on animals receiving a partial-width injury. Notably, tendons in the PT + IM group failed early during fatigue cycling 3 weeks post-injury ( $113 \pm 85$  cycles), prohibiting fatigue analysis (Fig. 2F). Surprisingly, a partial tear injury without immobilization had no effect on ankle range of motion through dorsiflexion at any time point, while PT + IM animals demonstrated diminished function at all post-injury time points (Fig. 1B). Additionally, immobilization after partial tear followed by return to cage activity revealed interesting differences compared to immediate cage activity post-injury, including increased ankle stiffness compared to animals not immobilized (Fig. 1A, C). While many mechanical properties were not different between these groups at 1 week post-injury, including dynamic modulus, modulus, stiffness, differences in these metrics became apparent at 3 weeks post-injury, and then returned to being largely comparable at 6 weeks (Fig. 2). These mechanical testing results along with residual joint stiffness suggest a delay in healing with immobilization after partial tear. Immobilization has been shown to be detrimental to intact tendon mechanics (Hettrich et al., 2013), and these time-dependent differences between PT + IM and PT tendons may be due to degeneration of intact portions of the Achilles with immobilization. PT + IM tendon cross-sectional area increased over time (Fig. S3A), but material properties including modulus (Fig. S3C) and dynamic modulus (Fig. S4B) significantly decreased, suggesting formation of poor quality scar tissue (Thomopoulos et al., 2003; Wang 2006). This was reflected in the comparison of PT + IM and PT tendons, which demonstrated that non-immobilized tendons

had significantly better mechanical properties (decreased stress relaxation, increased modulus, stiffness, and cycles to failure, Fig. S3) at three weeks compared to PT + IM. Together, these results indicate that even short-term immobilization may impair healing and increase ankle stiffness in partial Achilles tears in rats. We have previously shown different temporal healing responses when altering immobilization time and return to activity in complete rat Achilles tendon tears (Freedman et al., 2016; Freedman et al., 2017A; Freedman et al., 2017B).

All three models of Achilles injury could be useful for tendon healing investigations, chosen by the prospective applications of a potential therapeutic. Testing a therapeutic as both an adjuvant to surgical repair or as a stand-alone treatment for a non-operative partial tear would be valuable for defining target populations. Additionally, while the role of immobilization and early loading on Achilles healing is not entirely resolved, several clinical studies suggest that early loading is important for preventing ankle stiffness and aiding muscle recovery (Barfod et al., 2014; Barfod et al., 2015). This may be particularly true in a partial tear environment where tendon end apposition is not the foremost objective (Allenmark, 1992). Therefore, understanding the mechanisms of a therapeutic application with and without early loading is important. This study describes three potential models, though we acknowledge additional modifications could be made, including immobilization time and various post-injury activity regimens. Although some previous studies have been performed using a complete Achilles tear without immobilization (Hammerman et al., 2018), our unpublished studies utilizing this model resulted in significant gap formation between tendon ends, which correlates with poor clinical outcomes (Maquirriain, 2011).

This work also sheds light on the universal occurrence of HO after surgically-induced injury in a rat Achilles tendon. Previous studies have described endochondral ossification after Achilles tenotomy in a rat model (Lin et al., 2010). Clinical studies indicate that the incidence is lower in humans, with a rate of about 15% after open repair (Ateschrang et al., 2008). Tendon mineralization has clinically been shown to cause pain and weakness (Richards et al., 2008) and can occasionally require surgical resection (Ishikura et al., 2015). These models could be used to test prevention and treatment paradigms for post-injury tendon HO.

This study has several limitations. Our animal model likely does recapitulate the middle age demographic most affected by Achilles tendon rupture; aging rats to middle age may affect some rat Achilles tendon properties (Pardes et al., 2017). Second, our non-repair partial tear does require open surgery to create the injury and, for the sake of reproducibility, involves the formation of a sharp tissue excision through the tendon, therefore not mimicking the clinical parallel. Additionally, the study lacks biological data such as gene and protein expression analyses to explain injury and immobilization-dependent differences in tendon healing and function. Future studies will investigate mechanisms of healing as well as long-term effects of these injury models, as previous studies indicate lasting consequences of Achilles tendon injury in the rat (Freedman et al., 2017A).

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## Conflict of interest

Authors have nothing to disclose.

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## Author contributions

All authors were fully involved in the study and preparation of the manuscript. The manuscript has been read and approved by all of the authors.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbiomech.2019.03.029>.

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