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# Proceed with Caution: Mouse Deep Digit Flexor Tendon Injury Model

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**Background:** The purpose of this study was to determine the feasibility of using mouse models for translational study of flexor tendon repair and reconstruction.
**Methods:** Quantitative data detailing the gross anatomy, biomechanical characteristics, and microscopic structure of the deep digit flexor tendon (DDF) of the mouse hindpaw were obtained. Histological characterization of the DDF and the anatomy of the digit in the mouse hindpaw are detailed. Biomechanical testing determined the load-to-failure, stress, elastic modulus, and the site of tendon failure.
**Results:** In gross anatomy, the origins and insertions of the mouse deep digit flexor tendon are similar to those of the human digit, surrounded by a synovial sheath that is only 1- to 2-cells thick. A neurovascular network runs on each side of the digit outside the synovial sheath, but does not clearly penetrate it. The thickness of the DDF is 0.14 ± 0.03 mm and the width is 0.3 ± 0.03 mm. The thickness of the DDF is less than that of 9-0 nylon needle. The mean failure force of the deep flexor tendon was 2.79 ± 0.53N.
**Conclusions:** The gross anatomy of the mouse hindpaw digit is similar to that of the human digit except for key differences seen in the synovial sheath and vascular supply. The dimensions of the mouse DDF make it challenging to create a clinically translatable repair model using currently available surgical techniques. Despite the similarities between the human and mouse anatomy, and the powerful basic science tools available in murine models, mice are an unreliable model for assessing flexor tendon injury and repair. *(Plast Reconstr Surg Glob Open 2020;8:e3359; doi: 10.1097/GOX.0000000000003359; Published online 26 January 2021.)*

## INTRODUCTION

Tendons cannot effectively heal unless the ends are touching, which does not usually occur with a complete tear. Therefore, complete flexor tendon lacerations require repair. Unfortunately, 30% of these repairs are complicated by adhesion formation, resulting in disability.[1–5] In an effort to improve the clinical outcome of flexor tendon repair and reconstruction, clinician scientists, engineers, and basic scientists are expanding current translational research platforms to include the biological processes that govern tissue injury, regeneration, and repair. The enhanced characterization of injury, adhesion formation, fibrosis/scarring, and regeneration biology should contribute to regenerative surgical approaches that augment traditional surgical techniques on a tissue and cellular level. However, the development of effective therapeutics for improving flexor tendon healing postoperatively is impaired by the lack of a model that allows genetic manipulations to identify biological mechanisms that underlie the ineffective flexor tendon healing with associated adhesion formation.

Animal models offer an attractive method to investigate tendon regeneration, tendon healing, and the etiology of tendinopathy. In addition, they provide the opportunity to obtain tissue during all stages of the disease or healing process, unlike human tissue. A variety of animals have been used to study non-operative and postoperative flexor tendon healing in vivo, with the majority conducted on

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chickens, rabbits, and dogs.[4,6–9] The mouse model offers distinct advantages over other species due to the availability of numerous biological reagents, cost considerations, and, perhaps most importantly, the availability of genetically altered mice, which facilitate a detailed study of the cellular and molecular mechanisms of tissue pathology, healing, and therapeutics. The mouse model has been utilized to study musculoskeletal conditions, including osteoarthritis, tendon pathologies, and repair of the patellar tendon, Achilles tendon, and the rotator cuff.[10–13]

However, the mouse model has limitations and criticisms. Several studies have investigated mouse flexor tendon physiology and healing in vivo.[6,7,14–17] Other studies have investigated the repair of the distal deep flexor tendon (proximal to the digit) injuries in vivo.[18,19] Unfortunately, the mouse flexor tendon repair model described by Ackerman and Loiselle was not a translatable model because it required a transection of the flexor digitorum longus proximally at the myotendinous junction to protect the repair site from rupture. Although Wong et al and Freeberg et al have characterized a zone II mouse model of flexor tendon partial laceration injury,[5–7] no in vivo model has been developed for the investigation of mouse intrasynovial tendon repair (ie, zone II). Likely no model has been developed due to the dimensions of the mouse digit posing insurmountable surgical challenges, though these dimensions have yet to be exactly quantified.[20] Additionally, there has been a lack of characterization of the mouse digit's baseline biomechanical properties that has precluded its use from focused translational research of reconstructive flexor tendon procedures.

In this study, we sought to further evaluate the murine model as a suitable model for zone II flexor tendon injury and repair. By performing a biomechanical and anatomic evaluation of the mouse deep digital flexor tendon (DDF) in the hindpaw, we will determine the feasibility of the mouse model for translatable studies and serve as a baseline for further studies of biomechanical properties.

## METHODS

Use of mice in this study was approved by the Institutional Animal Care and Use Committee (APLAC Approval Number: 9999). In keeping with similar literature,[21] 20 hindpaws (10 left and 10 right) of skeletally mature male C57/BL6 mice, aged 8–10 weeks, were collected immediately following sacrifice. Eight paws were freshly dissected for mechanical testing of the deep digit flexor tendon and the remainder were used for imaging and histology.

Within 2 hours of sacrifice, the tendon specimens of the middle digit were used for mechanical testing. During the dissection and mechanical testing, samples were kept moist by regular spraying with 10% phosphate-buffered saline (PBS). Dimensional measurements of the deep flexor tendon were performed using a digital micrometer with fine tips and a resolution of 0.01 mm under microscope dissection. To reduce variability of measurements, the same person made the dimensional measurements. Custom grips were used with an Instron 5565 (Instron, Norwood, Mass.) and a 100N load cell to compressively grip proximally at the point of branching of the deep flexor tendon and distally at the osteotendinous junction. (**See figure 1, Supplemental Digital Content 1,** which displays gross anatomy of the mouse hindpaw. (A) Plantar surface of the mouse hindpaw with A3 pulley seen (within the bracket); (B) Longitudinally divided A3 Pulley (within the bracket); (C) The Superficial Digital Flexor Tendon (SDF) strands bifurcate and progress distally as 2 narrow tendon strips with Deep Digital Flexor Tendon DDF in between at the location of the white arrow in Figure 1A; (D) Dissected digit highlighting the DDF (yellow arrow) and the SDF (white arrow); (E) Dissected digit with flexor tendons present and 9-0 BV100-4 needle with nylon suture for size comparison. http://links.lww.com/PRSGO/B540.)

As per Beason et al, tendons were preloaded to 0.02N to remove slack, and the length of the tendon was measured.[16] The length of the tendon was then measured between the grip ends (ie, the distance of the entire tendon subjected to the displacement). Then, to provide a consistent strain history, the tendons were exposed to 10 cycles of preconditioning from 0.02N to 0.05N at a rate of 0.1%/s. After a 300-second hold, tendons were then loaded to 5% strain at a rate of 25%/second and then held for stress relaxation over 600 seconds. Finally, tendons were unloaded back to 0% strain and then immediately subjected to an extension test to failure at a rate of 2%/second.[16,22] (**See figure 2, Supplemental Digital Content 2,** which displays the setup for biomechanical testing using the Instron Mechanical Testing device. Custom grips were used to compressively grip at the osteotendinous junction and at the proximal aspect of the tendon. http://links.lww.com/PRSGO/B541.) (**See figure 3, Supplemental Digital Content 3,** which displays the representative preconditioning curve used for flexor tendon mechanical testing. The tendon was first exposed to 10 cycles between 0.02 and 0.05N (blue), followed by a 300-second hold (orange). Then, the tendon was loaded to 5% strain and held for stress relaxation for 600 seconds (green), before mechanical extension test until failure (red). To keep the graph in scale, the end of the extensional test is not shown in this graph. http://links.lww.com/PRSGO/B542.)

The Young modulus was calculated by taking a least-squares regression of the slope at the individual linear portion of the extensional test, which ranged between a strain of 0.05 and 0.15 for each sample. Yield force, stress, and strain were taken at the point of the curve when the tendon exited the linear deformation portion and began plastic deformation. Stress was calculated by dividing the force by the cross-sectional area. Strain was measured by dividing the displacement over the length of the tendon. Stress, strain, and modulus calculations were made using MATLAB (MathWorks, Natick, Mass.).

For gross imaging after dissection, anatomical structures were photographed using a Leica DFC310 FX camera on a Leica M205 FA side arm (Leica, Allendale, N.J.). After imaging, specimens were used for histology and were fixed in fresh 4% paraformaldehyde solution for 24 hours at 4°C, then transferred to RapidCal Immuno (BBC

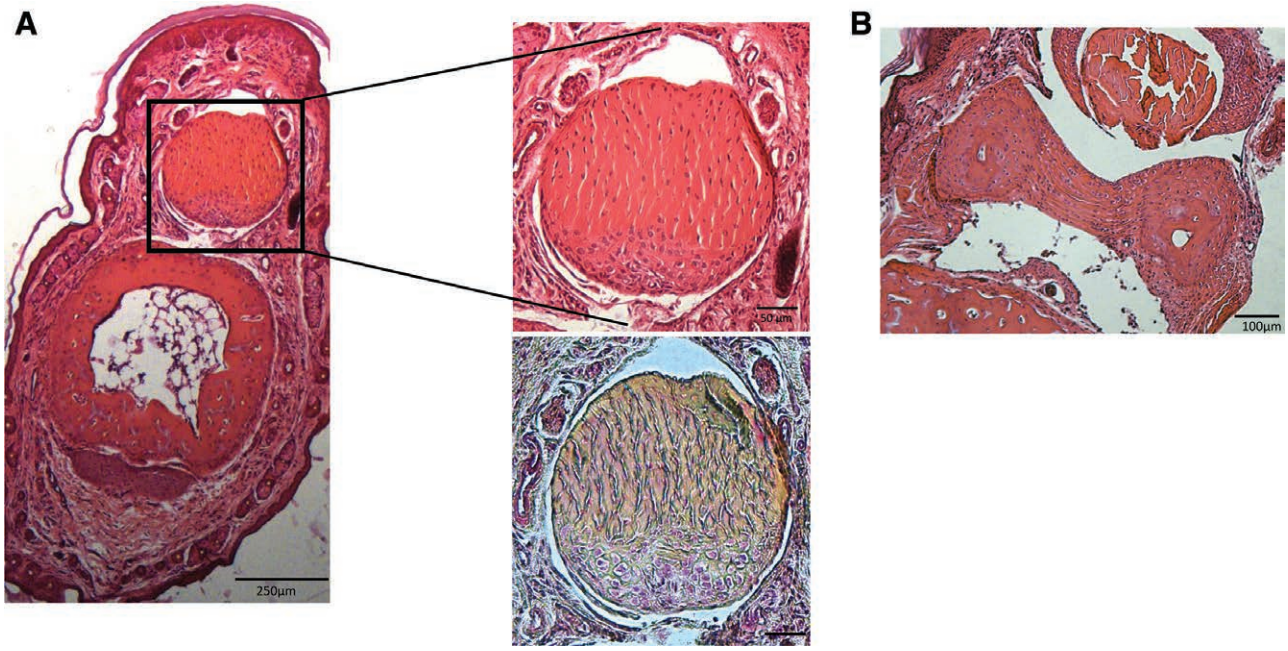


**Fig. 1.** Cross-sectional histology of murine digit. (A) Axial H&E section of the digit demonstrating a large fibrocartilage region on the dorsal aspect of the DDF and neurovascular bundles that run on each side of the tendon in the extrasynovial space at 10× and axial H&E and pentachrome sections at 20×. (B) Axial H&E section of digit the volar plate at 10×.

Biochemical, Vernon, Wash.) for 24 hours. Following this process, the decalcified hind feet were tissue-processed. Care was taken to prevent any shear or traction to the tendon on dissection. All tissue was processed using the STP 120 Spin Tissue Processor (Thermofisher, Waltham, Mass.) and embedded in paraffin wax. Digits were positioned in wells filled with molten wax, where 9 digits were placed in a longitudinal orientation and 3 in an axial orientation. Serial sections (thickness: 8 μm) were cut from the paraffin-embedded blocks. These sections were mounted onto slides, followed by drying at 37°C for 24 hours. Slides were stained with H&E and Trichrome. All brightfield images were captured using a Leica DFC 7000T (camera) mounted on a Leica DMI4000 B microscope.

## RESULTS

### Dissection of Hindfoot with Gross Evaluation

On removal of the plantar skin, a complex small network of vascular loops is clearly identified superficial to the tendon sheath, as previously described by Wong et al.[6] Deep to the vascular network, a thin membrane/film of translucent tissue covering the superficial digital flexor tendon (SDF) is apparent, consistent with that of the synovial sheath. The digital tendons lie within this sheath between the proximal and distal pulleys, which correspond to zone II (**See figure 1, Supplemental Digital Content 1**. http://links.lww.com/PRSGO/B540). Pulley fibers run perpendicular to longitudinal fibers of the tendon. The superficial tendon bifurcates around the deep tendon to attach to the middle phalanx and flex at the proximal interphalangeal joint, while the deep tendon acts on the distal phalanx, similar to that seen in the human digit (**See figure 1, Supplemental Digital Content 1**. http://links.lww.com/PRSGO/B540). The thickness of the DDF is 0.14 ± 0.03 mm and width is 0.3 ± 0.03 mm. The thickness of the DDF tendon is less than that of BV100-4 9-0 Ethilon Nylon Needle (Johnson and Johnson, New Brunswick, N.J.), which is approximately 0.2 mm (**See figure 1, Supplemental Digital Content 1**. http://links.lww.com/PRSGO/B540).

### Histological Evaluation

A neurovascular network runs on each side of the digit outside the synovial sheath (**See figure 1, Supplemental Digital Content 1**. http://links.lww.com/PRSGO/B540.) (Fig. 1). No vessels are identified within the sheath. On H&E staining, the synovial sheath is 1- to 2-cells thick and located directly between the subcutaneous tissue of the skin and flexor tendons (Fig. 1). Axial histological sections through the pulleys over the joints demonstrate the pulleys surrounding the tendon and inserting on the volar plate (Fig. 1). Of note, DDF tendons have fibrocartilaginous zones at areas of compression, which has been previously reported in humans as well[23] (Fig. 1).

### Biomechanical Testing of the Deep Flexor Tendon

The mean failure force of the deep flexor tendon was 2.79 ± 0.53N, the average yield stress was 61.25 ± 9.4 MPa, and the average Young's modulus was 418.19 ± 153.55 MPa (Fig. 2). An estimated 37% of the failures occurred at the mid-substance, 13% of the failures occurred at the tendon-to-bone junction, and 50% at the grip/tendon interface. No significant difference was observed between location of failure (ie, grip versus the mid-substance versus

tendon-to-bone interface (**See figure 4, Supplemental Digital Content 4,** which displays comparison of Ultimate Strength Deep Digital Flexor tendon based on location of failure. (A) Ultimate Failure Force Deep Digital Flexor tendon based on failure location. (B) Elastic Modulus of the Deep Digital Flexor tendon based on failure location. http://links.lww.com/PRSGO/B543). No slippage was observed during testing.

## DISCUSSION

In this study, we characterize the biomechanical and anatomic properties of the mouse hindpaw, with a focus on the deep digit flexor tendon. Interestingly, the elastic modulus is greater than what has been previously found more proximally of the same tendon.[5,16] However, this observation is in line with previous literature showing that biomechanical properties may vary along a tendon.[24,25] Our overall ultimate force and force-displacement curves are similar to those observed in Freeberg et al; however, our measurements of the tendon cross-sectional area were half that of the previous studies examining flexor tendons.[5] This 2-fold discrepancy in cross-sectional area explains the fold difference in elastic modulus observed between our studies and those by Freeberg et al.[5]

Despite the scientific benefits that a mouse model provides (genetically dissectible transgenics), including the ability to utilize gene manipulation and transgenic approaches to evaluate the cellular and molecular effects of specific targets, one should proceed with caution when investigating digital flexor tendon injury and repair in the mouse digit. With currently available surgical techniques, the dimensions of the mouse flexor tendon make it difficult to perform an accurate surgical repair. Although it is technically possible to perform a repair with a smaller caliber suture, this would compromise the strength of the repair and increase the likelihood of rupture during digit motion. This is supported by a prior work of Ackerman and Loiselle, who, in order to prevent a failure of a flexor tendon repair within the hindpaw, created a full-thickness laceration proximal to the repair at the myotendinous junction.[19]

The gross anatomy of the origins and insertions of the mouse deep digit flexor tendon are similar to those of the human digit, suggesting similar function as previously highlighted by Wong et al.[21] However, there are several key differences that should cause caution when using the mouse flexor tendon model for injury or repair. First, the mouse hindpaw flexor tendons begin as a single superficial and single deep digital flexor tendon in the hindpaw, as opposed to having independent muscle belly origins as seen in the human hand. Second, and most importantly, despite the mouse digit having an apparent synovial sheath, its thickness is proportionally thinner compared with that in humans, as evidenced grossly and histologically, making it difficult to have a truly translatable model.[26] Larger animal models have a more proportionally sized synovial sheath in comparison with humans.[27] Despite the mouse having paired neurovascular bundles in the middle digit, the vasculature networks that supply the mouse digit appear to differ from those of a human in that the vessels never appear to enter the synovial sheath. Given the thin nature of the mouse synovial sheath, it is possible that the intrasynovial tendon obtains nutrients via diffusion. Other animal models, such as the rabbit and chicken, have a more similar vasculature system when compared with a human digit. Previous studies have described the vincula as intrinsic vasculature specialized to intrasynovial flexor tendons along the dorsal aspect of these tendons.[28,29] These differences in the synovial sheath and vascular network of the mouse digit may result in differences in how cytokines, growth factors, cells, and nutrients appear in the area of injury/repair and facilitate healing.

We chose to conduct our surgeries in C57Bl6 because this is one of the most commonly used "wild type" mice. There may be some variations in tendon anatomy and biomechanics among other strains and transgenic mice. This is a potential limitation of this study. Another limitation was the use of grips for biomechanical testing. We initially attempted to avoid using grips given the risk of failure at the tendon-grip interface. However, there was significant slippage of the tendon secondary due to its limited width and thickness, thereby resulting in inconsistent and

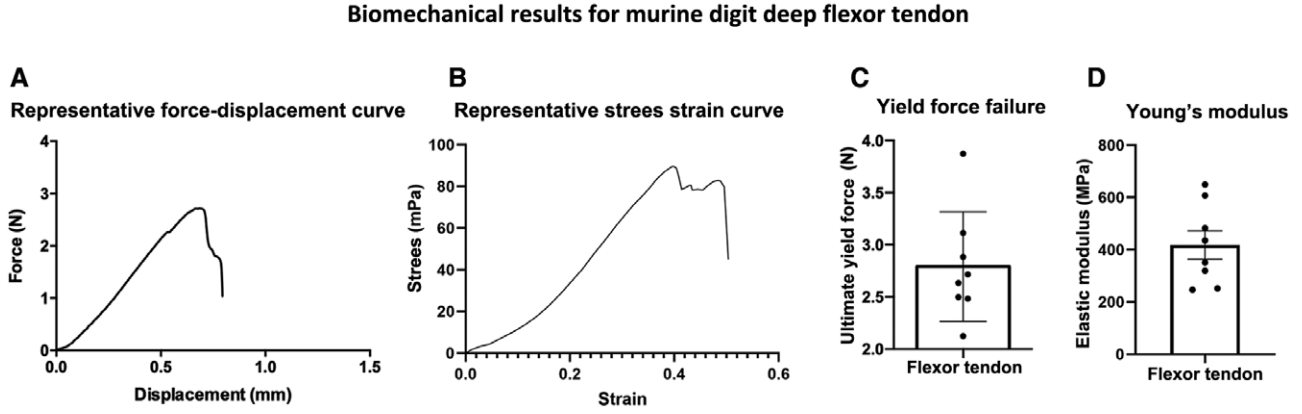


**Fig. 2.** Biomechanics of the deep digital flexor tendon. A, Representative force-displacement curve. B, Representative stress strain curve. C, Ultimate strength of the deep digital flexor tendon. D, Elastic modulus of the deep digital flexor tendon.

unreliable data. Therefore, we opted to use a technique with grips to ensure more reliable data and found no significant differences in the mechanical properties and the location of failure.

In conclusion, we have described the biomechanical and anatomic properties of the mouse hindpaw deep digit flexor tendon. This detailed analysis highlights concerns regarding the feasibility of the mouse tendon injury and repair model. The most notable limitations are issues of scale and associated difficulty of surgical repair, as well as the anatomically different synovial sheath and its associated blood supply. The mouse model can be a powerful tool for translational research that facilitates our understanding of the underlying biological processes involved in tendon healing postoperatively. However, given these anatomical and dimensional differences, one should proceed with caution in using this model for either injury or repair modeling.

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