Murine Flexor Tendon Repair Model with Noninvasive Assessment of Healing with Ultrasound

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Medical Illustration

*Murine Flexor Tendon Repair Model
with Noninvasive Assessment of Healing with Ultrasound*

by
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ABSTRACT

Flexor tendon injuries in the hands are relatively common, significantly disabling more than 100,000 people in the United States per year (Nichols 2019). The majority of flexor tendon injuries require surgical repair, but despite the advancement of technology and medicine, current treatments still yield poor outcomes. Even with prevailing treatments to improve or restore function to the affected digit(s), patients often suffer psychologically and financially in the long-term. A recent study gave a conservative estimate that flexor tendon lacerations cost the American healthcare system and taxpayers between US $240.8-409.1 million per year and emphasized the need to “focus our efforts to improve treatments and rehabilitation protocols” to decrease these direct and indirect costs (Mehrzad 2019).

Partially attributed to advancements in technology that have enabled increased study and understanding of cellular and molecular biology, attention has shifted from surgical techniques to focus on investigating the fibrotic healing mechanisms of tendons with the hopes of uncovering the switches that influence scar formation versus regenerative healing. However, progress has been somewhat limited due to the ability to only assess end-point outcomes of healing.

To address these limitations collaborators, Dr. Alayna Loiselle and Dr. Michael Richards have developed a murine (mouse) model to simultaneously study the fibrotic healing response in tendons and validate ultrasound algorithms to accurately quantify several physical and mechanical characteristics of healing tendons. The purpose of this project was to demonstrate to students and researchers in related fields the experimental setup of the mouse model and the application of ultrasound imaging techniques as a relatively accessible and inexpensive noninvasive method to reliably assess tendon healing in the form of a short 3D animation. A few future inquiries that expand upon the current murine flexor tendon repair model are also introduced.
INTRODUCTION

Flexor tendon injuries in the hands are relatively common, disabling more than 100,000 people in the United States per year (Mehrzad 2019). The majority of flexor tendon injuries require surgical repair, but despite the advancement of technology and medicine, current treatments still yield poor outcomes. Even with prevailing treatments to improve or restore function to the affected digit(s), patients often suffer psychologically and financially in the long-term. A recent study gave a conservative estimate that flexor tendon lacerations cost the American healthcare system and taxpayers between US $240.8-409.1 million per year and emphasized the need to “focus our efforts to improve treatments and rehabilitation protocols” to decrease these direct and indirect costs (Mehrzad 2019). More attention has turned to investigating the healing mechanisms in tendons and therapy methods to minimize adhesion growth and reduce the risk of tendon rupture.

In the form of a short (<3 minute) 3D animation and series of GIFs, this project aims to emphasize the potential applications of ultrasound (US) imaging technology as applied to a murine model to be a noninvasive, largely accessible, and cheaper method to accurately monitor flexor tendon healing. This imaging technique has the potential to be scaled to clinical applications and further adapted to assess healing in other tissue types and anatomic structures in the future but to date has been investigated in a few other similar studies including a healing tendon rabbit model of Achilles tendon transection (Yamamoto et al 2017). Other tissues such as the aorta and different tendons are currently being studied with US imaging to assess changes in mechanical properties that can be attributed to pathologies (Bah et al 2016; Chimenti et al 2016; Mix et al 2017; Yamamoto et al 2017). The animation demonstrates the ongoing development and evaluation of ultrasound algorithms and the resulting strain data as a way to visualize tendon healing, extent of adhesion masses, and infer mechanical properties of repaired tissue.

Portions of this project are anticipated to be included in grant proposals in order to continue the ongoing longitudinal investigation taking place at the University of Rochester Medical Center (URMC) and Rochester Institute of Technology (RIT). It was decided that it would be more effective to visually exemplify some of the current findings and briefly detail future inquiries in a few minutes, rather than compile pages of text. Sections of the project are to be incorporated into presentations for researchers and students to demonstrate the visualization of strain that the tendon undergoes over the course of flexion.

In order to effectively create the collective body of work for this project, collaborating researchers Dr. Michael Richards, the principal investigator at the Biomechanical Imaging Lab at RIT; and Dr. Alayna Loiselle, who is the principal investigator at her lab at URMC, were regularly consulted throughout the duration of this project. US strain maps were developed through detailed guidance from Dr. Richards, and accuracy of models and movements were approved by Dr. Richards and Dr. Loiselle.
SCIENTIFIC BACKGROUND

Prior to the 1960s, injury repair in Zone II of the hand was avoided and notoriously known as “no man’s land” due to a long history of unsuccessful surgical repair (Kotwal 2012). Zone II starts proximal to the metacarpophalangeal joint and extends to the insertion of the flexor digitorum superficialis (FDS) muscle on the middle phalanges of digits II-V. The majority of flexor tendon injuries require surgical repair to improve or restore function to the affected digit(s). Several recent studies have determined that the tendons of the flexor digitorum profundus (FDP) muscle in Zone II had the highest incidence of injury in cases involving flexor tendon injuries in the hand (de Jong 2014; Manninen 2017). Despite the advancements in medicine and technology, flexor tendon injuries in Zone II have among the poorest outcomes following surgical intervention. The main debilitating complications following repair are tendon rupture, occurring in an estimated 3.9% of cases, and scar tissue adhesions requiring tenolysis, which are more prevalent in around 10% of primary repairs of complete flexor tendon lacerations (Mehrzad 2019). However, a more inclusive analysis of flexor tendon injuries determined between 30-40% of patients developed adhesions following primary repair (Nichols 2019). These scar tissue adhesions can form at and around the repair site and tend to adhere to the synovial sheath that surrounds the tendons of both FDP and FDS muscles. Normally, this sheath facilitates movement by reducing friction, but the adhesions act like Velcro or if they are not actually fused with the sheath and/or the adjacent tendon, then their bulk impairs gliding of the tendons by occupying the already restricted space. Until a few decades ago treatment was based on surgical techniques that focused on factors including suture material, size, placement, technique, spacing, etc. to minimize scar tissue. Partially attributed to advancements in technology that have enabled increased study and understanding of cellular and molecular biology, attention has shifted from surgical techniques to focus on investigating the fibrotic healing mechanisms of tendons with the hopes of uncovering the switches that influence scar formation versus regenerative healing.

In the current investigation conducted by Dr. Richards and Dr. Loiselle, a mouse model was developed to use ultrasound imaging and image analysis as a means to noninvasively evaluate healing. Previous studies have found that the mouse flexor tendons in the hind paw are anatomically similar to the flexor tendons in Zone II in the human hand (Wong 2006; Charles 2016). Due to their anatomical and functional similarity, this makes mice a good experimental subject for studying tendon healing and adhesion formation. In the mouse model, the flexor digitorum longus (FDL) muscle in the hind limb is surgically transected and sutured back together. At specific intervals post-surgery (7, 14, 21, and 28 days), the repaired tendons undergo a series of US scans and mechanical testing (Ackerman 2019). The scans and mechanical test data are compared to the data of an uninjured healthy tendon control group as well as to data from other time-points.
US imaging is performed on the repaired FDL tendons as they are flexed, the data is then used to generate a strain map of the tendon at a certain point. The measure of strain correlates to the degree of compression deformation the tendon experiences over the course of flexion. Healthy uninjured tendons undergo substantial compression or high strain as they are pulled and glide past neighboring structures. This is due to their high water content being between 65-75% of wet weight and longitudinal arrangement of primarily type I collagen (Rumian 2006; Shvachkina 2018; Wu 2017). Water is essentially squeezed out of the tendon over the course of flexion and is then reabsorbed once the muscle is relaxed. Comparatively, repaired tendons are significantly less pliable, resulting from scar tissue growth that reduces the integrity of the native tendon at and surrounding the transection site. Bulky scar tissue leads to lower strain and decreased gliding function. Scar tissue deposits commonly adhere to surrounding tissues in the effort to provide stability, but in the limited space of the tendon sheath even a small adhesion can greatly impair normal movements and can compress neighboring tendons.

In an initial test in the mouse model, C57Bl/6J mice underwent surgical transection and repair of the FDL tendon (Ackerman 2019). All mice were subjected to US imaging once a week for four consecutive weeks following surgery. Half of the mice were sacrificed at day 14 for functional analyses (gliding function and mechanical properties) and the remaining mice were sacrificed at day 28 for function analyses. Histological analyses of the healing tendons were used to validate correct segmentation of scar tissue on ultrasound images. Gliding function was assessed by incrementally loading the tendon with a series of small weights (0-19 grams). Measurements of the flexion angle at the metatarsophalangeal (MTP) joint were measured from digital images taken after the application of each weight increment. MTP Flexion angle is the degree of flexion that results from the 19g weight, while Gliding Resistance (GR) is calculated over the range of applied loads. A decrease in MTP Flexion Angle and decrease in GR are indicative of reduced gliding function and increased adhesion formation. Mechanical properties were quantified after gliding testing using a ramp to failure tensile test (Ackerman 2019). These data demonstrated that static ultrasound imaging to quantify scar tissue volume (STV) was strongly correlated with gliding function, but no correlation was observed between static measures of scar tissue volume and mechanical properties, suggesting the need for a more dynamic ultrasound imaging protocol to define mechanical properties.

With US imaging, Dr. Richards and Dr. Loiselle are developing an algorithm to accurately estimate STV, which could be monitored over the course of healing and used to determine certain physical therapy exercises to improve range of motion. The STV measurement estimates the overall amount, form, and distribution of the adhesion and can provide insight on gliding function. However, it does not yield any significant correlation between the repair’s structural properties in predicting its mechanical attributes (Ackerman, 2019). The US transducer takes a series of images in the sagittal plane.
and a 3D model of the scar tissue is assembled by tracing the borders of the scar tissue masses in two dimensions and stacking them in order to create a volume segmentation. In order to make this measurement more applicable to human patients and also as a cost-effective therapeutic screening technique, the goal is to make the segmentation process semi-automated or completely automated. Dr. Loiselle’s lab is verifying the STV measurements through histological sectioning.
THE BODY OF WORK

OVERVIEW

The work completed for this project encompasses a short (<3 minute) 3D animation, a series of GIFs derived from the animation footage and separate illustrations, as well as illustrations of related investigations currently being conducted by Dr. Richards’ lab. Each of these works is intended to be incorporated into presentations for students and researchers of Dr. Richards’ and Dr. Loiselle’s respective research areas. The 3D animation serves to provide a brief background and context of the investigation to those not directly involved, while the GIFs lack audio or subtitles and are intended to demonstrate the mouse model in action, the application of ultrasound and potential data supporting the investigation’s objective.

GOALS OF THE ARTWORK

1. Create – a 3D animation that briefly describes the need for investigating mechanisms of flexor tendon healing to develop successful treatment options, demonstrates the application of ultrasound to assess tendon healing by comparing a healthy tendon to a repaired tendon, and discusses a few of the investigation’s end goals.

2. Demonstrate – the application of ultrasound to assess tendon healing through the generation of simulated strain maps that would be expected for a normal tendon and a healing tendon over the course of flexion.

3. Emphasize – that ultrasound is sensitive enough to be an effective method to monitor tendon healing and would be translatable to the human scale.
ILLUSTRATION PROCESS

Two illustrations that focus on two of Dr. Richards’ other ongoing projects were created and are currently displayed on his lab website: [https://pht180.rit.edu/bil/](https://pht180.rit.edu/bil/) and in Figures 23 and 24. These projects investigate: (1) Risk Assessment of Abdominal Aortic Aneurysms (AAA), working with Dr. Doran Mix at URMC; and (2) Ultrasound Elastography of Achilles Tendinopathy (IAT), in collaboration with Dr. Mark Buckley at URMC. Dr. Richards gave a brief overview of these investigations in the initial meeting. The objective of these illustrations was to emphasize the anatomy central to the study as well as show a representation of data that reveals some of the key findings determined in the ongoing investigation.

Thumbnail sketches were developed using the forms of the human body, leg, aorta, and US transducers were positioned in a Maya scene using 3D models already on hand, rendered as still frames, and served as a guide to paint over in Photoshop. The 3D model of the body was acquired from Adobe Fuse and the model of the aorta and probes had been built for other projects. A number of still frames from slightly different angles and orientations served as developed sketches and were sent to Dr. Richards. It was initially decided to use 3D models as a guide particularly for the AAA illustration, in order to maintain realistic perspective and proportion if the composition angle was changed later. The selected still frame served as a guide and was painted over in Adobe Photoshop and the layout completed in Adobe Illustrator. Images of the ultrasound data and strain plots for each of the IAT and AAA illustrations were provided by Dr. Richards and were edited in Adobe Photoshop following Dr. Richards’ specifications. Only one of the strain plots for the IAT illustration were edited due to there not being an image of the data at that specific point in the action sequence. The other images of strain data were cleaned up for sizing and resolution purposes. Both of the illustrations were rendered in a similar style to the work completed for this project in order to have a cohesive appearance.
ANIMATION PROCESS

It was decided to create the animation in 3D to better translate the anatomical forms of the mouse hind limb in relation to each other that are visualized with ultrasound (2D images). A 3D animation would also emphasize the necessity to assess healing in all planes of a damaged structure in order to determine tissue integrity and functionality. All of the 3D models are specifically of the right hind limb, since the transection/repair procedure and imaging were carried out exclusively on the right foot.

The original plan was to make a series of GIFs that would: (1) show and compare the mouse anatomy being studied and the human anatomy that the research would be applied to; (2) demonstrate normal flexion of a healthy tendon (mouse anatomy); and (3) demonstrate the impaired flexion of a repaired tendon (mouse anatomy). It was discussed whether to have a separate GIF that detailed the injury/repair procedure and mechanical testing procedure, and it was ultimately decided against because the surgical technique was not the primary feature or end goal of the study. There was agreement that the animation could be used to make a series of GIFs, which would be easier to incorporate into a lecture presentation.

Timeline

It was important to follow deadlines in order to have the animation largely completed to be presented at the end of April 2020. In-person meetings with Dr. Richards were planned to be at least once a month, and in-person meetings with both were held around once every two months depending on schedules. Email communication was frequent, with 1-4 correspondences per week. Storyboards, scripts, etc. and any rounds of revisions were sent at least a day in advance prior to an in-person meeting.

Script

A formal script was written for the second storyboard and significantly helped determine the flow and organization of information to include. The first draft of the script was written without serious consideration of the length in relation to the allotted time in mind, and was much too long. Background information and details of procedures were shortened to a minimum in order to provide enough context to make sense but not extensively to detract from the main points.

The script was approved by Dr. Loiselle and Dr. Richards after a couple rounds of revisions regarding wording and specific terms. Because students and researchers in the same or similar fields of study as the investigators themselves are the intended audience, it was decided that the word choice was appropriate if it included some specific terminology. Upon listening to the recorded script it was noted that some words, such as “outcomes” and “currently” or “differences” vs. “differently”, were used repeatedly in a sentence or in two consecutive sentences. Substituting one of the duplicate words would
have led to a smoother flow of sound and information. Despite this, the script was able to be concise, informative, and disclose only enough to invite further discussion. Brevity was achieved by keeping phrasing primarily vernacular and sticking to main points.

Storyboard

Two different storyboards were devised. The first was developed over the late summer (2019) and largely focused on a brief overview of the simulated injury/repair procedure, comparison of the uninjured/repaired strain, and Scar Tissue Volume procedures. It was completed with Adobe Illustrator with the 2D assets sketched in Adobe Photoshop. Each cell was accompanied with a description of the direction, action, and what assets would be included. This first storyboard lacked a formal script because the entire animation was intended to be divided into a series of 5 or more GIFs that would have no audio and only have minimal labels and captions. The storyboard was shared with Dr. Richards and Dr. Loiselle, so that each could comment on the flow of information, image accuracy, and clarity. However, as the project progressed it was decided that a brief background introduction and future applications segment would be beneficial to include for the viewer, particularly if they were not familiar with the investigation itself. As a result, the second storyboard included a script, which helped determine the flow and assets needed. After developing the script, it was necessary to create additional drawings for the added scenes. The new storyboard was presented in a Microsoft PowerPoint and utilized the majority of the cells and descriptions already constructed from the first storyboard.

One of the last cells in an early version of the scripted storyboard depicted a micropipette administering its contents to a cell culture along with a syringe being applied to the mouse model to suggest the investigation’s findings lead to the development of a new drug that could successfully shrink adhesions. It was advised to have the representation be more realistic, where the research could advise which known and available drugs might have a positive effect in reducing scar tissue mass and promote regenerative healing. This idea is portrayed as two different generic-looking pill capsules being “applied” to the mouse model, pills being recognizable to virtually all audiences as a representation of medicine compared to a micropipette (more symbolic of a laboratory than medicine) or even syringe.
Figure 1. Example of storyboard frame from the second draft of the first storyboard in Adobe Illustrator.

Figure 2. Example of storyboard frame from the second storyboard in Microsoft PowerPoint.
Narration

The script was recorded and edited in Adobe Audition. The initial storyboard developed over the summer lacked narration, but the revised storyboard completed in September incorporated a script. Recording was done in two separate takes with long pauses between sentences, to ease the editing process in order to align the timing during the animation. There were two different narrators, with the intention that the first narrator was only a placeholder. However, after recording the second narrator (completed in the same manner as the first) it was revealed that the pacing of the narration was remarkably slower than the animation and the first narrator. The audio was trimmed, background sound removed, time decreased, and pitch adjusted in Audition to better match the pacing already established in the animation. Further editing and time adjustment was achieved in Adobe After Effects.

Models and Development of Assets

3D models

All of the 3D models of the human forearm/hand and mouse hind limb bones, FDL and FDP muscles, and other assets such as the ultrasound transducer probe and pill bottles, some of which are shown in Figure 5, were modeled in Autodesk Maya. Arnold shaders were applied to all models and Adobe Photoshop was used to paint the FDL and FDP muscles. Human and mouse models were constructed and edited in separate scenes, and scenes were duplicated before proceeding with rigging, animating, and editing any animation.

For the 3D models of the mouse hind limb, the intention was to acquire a CT dataset of a mouse, however, there was difficulty in locating one that included complete hind limbs. It was also reasoned that building the model from scratch would result in a better understanding of the anatomic relationships as there was a lack of prior knowledge on mouse anatomy. The mouse hind limb bones were modeled from scratch and based off of assembled scans from the Micro-tomographic Atlas of the Mouse Skeleton as the primary reference (Bab 2007). The FDL muscle was likewise modeled using the interactive 3D PDF of the musculature of the mouse hind limb as the main reference (Charles 2016). Unlike the 3D mouse assets, the bases for the human forearm bones, hand bones, and FDP muscle were downloaded from the Japanese-originated open source site lifesciencedb.jp/bp3d/ (BodyParts3D 2008). These base models were subsequently cleaned-up, modified, and tessellated in Autodesk Maya. Constructive criticism of the human FDP muscle advised that the tendons to have tautness, which would reflect their position in a living person. A couple of the assets that were depicted in the storyboard, such as the Velcro ankle strap that would hold the mouse foot in place during testing and US imaging, were excluded due to the fact that it would block a portion of the anatomy from the viewer.
Paint layers and bump maps for the muscle textures and human hand skin were created by exporting a UV snapshot of their respective UVs, which were constructed and edited strictly in Maya, into Photoshop. The completed maps were exported as .tif files from Photoshop and linked to their corresponding models in Maya. All color and bump map edits were automatically updated due to the file path and overwriting of the older image file with the same naming convention. Bump maps were chosen over displacement maps for the human skin texture because of ease of linking, consistent rendering appearance, and the need to only simulate texture.

Rigging the models of the human forearm/hand bones and mouse hind limb bones was straightforward and based primarily on the structure of the skeleton itself. Some liberty was taken to simplify the number of joints, such as all of the tarsals and carpals were parented under a single joint in each respective rig. On the other hand, this step was less clear for aligning the FDL and FDP muscles to their corresponding skeleton and to get them to move together without the muscle model becoming warped. Both of the muscle models were initially constructed and edited in the y-plane, but the tendons needed to (1) be curved and aligned along the digits’ bones, (2) be able to be controlled by the joints in the rig, and (3) be editable with relative ease. Professor Craig Foster (RIT) recommended using a series of lattices and clustering specified lattice points to achieve these three functions. A large lattice was created on each of the muscles to arrange its belly and tendons into position. Several smaller lattices corresponding to general areas (e.g. calf, ankle, digit I, etc.) of the muscle then had groupings of four lattice points to form a cluster that was then designated to the nearest joint in the rig.

Figure 3. Plantar aspect of mouse foot. Comparison between (left) reference image from page 185 in the Micro-tomographic Atlas of the Mouse Skeleton, (middle) in-progress wire frame of rigged bones, and (right) completed 3D models of hind limb bones with joint rig (purple) and shader applied.
Illustration assets

A number of the 2D assets, including the mouse body, mouse skeleton illustrations, and arrow graphics were created in Adobe Photoshop and Adobe Illustrator respectively. Some of these are shown in Figures 6 and 7, and Figure 20 shows how they were layered in After Effects to achieve the desired ghosting effect. The style of the illustrations was made to mimic the appearance of the 3D models in order to be consistent in presentation and not distracting to the viewer. Conversely, arrow graphics and leader lines were created in Illustrator with the intent to be simple and have high contrast to direct the viewer to a particular detail.

The illustrations of the mouse body and its skeleton were specifically made for the FDL anatomy GIF since the short animation introduced the FDL in relation to the human FDP muscle. Early in the storyboard phase the FDL anatomy was presented by rotating the hind limb 360° in its anatomical position, followed by zooming in on its origin and panning down along its length to its insertion on the distal phalanges. However, this might disorient the viewer, so the FDL is shown in relation to where it is located on the mouse skeleton, and from a lateral view of the right side of the mouse to be consistent with the 3D model being of the right hind limb. It was decided that it would be considerably more efficient and just as effective to have 2D illustrations provide an anatomical frame of reference instead of having a full 3D model of the mouse and its skeleton.

Histological slices, date, and ultrasound footage

The two separate ultrasound movie clips were provided by Dr. Richards’ lab and feature: (1) an uninjured healthy tendon, and (2) a tendon with simulated injury repair 28 days post-repair. In both sequences the foot undergoes flexion and was aligned to match the position of the 3D models with the toes pointing towards the right of the frame. For the animation, the timing of the ultrasound sequences was slowed down in After Effects to match the pace of the 3D model flexing.

Photographs of example histological sections, scar tissue volume, and cell lineages were pulled from Dr. Loiselle’s presentations on Cellular Mechanisms of Tendon Regeneration and verified for context (Loiselle 2019). Labels of images were approved by Dr. Richards and Dr. Loiselle for wording and content.

Strain Maps

Strain maps were created in Adobe Illustrator using the gradient mesh function and developed under close guidance from Dr. Richards. They were originally based off of two single images of current data, one representing overall high strain of an uninjured healthy tendon and a separate one representing comparatively low strain of an injured and repaired tendon (Loiselle 2019). It was decided to flip the
strain scale colors to reflect that of a temperature scale, so that the higher strain values were red/orange/yellow, while the lower strain values were green/blue/violet. Dr. Richards had described what the expected strain sequence might be over the course of the flexion movement based on his lab’s current data. Five primary rounds of revisions yielded two separate sequences of nearly 80 plots each were devised for the normal flexion and the injury/repair flexion and aligned with the corresponding area of the ultrasound footage. The sequences’ duration was edited in Adobe After Effects to match the pacing of the US footage and the model movement. Towards the beginning of the storyboard process, there was a short-lived representation of the strain map being projected onto the 3D model itself, but it was reasoned to have the strain maps superimposed onto the US footage to keep the orientation of the US and strain map consistent with one another, making it easier to understand.

Figure 4. Original strain plots and strain scale from current data (left, circled in red) and duplicates with minimal changes beneath them to emulate changes in strain during flexion. Middle: finalized sequence of proposed strain in an uninjured healthy tendon; right: finalized sequence of proposed strain in a repaired tendon. Partway through this project it was decided to reverse the color values for strain, so that warmer colors indicate higher strain and cooler colors signify lower strain values. Keep in mind that these are a representation of expected results based on current data.
Animation

Once all of the 3D assets were constructed and rigged, the positioning and movement of the models were approved by Dr. Loiselle and Dr. Richards to reflect the setup of the experimental procedure. Initial flexion of both models was referenced from photos of mice at various points in their walk cycle, with flexion for the repair model adjusted based on the description of their flexion being slower and to a lesser degree. The intent was to have the motion be at a realistic pace but be slow enough for anatomical detail to be clear. A motion test was sent to both Dr. Richards and Dr. Loiselle to evaluate the animated movement after each round of revisions, in order to minimize the number of times the sequence needed to be rendered. Some of the assets were still in progress by the time the animation was being composed. Placeholder files of these assets, including strain maps and US movie footage, were imported into the composition, aligned with the timing, and subsequently replaced with the finalized version.

Adobe After Effects was used to compose the animation following rendering. It would have greatly eased organization if the composition was divided into seven pre-compositions that corresponded to sections of the storyboard instead of compiling all assets into a single composition for the main animation. For a couple of the GIFs, separate pre-compositions were created due to the need for adjusting the timing separately and replacing the same sequence from a different camera angle. However, designating specific color tags for narration layers, text layers, sequences, etc., helped categorize the massive list of layers. The seven sections of the storyboard were dubbed according to the main action occurring: (1) intro, (2) normal flexion, (3) experimental injury/repair simulation, (4) injury/repair flexion, (5) STV, (6) healing mechanisms, and (7) treatments.

The introduction section was key in providing a brief background to the viewer. It is important for the audience to understand the primary reason for the ongoing investigation - to study the healing mechanism in flexor tendons to develop more effective treatments. Part 2 presents normal flexion in a healthy tendon and a sample US scan and proposed strain map, as the control and desirable end goal of a healing tendon. In Part 3, an abbreviated version of the injury/repair procedure is described before demonstrating flexion of the repair model and its respective US scan and representative strain sequence in Part 4. Part 5 detailed the second US application in the study to quantify scar tissue volume and how it is being validated via histological sectioning. Both Parts 6 and 7 list a few of the potential future applications and inquiries that expand on the current findings. Artistic liberty was taken for Part 7, where the application of drugs is suggested by the depiction of two different pills arcing across the frame and fading once they make contact with the repaired tendon model. The camera then focuses on the scar tissue mass, which shrinks slightly following the second pill dissolving above its surface. This representation
had the aim of keeping the viewer engaged and is definitely not a realistic rendering of the mechanism of a drug.

While there was very little animated movement of the models in the Maya scene itself, some liberty was taken in the camera angles for the flexion of the normal and injury repair models in order to create some visual interest. In the short animation the camera zooms in and rotates partially around the mouse model while it is flexing, but for the GIFs each of these flexion sequences are rendered from a separate camera with a fixed position so that the model is seen only from the medial side of the right foot. The GIF flexion sequences are from a fixed angle in order to have more clarity for the viewer, which would most likely be a researcher or student.

Other measures similar to the arcing of the pills in Part 7 were taken to maintain the viewer’s interest. The ability to adjust any layer’s opacity was widely used to keep a consistent appearance and be easy on the eyes while leading into the next asset or statement. Text was kept to a minimum by being reserved for labels and summary bullet points. Subtitles were added to the animation to increase accessibility and clarity but were withheld from the GIFs as they were designed to be largely self-explanatory. Masks were applied to most 2D graphic layers of arrows, frames, and some 2D assets (still images of the repair model in the injury/repair simulation, etc.), so that the arrow would be able to be revealed across the frame and direct the viewer’s eye along with it to the figure or detail it was pointed towards.

In the storyboard and first final draft of the animation, the background was a flat black fill. This was originally chosen to be consistent with some of the research presentations and images that the animation and GIFs would likely accompany, as well as make the lighter bone and tendon models more visible. The presentations largely had white backgrounds and many of the images had black or dark grey backgrounds, common to many imaging techniques. To make the background more visually interesting but not compete with what was being shown, a noise texture was generated and applied to the black fill layer in After Effects. The dark blue from the UR logo was sampled and applied to another fill layer on top of the first layer, which then had its blending mode set to multiply for the noise texture to be visible beneath it.

GIFs were initially rendered from Adobe Photoshop after the After Effects movie was rendered as an MP4 via Adobe Media Encoder. In Photoshop the movie was cropped to the section length. Despite the effort to keep the lengths of the GIFs to a minimum, full-sized (1280x720 px) animated GIF files were between 72-108 MB, making them cumbersome or impossible to transfer, load, and play. Additional GIF tests were exported at 50% scale (640x360 px) and 30% scale (420x236 px) to reduce file size, 256-optimized color palette and various dithering settings. A higher dithering value (>80%) was ultimately selected in order to minimize altering colors and their distribution in the strain map and scale. 30% scale
GIFs yielded smaller file sizes for obvious reasons (between 9.3-14.4 MB) but were not optimal if viewed at full screen due to loss of detail. Final versions of the GIFs were exported using the Media Encoder due to faster rendering times. Each of the GIF file sizes are still significantly larger than GIFs commonplace to social media and forums (<1-2 MB). Reducing the frame rate to 15-20 fps was considered to decrease file size but was decided against since it would result in rough transitions of the strain maps.

**Measurement of Success**

The animation and GIFs were designed with the intent of being utilized in presentations for researchers and students in at least their 3rd and 4th years of undergraduate study or later. They also would be considered to be included in applications for research grants as supporting material demonstrating the purposes of the research being proposed. It is unknown how impactful the animation and/or the GIFs are to a grant committee as there have no grant proposals submitted to the knowledge of the author by the date of publication. Similarly, it is also unknown how effective the GIFs are in increasing students’ and researchers’ understanding, since classes have largely ended for the spring term. However, Dr. Richards had stated that GIFs and the like would be most helpful as an instructor, to be used as a visual aid that could be quickly and easily replayed. With the current popularity of GIFs being widely posted on social media platforms and online learning sites, it is likely that the GIFs will have a more neutral reception as a minimum or positive reception, than negative.

**Music**

Ambient music was acquired from [freemusicarchive.org](http://freemusicarchive.org) on an attribution license for the track *Headway* composed by the artist Kai Engel. While the majority of the track was incorporated into the animation, its entirety was slightly edited in Adobe Audition to reduce some of the contrast in decibel levels to minimize competing with the narration. Prior to exploring royalty free music sites, the intent was to create an original track using GarageBand available on macOS systems in order to avoid navigating licenses and filtering for an appropriate tone and track length. Due to a combination of time constraints and technology access as a result of the COVID-19 pandemic shutting down many facilities, it was decided to survey what music was readily available on royalty free music sites.
CONCLUSION

The ongoing investigation of the Murine Flexor Tendon Repair Model as a whole, is just one example that underscores the value in adapting and expanding upon “old” technology for new applications. Science as a whole follows this ideology of dynamic thinking, where advancements are made by branching off of current findings and studying the root of the question. Sharing research via publishing and presentations also allows for other researchers to be exposed to other areas that may connect the dots to an innovative idea.

The objectives of this project were to create a short 3D animation that summarizes the progress of the collaborative study at the Loiselle Lab, and aims to demonstrate and describe a few of the investigation’s end goals: (1) Validation of the ultrasound algorithm that is sensitive enough to distinguish differences in healing tendons; (2) utilize the ultrasound algorithm to generate strain maps of tendons to analyze mechanical properties of the healing tendon and scar tissue, and (3) use ultrasound and histological sectioning to quantify scar tissue volume and its relationship to surrounding tissues. Sections of the animation footage would be used to create a series of GIFs that would serve as a presentation visual aid to researchers and students. The animation would provide the audience with some background information for context, especially for those not directly involved with the study. Viewers would have a general understanding of the experimental procedure to simulate tendon injury and repair, as well as how the simulated strain correlates to the anatomical structures of the 3D model.

In addition to learning and employing many software programs including Maya, After Effects, Audition, etc., working with Dr. Richards and Dr. Loiselle provided insight on the collaborative effort of research. It was an opportunity to hear directly from them over the course of this project how their colleagues and students in their fields might interpret it and how to adjust for the most clarity.
Figure 5. Assets page featuring some of the 3D models part of the animation. From left to right: Miniature scale examination table where mouse subjects were strapped down; ultrasound transducer probe (MX 700; Fujifilm VisualSonics Inc.); sample pill bottles with generic pills for Part 7.

Figure 6. Mouse body illustration for FDL anatomy overview GIF. This illustration and the drawing of the entire mouse skeleton were rendered realistically enough to be identifiable and to match the overall appearance of the animation, but were simplified due to the reduced scale and brief time that the full image is presented in the GIF.
Figure 7. Edited still frame of rigged 3D model of lateral view of the right hind limb and FDL muscle for the FDL anatomy GIF. The bone texture, muscle origin and insertion, were rendered more fully in Photoshop, as they were not as detailed in the animation.

Figure 8. Still frame from animation introduction describing brief background of flexor tendon injuries and relevance of the investigation to human anatomy and medicine.
Murine Flexor Tendon Repair Model

Figure 9. Still frame from the animation, introducing why flexor tendon injuries can be tricky to treat.

Figure 10. Still shot from animation specifying boundaries of no-man’s land (Zone II) and other zones.
Figure 11. Still frame from animation comparing general anatomy of the mouse FDL muscle and tendon in the hind limb to the human FDP muscle and tendon in the forearm.

Figure 12. Still shot from animation of the uninjured healthy tendon flexion sequence. The data indicates that a healthy tendon transitions from having near zero strain to a relatively consistent distribution of high strain once the FDL is flexed.
Murine Flexor Tendon Repair Model

Figure 13. Still frame from animation summarizing the procedure to simulate tendon injury and repair. US imaging and testing of tensile properties follow a short period of uninterrupted healing.

Figure 14. Still shot from the animation of the repaired tendon flexion sequence. Current data indicates that scar tissue in the healing tendon results in lower and inconsistent distribution of strain.
Murine Flexor Tendon Repair Model

Figure 15. Still frame from the animation outlining measurement of Scar Tissue Volume via US imaging and validation process.

Figure 16. Still shot from animation briefly listing future inquiries that would expand the ongoing study.
Figure 17. Still frame from the animation listing future inquiries that would expand the study.

Figure 18. Still shot from the animation describing one of the future end goals of the study, where research would contribute to the development of a successful drug or other treatment and could be accurately assessed via ultrasound.
Figure 19. Still frame from the end of the animation showing an exaggerated shrinkage of scar tissue. Suggesting the application and testing of drugs to minimize scar formation or reduce scar tissue mass.

Figure 20. Still frame from the basic FDL anatomy and orientation overview GIF (50% scale). The mouse body illustration is layered over the skeleton illustration in After Effects.
Figure 21. Still shot from the basic FDL anatomy and orientation overview GIF (50% scale), highlighting origin and insertion.

Figure 22. Still frame from injury repair flexion GIF (50% scale). Labels are the only text in the GIFs.
Figure 23. Overview illustration for one of Dr. Richards’ projects with Dr. Doran Mix, involving US imaging techniques to assess risk of Abdominal Aortic Aneurysms (AAA), dissection, and rupture.

Figure 24. Illustration for one of Dr. Richards’ projects with Dr. Mark Buckley on Insertional Achilles Tendinopathy (IAT) with data comparison of compression strain visualized with ultrasound imaging over the course of dorsiflexion ensued by the subject moving into a partial squat.
REFERENCES


