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Literature Study on Energy-Storing Mice Tendons

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Spring 2022 MEMS Independent Study
Musculoskeletal Soft Tissue Laboratory

Principle Investigator: Dr. Spencer Lake
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I hereby certify that the lab report herein is our original academic work, completed in accordance with the McKelvey School of Engineering and Undergraduate Student academic integrity policies, and submitted to fulfill the requirements of this assignment:

Gustavo De Paiva  
Research Mentee
INTRODUCTION

The independent study I completed with the Musculoskeletal Soft Tissue Laboratory during the 2022 Spring semester involved an extensive literature search to determine the best course of action for testing the hypothesis that elastin helps reduce damage accumulation over time with cyclic loading of a tendon through a mouse model. This introduction is not a representation of what the lab is currently working on, but it’s to show how we’ve come to the point at which we’re at now: deciding between explant or in-vivo methods of testing the hypothesis.

Tendons are collagenous soft tissues that transmit loads between muscles and bones. Depending on their function, they can be anywhere on the spectrum from positional to energy storing tendons [1].

Throughout the semester, the goal was to determine which energy storing tendon would be optimal for testing the hypothesis. The prime energy storing tendon used in tests like this is the Achilles tendon. New information obtained at the most recent ORS conference indicates that the Achilles tendon is not suitable for the tissue culture the lab wanted to employ. The focus then turned to determining which tendons are considered energy storing ones and how compatible they are with the lab’s methodology. Because the Lake lab wants to use a tissue culture and subsequent CHP staining, it was imperative to determine whether potential tendons would be suited for these processes. It was found that the flexor digitorum longus (FDL) tendon and patellar tendon are energy storing tendons. Because the Achilles tendon is the most prominent for studying energy storing tendons, it was very difficult to find tissue culture and CHP staining on other energy storing tendons. Nonetheless, tissue cultures had been done on both tendons previously although not completely in murine models[2][3]. Additionally, CHP staining had been done on FDL tendons but the search came up inconclusive for patellar tendons[1]. In presenting the findings during weekly lab meetings, the patellar tendon and FDL were compared to determine which if either would be best to move forward with. Fruitful discussion determined that the experiment should consider pivoting towards an in-vivo model to evade the tissue culture and CHP staining process since they may be harmful to some tendons. Nonetheless, an explant study may be the path the lab takes as the procedures and equipment are well established in the lab.
METHODS

Procedure and Apparatus. Mice are unique because their tendons are uni-fascicular which allows researchers to isolate the role of elastin within a single fascicle. The preferred model for this experiment consists of Prx1Cre/ElnFlx mice. They will serve as our elastin knockout(KO) mice. The elastin gene has a flox site that the Cre gene recognizes and removes from the DNA. Because Cre is bound to the Prx1 gene which is only expressed in limbs, the Prx1Cre/ElnFlx mice should theoretically have no elastin in their tendons [4]. That’s specific to the tendons though as these mice should still have elastin in vital organs.

The lab has decided to examine three tendons: the tibialis anterior tendon (TBAT), the achilles tendon (AT), and the patellar tendon (PT). To test whether elastin reduces damage accumulation in tendons, we will compare elastin wild type (WT) and elastin KO mice through stress relaxation and fatigue mechanics, ECM images, cell images, and measured gene expression in either explant or in-vivo models. Recent conversations suggest the lab may be considering an in-vivo model supported by the research of Andarawis-Puri N, and EL Flatow [5]. The in-vivo model shown in fig. 1 may be used for one of the tendons given that Andarawis-Puri has fleshed out the methods and procedure for rat patellar tendons. Fig. 1 shows the model for in-vivo testing of a rat patellar tendon. The rat is anesthetized before its knee is cut open and clamped until cyclic loading is complete.
An in-vivo model has not yet been developed for TBAT and AT, and their viability in an in-vivo model has drawn skepticism from the lab because of their position and size within the mouse model. Nonetheless, explant studies can be conducted on all three tendons, and the procedure for doing that is commonplace in the Lake lab.

CONCLUSION AND NEXT STEPS

After deciding that CHP staining would not be the most effective method for characterizing the elastin content in the tendons chosen, it was concluded that additional research would have to be conducted to determine what methods have been employed in loading tendons in-vivo. As shown in fig. 1, a gripping or clamping method is used before directly loading the patellar tendon. Two other methods that should be considered include muscle stimulation by electric shock and cycling/moving the joint in question. Because of the variability in in-vivo methods and the position of tendons within the mouse, it’s unclear that this is the best course of action to start with this summer when the explant
method is already established. The lab will most likely reconsider in-vivo studies after completing explant studies. Additional steps I need to take before beginning this research include practicing dissections without damaging tendons, learning how to use the biax machine for stress testing, and learning how to do fatigue tests.
References


