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DEVELOPMENT OF A LOADING DEVICE FOR IMAGING RABBIT MCL ENTHESES WITH SECOND HARMONIC GENERATION MICROSCOPY

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INTRODUCTION

Entheses are a body's transitional structures between a flexible material and a much stiffer material: ligament and bone, respectively. A gradual transition of mineral content and collagen fibre organization over a short distance enables the enthesis to dissipate stress concentrations and transfer load between the adjoining elements, contributing to normal joint function [1]. Damage to this small region is associated with various conditions such as "tennis elbow". Enthesis tears result in long term weakness as the fibre organization in the enthesis and ligament is not recapitulated during healing. To date, the challenge of observing entheses under applied load has inhibited understanding of enthesis mechanics.

Second Harmonic Generation (SHG) microscopy is a technology used to image highly polarizable proteins (i.e. collagen) [2]. SHG does not require section fixation, which prevents researchers from testing those samples mechanically. Given that collagen fibre structure affects load transfer at entheses, SHG microscopy is an ideal tool to observe if and how the fibre organization changes with load.

The purpose of the project therefore was to develop a custom device to allow observation of the collagen fibre network of the rabbit medial collateral ligament (MCL) enthesis in the SHG microscope as tensile load is applied.

METHODS

Project requirements were defined and several alternative solutions reviewed. The option which fit the requirements best was created with CAD software (SolidWorks 2015). Where possible, the design was modified to optimize objectives – minimizing cost and maximizing movement accuracy. With CAD, it is possible to modify components of a model while assessing the impact of the change on the model as a whole.

RESULTS

In the final design, the rabbit bones are secured with bone cement in containers (pots) designed for that purpose. The MCL is held at a physiological angle of 70°, and is in the line of action of the applied force. One bone pot remains stationary as the other, sliding on rail guides which constrain pot movement, is pulled by a linear actuator. A custom load cell collects force data as the load is applied. For its light-weight property and potential to be scanned using MRI, Perspex is the material of choice for the device.

The completed design (Fig. 1) satisfies all established project requirements, allowing for a testing procedure simulating physiological conditions while maximizing accuracy of the recorded data.

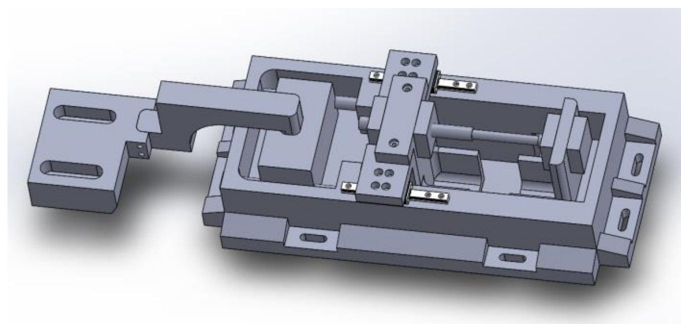

Figure 1: Final 3D model of loading device, with dimensions of approximately 280mm x 120mm x 110mm (length x width x height). The left side attaches to the linear actuator.

DISCUSSION AND CONCLUSIONS

The small size of entheses has stymied researchers' efforts to characterize their inhomogeneous material behaviour. However, the device developed in this work will enable the observation of enthesis collagen fibre behaviour under load. The results are expected to provide insight into the mechanisms of load transfer in the enthesis.

With some minor modifications to the bone pots, this device could be used as a reference product for the study of other soft tissues under load. However, it would be worthwhile to consider interchanging the bone pots between uses since the bone cement is difficult to remove.

REFERENCES

1. Thomopoulos et al., *J Orthop Res*, **21:**413-419, 2003.
2. Freund & Deutsch. *Opt. Lett,* **11**: 94, 1986.