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DESIGN AND MANUFACTURE OF A CUSTOM LIGAMENT LOADING DEVICE FOR USE WITH SECOND HARMONIC GENERATION MICROSCOPY

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INTRODUCTION

Ligament insertions into bone (entheses) are natural adaptations accomplishing the connection of severely mismatched materials. Entheses transfer load from relatively pliable connective tissue to relatively rigid bone over typically less than a millimeter [1]. Normal joint function and the prevention of injury depends on the proficient transfer of load at an enthesis [2]. Upon injury, healing entheses (surgically reattached or otherwise) are unable to recapture the structural and compositional characteristics that made them effective initially; as a result, healing entheses are prone to re-injury and represent a prominent clinical problem [3].

Several analytical and computer models have been employed to help elucidate some aspect of insertional mechanics, however, all suffer from limitations. Most importantly, given current imaging technologies and the small size of entheses, there has been an inability to observe their response under applied load. An accurate description of enthesis load transfer mechanics has thus been lacking.

A relatively new and powerful microscopic technology, second-harmonic generation (SHG), for which the University of Calgary has recently acquired an advanced microscope, has been shown to image movement on the microscale [4] and is a promising tool for the microscopic observation of entheses. The problem then remains as to how to load the ligament precisely during SHG imaging, highlighting the need for a custom-built device. Tensile testing of ligaments is not an unfamiliar procedure with commercial equipment available to do so. However, to our knowledge, there is nothing available for simultaneously loading and microscopically imaging entheses using SHG.

OBJECTIVE

Here we describe the process used in the design of a loading device particularly adapted to the rabbit medial collateral ligament (MCL). Emphasis is placed on the unique design constraints and objectives and the solutions conceived to meet them. This includes the consolidation of several custom-machined components and commercially available hardware.

DESIGN CHALLENGES AND SOLUTIONS

Microscope Sensitivity to Movement

The focal plane selectivity of SHG is similar to that of confocal and two-photon fluorescence

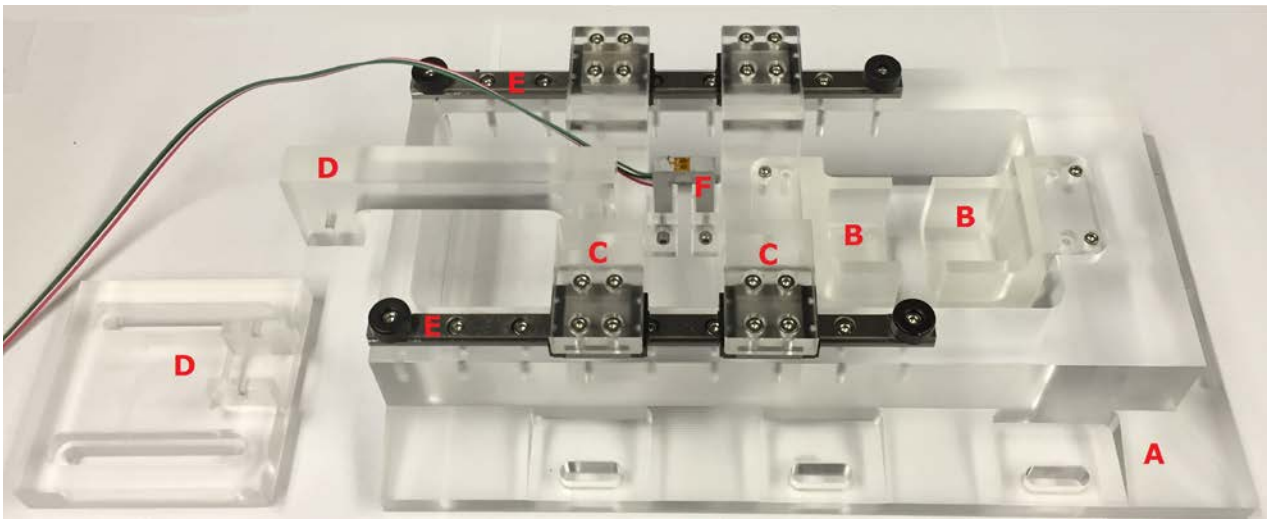

Figure 1: Device: A = base; B = bone pots; C = carriages; D = attachments for linear drive; E = linear rail guides; F = load cell.

microscopy [5]. Hence, optical sections are relatively thin and plane-specific. Any specimen movement parallel to the optical axis has the potential to put the plane under consideration out of focus, which is evidently problematic in mechanical loading applications. This problem can be partially addressed by capturing stacks of 2D images, typically up to 300 µm in depth, such that a small amount of vertical movement can be tolerated before the plane under consideration is lost from view. An additional benefit is 3D reconstruction of the section.

Nonetheless, it is desirable to minimize movement; thereby maximizing the consistent volume of tissue being imaged between loading conditions. This is accomplished in the design by the incorporation of linear rail guides (Thomson Industries Incorporated, Radford, VA, USA). Cementing one bone of the bone-ligament-bone specimen in a fixed position and the other bone to a carriage resting on the rails permits smooth, restricted (±10 µm) movement. Moreover, the low friction created by the rails enables accurate load measurement.

Dimensional, Weight and Immersion Lens Constraints

In order to function, the objective lens of the microscope must be submerged in solution. The microscope's stage has limited horizontal travel, usable area, bolt hole locations and clearance to the objective lens. Practically, the device cannot be exceedingly heavy or cumbersome for the stage or user.

No commercial hardware was found to satisfy these unique operating conditions and, as a result, a number of custom-machined components were designed and manufactured (Figure 1). The components consist of a base to secure the system to the stage and hold the solution; bone pots to carry whole joints; carriages to allow movement of the bone pots; and an attachment to a linear drive (Figure 2) to transmit load to specimens. Assembly is accomplished via screw connections. The final design was derived from numerous iterations using computer-aided software (SOLIDWORKS 2015, Dassault Systèmes SOLIDWORKS Corporation, Waltham, MA, USA), careful dimensional checks and consultation with staff of the Machine Shop in the Schulich School of Engineering at the University of Calgary. Acrylic was selected as the primary construction material for its light weight, machinability and rigidity.

Initial Specimen Preparation

Replicating and observing true physiological joint loading at the microscopic level is an exceedingly difficult task, and so was not an objective for the loading device. Given that the nature of the project is to characterize enthesis mechanics under load, the slow application of pure tensile loading was judged to be a reasonable first step.

Figure 2: Siskiyou 100cri Linear Drive

Figure 3: Specimen preparation jig

Figure 4: Siskiyou MC1100e Controller

Figure 5: Siskiyou DR1000 Digital Readout

However, to provide a level of consistency between observations, joints (rabbit stifle (knee) joints) were chosen to be fixed at 70º of flexion, an angle previously determined to place ligament midsubstance in tension [6]. To do so, a separate specimen "preparation jig" (Figure 3) was also manufactured from acrylic. The same bone pots as used in the device are placed in the jig and moved relative to one another until the appropriate angle is reached. The joint position is then secured with bone cement.

Measurement of Applied Load

While commercial load cells offer an obvious means of measuring the load applied to the ligaments, their cost can be prohibitive. In lieu of a commercial option, a load cell was designed. A piece of aluminum was custom-machined to a shape calculated to give a specified output for a given load when strain gauges (Vishay Intertechnology Incorporated, Shelton, CT, USA) were mounted and wired to create a full Wheatstone bridge (Figure 1). The strain gauges were mounted on the tension and compression faces of the bending portion of the aluminum piece. Following calibration using standard weights and coupled with an amplifier (Vishay Intertechnology Incorporated, Shelton, CT, USA) the load cell provides accurate measurements, sensitive to the small forces (up to 70 N in this case) involved in ligament loading.

Ligament Deformation

Rabbit MCLs are approximately 20 mm in length; their stress-strain curves depict a characteristic non-linear "toe" region until about 5% strain, after which point stress increases linearly with strain [7]. In order to describe enthesis mechanics fully, it is important to capture both the toe and linear regions, prescribing the need for a specialized deformation system.

Such a system is required to elongate ligaments incrementally up to 1 mm (or more). At each increment (~50 to 100 µm), the system must hold a static position while image stacks are collected. This was achieved through the use of a linear drive (Figure 2), controller (Figure 4) and digital readout (Figure 5) (Siskiyou Corporation, Grants Pass, OR, USA).

CONCLUSION

The overarching aim of the research project is to advance the understanding of enthesis mechanics via the novel application of SHG microscopy. Doing so will inform the biomaterial/tissue engineering and surgical communities and act as a foundation for

improving health outcomes of patients that have experienced enthesis injury.

To date, the key obstacle towards achieving this aim has been the construction of a specialized ligament-loading device that can be used with a sensitive imaging system. Analysis of relevant design constraints and objectives has led to the integration of the previously described components (rail guides, load cell and custom-machined parts). The end product is a device that adequately fulfills the unique role of ligament-loading with microscopy. While visualization of enthesis mechanics has been cited as the main reason for constructing the device, it is anticipated that the device will be useful for other tissues and areas of interest.

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