Biomechanical and Biochemical Study of Muscle-Tendon Junctions and Tendon-Bone Insertions

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Statement of Purpose: Tendons are responsible for transmitting contractile load from the muscles to the bones, thus enabling locomotion. Muscle-tendon junctions (MTJ) are functionally-graded connectivetissues whose anisotropic biomechanical functions depend intimately on the regional biochemical composition and structure [1]. In the muscle region, the elastic modulus or stiffness in the direction transverse to the fibers is much greater than that along the fibers, which is opposite to what is found in the tendon region. In fact the muscle region has a more pronounced anisotropy than the tendon near the interface, and a gradient in muscle stiffness exists as one approaches the interface [2]. On the other hand, the tendon-to-bone insertion site (i.e. the enthesis) is the tissue that connects these two dissimilar materials: tendon and bone [3]. The enthesis provides a gradual transition from soft tissue to hard tissue, functioning to alleviate stress concentration at the junction of these tissues. Specifically, dense and highly aligned collagen fibers are characteristic of the tendon side of the junction, and a high density of the mineral hydroxyapatite is characteristic of the bone side. Inhomogeneity and discontinuities in tissue-level properties at the enthesis manifest in the unique, stress concentration-reducing material behavior at the macro-scale. It has been observed that the unique transitional tissues existing between healthy MTJ and the enthesis are not faithfully reconstructed during tissue healing; instead, scar tissue is usually formed [4]. Therefore, surgical reattachments of MTJ and the enthesis often fail. As a result, a complete understanding of biomechanical and biochemical properties at these two interfaces will provide more information toward effective treatments for MTJ and the enthesis healing.

Methods: Collagen concentrations (collagen types I, III & V) of the MTJ and the enthesis are determined via an assay kit (Sircol; Accurate Chemical & Scientific Corp., Westbury, NY) using techniques adapted from our and other's published articles [5-7]. We have observed that collagens extracted at different time points at different locations in the samples play an important role in determining collagen concentration in native tissues, in which a minimum of 72 hours of acetic acid-pepsin extraction is required to obtain a saturated collagen concentration [5]. The extraction samples are obtained at five different locations over the length of the muscle-tendon-bone: muscle-tendonjunction, tendon mid-section (lower leg), tendon midsection (digital), fibrocartilage, and mineralized fibrocartilage region. The mechanical properties of native MTJ and enthesis tissue are critical to understand the physiological function and the design of tissue engineered MTJ and the enthesis scaffolds. Therefore, we have characterized the mechanical properties of native porcine MTJ and enthesis (i.e. the digital gastrocnemius muscle to flexor tendon). Six porcine trotters from large sows (i.e. greater than 180 lbs) are obtained from our local abattoir and from the NC State College of Veterinary Medicine (all tissues are obtained from animals already slaughtered for meat production or sacrifice) and are submitted to the laboratory within 60 minutes of sacrifice for dissection. Thirty native MTJ and enthesis specimens are prepared (i.e. five specimen from each trotter). Samples are stored in Hank's Balanced Salt Solution (HBSS) to allow for relaxation prior to testing.

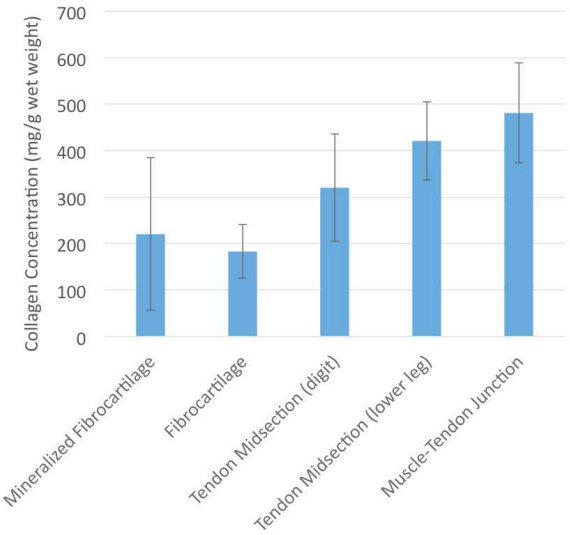

Figure 1: (Top) The collagen concentration over the length of the muscle-tendon-bone tissues. (Bottom) The BioTester is capable of applying physiologically plausible biaxial stretching or loading states to tissue samples.

Preloading and preconditioning of samples is anticipated to release the residual stresses inside the tissue samples and to establish a repeatable set of experiments, respectively. Samples are pre-loaded to 0.1N and then pre-conditioned at 10% strain/min to 0.5% strain for 5 cycles followed by a 5 min recovery period [8]. The tissue samples are tested to 35% strain in both axes with a 15 second stretch cycle and a 15 second recovery cycle (i.e.,

strain-rate = 2.33%/sec), with no hold time. Synchronized time lapse video for real-time monitoring and post-process analysis is provided by the charged-couple device (CCD) camera, which acquires images with a pixel resolution of 1280 × 960 at an acquisition rate of 15 Hz, using a lens focal length of 75 mm. Image tracking and analysis software (Labjoy, CellScale) is used to review and analyze the collected images during biaxial mechanical testing.

Results: The results reveal the sensitivity of collagen concentration along the muscle-tendon-bone tissues. The corresponding histological microphotographs are provided to support our collagen concentration results. The nonlinear anisotropy mechanical properties of tissues are correlated to the microstructure and the constituent of the tissues.

Conclusions: In the current study, a synergy approach is reported and aimed at understanding the interplay of muscle-tendon-bone tissue mechanical property and microstructures, with special emphasis on collagen content over different extraction time. This work provides an easy approach for quantifying biomechanical and biochemical properties of biological tissue, and potentially facilitates the development of tissue engineered MTJ and enthesis.

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