A STRUCTURAL AND CELLULAR EVALUATION OF SUB-FAILURE DAMAGE IN LIGAMENT

*Provenzano, P; *Hayashi, K; *Lakes, R; +Vanderby Jr., R
+Orthopedic Research Laboratory, University of Wisconsin, Madison, WI. Division of Orthopedics, 600 Highland Avenue, Madison, WI, 53792, 608-263-9593, Fax: 608-263-0454, vanderby@surgery.wisc.edu

Introduction: Microtrauma or subfailure injury in tendon and ligament, may occur either as the result of overuse or a single traumatic event [1] and is thought to play a role in tendinitis [2]. Sprains are classified by severity based upon clinical examination or imaging. Grade I sprains show no discontinuity of the ligament and no clinically detectable increase in joint laxity. Grade II sprains are moderate stretches in which some fibers are torn, but enough fibers remain intact so that the damaged ligament has not failed. These grade II sprains produce detectable laxity at the joint. Grade III sprains are severe and consist of a complete or nearly complete ligament disruption and result in significant joint laxity. Severe sprains involving complete disruption of the ligament and resulting in significant joint laxity (Grade III) constitute less than 15% of all ligament sprains [3]. This leaves more than 85% of the sprains where subfailure damage is the dominant issue. In one study of rabbit anterior cruciate ligaments, subfailure loadings (~80% of failure stretch) altered the shape of loading curves and thereby increased joint laxity [4]. In another study, dramatic reductions in peak force were observed in human cadaveric inferior glenohumeral ligament after repeated sub-failure loadings[5]. The purpose of this study is to evaluate sub-failure damage from a structural and cellular perspective.

Methods: This study meets N.I.H. guidelines for animal welfare. Medial collateral ligaments from Sprague-Dawley male rats (weight = 250 ± 25 g) were used. For the structural damage group (n=25) the gauge length (LO) was measured at 10 grams of preload. After preload the tissue was preconditioned and subjected to a sub-failure stretch in displacement control. The tissue was then unloaded and allowed to recover for 10 minutes (> 300X the test time), in order to ensure that changes in length were the result of irreversible elongation (damage) instead of a viscoelastic creep-relaxation response. After recovery the length (LS) of the MCL was measured at the preload loading of 10 grams. The measure of structural damage is based on a length difference and is expressed as a percent: DS = 100*(LS-LO)/LO. Cellular damage (n=22) was measured by stretching the ligaments, in the same fashion as the structural damage group, and using confocal microscopy with a cell viability assay to detect live and dead cells. In situ staining was done where live cells show green fluorescence and membranes of dead, and damaged (dying) cells show red fluorescence. Using a technique similar to areal density used to analyze bone density, image scans taken during this study. The tissue are compiled and overlaid. A histogram that counts the number of pixels of each color indexed was obtained and used to count the number of pixels representing dead cells. The area of the ligament was measured in units of pixels and used to normalize the number of necrotic cells. Therefore, the measure of cellular damage is a non-dimensional unit represented as a percent of the form DC = 100*(area containing damaged cells / tissue area) [pixels/pixels].

Results: Results indicate that structural damage and cellular damage are different for a particular strain. Structural damage (DS) was seen to be minimal below strains of ~5% and to increase at greater strains (Fig. 1). Cellular damage (DC) occurs below 5% strain where structural damage was not detectable (Fig. 2). Stress-strain curves of damaged ligaments (not shown) displayed an elongation of the toe region and decrease in stiffness, while undamaged ligaments follow the shape of the initial stretch.

Fig 1. Structural damage versus strain curve where DS = 100*[(LS-LO)/LO]. Note the slope change at ~5% strain.

Fig 2. Cellular damage versus strain where DC = 100*(area containing damaged cells / tissue area). Note the presence of cellular damage at lower strains than structural damage.

Discussion: Sub-Failure damage has been shown to lengthen the toe region of the force-displacement curve, which may result in increased joint laxity [4]. Results from this study indicate that medial collateral ligaments strained above ~5% do not regain their original length after significant recovery time. We speculate that this increase in elongation is the result of fiber damage in the form of torn or plastically deformed fibers. The resulting increase in tissue length can also be hypothesized to increase joint laxity. Results form this study show that small strains cause cellular damage. Since low tissue strains are seen in normal activity, mechanically induced cell death may be part of the natural remodeling process in ligaments. A threshold may exist before which natural remodeling takes place and after which symptoms of overuse may become present.

References:

Acknowledgement: The author’s would like to thank Yan Lu for his help with confocal microscopy. This work was funded in part by NSF (grant # CMS-9907977) and NASA (grant # NAG 9-1152).