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Research Paper

Multiscale mechanical integrity of human supraspinatus tendon in shear after elastin depletion

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ABSTRACT

Human supraspinatus tendon (SST) exhibits region-specific nonlinear mechanical properties under tension, which have been attributed to its complex multiaxial physiological loading environment. However, the mechanical response and underlying multiscale mechanism regulating SST behavior under other loading scenarios are poorly understood. Furthermore, little is known about the contribution of elastin to tendon mechanics. We hypothesized that (1) SST exhibits region-specific shear mechanical properties, (2) fiber sliding is the predominant mode of local matrix deformation in SST in shear, and (3) elastin helps maintain SST mechanical integrity by facilitating force transfer among collagen fibers. Through the use of biomechanical testing and multiphoton microscopy, we measured the multiscale mechanical behavior of human SST in shear before and after elastase treatment. Three distinct SST regions showed similar stresses and microscale deformation. Collagen fiber reorganization and sliding were physical mechanisms observed as the SST response to shear loading. Measures of microscale deformation were highly variable, likely due to a high degree of extracellular matrix heterogeneity. After elastase treatment, tendon exhibited significantly decreased stresses under shear loading, particularly at low strains. These results show that elastin contributes to tendon mechanics in shear, further complementing our understanding of multiscale tendon structure-function relationships.

1. Introduction

The supraspinatus tendon (SST), part of the rotator cuff in the shoulder, passes underneath the acromion such that interactions with surrounding bones/tissues cause multiaxial loading (i.e., tension, shear, compression) (Bey et al., 2002). Study of SST mechanical behavior under different loading scenarios could help determine how tendon modulates its structure and composition to support multiaxial loading, and potentially inform how degeneration or injury occurs due to such loading. Previous studies have reported unique and highly inhomogeneous mechanical, structural, and compositional properties of

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human SST under tensile loading (Lake et al., 2009, 2010; Ahmadzadeh et al., 2013; Bey et al., 2002). The medial SST exhibits properties typical of tendon while the lateral region (i.e., the area of likely multiaxial loading) shows planar mechanical isotropy, disorganized collagen fibers, and extracellular matrix (ECM) composition more similar to fibrocartilage (Ahmadzadeh et al., 2013; Buckley et al., 2013; Lake et al., 2010; Marturano et al., 2013). These data imply complex multiaxial *in vivo* loading of SST, however much remains unknown regarding SST properties under such non-tensile loads.

Biomechanical tensile testing combined with confocal microscopy imaging has been utilized to examine tendon mechanics and physical mechanisms underlying tendon behavior. For example, photobleached grids created on rat-tail tendons showed both rotation and stretch under tension, which indicated collagen fiber sliding and elongation as the predominant modes of microscale deformation contributing to tendon response in tension (Screen et al., 2004; Szczesny et al., 2015) Tendon viscosity and elasticity in tension are also facilitated by this microscale deformation (i.e. sliding between collagen fibers and fiber elongation) for avoiding injury (Cheng and Screen, 2007; Fang and Lake, 2015; Screen et al., 2004). In our previous work, sliding between collagen fibers and fiber reorganization were found to modulate the response of bovine deep digital flexor tendons under shear loading (Fang and Lake, 2015). Although strain transfer in tendon has been evaluated, it remains generally unknown which tissue constituents facilitate strain/force transfer, particularly under non-tensile and/or multiaxial loading.

While long known to be important in cardiovascular mechanics, recent work has suggested that elastin may play a mechanical role in orthopedic tissues such as tendon and ligament (Grant et al., 2015; Henninger et al., 2013, 2015; Jacobs et al., 2011; Michalek et al., 2009; Smith et al., 2008). For example, elastin was shown to sustain tensile loading in the toe-region and assist in load transfer of porcine ligament (Henninger et al., 2013). Additionally, decreased mechanical parameters of ligaments after elastin depletion under both shear and transverse tensile loading verified that elastin also provided support for transverse elongation and shear deformation (Henninger et al., 2015), which was also demonstrated for bovine annulus fibrosus under dynamic shear loading (Michalek et al., 2009). Interestingly, both rat-tail and human palmaris longus tendons showed lower tensile strength and failure strain following elastin degradation, and larger wavelength of crimp pattern under tension (Grant et al., 2015). To date, elastin has been shown to distribute along collagen fibers in tendon, with fibrillin-1 and fibrillin-2 coexisting with elastin as measured by immunohistochemistry (Feitosa et al., 2002; Grant et al., 2013; Ritty et al., 2002; Thakkar et al., 2014). However, it remains unclear how elastin is distributed within and throughout tendon, how it interacts with collagen fibers, and how elastin contributes to tendon mechanical behavior under multiaxial loading.

The aims of this study were (1) to quantitatively evaluate region-specific mechanical properties of human SST in shear using biomechanical testing in combination with multiphoton microscopy imaging, and (2) to examine the role of elastin in SST response to shear through targeted enzyme treatment. We hypothesized that (1) both anterior and posterior regions of SST would exhibit larger shear stresses and smaller microscale deformation than the medial region due to less organized collagen in anterior and posterior regions, and (2) SST would show smaller shear stresses but larger microscale deformation following elastin degradation, thereby demonstrating that elastin contributes to maintain tendon mechanical integrity under multiaxial loading.

2. Materials and methods

2.1. Elastase treatment protocol

The efficiency of elastase on SST was examined in concentration- and time-dependent testing. Small tissue segments ($n=15$), weighing 40–50 mg (approximate dimensions of $1 \times 0.5 \times 0.5$ mm), were obtained from the anterior region of a single SST. To hinder proteolysis caused by elastase impurity, all segments were equilibrated in 15 ml PBS with 0.1 mg/ml soybean trypsin inhibitor (SBTI, SB903, Elastin Product Company) for 15 min at room temperature. These segments were randomly divided into five groups ($n=3$ for each) and incubated in 15 ml PBS with 0, 1, 5, 10, and 20 units/ml elastase (trypsin-free porcine pancreatic elastase with high purity, EC134, Elastin Products Company) for 6 h at room temperature to evaluate the effect of elastase concentration. Similar small tissue segments ($n=15$) were prepared from the anterior region of another SST and treated similarly with soybean trypsin inhibitor, as stated above. After divided into five groups ($n=3$ for each), segments were incubated in 15 ml PBS with 5 units/ml elastase for 0, 1, 4, 6, 12 h at room temperature to evaluate the effect of incubation time. Following elastase treatment, the elastin content of all segments was quantified using the biochemical analysis described below. Results of the concentration- and time-dependent testing indicated an elastase treatment protocol of 15-min incubation in 0.1 mg/ml SBTI solution followed by incubation for 8 h in 5 units/ml elastase in PBS.

Due to the relatively large size of our samples for biomechanical testing and the method used to attach samples to shear clamps discussed below, the depth at which elastase could penetrate into the tissue for digestion was evaluated. Bovine deep digital flexor tendons (DDFTs), as a representative tendon tissue with a better known (and constant) distribution of elastic fibers (Grant et al., 2013), were used for this assessment. Samples ($n=4$) with the same size as the ones used for biomechanical testing were cut from the proximal region of four bovine DDFTs, similar to previous studies (Fang and Lake, 2015; Fang et al., 2014; Grant et al., 2013). An additional set of adjacent samples ($n=4$) was also obtained from the corresponding flexor tendons for control. Samples were glued to shear clamps and the clamps-sample assemblies were incubated in PBS for 8 h, consistent with the time used for sample preparation and biomechanical testing before elastase treatment. After PBS incubation, the clamps-sample assemblies were treated according to the elastase treatment protocol. The control clamps-sample assemblies were incubated in PBS but without elastase for the same amount of time. The surface of samples touching the bottom of beaker during treatment was defined as the bottom. Following treatment, thin pieces of