Acta Biomaterialia 25 (2015) 304–312

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Elastin governs the mechanical response of medial collateral ligament under shear and transverse tensile loading

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ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form 25 June 2015
Accepted 6 July 2015
Available online 7 July 2015

Keywords:
Ligament
Elastin
Transverse tensile
Shear
Elastase

ABSTRACT

Elastin is a highly extensible structural protein network that provides near-elastic resistance to deformation in biological tissues. In ligament, elastin is localized between and along the collagen fibers and fascicles. When ligament is stretched along the primary collagen axis, elastin supports a relatively high percentage of load. We hypothesized that elastin may also provide significant load support under elongation transverse to the primary collagen axis and shear along the collagen axis. Quasi-static transverse tensile and shear material tests were performed to quantify the mechanical contributions of elastin during deformation of porcine medial collateral ligament. Dose response studies were conducted to determine the level of elastase enzymatic degradation required to produce a maximal change in the mechanical response. Maximal changes in peak stress occurred after 3 h of treatment with 2 U/ml porcine pancreatic elastase. Elastin degradation resulted in a 60–70% reduction in peak stress and a 2–3× reduction in modulus for both test protocols. These results demonstrate that elastin provides significant resistance to elongation transverse to the collagen axis and shear along the collagen axis while only constituting 4% of the tissue dry weight. The magnitudes of the elastin contribution to peak transverse and shear stress were approximately 0.03 MPa, as compared to 2 MPa for axial tensile tests, suggesting that elastin provides a highly anisotropic contribution to the mechanical response of ligament and is the dominant structural protein resisting transverse and shear deformation of the tissue.

1. Introduction

Ligament and tendon are composed of up to 70% water, and the remaining constituents include type I collagen (~70% of dry weight), other collagens in significantly lesser amounts, proteoglycans and elastin, with lesser populations of fibrillin, fibrinogen, fibronectin and laminin [1]. The hierarchical structural organization and mechanical role of type I collagen in ligaments and other dense connective tissues is well appreciated, with its organization spanning multiple physical scales from tropocollagen molecules to fibrils, fibers, fascicles and ultimately the macro-scale tissue [2], where the mesoscale crimping and twisting of collagen leads to nonlinear material behavior [3]. Studies of the structure and function of normal ligament and tendon and alterations due to injury and disease have typically focused on the contributions of type I collagen due to its high percentage of tissue dry weight, highly aligned structure, stiffness and strength. However, the structure, organization and function of the remaining extracellular components of the matrix (the so-called "ground substance") have received considerably less attention. In order to understand the structure–function relationships in these biologic materials and to interpret the alterations in organization and mechanical response of ECM components due to disease or injury, we must first characterize their contributions to normal tissue mechanics.

Elastin constitutes approximately 5% of the dry weight of ligaments [4–6]. Assembled in the extracellular space, it consists of an elastin core of tropoelastin molecules surrounded by a fibrillin-rich microfibril scaffold [4,6]. Repeating α-helix segments composed of alanine and lysine oxidize to form highly stable covalent crosslinks between tropoelastin molecules [7,8]. Elastin stretches and recoils through both entropic and hydrophobic mechanisms [4,6]. We recently examined the role of elastin in ligament mechanics via selective degradation with elastase [9] and found that elastin provided a disproportionately high contribution during uniaxial tensile deformation along the primary collagen axis. Although elastin constituted only 4% of the tissue dry weight, it supported up to 30% of tensile stress under uniaxial strain [5]. In addition, elastin is localized between and along collagen fibers in

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http://dx.doi.org/10.1016/j.actbio.2015.07.011
1742-7061/

cruciate ligaments [10]. These factors led us to hypothesize that elastin may also resist transverse and shear tissue deformation relative to the primary collagen axis.

Therefore, the purpose of this study was to quantify the contribution of elastin to the quasi-static mechanical response of ligament when tested in elongation transverse to the primary collagen fiber direction and in shear along the fiber direction. We hypothesized that both stress and stiffness would decrease after selective enzymatic treatment to degrade elastin for both test protocols. In combination with our previous study, the results of this study provide a multiaxial characterization of both native and elastin-degraded ligament. These results clarify our understanding of the mechanical function of elastin, provide the basis for formulating constitutive models that include elastin for both normal and pathological dense connective tissues, and in the future, these results will help to interpret tissue pathologies that involve elastin in disease and injury.

2. Materials and methods

2.1. Experimental design

Forty-three porcine medial collateral ligaments (MCLs, age 5–8 mo., mixed sex) (Innovative Medical Device Solutions, Logan, UT) were harvested and frozen until testing. Porcine MCL was chosen as it is a readily available planar ligament that is large enough to allow isolation of multiple rectangular test specimens, and its native material properties are similar to human MCL [5,11]. The tissue was thawed and the ligament was fine dissected to remove overlying fascia. Tissue was kept moist with phosphate buffered saline (PBS) throughout dissection and experimentation. Tissue was refrozen to −70 °C and two rectangular specimens were punched from each ligament perpendicular to the primary collagen axis (Fig. 1) [11,12]. Specimen dimensions were 10 mm long × 14–20 mm wide (nearly the full width of the ligament). The width and thickness of each specimen were then recorded with a digital caliper (Mitutoyo, CA, accuracy ± 0.02 mm) for calculation of cross-sectional area. One of the two specimens was randomly selected to serve as a control and the other was treated with elastase enzyme to degrade elastin.

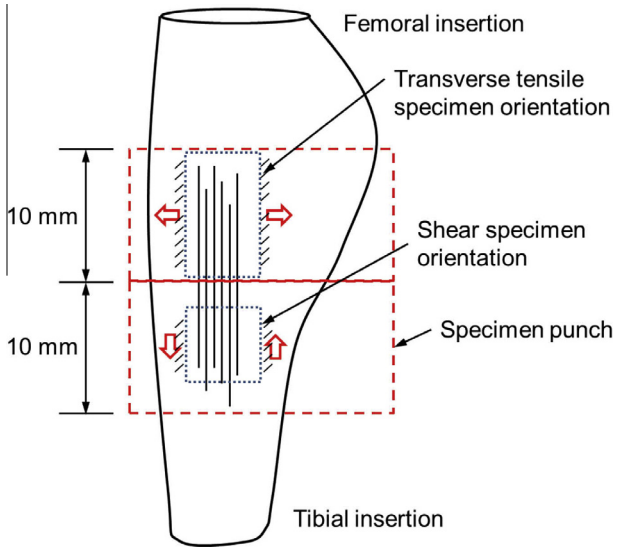

Fig. 1. Schematic of specimen harvest locations in porcine MCL. Two neighboring rectangular specimens were harvested at the midpoint of the ligament (punch footprint shown in red). A representative transverse tensile and shear specimen (blue) are shown as the area between the clamps with respect to the overall punch dimensions. Red arrows denote the axes of deformation relative to the collagen fibers. The shear specimen height was reduced to ensure the area between the clamps was nearly square, allowing for adequate tissue to be gripped in the clamps. Note that within a ligament both specimens were harvested for the same test protocol. Shear and transverse tensile are shown here in the same ligament for illustration only. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Elastin haploinsufficient [13–15] and human elastin knock-in [16] animal models have been developed to study the contributions of elastin to tissue development and mechanics, but these models cannot fully remove the influence of elastin. Animal viability suffers when total elastin levels drop below 30% of wildtype [16]. Alternatively, selective degradation of tropoelastin allows the study of tissue that has undergone normal development. Elastase enzymes cleave tropoelastin but leave the desmosine and isodesmosine crosslinks intact (Fig. 2) [9], resulting in a fragmented network that rapidly loses mechanical integrity as the level of elastin degradation increases [5].

Dose–response experiments were undertaken with transverse tensile tests to determine the elastase concentration and treatment time required to effect a maximal change in peak stress between control and treated specimens [5]. Fifteen specimens from 15 ligaments were used to test the influence of elastase concentration: 3 specimens treated for 3 h each at 0 (control), 0.1, 1, 2, and 10 U/ml elastase. Twelve pairs of specimens from 12 ligaments were used to examine the effects of treatment time: 3 pairs treated with control buffer or 2 U/ml elastase for 0.5, 1, 3, and 6 h. Eight pairs of specimens from 8 ligaments were used in both a transverse tensile and simple shear protocol (16 pairs total) once the optimal dose–response was determined.

2.2. Transverse tensile testing

The transverse tensile testing protocol was adapted from a prior study [12] where specimens were aligned with the primary collagen axis perpendicular to the test axis. Given the limited width of the porcine MCL (Fig. 1), harvesting a "dogbone" shaped specimen with at least an 8:1 aspect ratio (excluding tissue within the clamps) resulted in extremely thin, fragile specimens. Instead, a strip biaxial specimen shape was used to increase the cross

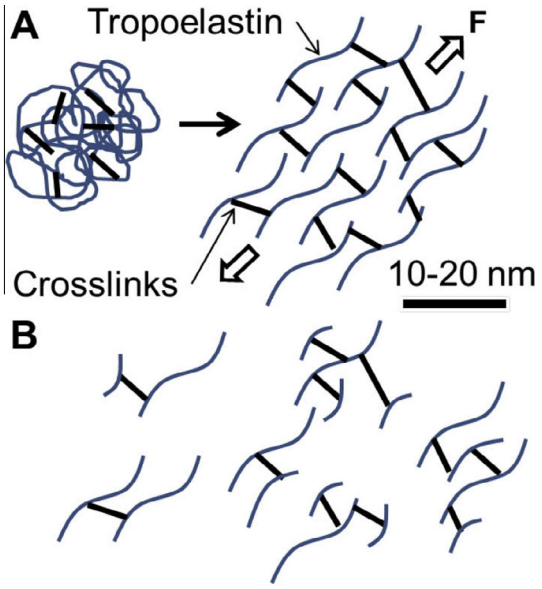

Fig. 2. Elastin structure and degradation products. (A) Elastin recoils due to hydrophobic and entropic forces, but extends under applied force (F). Desmosine and (iso)desmosine crosslinks resist network deformation. (B) Elastase degrades elastin via cleavage of tropoelastin, leaving fragments with crosslinks intact.