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Mechanical properties and morphological analysis of the transitional zone between meniscal body and ligamentous meniscal attachments

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ABSTRACT

In recent years, an increasing number of studies reporting on meniscal root tears have been published. While the meniscus and its ligamentous meniscal attachments have been studied before, little is known about the transitional zone between these two structures. Therefore, the aim of this study was to mechanically and morphologically characterize the transitional zone between meniscus and its meniscal attachments.

Dumbbell-shaped specimens were obtained from the transitional zone between meniscus and its meniscal attachments of 6 knee joints. Samples were divided into tibial and central layers of the anterior lateral (AL), anterior medial (AM), posterior lateral (PL) and posterior medial (PM) transitional region. Testing was performed to obtain the dissipated energy during hysteresis as well as the linear modulus (E_{lin}), the maximum strain ($\varepsilon_{\max}$), the maximum engineering stress ($\sigma_{\max,\mathrm{eng}}$) and location of rupture during tensile test to failure. Two additional knee joints were used to investigate morphological differences between meniscus, transitional zone and meniscal attachments in 8 μm transverse slices.

The central layer of the AL, AM and PL dissipated up to 48% less energy than the tibial layer. E_{lin} was highest in the tibial layer of the PM with 107.4 ± 61.1 MPa and lowest in the central layer of the PL with 56.0 ± 20.5 MPa. The maximum strain was higher in the central layer than in the tibial layer at the AL, AM, and PL locations. The average $\sigma_{\max,\mathrm{eng}}$ was 12.7 ± 9.9 MPa over all location and layers. 78% of the samples ruptured during tensile test to failure in the transitional zone. The morphological evaluation showed a smooth transitional zone with a transitional curve which was either linear or bell-shaped. The strength found in the transitional zone was lower than in the meniscus and the meniscal attachments, which corresponds well to clinical findings.

1. Introduction

The knee menisci are essential for the load transfer between femur and tibia (Bullough et al., 1970; Walker and Erkman, 1975; Masouros et al., 2008). Each meniscus is firmly attached to the tibia via its ligamentous anterior and posterior meniscal attachment (Masouros et al., 2008). Consequently, axial loading of the knee joint creates hoop strain in the menisci, preventing the menisci from completely protruding from the joint.

Meniscal root tears diminish the function of the meniscus and it has been shown that this increases the contact pressure between femur and tibia, and decreases the contact area (Marzo and Gurske-DePerio, 2009). Long term, this leads to degenerative changes of the adjacent articular cartilage (Andriacchi et al., 2004; Felson, 2013).

The native mechanical properties of the entity (meniscal body, transitional zone, meniscal attachments) are important biomechanical parameters for a number of reasons. Recently, an increasing number of studies on meniscal root tear (Bin et al., 2004; Brody et al., 2006; Jones et al., 2006; Navarro-Holgado et al., 2007; Choi et al., 2008; Ahn et al., 2009; Kenny, 2009; Marzo, 2009; Feucht et al., 2015; Iversen and Krogsgaard, 2014) have been published. To effectively restore the function of the meniscus after a meniscal root tear, reestablishing the native mechanical properties of the transitional zone is important. Moreover, it can also be useful for the improvement and development of total meniscal implants and to more precisely model the transitional zone between meniscus and its meniscal attachments in finite element models. Previous studies have reported the tensile properties of the human meniscal body (Bullough et al., 1970; Fithian et al., 1990; Tissakht and Ahmed 1995; Lechner et al., 2000) as well as the properties of the human meniscal attachments (Hauch et al., 2010).

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However, to the best of our knowledge, the mechanical properties of the transitional zone have not yet been characterized.

Typically, the mechanical properties of the tissue correlate with its morphology. The fibrocartilagenous menisci and their meniscal attachments are primarily composed of Type I collagen fibrils (McDevitt and Webber, 1990; Villegas et al., 2008). The microstructure of the human meniscus shows three distinct layers of collagen arrangement. The tibial or femoral superficial layer combined with the lamellar layer is approximately 200 μm thick with no predominant fiber direction (Petersen and Tillmann, 1998). The primary and middle layer has predominantly circumferential fibers (Petersen and Tillmann, 1998). These circumferential fibers of the meniscus continue into the ligamentous meniscal attachments (Villegas et al., 2008). The meniscal attachment is then firmly fixed to the underlying tibial bone (Messner and Gao, 1998). The microstructure of the ligamentous meniscal attachments is similar to tendons and other ligaments (Benjamin et al., 1986, 1991), with the primary fiber orientation aligned with the tensile direction. However, to the best of our knowledge, the transition between meniscus and ligamentous meniscal attachments has not yet been investigated.

The aim of this study was to mechanically characterize the transitional zone between meniscus and its ligamentous meniscal attachments. Moreover, the morphological analysis should support the mechanical findings.

2. Material and methods

2.1. Study outline

Eight unpaired human knee joints (4 male, 4 female, 62.5 ± 12.3 years, Southeast Tissue Alliance, Gainesville, FL, USA) were tested. Six knee joints were used to explore dissipated energy (ΔW), linear modulus (E_{lin}), maximum strain ($\varepsilon_{\max}$) and maximum engineering stress ($\sigma_{\max,\text{eng}}$) during biomechanical testing of the transitional zone. The remaining two joints were prepared for morphological analysis of the transitional zone between meniscal body and ligamentous meniscal attachments.

2.2. Biomechanical testing

The fresh frozen human knee joints were thawed at 4 °C before preparation. The medial and lateral menisci and their adjacent meniscal attachments were carefully excised at the bony insertion. Two dumbbell-shaped samples were punched out of the transitional zone between the meniscal body and its anterior and posterior meniscal attachments, respectively (Fig. 1a). The punch was oriented parallel to the main fiber direction of the meniscal attachments and placed such that the transitional zone was located in the middle of the sample. From each dumbbell-shaped sample, two slices were obtained with a thickness of approximately 1 mm using a custom microtome. One slice contained the tibial surface of the meniscus while the other was cut out of the central part of the dumbbell shaped specimen (Fig. 1a). This resulted in 96 specimens from two layers (central and tibial) of the anterior lateral (AL), anterior medial (AM), posterior lateral (PL) and posterior medial (PM) location.

The width and depth of the dumbbell shaped samples was measured with digital calipers prior to testing, since small variations occurred by punching the specimens. Each specimen was then clamped into a material testing machine (Zwick Roell, Ulm, Germany; Fig. 1b) and testing was performed. To ensure proper fixation of the specimen and to enhance friction, sand paper was glued to both ends of the specimen using superglue (UHU Sekundenkleber, UHU GmbH & Co. KG, Bühl, Germany). Care was taken that no glue penetrated the testing area, which was verified under the microscope during pretestings.

Each specimen was preconditioned with a stress relaxation test at 10% strain for 10 min (Chia and Hull, 2008). Afterwards, a hysteresis test was performed over 5 cycles with a maximum strain of 20% under a strain rate equal to 100% of the specimen's initial length per minute (deflection rate similar to: (Villegas et al., 2007; Hauch et al., 2010)). Following the hysteresis test, each specimen was pulled to failure under the same strain rate.

From the hysteresis test, the dissipated energy (ΔW) was determined by the enclosed area between loading of the first cycle and unloading of the fifth cycle in the load displacement curve. During the tensile test to failure, the linear modulus (E_{lin}) between 10% and 15% strain, the maximum strain ($\varepsilon_{\max}$) and the maximum engineering stress ($\sigma_{\max,\text{eng}}$) were evaluated. $\sigma_{\max,\text{eng}}$ was defined as the maximum force divided by the initial cross-sectional area of the specimen. Moreover, the rupture region was noted.

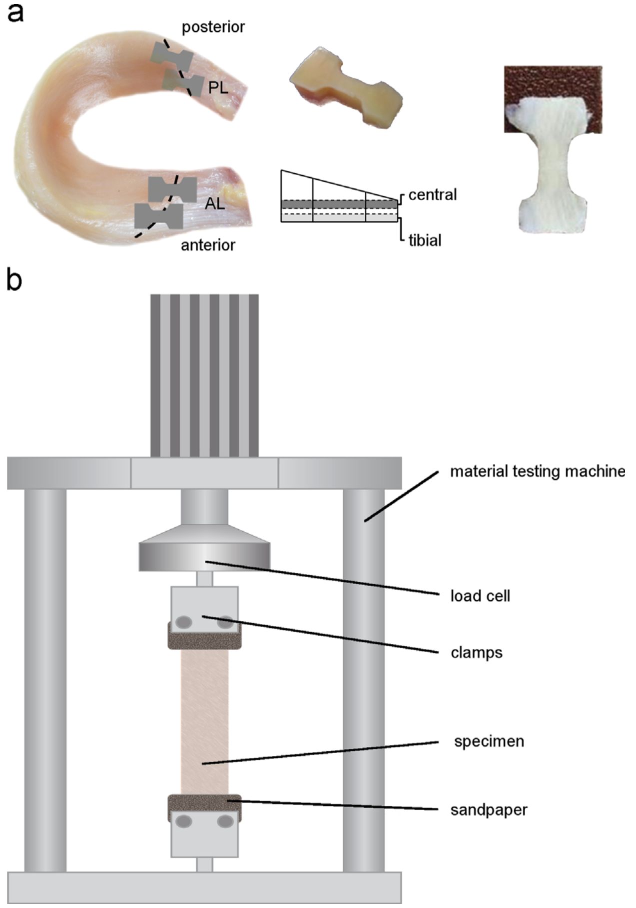

Fig. 1. Sample locations for tensile testing within a lateral meniscus with its anterior (AL) and posterior (PL) meniscal attachment, location of central and tibial layer and sample with sandpaper (a; from left to right) as well as schematic test setup with the sample mounted in the material testing machine for biomechanical testing (b).

2.3. Histological analysis

From two additional knee joints, samples 40 mm in length which included parts of the ligamentous meniscal attachments and meniscal body, with the transitional line in the center, were dissected from the medial and lateral meniscus (8 samples overall). These samples were then preconditioned under a tensile load of 0.16 N in a special loading-apparatus and frozen at −80 °C in order to maintain the stretched condition. Afterwards the sample length was reduced to a length between 20 mm and 25 mm and adjusted onto sample stubs with embedding compound (Tissue-Tek® O.C.T.™ Compound, Sakura Finetek Europe B.V, Alphen aan den Rijn, The Netherlands) in order to obtain transversal cryoslices of 8 μm (Kryotom Leica CM 1950, Leica Microsystems GmbH, Wetzlar, Germany). The center slices of each transitional zone was stained with hematoxylin and eosin (HE stain) to enhance contrast. Afterwards, each slice was digitized under a microscope (Leica DMI600 B, Leica Microsystems GmbH, Wetzlar, Germany) at a 12.5-fold magnification.

The slices were quantitatively evaluated with a custom matlab routine using the embedded matlab function regionprops (v. R2013b, MathWorks, Natick, USA). For each slice the number of non-collagenous spaces were calculated in order to determine the degree of cross-linking in the tissue slice. Moreover, the principal axis length of the non-collagenous spaces was determined as well as the level of organization, which was based on the relative orientation of the principal axes of the non-collagenous spaces to each other (Fig. 2). The data was obtained for the meniscus, transitional zone, and meniscal attachments (Fig. 3).

2.4. Statistical analysis

Samples from the same layer, location and specimen were averaged and normal distribution of all data was checked using a Shapiro–Wilk test (Shapiro and Wilk, 1965). A hierarchical linear model (Raudenbush and Bryk, 2002) was used to account for paired meniscus samples. Fixed effects were defined as location (anterior lateral (AL), anterior medial (AM), posterior lateral (PL), posterior medial (PM)) and layer (central and tibial). A significance level of $p < 0.05$ was used. The analysis was performed using a statistics software package (SPSS, v.21, SPSS Inc., Chicago, USA). The morphological analysis was evaluated descriptively.

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