Journal of the Mechanical Behavior of Biomedical Materials 41 (2015) 108–114

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Research Paper

Regional and depth variability of porcine meniscal mechanical properties through biaxial testing

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ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form
7 October 2014
Accepted 8 October 2014
Available online 19 October 2014

Keywords:
Meniscus
Mechanical properties
Biaxial testing

ABSTRACT

The menisci in the knee joint undergo complex loading in-vivo resulting in a multi-directional stress distribution. Extensive mechanical testing has been conducted to investigate the tissue properties of the knee meniscus, but the testing conditions do not replicate this complex loading regime. Biaxial testing involves loading tissue along two different directions simultaneously, which more accurately simulates physiologic loading conditions. The purpose of this study was to report mechanical properties of meniscal tissue resulting from biaxial testing, while simultaneously investigating regional variations in properties. Ten left, fresh porcine joints were obtained, and the medial and lateral menisci were harvested from each joint (twenty menisci total). Each menisci was divided into an anterior, middle and posterior region; and three slices (femoral, deep and tibial layers) were obtained from each region. Biaxial and constrained uniaxial testing was performed on each specimen, and Young's moduli were calculated from the resulting stress strain curves. Results illustrated significant differences in regional mechanical properties, with the medial anterior (Young's modulus (E)=11.14±1.10 MPa), lateral anterior (E=11.54±1.10 MPa) and lateral posterior (E=9.0±1.2 MPa) regions exhibiting the highest properties compared to the medial central (E=5.0±1.22 MPa), medial posterior (E=4.16±1.13 MPa) and lateral central (E=5.6±1.20 MPa) regions. Differences with depth were also significant on the lateral meniscus, with the femoral (E=12.7±1.22 MPa) and tibial (E=8.6±1.22 MPa) layers exhibiting the highest Young's moduli. This data may form the basis for future modeling of meniscal tissue, or may aid in the design of synthetic replacement alternatives.

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http://dx.doi.org/10.1016/j.jmbbm.2014.10.008
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1. Introduction

The meniscus, which is composed of two C-shaped fibrocartilagenous tissues, is interposed between the articulating surfaces of the femur and tibia in the knee joint. Its functions have been described as load distribution, joint stabilization, and joint lubrication (Allen et al., 2000; Arnoczky and McDevitt, 2000; Favenesi et al., 1983; Radin et al., 1984), making the meniscus an integral component in knee biomechanics. It has been established that meniscal injuries and deterioration of this tissue can lead to osteoarthritis (Rangger et al., 1995; Ratzlaff and Liang, 2010). For this reason the standard of care in orthopedics is currently to salvage and restore damaged meniscus whenever possible (Hutchinson et al., 2014). As a result, an in-depth understanding of the indigenous structure and biomechanical functioning of the native meniscus is crucial to our progression in developing improved repair strategies and transplant alternatives.

Tensile material properties of the meniscus have been studied extensively (Proctor et al., 1989; Fithian et al., 1990; Lechner et al., 2000; Goertzen et al., 1997; Skaggs et al., 1994; Tissakht and Ahmed, 1995). Although these studies vary in their methodology, including type of species, specimen dimensions and the region from which samples were extracted; one common factor is that separate uniaxial tests in the circumferential and radial directions have been performed. However, the meniscus experiences stresses simultaneously in both the circumferential and radial direction during in vivo loading-phases (Bylski-Austrow et al., 1994; Mow et al., 1992; Walker and Erkman, 1975), suggesting that a simultaneous multi-axial loading regime is more physiologically relevant. The uniaxial and biaxial tissue responses have been shown to be significantly different in other tissue types (Eilaghi et al., 2010; Gregory and Callaghan, 2011). A number of studies have looked at the biaxial tensile response of collagenous tissues (Eilaghi et al., 2010; Gregory and Callaghan, 2011; Holmes et al., 2012) but none have investigated this response in the meniscal tissue. Given the current focus on salvaging meniscal tissue and developing synthetic alternatives, a thorough investigation into the biaxial response of meniscal tissue is warranted.

The distinct layers of the ultrastructure in the meniscus can be identified by the differences in the collagenous fiber alignment (Mow et al., 1992; Petersen and Tillmann, 1998). The superficial layer at the femoral and tibial surfaces consists of a smaller fibril network (bundles of approximate diameter 10 μm, fibrils of approximate diameter 35 nm) without any distinct orientation. Below this surface is the lamellar layer, identified with a larger fibril network (bundles: 20–50 μm, fibrils: 120 nm), that has mostly randomly oriented fibers except at the periphery of the anterior and posterior segments where the fibers are radially oriented. The largest volume of the meniscus is the deep zone which has predominately circumferentially oriented fibers intermixed with radially aligned tie fibers (Mow et al., 1992; Petersen and Tillmann, 1998). Skaggs et al. (1994) found regional differences in the distribution of these radial tie fibers in medial bovine menisci with corresponding regional differences in the uniaxial tensile moduli directly correlating to the size of the radial tie fiber. Tissakht and Ahmed (1995) investigated the regional and depth variations of the uniaxial tensile material properties in human meniscal tissue. With similar objectives Proctor et al. (1989) investigated bovine meniscus by examining these properties from the femoral and deep regions. The results of these previous studies suggest the mechanical response in meniscal tissue is non-homogenous. However, current finite element models (FEM) of the meniscus have modeled the meniscus as transversely isotropic without accommodating regional variation in properties (Haut Donahue et al., 2003; Bao et al., 2013; Dong et al., 2014; Mononen et al., 2013). Moreover, while little has been reported on the mechanical response of the two scaffolds currently commercially available (Spencer et al., 2012), there may be benefits to gain from synthesizing scaffolds of non-homogenous properties. This approach to synthetic meniscal substitute design could result in better clinical outcomes following surgery.

Therefore, an in-depth study of the biaxial material properties will be investigated to gain understanding of the more physiological material response of the meniscus. Furthermore, regional differences (anterior, central, and posterior) and depth variations (femoral lamellar, deep, and tibial lamellar) of porcine lateral and medial meniscus will be examined. The resulting data may be used in improve models of meniscus under load to better understand physiologic response. This may, in turn, lead to improved surgical repair method and development of synthetic alternatives and tissue engineered scaffolds.

2. Material and methods

2.1. *Specimen preparation*

Ten fresh-frozen left porcine knee joints were obtained from a local abattoir. On the day prior to dissection, the joints were thawed overnight. The lateral and medial menisci from each knee joint were carefully harvested by sharp dissection with a scalpel. Each menisci was carefully inspected for evidence of degeneration or damage from the dissection.

Each menisci was then divided with a scalpel into three regions (anterior, central, and posterior) (Fig. 1). In order to maintain consistency for choosing the region, a radial center line dividing the menisci into two halves was drawn. The size of the central block was selected to be large enough to fit two $7 \times 7\ mm^2$ square blocks on the femoral surface with the anterior and posterior regions being adjacent to these blocks. The anterior and posterior horns were then removed. Each region was then cut into a smaller block with an approximate inner arc length of 10 mm without removing any inner or peripheral tissue. The membranes from the femoral and tibial surfaces of each block were removed. To ensure that the slices were obtained from both the femoral and tibial surfaces each block was then carefully cut circumferentially using a scalpel into three further depth regions (femoral, middle, and tibial) (Fig. 1). Finally, using a square guide, $7 \times 7\ mm^2$ slices from each layer of approximate thickness of 1 mm were obtained. In total, nine slices per menisci were removed. Extra care was taken to ensure that the inner and