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Comparison of nonlinear mechanical properties of bovine articular cartilage and meniscus

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ABSTRACT

Nonlinear, linear and failure properties of articular cartilage and meniscus in opposing contact surfaces are poorly known in tension. Relationships between the tensile properties of articular cartilage and meniscus in contact with each other within knee joints are also not known. In the present study, rectangular samples were prepared from the superficial lateral femoral condyle cartilage and lateral meniscus of bovine knee joints. Tensile tests were carried out with a loading rate of 5 mm/min until the tissue rupture. Nonlinear properties of the toe region, linear properties in larger strains, and failure properties of both tissues were analysed. The strain-dependent tensile modulus of the toe region, Young's modulus of the linear region, ultimate tensile stress and toughness were on average 98.2, 8.3, 4.0 and 1.9 times greater ($p < 0.05$) for meniscus than for articular cartilage. In contrast, the toe region strain, yield strain and failure strain were on average 9.4, 3.1 and 2.3 times greater ($p < 0.05$) for cartilage than for meniscus. There was a significant negative correlation between the strain-dependent tensile moduli of meniscus and articular cartilage samples within the same joints ($r = -0.690$, $p = 0.014$). In conclusion, the meniscus possesses higher nonlinear and linear elastic stiffness and energy absorption capability before rupture than contacting articular cartilage, while cartilage has longer nonlinear region and can withstand greater strains before failure. These findings point out different load carrying demands that both articular cartilage and meniscus have to fulfil during normal physiological loading activities of knee joints.

1. Introduction

Articular cartilage and meniscus play crucial roles in load bearing and load distribution, providing stability and lubrication during knee joint motion (Lai and Levenston, 2010). Compared to articular cartilage, meniscal fibrocartilage has lower proteoglycan content (1–2% vs. 5–10% by mass), lower water content (60–70% vs. 68–85%) and higher collagen content (15–25% vs. 10–20% by mass) (Mow and Huiskes, 2005). Proteoglycans are mainly responsible for hydration and compressive properties of the tissues (Scott et al., 1997; Adams and Hukins, 1992). The entrapment of water and its interactions with the extracellular matrix components provides the tissue its ability to resist fast-rate compression and return the tissue to normal shape after deformation (Buckwalter, 1983; Buckwalter and Mankin, 1998). Based on experimental evidence, collagen network mainly modifies the dynamic (instantaneous) compressive and tensile properties of the tissue (Roth and Mow, 1980; Mow et al., 1990; Bader et al., 1992; Laasanen et al., 2003). Collagen also defines the nonlinear behaviour of fibril reinforced tissues in tension, primarily caused by different recruitment of collagen fibrils (Woo et al., 1976; Roth and Mow, 1980).

Under physiological loading conditions, both articular cartilage and meniscus experience tensile stretching. With weight bearing, the curved femoral condylar cartilage surface displaces the meniscus radially in the knee joint, creating circumferential hoop stresses (Fox, 2007). These hoop stresses are characterised by tensile stretching of the tissue and collagen fibres in the meniscus which are mostly arranged circumferentially (Bullough et al., 1970). Generally, the lateral meniscus is displaced more than the medial meniscus during joint loading (Bylski-Austrow et al., 1994; Kawamura et al., 2003). With articular cartilage, the superficial zone with parallelly oriented collagen fibrils is strong in tension, providing the integrity to cartilage and resisting lateral tissue expansion (Kempson, 1979). Due to this functional role of collagen,

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it has been suggested that tensile stiffness plays an essential role even in compression of cartilage during physiological loading (Korhonen et al., 2003; Charlebois et al., 2004). Tensile properties of articular cartilage and meniscus are therefore essential for the physiological load bearing capability of the tissues.

Although there are several studies on the tensile and compressive properties of articular cartilage and meniscus, there still remain significant gaps in the literature in terms of comparison between these tissues located in contact with each other within knee joints. Rather, tensile properties of meniscal tissue (Proctor et al., 1989; Fithian et al., 1990; Tissakht and Ahmed, 1995; Goertzen et al., 1997; Lechner et al., 2000; Muratsu et al., 2000; LeRoux and Setton, 2002; Stärke et al., 2009) have been typically studied in isolation from those of articular cartilage (Kempson et al., 1973; Woo et al., 1976; Roth and Mow, 1980; Elliott et al., 1999; Williamson et al., 2003; Sasazaki et al., 2006). Also, there are separate studies on the compressive properties of meniscal (Chia and Hull, 2008; Bursac et al., 2009) and cartilage tissues (Korhonen et al., 2002; Julkunen et al., 2008). In a recent study (Eleswarapu et al., 2011), tensile properties of cartilage, meniscus, ligament and tendon were studied. In that study, both Young's modulus and ultimate tensile strength of femoral condylar cartilage were found to be approximately 3 times less than those of lateral meniscus. However, nonlinear characteristics of both cartilage and meniscus due to collagen recruitment and energy absorption properties of the tissues were not investigated.

Nonlinear properties of different fibril-reinforced tissues in tension have been traditionally modelled by an exponential formula (Fung, 1967). On the other hand, since the nonlinear behaviour is thought to result from fibre recruitment, i.e., progressive amount of fibres are fully aligned as a function of tensile strain, the nonlinear toe region is typically followed by a linear region in which all fibres are thought to be stretched (Viidik, 1968). Stress–strain relationship of articular cartilage has also been modelled with a second order polynomial formula, resulting in linearly increasing Young's modulus as a function of tensile strain (Li et al., 1999; Korhonen et al., 2003). However, nonlinearities of articular cartilage and meniscus have not been characterised simultaneously with different aforementioned models, especially for cartilage and meniscus from the same joint. Furthermore, nonlinear tensile strength of cartilage or collagen fibril network properties have earlier been characterised indirectly from mechanical tests in compression using finite element modelling (Li et al., 1999; Korhonen et al., 2003), while a direct determination from tensile testing experiments is lacking.

The aim of this study was to experimentally characterise and compare nonlinear and linear tensile properties, as well as failure properties, of articular cartilage and meniscus in contact with each other within knee joints. We hypothesised that (1) the nonlinear increase in the tensile stiffness in the toe region is significantly greater for meniscus than cartilage, while the stiffness values differ from those characterised indirectly earlier using finite element analysis, (2) significantly more energy is absorbed by meniscus than cartilage before tissue rupture, and (3) cartilage can withstand significantly greater strains than meniscus before failure. We further hypothesised that (4) tissue adaptation varies between knee joints, leading to a significant correlation between the tensile properties of cartilage and meniscus.

2. Materials and methods

2.1. Sample preparation

Lateral femoral condyle cartilage and lateral meniscus were obtained from healthy bovine knee joints (21 ± 3 months old, 10 animals). A large piece of cartilage was removed from each joint surface using a razor blade, while entire menisci were released from joints for further processing. The samples were placed in an isotonic phosphate buffered saline (PBS) (pH 7.4) with enzyme inhibitors (ethylenediaminetetraacetic acid (EDTA VWR International, Radnor, PA, USA) and benzamidine HCl (Sigma-Aldrich Co., St. Louis, MO, USA)) and then frozen at −20 °C. Prior to the sample preparation, the frozen samples were thawed in water bath at room temperature (about 21 °C). Rectangular articular cartilage (n=29) and meniscus (n=30) (Table 1) samples were then prepared (Fig. 1). The samples were prepared from the regions where the collagen fibres were assumed to be oriented along the length of the rectangular samples. This was verified from the literature (Petersen and Tillmann, 1998; Below et al., 2002). For more details, see Supplementary material.

Table 1
Dimensions (mean ± SD) of lateral meniscus and lateral femoral condyle cartilage samples used in this study.

	n	Thickness (mm)	Width (mm)
Cartilage	29	0.28 ± 0.07	2.94 ± 0.50
Meniscus	30	0.42 ± 0.11	3.12 ± 0.22

The samples were ~2 cm in length.

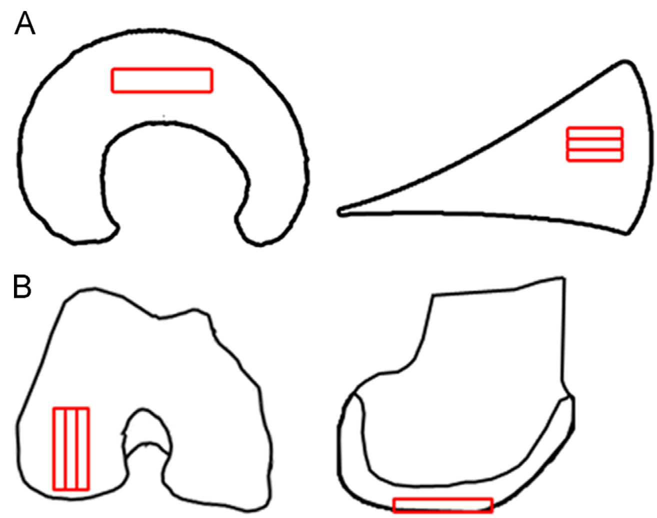

Fig. 1. Schematic diagram of the location of the prepared meniscus (A) and articular cartilage (B) samples.

2.2. Mechanical tests

Tensile tests were carried out immediately after the sample preparation using LFPlus Lloyd mechanical testing instrument (Lloyd Instruments, Inc., Amtec, Paoli, PA, USA). First, sandpaper was glued on both ends of the clamps to prevent tissue sliding, and the device was calibrated. Subsequently, the upper clamp was driven in contact with the lower clamp. The upper clamp was pulled back and the sample was placed firmly in-between the clamps. Finally, the samples were elongated until tissue rupture using a loading rate of 5 mm/min to obtain force–elongation curves. For representation of the biomechanical behaviour of primarily the solid phase of the tissues, slow loading rate was used in the testing to ensure that the frictional drag of the fluid flow does not significantly contribute to the results (Lechner et al., 2000). The measurements of those samples that failed at the grips were discarded and only the samples that experienced the failure at the gauge region were used in the final analysis. From the force–displacement data, stress–strain curves were calculated. Engineering stress was calculated as the force divided by the average cross-sectional area of the tissue and engineering strain as the elongation divided by the initial length of the sample (distance between clamp-ends). The initial length of the sample was defined from the point where the force in the experiments started to increase.

The nonlinear tensile behaviour of cartilage and meniscus was modelled by three-phase behaviour. In the first phase with negative strains, zero stress was assumed. In the second phase, the toe region was characterised with an exponential formula (Fung, 1967). In the third phase, the linearly elastic region was in-between the toe region and the yield point. When these phases are combined, we obtain the following equation for tensile stress (σ):

$$\sigma = \begin{cases} 0, & \varepsilon < 0 \\ A(e^{B\varepsilon} - 1), & 0 < \varepsilon < \varepsilon_{toe} \\ C\varepsilon + D, & \varepsilon_{toe} < \varepsilon < \varepsilon_{yield} \end{cases}, \tag{1}$$