**Interspecies Comparison of Meniscus Structure in Human, Sheep and Rabbit**

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**Introduction:** Menisci play a central load-bearing role in knee biomechanics and pathologies of meniscus are associated with cartilage damage and osteoarthritis. The purpose of this study was to compare human menisci to sheep and rabbit in order to develop appropriate animal models for meniscus repair.

**Materials and Methods:** Menisci were from NZW rabbits (n = 26, age 6-8 months), Suffolk-Dorset sheep (n = 18, age 2-3 years) and human (n = 12, age 34-84 years). Cryosections (6 μm) were collected in a radial orientation. For histostaining, cryosections were immersed in Fast Green and Safranin O. For immunostaining, cryosections were subjected to antigen retrieval and enzymatic digestion prior to incubation with the primary antibody of interest 1) anti-collagen type I IgG2a, 2) anti-collagen type II IgG1, or 3) anti-collagen type VI IgG1. Histochemical detection was performed with the Vectastain ABC-Alkaline Phosphatase (AP) system and AP Red Substrate kit.

**Results:** Safranin O staining was present in the inner portion of menisci (Fig. 1a). The % of meniscus that was Safranin O-positive varied substantially within all species but was greater on average for rabbit and sheep menisci than for human (41.9 ± 27.5 % and 62.9 ± 16.5 % and 3.7 ± 9.6 % respectively). Large round chondrocyte-like cells were more prevalent in the inner portion of menisci (empty arrowhead in Fig. 1b) while near the surface, cells were more fusiform.

Blood vessels were found in the adipocyte-rich tissue peripheral to the meniscal body. Blood vessels were additionally identified in the outer meniscal body in all species (the outer 1 ± 2%, 11 ± 6% and 14 ± 6% was vascularized in rabbit, sheep and human respectively).

Collagen I immunostaining was found throughout rabbit, sheep and human menisci as well as in the adipose-rich tissue peripheral to the menisci (Fig. 1c). Staining appeared punctuate (red arrowhead in Fig. 1d) and as a network of strands (black arrowhead in Fig. 1d).

Collagen II immunostaining was found in the inner main body of rabbit, sheep and human menisci (Fig. 1e) appearing as an intricate network of thick strands (Figs. 1f). Collagen II immunostaining did not necessarily overlap with Safranin O staining.

Collagen VI immunostaining was found throughout rabbit and human menisci as well as in the adipose-rich tissue peripheral to the menisci (Figs. 1g, h). Cellular and pericellular staining was found in and around the fusiform cells, the chondrocyte-like cells (black arrow in Fig. 1h) and some cells in the adipose tissue adjacent to the menisci. A network of fine fibers was also immunostained for collagen VI in the main meniscal body. Collagen VI immunostaining in sheep menisci has not been positive to date.

**Discussion:** In the current study, Safranin O staining indicating the presence of glycosaminoglycans and collagen II immunostaining were found in the inner main body of the menisci in all species, as has been reported in mature animals (1, 2). However, Safranin O staining was lower in human menisci, suggesting either an interspecies variation in meniscus GAG content or effectively older human menisci versus sheep and rabbit. Our data agreed with previous work that has identified 2 cell types in menisci: one with a fusiform morphology at the surface and second with a rounder morphology in central regions (1, 3, 4).

It has been reported that the outer 10-30% of menisci are vascularized in mature rabbit and human (4, 5). Our data agreed with these observations. However, only a small portion of the outer rabbit menisci was vascularized compared to human and sheep, suggesting a fundamental difference in vascularisation pattern in smaller species compared to larger. Vascularization may also vary longitudinally and differ in the middle of the menisci versus the posterior or the anterior or horn, the latter being the only site we have fully analyzed to date.

There have been conflicting reports regarding collagen immunostaining of menisci. In one study of mature rabbit, little overlap was found between the regions of collagen I and collagen II staining (2) while in another study, collagen I and II staining were uniform throughout the menisci (6). In the current study, patterns of collagen typing were similar for all species. Collagen I appeared throughout the menisci with both punctuate staining suggesting fibril cross-sections of circumferential collagen bundles and a network of strands suggesting radial tie fibers. In contrast collagen II was restricted to the inner portion of menisci, where chondrocyte-like cell morphology was also present. Collagen VI was found in and around the cells of the menisci and in the main meniscal body.

Use of animal models to assess meniscus repair techniques for human applications requires detailed analyses of interspecies characteristics. The sheep model appears to be more appropriate than rabbit for the study of meniscus repair. In addition to similar sizes between sheep and human menisci, vascularisation patterns in sheep are also similar to that of human allowing better replication of human injury and repair patterns. Work is ongoing to complete comparison of anterior, central and posterior regions of the medial and lateral meniscus.


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