Introduction  The meniscus is a fibrocartilage composed predominantly of Type I collagen (1). Small amounts of type II collagen have been reported (2-4). The spatial distribution of type II collagen in the meniscus is unknown. The purpose of this study was to determine the distribution of type II collagen in the meniscus with emphasis on its spatial relationship to type I collagen.

Materials and Methods  Whole knee joints were obtained from ten skeletally mature 25 Kg dogs. This protocol received prior approval from the Animal Research Committee at our institution. Frozen sections from the mid-center of medial and lateral menisci were embedded in OCT compound and cut either in the dorsal or coronal planes. Articular cartilage served as positive controls for the Type II collagen staining.

Sections (6 micron) for type I collagen staining were pretreated with testicular Streptomyces hyaluronate lyase and bacterial protease type XXIV (EC 3.4.21.62; Sigma Chem Co., St. Louis, MO) for 15 minutes. Sections were blocked using 3% normal goat serum. Indirect immunohistochemistry was performed using monoclonal antibodies directed against either type I collagen (CP17L, Oncogene Research Products Boston, MA) or type II collagen, (II-II6B3, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) and FITC conjugated goat anti-mouse IgG. Cell nuclei were visualized using Vectashield with Dapi. Incubation of tissues without primary antibody served as negative controls.

Results  Type I collagen appeared in coronal sections as an interconnected meshwork and also as dots within the space of the meshwork in the inner tip and main body (Fig 1A) of the meniscus. Intense staining was seen in the outer layer of the superficial zone at the femoral and tibial surfaces (Fig 1A). Staining for type II collagen was evident in coronal sections as diffuse patches in the inner tip; as a meshwork colocalizing with type I collagen in the main body of the tissue (Fig 1B); and as a thin layer in the deeper superficial zone at the femoral and tibial surfaces (Fig 1B). The staining of the radial tie fibers was more intense for collagen type II than for collagen type I (Fig 1C, 1D). The staining of type II collagen in the tie fibers of the lateral meniscus was pronounced. No positive labeling was found in the negative controls.

Discussion  The presence of type II collagen in the inner tip region of the meniscus is consistent with the high compressive forces that this region of the tissue experiences. The central body of the meniscus appears to contain two fibrillar systems: (a) circumferential bundles of type I collagen fibrils that stain in cross section as dots in coronal sections; and (b) surrounding each bundle is a fibrillar system composed of both type I and type II collagen whose staining suggests that the fibril orientation is perpendicular to the main circumferential bundles. It is notable that collagen type II was present not only in the inner meniscus where the loads are predominantly compressive but also throughout most of the remainder of the tissue. The complex organization of collagen type I and II in the superficial zone is interesting particularly as flattened cells populating this zone appear to express α-smooth muscle actin (α-SMA) and play a critical role in wound healing (5). The significance of the predominance of collagen type II in the radial tie fibers is unclear. The organization of the collagen fibrils in the meniscus appears to more complex than the simple conventional model of bundles of circumferential fibers.

References:

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Figure 1  Immunolocalization of collagen type I (A, C) and collagen type II (B, D) at the femoral surface and the tie fibers of the medial meniscus.