Human Menisci Evaluation; A macroscopic, histological and histomorphometric analysis from aged normal and diseased knee joints

INTRODUCTION
Numerous studies describe the degenerative changes of the menisci of the knee joint following damage or due to aging and disease. However, limited studies have been conducted to systematically harvest, process and evaluate human meniscus in normal, aged and diseased tissue. We therefore i) reviewed existing methods/systems used to evaluate meniscus, ii) established protocols for harvest and processing meniscal samples that provide a representative overview of tissue health and structure; and iii) developed a systematic method for the macroscopic and histologic evaluation of the meniscus that would be useful to document changes related to aging and disease processes.

METHODS

Human menisci procurement Medial and lateral menisci of a total of 104 fresh human knees from 52 donors (25 males, 26 females; age range = 92 -24) from tissue banks were harvested.

Fig 1. Macroscopic assessment: Grade 1: normal intact meniscus, attached at both ends with a sharp inner border, no tibial or femoral surface changes. Grade 2: fraying at inner border, surface fibrillations on tibial or femoral surface, no tears. Grade 3: partial substance tears, fraying, tibial or femoral fibrillations. Grade 4: full/complete substance tears, defects due to loss of meniscal tissue. Presence of calcification was also recorded.

Fig 2: Representative histologic images of the grades described.

Histological processing and scoring After macroscopic scoring, meniscal tissue was cut in two different planes (radial cross-section and horizontal parallel to tibial surface) in three separate locations (anterior, middle, and posterior). Tissue was fixed for multiple staining with Haemotoxylin Eosin (H&E), Safranin O – Fast Green, Alizarin Red, and Alcian Blue. Sirius Red was used for qualitative polarized light microscopy to identify and quantify early changes in the collagenous matrix. Sections were histologically graded using a new standardized system with subscores in the following categories i) surface topology, ii) cellularity, iii) matrix/fiber organization and collagen alignment, and v) Safranin O staining intensity. The range of possible total scores is 0-18 with 18 denoting maximum degeneration. This total score can be converted to a grade as follows: G1=0-4, G2=5-9, G3=10-14, G4=15-18 (Fig 2)

RESULTS
Intra-observer reliability: Agreement between four different readers for the macroscopic scoring system was excellent with a intra-class coefficient of 0.96 for the medial menisci and 0.95 for the lateral menisci. Two different readers performed the histologic scoring system and their agreement for the total histologic score showed a high intra-class coefficient of 0.97 while the intra-class coefficient for the histologic grades was 0.93.

Changes with aging: From joints with minimal signs of osteoarthritis (Gr I or Gr II Outerbridge classification) we analyzed changes in meniscus cellularity, and matrix structure and integrity. All histologic sections included the vascular and avascular regions. The major changes with age include increased Safranin O staining intensity, decreased cell density and appearance of acellular zones, and evidence of mucoid degeneration with some loss of collagen fiber organization. The earliest changes in the surface, cellularity and matrix organization were observed mainly along the inner rim.

Changes with osteoarthritis: In menisci from arthritic joints (Gr III or Gr IV Outerbridge classification) we observed severe fibrocartilaginous separation of the matrix and extensive fraying with increased cell cluster formation in the avascular zone. Abnormal cell clusters were only found close to the meniscus surface, around tears, and in the frayed areas. Overall, the anterior horns of both medial and lateral menisci appeared to be macroscopically as well as microscopically less affected in aging related and disease changes of the tissue.

DISCUSSION
We conducted a careful evaluation of changes in meniscus at a macroscopic and microscopic level. We developed and validated a grading system that is comprehensive and includes macroscopic, histologic and histomorphometric evaluation. The grading system is reproducible and can be readily learned.

Several characteristics appear to be specific to aging in the absence of significance osteoarthritis. With aging the meniscal surface remained macroscopically intact while changes in matrix stain and cellularity were observed within the meniscal substance. This is in distinct contrast to degeneration in articular cartilage which almost invariably progresses from the surface inward. The increase in Safranin-O stain with aging suggests a shift from fibroblastic to chondrocytic phenotype during early degeneration and warrants more investigation.

In the presence of moderate or severe osteoarthritis there was severe disruption of the matrix in one or more of the regions (anterior, middle, or deep). Macroscopically the anterior horn was consistently less affected. This possibly suggests either that the anterior horn could be more resistant to degeneration or that the anterior horn is exposed to less biomechanical loading than the rest of the meniscus. Abnormal cell clusters, reminiscent of those found in osteoarthritic cartilage were also found in regions of severe matrix disruption.

Future directions include automating the histomorphometric grading via computerized image analysis to reduce variability in observer grading and the application of adjunct imaging techniques such as polarized light microscopy to identify and quantify early changes in the collagenous matrix.

Funded by NIH/NIAMS grant P01 AG007996

REFERENCES

Poster No. 1928 • ORS 2011 Annual Meeting