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Atomic force microscopy reveals age-dependent changes in nanomechanical properties of the extracellular matrix of native human menisci: implications for joint degeneration and osteoarthritis

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Abstract

With aging, the menisci become more susceptible to degeneration due to sustained mechanical stress accompanied by age-related changes in the extracellular matrix (ECM). However, the mechanistic relationship between age-related meniscal degeneration and osteoarthritis (OA) development is not yet fully understood. We have examined the nanomechanical properties of the ECM of normal, aged, and degenerated human menisci using atomic force microscopy (AFM). Elasticity maps of the ECM revealed a unique differential qualitative nanomechanical profile of healthy young tissue: prominent unimodal peaks in the elastic moduli distribution in each region (outer, middle, and inner). Healthy aged tissue showed similar regional elasticity but with both unimodal and bimodal distributions that included higher elastic moduli. In contrast, degenerated OA tissue showed the broadest distribution without prominent peaks indicative of substantially increased mechanical heterogeneity in the ECM. AFM analysis reveals distinct regional nanomechanical profiles that underlie aging-dependent tissue degeneration and OA.

Key words: Atomic force microscopy; Meniscus; Nanomechanics; Osteoarthritis

Background

Aging is the leading risk factor for osteoarthritis (OA), a whole joint degenerative disease that affects all articulating tissues, including articular cartilage and menisci in the knee. The menisci play a crucial role in joint loading by providing mechanical stability, smooth articulation, shock absorption, load bearing and transmission in the knee.[1-7] Damage or degeneration due to aging of the menisci leads to unfavorable changes in joint loading that significantly affect overall joint health by precipitating the onset of OA development. The role of meniscal injuries and surgical removal of the meniscus on the development of post-traumatic OA is well established.[8-10] However, the mechanistic relationship between age-related meniscal degeneration and OA development is not yet fully understood.

The menisci are two crescent shaped tissue structures wedged between the articulating surfaces of the femoral condyle and tibial plateau. During normal joint loading, the femoral condyle bears down on the menisci concentrating the highest compressive loads in the inner tapered end of the tissue. The forces are transmitted to the outer periphery due to its geometry, which causes the tissue to expand circumferentially. The extrusion is resisted by the anterior and posterior attachments, creating circumferential

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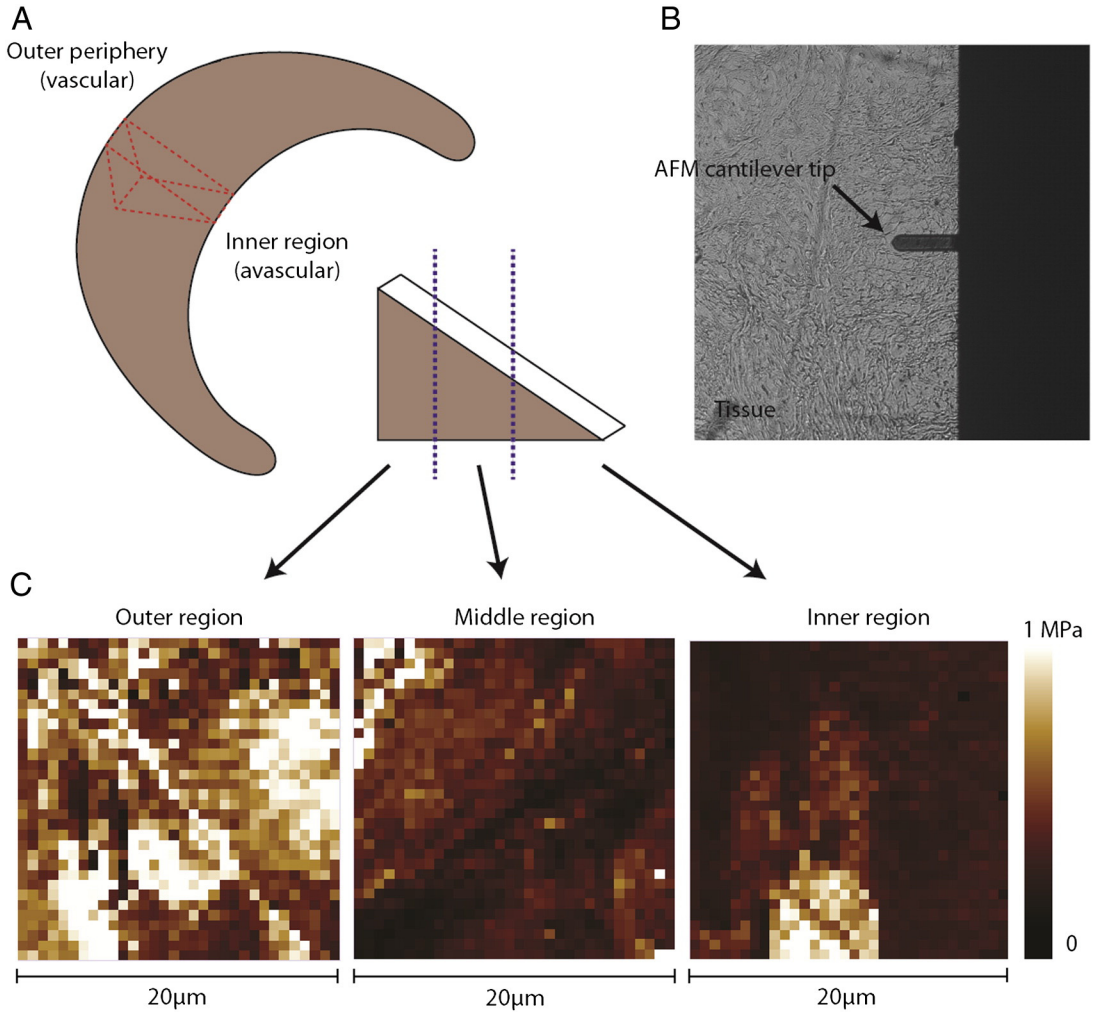

Figure 1. AFM schematics for force mapping of human meniscal tissue. **(A)** Central portion of medial meniscus was sectioned in the sagittal direction (red). The sagittal tissue portion was cut by a cryostat microtome at a thickness of 10 μm. The regions were defined by the purple line as the outer one-third, middle, and inner one-third of the tissue. **(B)** AFM cantilever tip with a glass microsphere attached (5 μm in diameter) was used to probe the tissue sample surface of the ECM. **(C)** Representative force maps (scan size of 20 μm × 20 μm) of outer, middle, and inner regions were generated from which the elastic moduli was extracted and plotted in histogram distributions.

tensile hoop stresses that distribute the compressive load. This anisotropic biomechanical response is supported by a regionally heterogeneous network of collagenous extracellular matrix (ECM) that varies in microvasculaturity, microstructure, and biocomposition.[11,12] The inner region is composed primarily of randomly aligned collagen type II fibrils embedded within an abundance of proteoglycan molecules that help resist high compressive loads.[13,14] The outer region is more fibrous consisting of both collagen types I and II that form mostly circumferentially aligned fibrils to uphold tensile strength and carry out anisotropic load transmission.[13,14]

Age-related changes are accompanied by molecular and structural changes in the ECM that lead to matrix degeneration.[15-17] This degenerative process is characterized by an imbalance between anabolic and catabolic processes as cells reduce production of important growth factors and increase production of matrix degrading enzymes and inflammatory cytokines.[18,19] These age-related biochemical changes have been identified by histological analyses which demonstrate increased Safranin-O staining, reduced cellularity, and loss of collagen fiber orientation.[20] While the biochemical changes have been investigated by histological and immunohistochemical analyses, age-related changes in biomechanical properties have not been documented at the microstructural level in human menisci. Our hypothesis is that aging alters the biomechanical properties of meniscal ECM thus changing the tissue response during joint loading thereby contributing to OA development.

To define the earliest age-related biomechanical changes of the ECM, a high resolution force scanning technique is required to measure mechanical properties at the nano-to-microscale, the native length scale of the cells and ECM. Recent studies have examined tissue mechanical properties at the nano-to-microscale utilizing atomic force microscopy (AFM).[21] Cartilaginous tissues, including articular cartilage and porcine menisci, have been evaluated by AFM demonstrating depth-dependent and regional variations in nanomechanical properties.[22,23] However,