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Biomechanical, structural, and biochemical indices of degenerative and osteoarthritic deterioration of adult human articular cartilage of the femoral condyle

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Summary

Objective: To compare the tensile biomechanical properties of age-matched adult human knee articular cartilage exhibiting distinct stages of degenerative or osteoarthritic deterioration and to determine the relationships between tensile properties and biochemical and structural properties hypothesized to underlie functional biomechanical deterioration.

Methods: Age-matched articular cartilage samples, obtained from the lateral and medial femoral condyles (LFC and MFC), exhibited (1) minimal fibrillation, characteristic of normal aging (NLA), (2) overt fibrillation associated with degeneration (DGN), or (3) overt fibrillation associated with osteoarthritis (OA). DGN samples were from knees that exhibited degeneration but not osteophytes while OA samples were from fragments removed during total knee arthroplasty. Cartilage samples were analyzed for tensile properties, cell and matrix composition, and histopathological structure.

Results: Differences in tensile, compositional and surface structural properties were indicative of distinct stages of cartilage degeneration, early (OA) advanced (DGN) and late (OA) with early degenerative changes in NLA samples being more advanced in the MFC than the LFC, including higher surface fibrillation, lower intrinsic fluorescence, and lower mechanical integrity. The transition from early to advanced degeneration involved a diminution in mechanical function, surface integrity, and intrinsic fluorescence. The transition from advanced to late degeneration involved an increase in cartilage water content, an increase in degraded collagen, and loss of collagen.

Conclusions: These results provide evidence of coordinated mechanical dysfunction, collagen network remodeling, and surface fibrillation. Even in the cartilage of knees exhibiting overt fibrillation but not extensive erosions characteristic of clinical osteoarthritis, most features of advanced cartilage degeneration were present.

Key words: Cartilage degeneration, Tensile properties, Wear, Fluorescence, Human articular cartilage.

Introduction

Aging, cartilage fibrillation and osteoarthritis may be contributing factors to the site-specific deterioration of the biomechanical function of articular cartilage in the knee joint. The effects of these factors can be difficult to separate because the incidence of both cartilage fibrillation and clinical osteoarthritis increases with aging[1]. The tensile biomechanical properties of human knee articular cartilage vary between macroscopically normal[2–4], fibrillated[4], and fibrillated osteoarthritic[4] states due to pathological state, age, and site within the knee (Table I and Supplement). Normal aging is associated with marked decreases in tensile modulus and strength in the LFC, but not in the MFC where tensile properties were already low in young adults.

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Biomechanical failure of articular cartilage in degeneration and in osteoarthritis has been hypothesized to be due to (1) degradation and loss of collagen and proteoglycan matrix components, (2) abnormal collagen network remodeling, (3) consequences of decreased cellularity, and (4) mechanical wear. The tensile stiffness and strength of cartilage depend on the organization of the collagen network, with highest values normally at the articular surface where collagen fibers are aligned along the tangential axis of testing[5,6]. Fragmentation and loss of collagen molecules are increased at sites adjacent to focal cartilage lesions[7]. Collagen degradation reduces the tensile stiffness and strength of articular cartilage[8], whereas proteoglycan extraction reduces compressive[9] but not tensile stiffness[10]. Together, these results suggest that collagen degradation and loss contribute primarily to the tensile biomechanical weakening of human cartilage.

Abnormal collagen network remodeling, comprised of both synthesis and degradation of collagen, has also been postulated to result in cartilage weakening. With the upregulated synthesis of matrix molecules including

Table I
Summary of studies of adult human knee articular cartilage examining association between tensile properties of cartilage surface layer with cartilage degeneration and joint OA, age and site

Reference	Condition of joint or articular cartilage	Site	# Knees	Age (yrs)	Tensile properties of surface layer (MPa)	
					Strength	Modulus
Kempson[2]	Normal (Indian Ink)[15]	Averaged for LFC and MFC	5	~24–40	~35	
			3	~41–60	~20	
			7	~61–80	~15	
Akizuki *et al.*[4]	Normal (Indian Ink)	MFC in low weight	4	24–42		10.1
	Partial fibrillation	bearing area	2	52, 60		8.5
	Osteoarthritic		3	65, 68, 74		1.4
Temple *et al.*[3]	Normal (Indian Ink	Anterior LFC	9	21–39	19	24
	& Histopathology)		9	40–59	12	19
			10	60–91	11	17
	Normal (Indian Ink	Anterior MFC	9	21–39	9	11
	& Histopathology)		10	40–59	8	11
			9	60–91	10	12

collagen in osteoarthritic cartilage[11], collagen content can be maintained despite collagen degradation and be manifest as diminished intrinsic cartilage fluorescence in clinical osteoarthritis[12] and experimental animal models[13]. This rapidly metabolized collagen may result in a network that has a reduced ability to withstand tensile loads in early cartilage degeneration.

Alteration in the cellular content of cartilage has also been implicated in cartilage weakening associated with cartilage degeneration and development of osteoarthritis. The cell density of adult articular cartilage is decreased with cartilage fibrillation[14]. Such decreased cell density may be detrimental to matrix homeostasis and lead to tissue deterioration and, thus, cartilage biomechanical function.

Finally, mechanical wear could directly cause cartilage weakening at the articular surface. India ink staining highlights alteration of the articular cartilage surface which is slight with normal aging[3]. With severe wear and erosion of the cartilage surface that are characteristic of osteoarthritis, India ink staining of the articular surface is considerable[15].

Thus, these proposed mechanisms of biomechanical weakening may, individually or in concert, contribute to the deterioration of cartilage biomechanical function and the progression of osteoarthritic disease. The hypothesis of this study was that aged human articular cartilage exhibits tensile weakening that is associated with variations in tissue composition and structure, in a depth- and site-associated manner, indicative of one or more of the postulated mechanisms of cartilage deterioration. The specific aims of this study were to characterize and compare adult human knee articular cartilage exhibiting distinct stages of degenerative or osteoarthritic deterioration, isolated from different depths at the LFC and MFC sites in terms of (1) tensile biomechanical properties, (2) density of cells, (3) content of extracellular matrix components, and (4) structure of the articular surface. By examining these samples, the results of this study were interpreted in terms of stages of cartilage degeneration.

Materials and methods

SAMPLE SELECTION AND PREPARATION

Age-matched samples (mean ± SEM, 68 ± 2 y, range 50–91 y, Table II) in the form of 10-mm osteochondral cores were isolated using a surgical instrument (Osteochondral Autograft Transfer System; Arthrex, Naples, FL) from the anterior region of the MFC ($n = 24$ cores) and LFC ($n = 24$ cores) approximately 1.5 cm lateral or medial to the intercondylar notch. The accuracy of the core position within a knee was ~0.5 cm. The cores displayed (1) mild age-associated surface roughening of the articular cartilage surface, grade 1 as described in[16] (NLA, $n = 23$ cores from 14 donors), (2) overt fibrillation, grade 3[16] associated with degeneration but not osteoarthritis (DGN, $n = 12$ cores from eight donors), or overt fibrillation, also grade 3[16], but associated with osteoarthritis (OA, $n = 13$ cores from 11 donors). NLA and DGN samples were from 22 cadavers obtained from tissue banks with donation areas in the Western and Southern areas of the United States. Cadaveric knee joints were stored at 4°C prior to shipment, shipped on wet ice, and obtained within 48 h of death. OA samples were obtained with Institutional Review Board approval from 11 patients undergoing total knee replacement (TKR), stored at 4°C, and obtained within 16 h. In all, samples were from one knee of each of 33 donors, from both the LFC and MFC of most (9/14) NLA knees and many (4/8) DGN knees, but relatively few (2/11) OA knees.

While the fibrillated cartilage surfaces of DGN and OA samples appeared grossly similar, the above criteria clearly distinguished the status of the knee joints, with the OA knee joints having cartilage degeneration and erosion that was much more extensive overall than DGN knee joints. The extent of cartilage erosion and OA disease was characterized by the overall joint grade and presence of osteophytes (Table II) and further quantified as the area of full thickness cartilage erosion (as measured from digitized gross images of the joint surfaces, Table II). Because of the presence of osteophytes in most joint from which OA samples were obtained, and because the area of cartilage erosion on the femoral condyles and the joint overall was higher in OA samples than DGN or NLA samples (Supplement), these experimental groups were considered to represent distinct stages of cartilage degeneration.

Tissue samples were graded macroscopically[16], isolated, and immersed in phosphate buffered saline with proteinase inhibitors (PBS with PI)[17] at 4°C for 1 h, and then stored at −70°C until the time of testing. Samples were thawed in ~1 ml of PBS with PI for 15 min at room temperature prior to analysis. Previous studies indicate that cartilage mechanical properties are not affected by a single freeze-thaw cycle[18].

STRUCTURAL INDICES OF FIBRILLATION

Samples were analyzed for cartilage thickness and surface roughness (reflectance score after India ink staining) as described previously[3,17]. An osteochondral fragment was isolated for histopathological analysis (Mankin–Shapiro score including surface irregularity) from a region adjacent to cartilage used for biomechanical and biochemical analyses[17].

BIOMECHANICAL PROPERTIES

The remaining cartilage of each core was sliced into ~0.3-mm thick layers, at a distance from the articular surface of 0% (superficial layer, including the articular surface), 30% (middle layer), and 60% (deep layer) of the average cartilage thickness. A portion of the slices were cut into tapered specimens with the gage region oriented in the medial-lateral direction, parallel to the split-line direction typical for this site[5] for equilibrium and constant strain-rate tensile testing, which was performed as described previously[3,19].

BIOCHEMICAL PROPERTIES

The remainders of the tissue slices, adjacent to tensile samples, were analyzed for cell and matrix components. A portion was weighed wet, lyophilized, weighed dry, solubilized with proteinase K and analyzed for DNA[20],