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Altered cartilage mechanics and histology in knee osteoarthritis: relation to clinical assessment (ICRS Grade)[1]

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Summary

Objective: Substantial changes in articular cartilage composition and mechanical properties occur during the development of osteoarthritis (OA). While softening in the initial stage is reported and sometimes used as an indicator of early OA, there is a lack of data relating the macroscopic appearance of cartilage to its mechanical and histological properties in all stages of degeneration. Knowledge about the mechanical quality of the tissue is important for diagnostic reasons and the understanding of the development of OA.

Design: The cartilage areas of 21 osteoarthritic human cadaver tibia plateaus were classified using the International Cartilage Repair Society (ICRS) system. A material testing device determined the Young's modulus of the cartilage by unconfined compression. Histological analysis used haematoxylin and eosin staining and Safranin-O staining for the evaluation of the Mankin score.

Results: A correlation between increasing ICRS Grade and stiffness reduction was found ($R^2 = 0.69$). Stiffness values were for ICRS Grades 1, 2 and 3: $E_1 = 0.50 \pm 0.14$ MPa, $E_2 = 0.37 \pm 0.13$ MPa and $E_3 = 0.28 \pm 0.12$ MPa, respectively. The histological evaluation confirmed the ICRS classification ($R^2 = 0.74$). A moderate correlation between Mankin score and cartilage stiffness was observed ($R^2 = 0.47$).

Conclusions: The results indicate a relation between structural, mechanical and histological changes in all stages of the degeneration. With increasing ICRS Grade the cartilage stiffness, which is primarily influenced by the integrity of the extracellular matrix, decreases. Therefore, methods of stiffness determination such as indentation may be used to characterize cartilage in all stages of OA. However, the data suggest that differentiating between healthy cartilage and ICRS Grade 1 may be difficult using mechanical testing alone.

Key words: Articular cartilage, Arthritis, Cartilage mechanics, Unconfined compression, ICRS score.

Introduction

Articular cartilage has unique material properties which enable it to distribute and support forces generated during joint loading. The loading of articular cartilage causes surface deformation resulting in increased joint contact areas and decreased contact stresses. The physiological function of articular cartilage depends on the structure, the composition and the integrity of the extracellular matrix (ECM). With injury or degeneration such as osteoarthritis (OA), changes in structure and composition will occur[1]. These changes are associated with a significant loss of mechanical function that may cause further progressive degeneration of cartilage. In the initial stage, OA provokes a loss in cartilage volume making the tissue more easily vulnerable to be damaged by injury or excessive use[2]. The underlying bone thickens and may develop fluid-filled cysts near the joint[3]. Particles of bone or cartilage may float loosely in the joint space causing further mechanical abrasion. Finally, the synovium becomes inflamed as a result of the cartilage degeneration[4]. The specific pattern and path of OA development is also influenced by heredity, age, gender, obesity, trauma to the joint and other factors[5].

In clinical assessment, the status of the cartilage in the knee joint is determined by macroscopic examination. The International Cartilage Repair Society (ICRS) recommends that the clinical evaluation of the tissue condition is performed using an enhanced Outerbridge Score[6,7]. The classification ranges from healthy cartilage (ICRS Grade 0) to the absence of cartilage with exposed subchondral bone (ICRS Grade 4). The score criteria are the quantity and depth of lesions, either visually inspected by arthroscopy or non-invasively by magnetic resonance imaging (MRI). In arthroscopy, the method relies on visual inspection and physical probing of the cartilage surface to find abnormalities in texture or hidden defects within the midsubstance of the tissue. MRI offers an excellent soft tissue contrast and multiplanar imaging, and therefore, it has been used recently to diagnose cartilage diseases. As an enhanced method, the functional MRI (dGEMRIC)[8–10] determines the *in vivo* proteoglycan content by using a contrast agent, and consequently gives an impression of the tissue's mechanical properties. Quantitative information about the mechanical properties of articular cartilage can be obtained by cartilage indentation, a technique that has frequently been used in *in vitro* studies[11–14] and *in vivo* studies[15–19].

However, the relationship between the various stages of cartilage degeneration described, e.g., by means of ICRS Grade and changes in tissue mechanical property in the

[1]No benefit of any kind will be received either directly or indirectly by the authors.

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osteoarthritic human knee has not been determined previously. Especially, it is unclear how sensitively changes in stiffness reflect the different ICRS Grades.

Therefore, the aim of our study was to determine quantitatively the mechanical changes in articular cartilage at different stages of OA degeneration. This was achieved by a comparison of the mechanical properties and the degeneration of human tibial plateau cartilage, assessed both macroscopically and histologically. Improved knowledge of these relationships will allow a better understanding of the progress and development of OA and its symptomatic appearance and enable the development of diagnostic tools.

Method

After written approval from the local Ethic Committee, human tibia plateaus were collected during total knee replacement surgery from patients with knee joint osteoarthritis ($n = 21$). All specimens were immediately rinsed in 0.9% saline solution and tested within 24 h. The cartilage areas were classified using the score developed by the International Cartilage Repair Society (ICRS Grade, Table I)[7]. Grading was performed independently by three blinded surgeons. Cartilage areas presenting different stages of degeneration were selected, and two half-overlapping osteochondral plugs (6 mm in diameter) were taken using an autograft tool. The cylindrical shaped plug was taken for mechanical analysis, and the crescent shaped plug was taken for histological analysis. This method was chosen to avoid possible effects from the biomechanical tests on the histological analysis. The thickness of each cartilage sample was determined using a micrometer screw under microscopic assessment.

BIOMECHANICAL TESTING

A custom made high-precision material testing device (resolution 0.1 μm, 0.005 N for deformation and force, respectively) was used to determine the mechanical properties of the cartilage in unconfined compression[11,20,21]. Displacement and force are recorded using an LVDT (Wuntronic, Munich, Germany) and a load cell (Burster, Gernsbach, Germany). Each specimen was compressed uniaxially between two parallel polished stainless steel platens in a testing chamber filled with phosphate buffered saline. After surface contact, a pre-load of 0.003 N was applied and allowed to equilibrate for 10 min. Then, stepwise loads of 0.019 N were applied up to 25% strain using 20 g weights, and the creep behavior of each sample was recorded. When the displacement rate fell below $v_{disp} = 0.1$ μm/s, equilibrium in stress and strain was assumed. The Young's modulus was calculated with a custom-designed software algorithm from the linear range of the stress–strain curve. Isotropic-elastic behavior of cartilage with no fluid flow out of the tissue at equilibrium was assumed. Each test lasted 1–2 h.

Table I
ICRS grading based on the Outerbridge score[6]

Grade	Property
1	Superficial lesions, fissures and cracks, soft indentation
2	Fraying, lesions extending down to <50% of cartilage depth
3	Partial loss of cartilage thickness, cartilage defects extending down >50% of cartilage depth as well as down to calcified layer
4	Complete loss of cartilage thickness, bone only

HISTOLOGICAL ANALYSIS

For histological analysis, specimen plugs were fixed in a neutral buffered, isotonic formalin–alcohol solution for 24 h. The probes were decalcified in 20% ethylenediaminetetraacetic for 14 days and embedded into paraffin. The specimens were then sliced in 6-μm serial slices using a hard-cutting microtome (Polycut, Leica, Cambridge, England), and stained with haematoxylin and eosin for morphological measurements, and with Safranin-Orange staining to assess glycosaminoglycan (GAG) content. The histological appearance of the knee joints was evaluated by three blinded, independent investigators using a modified Mankin scoring system[22] (Table II). The inter-observer variance was calculated from the difference between observer scores as compared to the mean for each section. The kappa value was determined as an index for inter-observer agreement. Areas including denuded bone (ICRS Grade 4) were excluded from the measurement.

STATISTICAL ANALYSIS

Results were expressed as mean values ± standard deviation for each parameter. Comparison of the mean values between ICRS Grades was done using a one-way analysis of variance (ANOVA), and specific inter-group differences between mean values were identified using the *post hoc* Bonferroni test ($P < 0.05$). The degree of association between Mankin score, ICRS Grade and stiffness was expressed by the coefficient of determination R^2. The statistical analysis was performed using SPSS Software V.11 (SPSS Inc., Chicago, Illinois).

Table II
Histological and histochemical grading system for evaluation of articular cartilage degeneration (Mankin et al.[40]*)*

		Grade
I	Structure	
	a. Normal	0
	b. Surface irregularity	1
	c. Pannus and surface irregularity	2
	d. Clefts to transitional zone	3
	e. Clefts to radial zone	4
	f. Clefts to calcified zone	5
	g. Complete disorganization	6
II	Cells	
	a. Normal	0
	b. Diffuse hypercellularity	1
	c. Cloning	2
	d. Hypocellularity	3
III	Safranin-Orange staining	
	a. Normal	0
	b. Slight reduction	1
	c. Moderate reduction	2
	d. Severe reduction	3
	e. No dye noted	4
IV	Tidemark integrity	
	a. Intact	0
	b. Crossed by blood vessels	1