OsteoArthritis and Cartilage (2005) **13,** 958–963

doi:10.1016/j.joca.2005.06.008

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

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

R. U. Kleemann M.S., D. Krocker M.D., A. Cedraro M.S., J. Tuischer M.D. and G. N. Duda Ph.D.*
Center for Musculoskeletal Surgery, Charité – University Medicine Berlin, Free and Humboldt-University of Berlin Augustenburger Platz 1, D-13353 Berlin, Germany

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.

*Address correspondence and reprint requests to: Professor Dr Georg N. Duda, Center for Musculoskeletal Surgery, Charité – University Medicine Berlin, Free and Humboldt-University of Berlin, Research Laboratory PF 24, Augustenburger Platz 1, D-13353 Berlin, Germany. Tel: 49-30-450-559079; Fax: 49-30-450-559969; E-mail: georg.duda@charite.de

Received 19 January 2005; revision accepted 21 June 2005.

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

Results

GROSS EXAMINATION

Twenty-one subjects entered the study (15 females, 6 males) with an average age of 70 ± 13 years. Cartilage degeneration and abrasion were evident in all specimens. In most cases, a varus-gonarthrosis and areas of denuded bone were prominent. As expected, the cartilage degeneration was extremely severe in the medial tibial condyle, whereas in most cases degeneration had reached only a moderate stage in the cartilage on the lateral tibial condyle.

BIOMECHANICAL TESTING

Values obtained from the mechanical tests showed a correlation between increasing ICRS Grade and stiffness reduction ($R^2 = 0.69$, $P < 0.01$; Fig. 1). Stiffness values were $E_1 = 0.50 \pm 0.14$ MPa for ICRS Grade 1, $E_2 = 0.37 \pm 0.13$ MPa for ICRS Grade 2 and $E_3 = 0.28 \pm 0.12$ MPa for ICRS Grade 3. The average thickness of the cartilage samples was 1.96 ± 0.44 mm. A progressive thinning of the cartilage layer was observed in the severe cases of the disease (classified ICRS Grade 3).

HISTOLOGICAL RESULTS

In the tibial plateau, higher Mankin score values were obtained with increasing ICRS Grade (Fig. 2). The Mankin score in areas macroscopically evaluated as ICRS Grade 1 was 3.2 ± 1.5 points. The superficial and intermediate layer showed deterioration by means of fissures and cracks, hypercellularity and a decrease in Safranin-Orange staining. The Mankin score for ICRS Grade 2 areas averaged to 5.7 ± 2.0 points. At this stage of degeneration cell-clustering of chondrocytes appeared, and deep cracks penetrating to the middle zone were apparent. The clefts and disruption of the matrix increased in ICRS Grade 3 areas for which the Mankin score was 7.6 ± 1.7 points (Fig. 3). In this stage of degeneration, both chondrocyte cell-clustering (Fig. 4) and hypocellularity were present and the thickness of the subchondral bone layer increased.

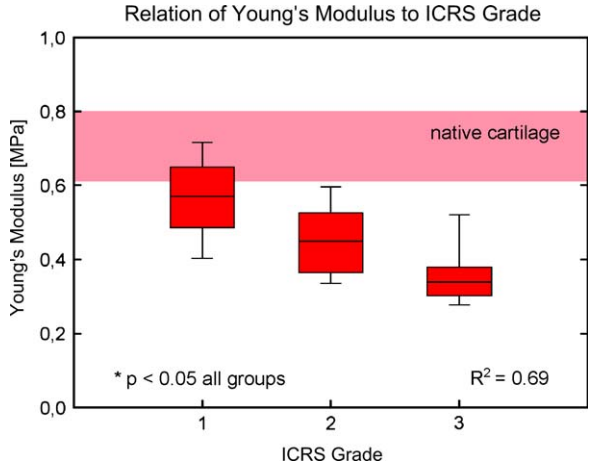

Fig. 1. Stiffness reduction of degenerated cartilage (increasing ICRS Grade) related to Young's modulus; the band layer represents native human articular cartilage (Athanasiou *et al.*[39]). Boxplots displaying median values and interquartile range.

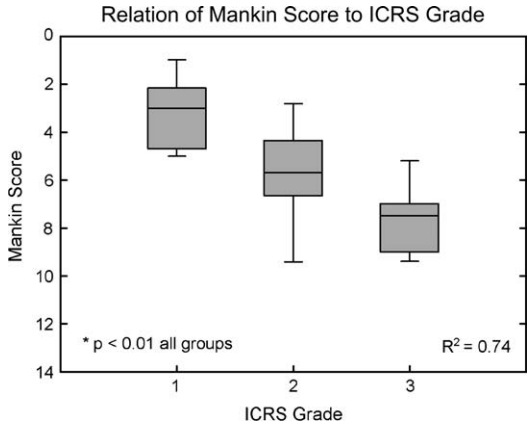

Fig. 2. Stiffness reduction in degenerated cartilage (increasing ICRS Grade) related to histological appearance (Mankin score).

The mean inter-observer variance was 0.8, 1.1 and 1.1 score points of a possible 14 Mankin score for ICRS Grades 1, 2 and 3, respectively. The kappa value for the inter-observer reliability was 0.45.

Overall, the histological evaluation showed clear structural disorganization in the more degenerated cartilage, and fibrillation and derangement increased from ICRS Grades 1 to 3 (Fig. 3). A significant correlation was found between the Mankin score and the ICRS Grade ($R^2 = 0.74$, $P < 0.01$). A moderate correlation was found between the Mankin score and the Young's modulus ($R^2 = 0.47$, $P < 0.02$).

Discussion

The aim of this study was to describe the mechanical alterations of articular cartilage at different stages of degeneration in the course of OA, and to investigate the interrelation between mechanical properties and macroscopic (ICRS Grades) and histological (Mankin score) appearance of osteoarthritic articular cartilage.

The results indicate that the mechanical stiffness of cartilage decreases as its degeneration progresses. The relationship between cartilage stiffness and ICRS clinical scoring predicts a stiffness loss of about 25% for each ICRS Grade. The classification into different ICRS Grades is based on macroscopic markers like the loss of glistening and shiny cartilage surface appearance in the mild stage, and fissures and deep clefts in the moderate and severe stage of OA. These markers monitor the loss of the structural integrity of cartilage in the process of the degeneration. The structural derangement of the matrix seems to have a direct influence on cartilage mechanics. As a consequence, the visual surface appearance of cartilage gives an impression of the mechanical behavior of the tissue − more structural disorder means less stiffness. The Mankin score, which describes the histological deterioration, confirmed the ICRS classification of the specimens. The findings of the Mankin score are consistent with recent studies[14,22,23]. The inter-observer reliability was only moderate compared to other studies[24]. In consequence, macroscopic assessment is a reliable method for the classification of degenerated cartilage. As a limitation, the initial stage of the disease can hardly be detected by visual assessment only, because of the inconspicuous changes of the surface appearance between ICRS Grade 1 and native cartilage.

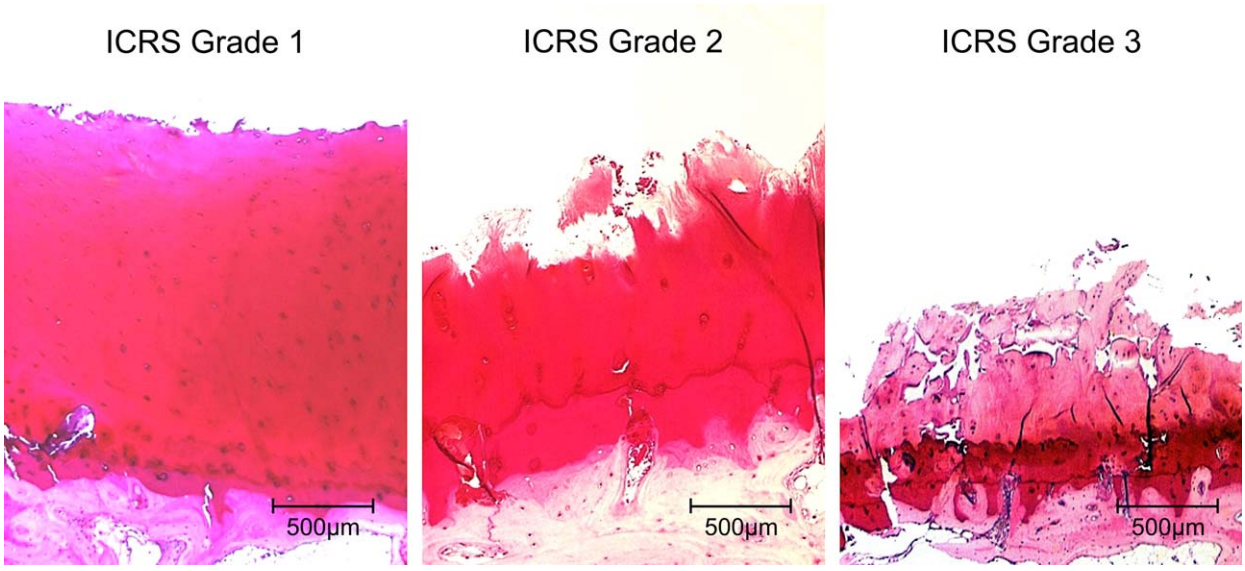

Fig. 3. Histologic views of Safranin-O-stained cartilage examples for ICRS Grade 1 including surface irregularities (left), ICRS Grade 2 showing thinning and clefts of cartilage (middle) and ICRS Grade 3 with complete disorganization of cartilage (right).

A qualitative stiffness decrease of cartilage in the course of OA was likewise observed in previous studies, which analyzed the mechanical properties of native and osteoarthritic cartilage in humans or animals[1,18,19,25–27]. Mechanical softening of the tissue during the degeneration process is caused by changes in the composition and integrity of cartilage. The water content, the collagen integrity, and the amount of GAG are the major cartilage constituents affecting the mechanical properties of cartilage, whereas the mechanics of chondrocytes remain unchanged in the degeneration[28]. In the progress of OA, the water content increases due to a disruption of the collagen network, and the synthesis rate and GAG content decreases[29]. With the loss of the ECM integrity, superficial irregularities and fissures of articular cartilage appear in the mild stage, and deep clefts, matrix disorganization and extended erosion in the advanced stages of OA. Only minor changes in composition between native and degenerated cartilage are apparent while large changes in structure can be described. As reported by others, it seems that structural changes are superior to alterations in composition[25], consequently causing a stiffness reduction in articular cartilage.

Fig. 4. Surface clefts and focal clustering of chondrocytes can be observed in degenerated cartilage classified ICRS Grade 2.

Beside the observed progressive thinning of cartilage, which is consistent with previous data[23,30], the thickness of the subchondral bone seems to adapt in the progress of OA. The behavior and interaction of cartilage and subchondral bone were previously analyzed[1,21–23,31–33], and a mechanical interdigitation for both materials during the development of OA is assumed. The underlying subchondral bone seems to change its thickness, density and its architecture[26,34]. Although the thickness of the bone plate increases, its stiffness, the bone density and the mass fraction of mineral are reduced[35]. It is suggested that the thickening of the bone plate increases the internal cartilage stresses, which may cause a progressive thinning of the cartilage[36]. Other studies reported that an initiation of cartilage fibrillation is related to a significant stiffness gradient in the underlying bone[3]. As a consequence, a stiffness reduction in cartilage appears together with a thicker subchondral bone plate[22]. In summary it has not been proven, which of both alterations is initiated at first and how they influence themselves in the early stage of OA. However, the biomechanics of the tissue seems to play a decisive role only at the tissue level in the progress of OA. The finding of the predominant cartilage degeneration on the medial rather than the lateral plateau side suggested that there is a close relation to the varus-gonarthrosis present in most of the cases. This observation is in agreement with investigators who found that knee–hip–ankle alignment influences the load distribution at the knee and a varus alignment increases the risk of medial condyle OA degeneration[22,23].

The correlation between the mechanical properties and macroscopic and histological results suggests that

mechanically based testers may be used to identify the stage of OA in clinical diagnostics. The relation of mechanical properties to certain indices of degeneration is consistent with previous studies, where the indentation stiffness of cartilage decreased with OA degeneration[37,38]. The present study supports the assumption that mechanical indenters[14–17,19] may be a potential enhancement to classify the stage of degeneration in OA. However, in the light of the small stiffness decrease for ICRS Grade 1 with respect to intact cartilage (ICRS Grade 0) and the inter-individual stiffness variability of the specimens, it appears questionable if the initial stage of OA (ICRS Grade 1) may be detected by mechanical stiffness measurements alone.

In conclusion, increasing ICRS Grades are related to a significant stiffness reduction of cartilage in the osteoarthritic human tibia plateau. The close relationship between macroscopic and histological examination has proven that the macroscopic assessment (e.g., in arthroscopy) is a reliable method for the classification of degenerated cartilage and detection of OA, except for the initial stage of the disease. Further, the results indicate that mechanically based diagnostic instruments like indenters may be a likely enhancement to classify the stage of degeneration in OA. However, the data suggest that a differentiation between healthy cartilage and ICRS Grade 1 may be hardly possible by mechanical testing alone.

Acknowledgements

Thanks to Mrs. Camilla Bergmann for technical assistance in the histological preparation. This study was supported by the DFG (Deutsche Forschungsgemeinschaft) DU 298/8-1.

References

1. Setton LA, Elliott DM, Mow VC. Altered mechanics of cartilage with osteoarthritis: human osteoarthritis and an experimental model of joint degeneration. Osteoarthritis Cartilage 1999;7:2–14.
2. Jones G, Ding C, Scott F, Glisson M, Cicuttini F. Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. Osteoarthritis Cartilage 2004;12:169–74.
3. Radin EL, Abernethy PJ, Townsend PM, Rose RM. The role of bone changes in the degeneration of articular cartilage in osteoarthrosis. Acta Orthop Belg 1978;44: 55–63.
4. Sokoloff L. Biology of Degenerative Joint Disease. Chicago: University of Chicago Press 1969.
5. Mainnen P, Riihimaki H, Heliovaara M, Makela P. Overweight, gender and knee osteoarthritis. Int J Obes Relat Metab Disord 1996;20:595–7.
6. Outerbridge RE. The etiology of chondromalacia patellae. J Bone Joint Surg Br 1961;43B:752–67.
7. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, *et al*. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg Am 2003; 85-A(Suppl 2):45–57.
8. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: *in vivo* assessment with delayed Gd(DTPA)$^{2-}$enhanced MR imaging. Radiology 1997;205:551–8.
9. Burstein D, Velyvis J, Scott KT, Stock KW, Kim YJ, Jaramillo D, *et al*. Protocol Issues for Delayed Gd(DTPA)$^{2-}$enhanced MRI (dGEMRIC) for clinical evaluation of articular cartilage. Magn Reson Med 2001;45:36–41.
10. Tiderius CJ, Olsson LE, Leander P, Ekberg O, Dahlberg L. Delayed gadolinium-enhanced MRI of cartilage. Magn Reson Med 2003;49:488–92.
11. Mak AF, Lai WM, Mow VC. Biphasic indentation of articular cartilage-I. Theoretical analysis. J Biomech 1987;20:703–14.
12. Mow VC, Gibbs MC, Lai WM, Zhu WB, Athanasiou KA. Biphasic indentation of articular cartilage-II. A numerical algorithm and an experimental study. J Biomech 1989;22:853–61.
13. Jurvelin JS, Kiviranta I, Arokoski JP, Tammi M, Helminen HJ. Indentation study of the biomechanical properties of articular cartilage in the canine knee. Eng Med 1987;16:15–22.
14. Saarakkala S, Laasanen MS, Jurvelin JS, Törrönen K, Lammi MJ, Lappalainen R, *et al*. Ultrasound indentation of normal and spontaneously degenerated bovine articular cartilage. Osteoarthritis Cartilage 2003;11: 697–705.
15. Niederauer MQ, Cristante S, Niederauer GM, Wilkes RP, Singh SM, Messina DF, *et al*. A novel instrument for quantitatively measuring the stiffness of articular cartilage. Trans Orthop Res Soc 1998:905.
16. Lyyra T, Jurvelin J, Pitkanen P, Vaatainen U, Kiviranta I. Indentation instrument for the measurement of cartilage stiffness under arthroscopic control. Med Eng Phys 1995;17:395–9.
17. Laasanen MS, Töyras J, Hirvonen J, Saarakkala S, Korhonen RK, Nieminen MT, *et al*. Novel mechano-acoustic technique and instrument for diagnosis of cartilage degeneration. Physiol Meas 2002;23:491–503.
18. Appleyard RC, Burkhardt D, Gosh P, Read R, Cake M, Swain MV, *et al*. Topographical analysis of the structural, biochemical and dynamic biomechanical properties of cartilage in an ovine model of osteoarthritis. Osteoarthritis Cartilage 2003;11:65–77.
19. Bae WC, Lewis CW, Sah RL. Intra-tissue strain distribution in normal human articular cartilage during clinical indentation testing. Trans Orthop Res Soc 2003:254.
20. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. J Biomech 1972;23:541–51.
21. Barker MK, Seedhom BB. Articular cartilage deformation under physiological cyclic loading – apparatus and measurement technique. J Biomech 1997;30: 377–81.
22. Bobinac D, Spanjol J, Zoricic S, Maric I. Changes in articular cartilage and subchondral bone histomorphometry in osteoarthritic knee joints in humans. Bone 2003;32:284–90.
23. Matsui H, Shimizu M, Tsuji H. Cartilage and subchondral bone interaction in osteoarthrosis of human knee joint: a histological and histomorphometric study. Microsc Res Tech 1997;37:333–42.
24. Moojen DJF, Saris DBF, AuwYang KG, Dhert WJA, Verbout AJ. The correlation and reproducibility of histological scoring systems in cartilage repair. Tissue Eng 2002;8:627–34.
25. Franz T, Hasler EM, Hagg R, Weiler C, Jakob RC, Mainil-Varlet P. *In situ* compressive stiffness, biochemical composition, and structural integrity of

articular cartilage of the human knee joint. Osteoarthritis Cartilage 2001;9:582–92.
26. Pastoureau P, Leduc S, Chomel A, De Ceuninck F. Quantitative assessment of articular cartilage and subchondral bone histology in the meniscectomized guinea pig model of osteoarthritis. Osteoarthritis Cartilage 2003;11:412–23.
27. Appleyard RC, Ghosh P, Swain MV. Biomechanical, histological and immunohistological studies of patellar cartilage in an ovine model of osteoarthritis induced by lateral meniscectomy. Osteoarthritis Cartilage 1999;7: 281–94.
28. Jones WR, Ting-Beall HP, Lee GM, Kelley SS, Hochmuth RM, Guilak F. Alterations in the Young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage. J Biomech 1999;32:119–27.
29. Brocklehurst R, Bayliss MT, Maroudas A, Coysh HL, Freeman MA, Revell PA, *et al*. The composition of normal and osteoarthritic articular cartilage from human knee joints. With special reference to unicompartmental replacement and osteotomy of the knee. J Bone Joint Surg Am 1984;66:95–106.
30. Radin EL, Rose RM. Role of subchondral bone in the initiation and progression of cartilage damage. Clin Orthop 1986;213:34–40.
31. Eckstein F, Muller-Gerbl M, Putz R. Distribution of subchondral bone density and cartilage thickness in the human patella. J Anat 1992;180:425–33.
32. Wu JZ, Herzog W, Epstein M. Joint contact mechanics in the early stages of osteoarthritis. Med Eng Phys 2000;22:1–12.
33. Felson DT, Neogi T. Osteoarthritis: is it a disease of cartilage or of bone? Arthritis Rheum 2004;50: 341–4.
34. Shimizu M, Tsuji H, Matsui H, Katoh Y, Sano A. Morphometric analysis of subchondral bone of the tibial condyle in osteoarthrosis. Clin Orthop 1993: 229–39.
35. Li B, Aspden RM. Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. Ann Rheum Dis 1997;56:247–54.
36. Bailey AJ, Mansell JP. Do subchondral bone changes exacerbate or precede articular cartilage destruction in osteoarthritis of the elderly? Gerontology 1997;43: 296–304.
37. Kempson GE, Freeman MA, Swanson SA. The determination of a creep modulus for articular cartilage from indentation tests of the human femoral head. J Biomech 1971;4:239–50.
38. Bae WC, Temple MM, Amiel D, Coutts RD, Niederauer GG, Sah RL. Indentation testing of human cartilage: sensitivity to articular surface degeneration. Arthritis Rheum 2003;48:3382–94.
39. Athanasiou KA, Rosenwasser MP, Buckwalter JA, Malinin TI, Mow VC. Interspecies comparisons of *in situ* intrinsic mechanical properties of distal femoral cartilage. J Orthop Res 1991;9:330–40.
40. Mankin H, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.