Correlation of dynamic impact testing, histopathology and visual macroscopic assessment in human osteoarthritic cartilage

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Received: 24 November 2010 / Accepted: 18 December 2010 / Published online: 18 January 2011
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Abstract

Objective Improved staging of cartilage degeneration is required, particularly during the early stages. We correlated mechanical properties with histological and macroscopic findings.

Methods One hundred and twenty cartilage samples were obtained during total knee arthroplasty. Two adjacent plugs were harvested—one for histological classification and one for macroscopic and biomechanical purposes. Dynamic impact testing was performed; normal stress, dissipated energy (ΔE), tangent modulus and stiffness were evaluated. **Results** Samples were classified according to six categories of the ICRS histological scale. Mechanical

characteristics revealing significant differences between the groups ($p < 0.01$) were specific damping and related absolute ΔE . A significant correlation was found between the macroscopic score and specific damping, as well as absolute and relative ΔE ($p < 0.01$). A strong relation was revealed between relative ΔE and cartilage thickness ($p < 0.001$; $R^2 = 0.69$).

Conclusions Only ΔE correlated with the condition of the cartilage—the value increased with decreasing quality—and is the most suitable characteristic. This change appears substantial in initial stages of cartilage deterioration.

Introduction

Degradation of the articular cartilage in osteoarthritis influences its functional properties. Cartilage mechanical properties may provide a quantitative method for monitoring degenerative changes. Articular cartilage displays unique biomechanical properties due to its extracellular matrix that facilitates transmission of load to the subchondral bone while providing near frictionless articulation.

Water makes up the majority of native cartilage and plays the principal role in the ability to withstand compressive forces. Solid cartilage structures are thus exposed to hydrostatic pressure. In diarthroidal joints this interstitial pressure ranges between 5 and 10 MPa and it is applied periodically during normal activities [1]. The human hip and knee joint may sustain loads up to ten times body weight, which may increase up to a value of 18 MPa of compressive stress [1, 2].

During normal gait the acting force reaches its peak in approximately 100 minutes. Corresponding loads in the tibio-femoral joint are about two to three times body

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weight, with loading rates in the order of $20 \text{ kN}\cdot\text{s}^{-1}$ and strain rates in the order of 5 s^{-1} [3]. During running, the loading time shortens to about 30 minutes, acting forces are about 2 kN and the loading rate is in the order of $200 \text{ kN}\cdot\text{s}^{-1}$ [4].

Articular cartilage can withstand a wide range of applied stresses. For impact loading, the accepted critical stress value causing chondrocyte death and ruptures in the extracellular matrix is 15–20 MPa [5]. Recently measured ultimate stress values causing irreversible damage in impact testing range from 16 to 237 MPa, depending on the impact energy and strain-rate applied [6, 7].

Referring to animal experiments [8, 9], it would be expected that there is a significant relation between cartilage biomechanics and histopathological alteration. The biomechanical properties of human cartilage have already been extensively studied, but only few studies correlating these results to the clinical examinations can be found [e.g. 10] and the elastic modulus usually serves as a representative mechanical characteristic. Studies relating dynamic mechanical properties with histopathology are even more rare [11]. The measurement of mechanical cartilage parameters in situ has been already pioneered [12]. This is a promising alternative approach in cartilage diagnosis; indentation instruments that could be used for such purposes have been designed and tested [e.g. 13, 14].

Improved staging of cartilage degeneration is required, particularly during the early stages when minimal surface damage is visible and could aid prognosis and therapy. In our study we determined whether the dynamic mechanical properties correlate with histopathology and macroscopic visual scores. Mechanical characterisation, though extensively developed, does not yet serve for diagnostic purposes. The current standard for staging cartilage degeneration is histopathological scoring based on a scheme described by Mankin et al. [15]. Another scoring system was reported by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS) [16]. These approaches require invasive sampling and therefore are not suitable in vivo. Clinical methods evaluating cartilage status in vivo are based on arthroscopic visual examination. The ICRS macroscopic classification is the one most often used [17]. However, this assessment gives no information concerning the cartilage morphological status. In cartilage with a low grade (ICRS 0–1) of macroscopic degradation, the severity of the histopathological findings may vary considerably (Mankin 1–8 of 14) [11]. This is why the accuracy of visual macroscopic classification of early-stage cartilage degradation is under discussion [10, 12].

Standard diagnostic techniques including X-ray, MRI, CT and ultrasonography are somewhat subjective and mostly presume macroscopic tissue alteration. Recently, expert techniques based on described imaging modalities

are being developed to facilitate quantitative interpretation [18–21].

Developing a minimally invasive technique for in vivo measurement of cartilage biomechanics could assist in effective early impairment diagnosis. A detailed classification of biomechanics in different stages of deterioration would be essential, as alterations in biomechanical properties can be observed before any gross morphological change is apparent [22].

Materials and methods

Fifteen patients were included in our study (mean age 71.2 years, range 56–84 years, five males, ten females, four right knees, 11 left knees) and 120 hyaline cartilage samples were obtained during knee joint arthroplasty. The osteochondral samples were harvested from the femoral and tibial lateral and medial condyles. Each sample with layer of subchondral bone was cylindrical with a diameter of exactly 6 mm, and the cartilage layer thickness ranged from 0.6 to 4.2 mm. Two corresponding adjacent plugs were harvested from the same place in all cases—one for histological and one for biomechanical purposes (see Fig. 1).

The samples for biomechanical testing were delivered to the laboratory in 0.9% NaCl and stored in glass containers at 4°C. The measurements were performed at room temperature (21°C).

The dynamic biomechanical property testing method used in our study employs a novel approach, based on the examination of the drop-weight-impact sample's deformation. A pendulum-like apparatus setup permits tracking of the material's response to a single impact. The rapid increase in the acting force should resemble physiological joint cartilage loading. The bone tissue of the cartilage-on-bone samples was firmly attached in the stand's chuck fitting. When the impactor hit the sample in the direction perpendic-

Fig. 1 Hyaline cartilage sample harvesting

ular to its surface, the sample's deformation was read simultaneously by a piezoelectric accelerometer and a laser Doppler vibrometer and then processed by computer. The blunt impact dynamic setup was employed. It permits effective acquisition of data with a high information yield [23].

In our study, a loading resembling a typical physiological value was used rather than extreme values. The impact velocity used was $0.25 \text{ m}\cdot\text{s}^{-1}$, resulting in strain rates in the order of 10 s^{-1} . Maximal stresses achieved ranged from approximately 10 MPa to 20 MPa.

The acquired data was used to determine force–deformation loading diagrams characterising each sample (examples in Fig. 2). A preload of 1 N was established as the standard. Several mechanical characteristics were evaluated including stiffness, tangential modulus, absolute and relative dissipated energy as well as specific damping.

Stiffness was evaluated as the ratio dF/dl , where F is the normal acting force and l the actual cartilage deformation, while the tangential modulus of elasticity is $d\sigma/d\varepsilon$, where σ is normal stress and ε actual strain ($\varepsilon=l/l_0$, l_0 is the original cartilage thickness). Both the stiffness and tangential modulus, which are strain dependent, were evaluated at three specific points of the loading diagram: at the very beginning of the loading process (0.2 mm deformation), in the medial phase ($\sigma=1 \text{ MPa}$) and just before reaching the maximum (individual for each diagram).

Dissipated energy is understood as a portion of the impact energy that was absorbed in the sample during the impact-rebound process. The integral of acting force was

used to evaluate the absolute dissipated energy value ΔE [mJ].

$$\Delta E = E_1 - E_2 = \int_0^{l_{\max}} F_1(l)dl - \int_{l_{\max}}^0 F_2(l)dl = \frac{1}{2}m(v_1^2 - v_2^2)$$

where E_1 is the kinetic energy and v_1 the velocity of the impactor just before the impact, E_2 and v_2 are its energy and velocity immediately after rebounding, F_1 is the actual force acting during the loading phase of the impact process and F_2 is the force during the unloading phase.

Value ΔE divided by the cartilage sample volume was denoted as relative dissipated energy ΔE_r [mJ/mm³]. Specific damping (energy absorption ratio) is an intrinsic material characteristic defined as the ratio of absolute dissipated energy ΔE and the total energy of the impact E_1 (kinetic energy of the impactor just before contact).

Prior to biomechanical testing, all the samples were visually classified according to the International Cartilage Repair Society (ICRS) macroscopic classification [17]. A study on the relationship between ICRS grades and mechanical properties was reported recently [10]. Cartilage thickness was measured digitally from samples' photographs shot prior to mechanical testing.

Histological examination was performed on formalin-fixed paraffin-embedded decalcinated samples and stained using haematoxylin-eosin and classified according to the ICRS score [16] (Table 1). In recent studies, there was none or poor correlation between biomechanics and numerous biochemical properties or gene expression in articular cartilage tissue; the only significant relation was found to the ICRS score [10, 11].

Biomechanical classifications data were compared using the one-way ANOVA test (diversity of groups) and the unpaired T-test (comparing two sets of results).

Results

Three sample pairs were excluded from the study due to incomplete histological data; thus, 57 were involved.

Examples of loading diagrams for samples of different location and histologically assigned quality are given in Fig. 2.

Considering individual histological features separately, significant differences were found in specific damping and proportionally-related absolute ΔE , as well as in relative ΔE_r for features 1, 2 and 3 of histological scoring. No other mechanical characteristics correlated significantly with the histological score (T-test, $p>0.05$) (Fig. 3a, b).

Evaluation of the samples' quality according to the ICRS macroscopic score is shown in Table 2. A significant

Fig. 2 Examples of force–deformation loading diagrams for corresponding samples of different thickness and histological score. **a** Medial (thickness 2.1 mm, total ICRS=9, group IV). **b** Lateral (4.2 mm, ICRS=14, group II) tibial condyle of single joint. **c** Medial (1.15 mm, ICRS=12, group III). **d** Lateral (3 mm, ICRS=18, group I) femoral condyle of single joint

Table 1 Histological classification of cartilage according to International Cartilage Repair Society (ICRS) score, with the number of samples fitting individual criteria

ICRS visual histological assessment scale and numbers of samples used in this experiment			
Category	Feature	Score	Number of samples
I.	Surface		
	Smooth/continuous	3	19
	Discontinuities	0	38
II.	Matrix		
	Hyaline	3	34
	Mixture: hyaline/fibrocartilage	2	23
	Fibrocartilage	1	n/a
	Fibrous tissue	0	n/a
III.	Cell distribution		
	Columnar	3	7
	Mixed/columnar-clusters	2	44
	Clusters	1	6
	Individual cell/disorganised	0	n/a
IV.	Cell population viability		
	Predominantly viable	3	57
	Partially viable	1	n/a
	<10% viable	0	n/a
V.	Subchondral bone		
	Normal	3	49
	Increased remodeling	2	8
	Bone necrosis/granulation tissue	1	n/a
	Detached / fracture / callus at base	0	n/a
VI.	Cartilage mineralisation		
	Normal	3	48
	Abnormal/inappropriate location	0	9

correlation was again found between the score value and specific damping and proportionally-related absolute ΔE , as well as relative ΔE_r (ANOVA, $p < 0.01$). Neither stiffness nor elasticity modulus values followed the ICRS macroscopic classification (Fig. 4a, b).

As the samples were harvested from the same sites in each examined knee with no regard to cartilage condition, more severe deterioration of the medial condyle samples was proven, as shown in Fig. 5a, which relates the macroscopic evaluation to the location of the samples. The pattern is followed by values of specific damping (Fig. 5b).

Though no correlation between sample thickness and total histological score was proven, thickness can be considered as a marker of cartilage condition (e.g. as it partially determines the ICRS macroscopic score). Related to mechanical properties, a strong relation was revealed between energy dispersed in unit volume (relative ΔE_r) and cartilage thickness ($p < 0.001$; $R^2 = 0.69$ for linear correlation). On the other hand, no relation was found between cartilage thickness and specific damping, absolute ΔE , stiffness or tangential modulus.

Discussion

Based upon the histological classification of the mechanical features, none of the differences between the individual groups could be considered substantial. Even the difference in specific damping was ambiguous. This might be caused by the fact that though a decreasing value in each histological feature's score indicates more serious cartilage damage, from a mechanical point of view, these degenerative changes do not necessarily have to have the same consequences. Fibrotisation (feature II) and mineralisation (feature IV) could be expected to increase the stiffness. However, disorganization in chondrocyte distribution (feature III) would be expected to hamper the tissue's integrity and thus its stiffness. The effects of uneven cartilage surface (feature I) on measured stiffness would be quite unpredictable. Also, the effects of individual histologically-revealed impairments on the amount of impact energy dispersed in the sample is beyond simple rational assessment.

When comparing the biomechanical properties on the basis of individual evaluated histological features, only the values characterising ΔE were found to be significantly related. This was proven for histological features I–III, i.e.

Fig. 3 **a** Specific damping for scores achieved in individual features of histological classification, mean values with standard deviations shown. For feature number 4 all samples scored the same and are thus not provided. **b** Tangential modulus of elasticity at 1 MPa normal stress for scores achieved in individual features of histological classification; mean values with standard deviations shown. For feature number 4 all samples scored the same, and are thus not provided. Column description corresponds to specific feature score for each category of the International Cartilage Repair Society (ICRS) scale

surface quality, degree of fibrotisation and cell distribution. The fact that features V and VI did not show significant correlation might be partially caused by the uneven distribution of samples for the individual score values (49:8 and 48:9). Generally, having only two subgroups with a continuous qualitative transition for each histological

Fig. 4 **a** Specific damping for individual classes according to the International Cartilage Repair Society (ICRS) macroscopic score; mean values with standard deviations shown (in class 4 two samples only, thus no standard deviation shown). **b** Tangential modulus of elasticity at 1 MPa normal stress for individual classes according to the ICRS macroscopic score; all measured values except two outliers shown

score makes it difficult to establish any statistical significance in their discrepancies.

The ICRS macroscopic evaluation correlates with histological examinations, and is thus believed to be a reliable method for classifying cartilage [10]. We have confirmed that biomechanical parameters follow similar patterns,

Table 2 Macroscopic classification of cartilage according to the International Cartilage Repair Society (ICRS) with number of samples fitting individual criteria

ICRS class	Description of cartilage deterioration	Number of samples
0	Normal surface	11
1	Almost normal surface with fibrillation or softening, fissures, cracks	23
2	Abnormal surface with damage up to 50% of cartilage thickness, fraying	19
3	Substantially damaged surface with damage to more than 50% of cartilage thickness, but not up to bone	5
4	Severely abnormal surface with lesion up to subchondral bone, complete cartilage loss	2

Fig. 5 **a** Location of harvested samples related to the International Cartilage Repair Society (ICRS) macroscopic score. **b** Specific damping according to location of harvested samples. Mean values with standard deviations shown; *FL* lateral femoral condyles, *FM* medial femoral condyles, *TL* lateral tibial condyles, *TM* medial tibial condyles (**a**, **b**)

whether macroscopic or histological quality assessment is used [24].

In all of the results presented, ΔE values revealed a correlation to the cartilage condition. Decreased quality has always meant an increase in absolute and relative ΔE as well as specific damping value. An equivalent evaluation of cartilage quality can thus be given in terms of ΔE , e.g. specific damping.

Generally, difficulties have been reported in distinguishing between healthy cartilage and the initial stages of degradation [10, 25]. Young et al. [11] evaluated the dynamic mechanical properties (shear modulus and phase lag) of human tibial cartilage using a special indenter. They only involved cartilages of grade 0 or 1 ICRS macroscopic scores and related results to histological findings, concluding that mechanical analysis is sensitive enough to aid in determining the stage of cartilage disease. Evaluating indentation stiffness led [12, 26] to similar conclusions. Our results showing differences of more than 10% in values of specific damping

between groups scoring 0, 1 and 2 in the ICRS macroscopic classification. With further research, it may be possible to use ΔE as an indicator for early osteoarthritis recognition.

Kleeman et al. [10] reported stiffness reduction with cartilage degeneration—an increasing ICRS macroscopic score and histological appearance according to Mankin et al. [15]. The biomechanical characteristics were acquired in a quasi-static setup. Our results in dynamic impact testing have not confirmed this relation.

No correlation between thickness and stiffness or tangential modulus was demonstrated in our study, which accords with Bae et al. [26], while Shepherd et al. [27] reported compressive moduli correlating with thickness, but the condition of cartilage is not mentioned. As stiffness and the elasticity modulus are determined by the material's composition and its condition rather than by its amount, we find our results reasonable.

Dissipated energy has not yet been assessed as a representative feature of cartilage status. Increasing mechanical damage of cartilage leads to greater energy absorption [6]. In drop-tower dynamic impact testing, no correlation between energy of deformation per unit volume (relative ΔE_r) and cartilage thickness was found. Our findings showed significant correlation of relative ΔE_r and cartilage thickness. In the above-mentioned study, cartilage quality was not assessed, only its mechanical properties under different conditions were evaluated. Furthermore, much higher impact energies and strain rates were used. These might be the reasons for substantial anticipation for our results. Once we accept the amount of ΔE as a quantifier of cartilage condition, this relation proves that the cartilage thickness describes cartilage quality as well.

Being conscious of the range of variation in the acquired data, we attribute this mainly to the not totally precise relationships of the samples, e.g. cartilage-bone interface not ideally perpendicular to the acting force direction, uneven surface, borderline cases in histological or macroscopic evaluation, etc. These limitations can hardly be avoided when examining native tissue samples. The measurements were taken in unconfined compression, though substantially higher interstitial fluid leakage was possible compared to in situ circumstances.

Conclusion

Among all the evaluated mechanical characteristics of cartilage tissue, only values related to ΔE showed a consistent relation to the condition of the examined tissue. According to our study, the value of ΔE increases with decreasing cartilage tissue quality. This appears substantial even in initial stages of articular cartilage deterioration. No significant relation of stiffness or elasticity modulus was found.

Therefore we conclude that the amount of energy dissipated during a mechanical loading examination (or specific damping as a material characteristic) is the most suitable biomechanical characteristic to assess actual cartilage quality.

However, more extensive study could develop more precise ranges of ΔE values for the particular classification of examined cartilages.

Acknowledgments We would like to thank the staff of the Institute of Pathological Anatomy for histological staining and the staff of the International Laser Centre for help with biomechanical testing. We would also like to thank the staff of the Orthopaedic Clinic where the total knee joint replacements were performed. Special thanks to the staff of the Department of Biophysics for their excellent cooperation.

Role of the funding source This work was supported by Grants FI IM4/205 and FT TA5/020 from the Ministry of Industry of the Czech Republic.

Conflict of interest There were no financial or personal relationships with other people or organisations that could influence our work and conclusions.

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