

# Changes in the stiffness of the human tibial cartilage-bone complex in early-stage osteoarthritis

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Cylindrical human tibial cartilage-bone unit specimens were removed from 9 early-stage medial osteoarthrotic (OA) tibiae (mean age 74 years) and 10 normal age-matched tibiae (mean age 73 years). These specimens were divided into 4 groups: OA, lateral comparison, medial age-matched, and lateral age-matched and were tested to 0.5% bone strain with a novel technique to obtain the stiffnesses of both cartilage and bone simultaneously.

We found a pronounced reduction in the stiffnesses of OA cartilage and subchondral bone when compared with the medial age-matched group. OA carti-

lage was significantly thinner than that of the lateral comparison and the medial age-matched control groups. However, this reduction in thickness was not correlated with the reduction in stiffness for OA cartilage. The stiffnesses did not correlate between OA cartilage and bone, whereas the stiffness relationships between cartilage and bone remained significant in the three control groups.

Our findings suggest that both cartilage and bone in early-stage OA are mechanically inferior to normal, and that OA cartilage and bone have lost their unit function to mechanical loading.

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Studies on the variation in the stiffness of articular cartilage in different species, different joints and different locations within the joint have shown the same pattern (Armstrong and Mow 1982, Mow et al. 1991) as that of trabecular bone (Hvid and Hansen 1985, Hodgkinson and Currey 1990). This relationship indicates that cartilage and subchondral trabecular bone may function as a mechanical unit. A novel technique for simultaneous mechanical testing of articular cartilage and subchondral trabecular bone has been developed by Røhl et al. (1997). Using this technique, further evidence has been added that similar age-related trends were observed in cartilage and bone (Ding et al. 1998), as if they functioned as a single mechanical unit (Ahn et al. 1995).

A debate has gone on for several years whether the initial changes in osteoarthritis (OA) occur first in cartilage or in the subchondral trabecular bone. Both hypotheses assume that the unit function of the cartilage-bone complex is disrupted. The object of this study was therefore: (1) to compare the stiffness relationships of the cartilage-bone complex in early-stage OA with normal age-matched groups, and (2) to test the hypothesis that the unit function of cartilage and bone alters under pathological conditions.

## Material and methods

### Specimens

Cylindrical cartilage-bone composite specimens were removed from 9 human early-stage OA proximal tibiae (mean age 74 (63–81) years, 7 men), together with 10 normal human age-matched proximal tibiae (mean age 73 (60–85) years, 7 men). All these donors were Caucasian. One tibia was removed from each donor, and 3 specimens were taken from each medial and lateral condyle. These specimens were divided into 4 groups: medial OA, lateral comparison, medial age-matched, and lateral age-matched. All specimens were stored in plastic tubes filled with physiological saline at  $-20^{\circ}\text{C}$ .

The normal tibiae had no diseases, and all the cartilage surfaces of the specimens were intact. These patients had died suddenly from trauma or acute disease.

The early-stage OA was defined as macroscopically degenerated fibrillated cartilage and was confirmed histologically. According to criteria described by Mankin et al. (1971), the OA group was graded as 4.9 (3–7), lateral comparison as 1.7 (1.5–3), medial age-matched as 0.8 (0–2), and lateral age-matched as 0.5 (0–1) (graded independently by 2 persons). The OA tibiae showed visual degeneration with slight fissures

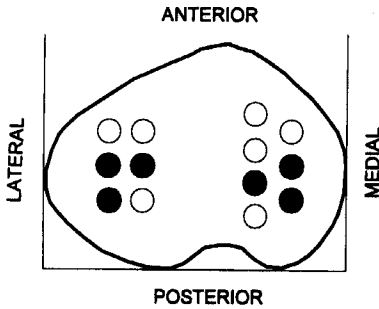


Figure 1. Schematic drawing illustrating the plastic templates which were used to obtain specimens from standardized locations (filled circles) where cartilage surfaces were most plane. The size of the template was chosen according to the width of the cartilage-bone slice. The template was aligned on the line formed by the posterior margin of both condyles.

on the surface (superficial zone) of the medial condyle cartilage, whereas the surface of the lateral condyle cartilage was intact. The cell clusters in the superficial zone and reduction of safranin O staining of OA cartilage could be seen.

**Specimen preparation**

From each proximal tibia, the cartilage and the underlying 12 mm trabecular bone were cut off parallel to the subchondral bone plate, using an EXAKT-Cutting

Grinding System. 6 cylindrical cartilage-bone unit specimens, 3 from each medial and lateral condyle, were removed. A plane section perpendicular to the longitudinal axis of the tibia was made to obtain a surface which was relatively parallel to the cartilage surface of the tibia. Then a 7.5 mm inner diameter trephine, parallel to the longitudinal axis of the tibia, was used to drill out specimens from standardized locations of the composite slice where cartilage surfaces were most plane (Figure 1). After this, the distal part of the trabecular bone was cut off, using a Leitz Saw Microtome 1600 to achieve a bone length of 8.5 mm. The exact dimensions of cartilage and bone were measured afterwards with a caliper, using the mean of three measurements from different positions of the cylinder. Before testing, the specimens were kept at room temperature for 2 hours and moist during testing.

**Mechanical set-up**

The mechanical set-up for combined testing was done as described by Røhl et al. (1997) (Figure 2). The specimen was mounted in an aluminum cage by 3 fixation screws fixed onto the subchondral bone plate. The cage has a broad midplate, which forms a reference plane between cartilage and bone. The cartilage was directed downwards and submerged in a bath

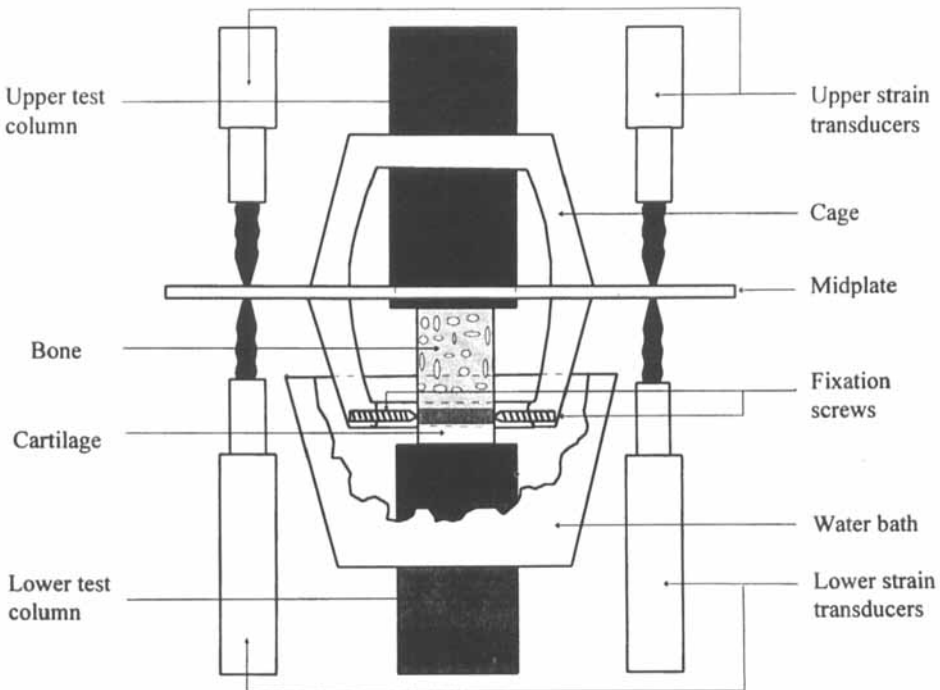


Figure 2. Mechanical set-up for combined testing (see detail in text).

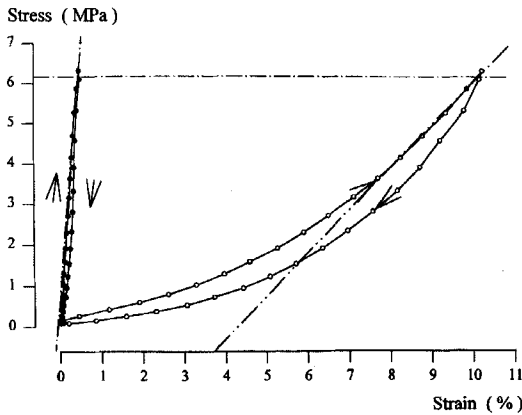


Figure 3. A typical combined testing curve. Cartilage and bone were tested to 0.5% bone strain. The stiffnesses of cartilage and bone were determined as the tangent to the point on the cartilage or bone loading-curve intersecting the cartilage strain-line (corresponding to 0.5% bone strain) and the 0.5% bone strain-line.

filled with physiological saline. The top 2.5 mm of the lower column was made of porous sintered steel, with a porosity 30%, mean pore size of 20  $\mu\text{m}$ , to allow outflux of water from the cartilage. The specimens were tested in compression between 2 steel columns. Deformation of the cartilage and trabecular bone was measured simultaneously as the average between the data of the 2 lower strain transducers and the 2 upper strain transducers, respectively.

### Combined testing

The mechanical tests were performed on an 858 Bio-nix MTS hydraulic material testing machine (MTS Systems Cooperation, Minneapolis, Minnesota, USA), using a 1 kN load cell. A pre-load of 4 N (corresponding to 0.09 MPa) was applied to the specimens to ensure full contact between the two steel columns and the ends of the specimen. At this load, the channels from the 4 strain transducers were reset to zero, thus defining the zero strain. For each testing, 20 preconditioning cycles with a bone strain rate of 0.002/sec were performed between pre-load and 0.5% bone strain to reach a viscoelastic steady state of the bone before the final test was performed. Only the actual test data were collected for analysis. The specimens were tested by non-destructive compression to 0.5% bone strain, to obtain the stiffness in cartilage and bone (Røhl et al. 1997).

Force-deformation curves were recorded. The force-deformation data were converted into stress and strain data, using the cross-sectional area and the original length of the specimens. The normalized stiffness of bone was determined as the tangent to the point on the bone loading curve intersecting the 0.5% bone

strain line. The normalized stiffness of cartilage was determined as the tangent to the point on the cartilage loading-curve intersecting the cartilage strain-line corresponding to 0.5% bone strain (Figure 3).

### Statistical evaluation

A t-test, based on the mean values for each condyle (each condyle represented by one set of values), was used to compare these properties between groups. Due to non-normal data but equal variance, the Wilcoxon matched pairs signed-rank test was used to compare the stiffnesses of cartilage between groups. Linear regression analysis was used to assess the association of different properties between groups. A  $p$ -value  $< 0.05$  was considered significant.

### Results

The average values of the stiffnesses of cartilage and bone in OA, lateral comparison, medial age-matched, and lateral age-matched groups are summarized in the Table.

The stiffness of OA cartilage was lower than that of the medial age-matched cartilage (reduced by 29%,  $p = 0.04$ ), whereas the stiffness of OA cartilage did not differ significantly from that of the lateral comparison cartilage ( $p = 0.8$ ). Likewise, the stiffness of OA bone was 24% lower than that of the medial age-matched bone. However, due to large variation in data, this difference was not statistically significant ( $p = 0.4$ ).

The mean thickness of OA cartilage at the test location was 2.3 (1.8–3.0) mm, which was thinner than the lateral comparison cartilage at the test location (2.8 (1.8–4.2) mm),  $p = 0.03$ . OA cartilage was also thinner than the medial age-matched cartilage at the test location (2.5 (1.7–3.6) mm),  $p = 0.3$ . This reduction in thickness, however, was not correlated with the reduction in stiffness for OA cartilage, and no correlation was found between the thickness and the cartilage stiffness in the other three control groups.

The stiffness of OA cartilage was not correlated with bone stiffness ( $p = 0.5$ ), whereas the stiffness of the medial age-matched cartilage correlated with bone stiffness ( $p = 0.006$ ). The significant correlations between cartilage and bone were also found in the lateral comparison group and the lateral age-matched group (Table).

### Discussion

The specimens we used were removed from human

The mean values and the relationships of the stiffnesses between cartilage and bone in the four groups: osteoarthritis (n 9), lateral comparison (n 9), medial age-matched (n 10) and lateral age-matched (n 10). The determination coefficients ( $r^2$ ) and p-values were derived from linear regression analysis between cartilage and bone stiffnesses within the same group

Stiffness (MPa)	Cartilage mean (range)	Bone mean (range)	Cart. vs. Bone $r^2$	p-value
Osteoarthritis	58 (15-180)	237 (53-646)	0.07	0.5
Lateral comparison	61 (11-221)	218 (23-608)	0.57	0.02
Medial age-matched	82 (34-235)	311 (53-887)	0.63	0.006
Lateral age-matched	67 (16-170)	292 (30-723)	0.49	0.02

early-stage medial OA proximal tibiae. The medial condyle of OA cartilage surface showed visual degeneration with slight fissures. Histological analysis was done to confirm these changes (Mankin et al. 1971). Brocklehurst et al. (1984) and van Valburg et al. (1997) found a good correlation between the results of histology and the visual appearance of human knee articular cartilage observed at autopsy, and these changes in cartilage surface are suitable for studying the process of cartilage degeneration in OA.

The initiation and progression of cartilage damage are distinct phenomena of OA, which have been proposed to be multifactorial, such as biomechanical, biochemical, and biophysical changes. It has been found that the water content of human OA articular cartilage was above normal and was highly correlated with the intrinsic equilibrium modulus and permeability (Armstrong and Mow 1982). As the water content increases, the matrix of cartilage tissue becomes more permeable and softer, and the decrease in modulus is balanced by the increase in permeability. In a canine OA model, breakdown of the collagen network, decrease of tensile stiffness and increase of swelling were observed (Altman et al. 1984). It has also been suggested that the structure of cartilage in canine OA may play a more important role than the composition in determination of its mechanical properties (Guilak et al. 1994).

The structural integrity of the solid matrix, such as collagen and proteoglycans, provides cartilage with the ability to withstand mechanical loading. In our study, the slight fissuring of the cartilage superficial zone due to early-stage OA is the result of damage to its collagen network, and hence, the cause of the reduction of cartilage stiffness. The marked thinning of cartilage is one of the early features of OA. While it is not surprising that OA cartilage shows a reduction in thickness, the cartilage thickness did not correlate with the cartilage stiffness in any group. These results are in accordance with previous findings (Athanasios et al. 1991). It appears that the changes in cartilage

due to early OA mainly cause disruption of cartilage collagen fiber network (Guilak et al. 1994), and consequently disrupt the unit function of cartilage and subchondral bone in response to mechanical loading.

The stiffnesses of normal cartilage and bone from medial tibial condyles are generally higher than those from lateral condyle (Hvid and Hansen 1985, Ding et al. 1997, 1998). However, during the development of early-stage OA, both OA cartilage and bone showed a reduction in stiffness compared to the lateral comparison and medial age-match groups. These results support previous findings based on testing of cartilage (Obeid et al. 1994) or bone (Hvid and Hansen 1986) separately, however, differ from those of Radin and Rose (1986) who hypothesized that initiation of cartilage lesions was due to steep stiffness gradients in the underlying subchondral bone. The present data suggest that both cartilage and subchondral bone are mechanically inferior to normal in early-stage OA.

Mechanical factors, such as normal loading, play an important role in the maintenance of normal articular cartilage. It has been demonstrated that stiffnesses correlate significantly between normal cartilage and bone (Ahn et al. 1995, Ding et al. 1998). Hence, it is an interesting finding that the stiffness correlation between cartilage and bone for the OA group was lost, whereas for the other three groups, these correlations between cartilage and bone remained significant. Therefore, it is reasonable to suggest that, in early-stage OA, the cartilage and subchondral bone have lost their unit function in response to mechanical loading.

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