Mechanical behaviour of articular cartilage under tensile cyclic load

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Abstract

Introduction. Although fatigue has been implicated in cartilage failure, there are only two published studies in this area, by the same author. However, in these previous studies cartilage was tested in the direction parallel to that of collagen orientation in the superficial layer, where it possesses greater tensile strength. In the present work, articular cartilage was also tested along the direction perpendicular to that of the collagen. Furthermore, the study investigated topographic and zonal variations in the fatigue behaviour of cartilage from the human knee.

Methods. Specimens were tested in a specially constructed apparatus that allowed the number of cycles at specimen failure, as well as the load and elongation of the specimen, to be monitored for each specimen. To date, some 72 specimens have been tested, all from the same knee joint, though from different sites and at different depths within the cartilage layer.

Results and conclusions. The most impressive of the outcomes of this study is the scatter of the data. Considering all the specimens used, the range of number of load cycles to failure was between 2 and 1.5 million. The zonal variation in fatigue behaviour was similar to that in tensile modulus reported previously; the surface and deep layers seemed to have better fatigue properties whether tested in the direction parallel or perpendicular to that of the collagen in the superficial layer. The middle layer was far weaker, suggesting that highly packed and ordered fibres in the surface and deep zones have better mechanical properties than the more random and loose fibres in the middle zone. The variation in fibre organization through the cartilage thickness was also reflected in the differences observed in the elongation of the specimen during the test. The surface and deep zones had a higher stiffness than the middle zone. Cartilage had better fatigue resistance when the specimen was loaded in a direction parallel rather than perpendicular to the collagen within the surface layer. This was true whether specimens were harvested from the superficial, intermediate or deep layer. There were many factors that confounded attempts to estimate the likely fatigue life from the data obtained in such a study.

KEY WORDS: Articular cartilage, Fatigue, Tensile cyclic loading.

It has been reported that the average person takes approximately 2 millions steps per year. Thus, every joint in the lower limbs undergoes 1 million load cycles during this time [1, 2]. Furthermore, the load supported by the human knee can peak at values of 4–5 times body weight during walking [3, 4], producing peak stresses of 4–9 MPa [5–9]. Hence, because of the presence of cyclic stress, it is important to study joints from a fatigue point of view and the relationship of this stress with osteoarthrosis (OA).

OA is often referred to as a disease of ageing. It increases the degradation of the tissue with age, which reaches a maximum in the seventh decade [10]. Freeman [11] suggested that the primary event in the pathogenesis of OA could be a fatigue failure in articular cartilage. As osteoarthritic damage to cartilage is progressive, the damage mechanisms associated with gradual accumulation of microdamage, of which fatigue is typical, are likely to be operative.

Fatigue is a term that applies to changes in properties that occur in metallic and non-metallic materials due to the repeated application of stresses or strains, although the term applies generally to those changes that lead to cracking or failure of the material.

Two studies have thus far investigated the fatigue behaviour of cartilage; both studies, from the same institution, used the same method. In the first of these studies, Weightman [1] studied the fatigue properties of articular cartilage of the femoral head, carrying out a zero-to-tension fatigue test. He applied cyclic tensile load on cartilage specimens from the surface layer only, along the direction of the collagen fibrils, which he...
identified by the splits produced by pricking the surface with a pin. The load was applied for 1 s followed by 20 s of resting, with a rise time of approximately 0.1 s. The apparatus was refrigerated at +4°C throughout the tests in order to reduce the rate of cartilage degeneration. We tested 30 post-mortem human femoral heads, from subjects aged 9–80 yr, and three specimens were obtained from each femoral head. In order to obtain more data, Weightman et al. carried out a second study in which 20 femoral heads from subjects aged 9–82 yr were tested. The main conclusions drawn from both studies were that cartilage exhibited typical fatigue behaviour, and that as the magnitude of the applied cyclic stress decreased an increasing number of load cycles was required to produce fracture. The data also showed that the fatigue resistance of cartilage varied widely with age and from one femoral head to another. Furthermore, in both of his studies Weightman attempted to estimate the fatigue life of joints and made predictions from his data. In the first study, he predicted a fatigue life beyond the human lifespan (130–150 yr). In the second study he used a different analytical approach to the data, which were not significantly different from those obtained in the first study, and estimated a fatigue life within that of the human lifespan. However, the abstracts of both publications seem to conclude that fatigue failure of cartilage could occur in life.

Cartilage fatigue, which has been investigated in the above pioneering and labour-intensive studies, is revisited here on methodological grounds, which we believe may influence the results. The first, and perhaps most important, of these grounds is the direction of testing of the cartilage specimens. In both studies by Weightman et al., specimens from the superficial layer were subjected to tensile load along the direction of the collagen fibrils. As cartilage possesses a much lower tensile strength in the perpendicular than in the parallel direction, its resistance to fatigue in the former direction is also likely to be weaker than it is along the direction of the collagen fibrils. Secondly, the protocol of loading the specimen for 1 s and then allowing it to recover for 20 s differs greatly from what happens under physiological conditions.

To improve our understanding of cartilage fatigue, we have now revisited the issue. We investigated the regional and zonal variations in the response of cartilage to prolonged cyclic tensile loading, applied under physiological conditions, and to monitor the elongation of the test specimens through the entire test to specimen failure.

Materials and methods

Materials

Specimens and their preparation. Only one human knee, 48 yr old, has been used so far in this study. The knee was kept frozen at −20°C until the day before the experiment, when it was thawed at room temperature. Cartilage was assessed visually to identify fibrillated regions. Then, osteochondral plugs were harvested with a reamer that allowed the cutting and extraction of cylindrical specimens. A total of 16 plugs were harvested from different regions: the medial and lateral femoral condylar surfaces, tibial surfaces covered by menisci and tibial surfaces not covered by menisci (i.e. those that come into direct contact with the femoral condyles) (Fig. 1). Some of the plugs had fibrillated areas, especially plugs from areas on the tibial plateau that were not covered by menisci.

Apparatus

Two pieces of apparatus were used in this study. The core piece of apparatus was for the application of cyclic tensile load. The second apparatus was specifically designed for measuring the thickness of the tensile cartilage specimen.

Apparatus for cyclic tensile load application. This was a specially designed apparatus to apply a cyclic tensile load onto dumbbell-shaped cartilage specimens (Fig. 2). The load was applied using a very soft spring maintained in tension and the specimen was unloaded with a lever arm driven by a cam mounted on the shaft of an electric motor. The cam profile controlled the period of loading and recovery of the cartilage specimens. The extension of the spring (and hence the load applied) could be adjusted with a screw thread mounted on the frame of the apparatus. By the use of a spring of low stiffness, we ensured that the load that was applied remained almost constant for a large extension of the specimen. The rise time and frequency of load application were varied with the rotational speed of the motor driving the cam, which was controlled with an electric circuit. A dashpot containing
silicon oil was used to damp vibration and minimize load overshoot. In this way the system provided a near square-wave load cycle with a rise time of approximately 150 ms without any appreciable dynamic peak. A piezoelectric load cell monitored the tensile load applied and the resulting elongation of the specimen was monitored with a linear variable differential transformer (LVDT) transducer. Both transducers were subjected to static calibration while mounted in the apparatus and the accuracy of each was then determined at a level of confidence of 95%. The accuracy of the load cell was 0.025 N and that for the LVDT was 0.042 mm. During the test, data from both the load cell and LVDT were collected with Microlink 4000 (Biodata, Ltd), an apparatus for data acquisition, and a PC controlled the data collection system.

**Specimen thickness measurement.** The thickness of each specimen was measured with a purpose-built apparatus (Fig. 3). The measurement utilised the electrical conductivity of the cartilage. The cartilage specimen was placed on a metallic plate and voltage was applied across it. A blunt needle, which was attached to a micrometer, was slowly advanced towards the surface of the specimen; when it touched the latter, thus closing the circuit, a signal was obtained from an amperometer. It was thus possible to determine the specimen thickness with an estimated accuracy of 20 \( \mu \text{m} \). Accuracy was estimated by repeating the measurement, which was hindered by the exudation of a thin film of fluid as the needle touched the surface and indented it slightly.

**Methods**

**Preparation of tensile specimens and test conditions.** For each osteochondral plug, the predominant orientation of the collagen fibres in the surface layer was determined with a pinpricking technique [14]. This was done near to but outside the region that was subjected to tensile load. The cartilage was then sliced with a microtome and a number of cartilage layers 200 \( \mu \text{m} \) in thickness were obtained from each osteochondral plug (Fig. 1). Once the superficial layer had been cut, acid haematoxylin was used to mark the direction of collagen in the superficial layer on all the slices from each plug. The number of cartilage slices obtained from an osteochondral plug depended on the thickness of the cartilage layer at a particular site and ranged from three to 10. Moreover, because of the curvature (especially convexity) of the cartilage surface, the first slice was not always used. In some cases the first layer was shaved off to flatten the surface in order to obtain subsequent layers of a constant thickness with flat parallel surfaces. A dumbbell-shaped test specimen was then cut from each layer using a specially designed cutter, similar to that used by Kempson [13]. The test specimens were prepared with their long axis either parallel or perpendicular to the predominant orientation of the collagen in the surface layer, as determined previously. The specimens were approximately 0.2 mm thick, 1 mm wide and 9 mm long (Fig. 1). All the specimens were then frozen again at \(-20^\circ\text{C}\) until required for the test.

When tested, the specimen was clamped within specially designed jaws (Fig. 4). A small plastic block was fixed at each end of the specimen with a special adhesive (Indermil; Loctite, Dublin, Ireland) to prevent slippage of the specimen from between the blocks, which were then attached to the loading apparatus. During the
whole testing period, the specimen was immersed in a buffer solution at room temperature.

At the end of the test, i.e. on failure of the specimen, the thickness of the specimen was measured again. All the specimens were then frozen again at $-20^\circ\text{C}$ until required for further investigation.

**Loading of the specimen.** In order to obtain an $S$–$N$ curve (relating $S$, the stress applied on cartilage to $N$, the number of cycles at failure), it was decided to test the cartilage using loads in a range close to the physiological one. Nominal tensile stress ranging between 1.5 and 3 MPa was applied to the test specimen. Approximately half of the specimens were tested in the direction parallel to the collagen in the superficial layer and the other half in the perpendicular direction. The same direction of testing was used for all the dumbbell specimens that were prepared from the same site. The load applied was varied between the specimens to determine the relationship between the stress applied and the number of load cycles to failure. Specimens from a corresponding region on the lateral and medial sides were tested in the other direction in order to compare the fatigue behaviour of cartilage from two plugs, possibly with the same mechanical characteristics.

**Data collection from the tensile fatigue test.** The data collection system, together with software that was written specifically for this study, controlled the apparatus and collected and stored the data. The data were (i) the number of load cycles to failure, and (ii) load and extension for each specimen. The number of load cycles to failure was determined with an electronic counter. After each 15 min (900 cycles at 1 Hz), the counter sent an impulse to the Microlink. When failure occurred, an electric switch stopped the apparatus automatically and the number of load cycles was counted after the last impulse had been recorded. The load and extension of the specimen were sampled and recorded (sampling frequency 1 kHz) every 900th cycle. A stack of load and displacement data was stored continually over 64 consecutive cycles. Thus, when the 65th cycle was recorded, the first of the 64 was dropped off from the stack, and so the last 64 load cycles (i.e. including that at which specimen failure occurred) were always available for analysis. This was in addition to the load/displacement data collected at every 900th load cycle.

**Preliminary results**

To date, 72 tensile specimens from various depths at 16 sites on one knee specimen, aged 48 yr, have been tested. The data collected during the test were analysed and presented as follows.

The applied tensile stress vs the number of cycles to failure recorded for each cartilage specimen was plotted on a semi-logarithmic scale, first for all the test specimens (i.e. those from all sites and all zones) separated into two groups according to the orientation of load application with respect to that of the collagen in the superficial layer (Fig. 5). Next, the data were divided into three groups according to the origin of the specimen: the first group of data related to the specimens from the superficial layer, the second to the specimens from the middle layer, and the third to the specimens from the deep layer (Figs 6–8). In this way it was possible to compare the fatigue properties of each specimen with the different cartilage structures through the thickness of the specimen. For each graph, a linear relationship representative of the fatigue behaviour was considered.

The elongation of every specimen was plotted against the tensile load applied in order to evaluate changes in tissue structure during the test (Fig. 9). The same
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Elongation was plotted against the number of load cycles to failure (Fig. 10).

Number of load cycles to failure at different stress levels

The number of load cycles to failure varied from 20 to 1.5 × 10⁶ over a range of applied stresses between 1 and 3 MPa. The most striking finding was the extremely wide scatter of the number of cycles to failure recorded for different specimens tested at the same tensile stress.

The entire data set. For all data collected to date, regardless of the zone or the site where the specimens were harvested, a statistically significant linear correlation was found for the specimens tested in the perpendicular direction to that of the collagen in the surface layer \( r = -0.569; P = 0.004 \). For specimens tested in the parallel direction, the relationship was statistically much weaker \( (r = -0.273; P = 0.159) \). Fatigue resistance was better for specimens tested in the parallel than those tested in the perpendicular direction. This is evident by the smaller slope of the regression line for the former set of data in comparison with that for the latter. In addition, specimens tested in the parallel direction reached a higher number of cycles than those tested in the perpendicular direction under the same load condition, again indicating superior resilience against fatigue.

Zonal variation. When the data were divided into groups from different zones through the cartilage thickness, the same characteristics were found. For all tests along the parallel direction, the statistical

**Fig. 6.** Cyclic tensile stress vs number of cycles to failure for specimens from the superficial layer, from all sites. (A) Specimens tested in a direction parallel to the collagen fibres. (B) Specimens tested in a direction perpendicular to the collagen fibres.

**Fig. 7.** Cyclic tensile stress vs number of cycles to failure for specimens from the middle layer, for all sites. (A) Specimens tested in a direction parallel to the collagen fibres. (B) Specimens tested in a direction perpendicular to the collagen fibres.
The correlation between the applied stress and the number of cycles to failure was very weak ($r$ ranging from $0.37$ to $0.10$ and $P$ from $0.25$ to $0.84$). The weakest correlation was found for data from the deep layer, whether tested in the parallel or the perpendicular direction ($r = 0.075$, $P = 0.847$ and $r = 0.155$, $P = 0.77$ respectively). A significant correlation was found for specimens from the superficial layer tested in the perpendicular direction, ($r = 0.823$, $P = 0.012$).

The greatest difference in behaviour between specimens tested in the parallel or the perpendicular direction was found for specimens from the superficial layer. The slope had a value of $-0.09$ for specimens tested in the parallel direction and $-0.33$ for those tested in the perpendicular direction. This difference decreased with depth.

Adjacent specimens. Another surprising result was the large variation in fatigue properties between two adjacent specimens from the same osteochondral plug. Adjacent specimens under the same tensile stress could fail at two numbers of cycles that differed by up to two orders of magnitude. For example, a specimen from the superficial layer of the tibial plateau reached 672 987 cycles under a stress of 2 MPa, while the adjacent specimen failed at 15 359 under the same stress. In another case, two adjacent specimens from the middle layer reached 559 120 and 101 cycles at a stress of 2.5 MPa. These large differences in the number of load cycles to failure for adjacent specimens were common within each zone.

Regional variation. Because of the multitude of parameters that influenced the results (the stress applied, the orientation of the specimen with respect to the split pattern, and the particular structure of each cartilage layer) it was not possible to find any qualitative correlation between the fatigue behaviour of cartilage from the different joint regions.

Fracture stress

The fracture load of each specimen tested was determined as the intercept of the regression line with the vertical axis, i.e. the load axis. The value of the intercept represents the load needed to break the
specimen in one application. An average value of 2.55 MPa was recorded.

**Load–elongation behaviour**

The load–elongation relationship tended to be of a similar shape for all specimens, and it was possible to identify three distinct regions: a toe region, a heel region and, immediately after this, a sudden linear increase in stiffness, evidenced by a small increase in elongation for a large increase in load. Due to the repeated application of load, the load–elongation graph shifted continually to the right. Because of the increased elongation, the graphs changed shape in time. Thus, the toe region flattened further whereas the heel region increased in convexity and the third region (the linear part) became steeper. Consequently, the tensile modulus changed during the test. This trend was particularly pronounced for specimens from the middle and deep layers. Furthermore, specimens from the middle layer had the greatest elongation irrespective of whether the load was applied in the parallel or the perpendicular direction (to that of the collagen in the superficial layer), while specimens from the surface and deep layer were much stiffer.

**Reduction in thickness of the specimen**

The specimen thickness at failure was 20–70% lower than its initial thickness. Specimens from the middle layer had the greatest reduction in thickness. No association was found between thickness reduction and specimen orientation.

**Discussion**

**Directional differences in cartilage fatigue properties**

The data obtained so far show that the specimens tested in the parallel direction to the split pattern (i.e. along the direction of fibre orientation in the superficial layer) had better fatigue properties than those tested in the perpendicular direction. This finding was consistent throughout the depth of cartilage and lends support to the hypothesis that the weakest direction of articular cartilage is that perpendicular to the split pattern left by the pinpricking technique.

Examination of the semi-logarithmic plots of stress vs number of load cycles to failure (Figs 5–7) revealed a statistically significant linear relationship in only two cases. In the other four cases, the data had too much scatter. As the data showed no other trend, we decided to use the fitted regression lines that were calculated to make comparisons with existing data. This decision was based on the fact that linearity is normally observed in such fatigue data. On comparing the present data with those of Weightman et al. [1, 12], the fatigue properties of cartilage found in this study seemed to be better than those shown by Weightman’s data irrespective of whether the cartilage was tested in the parallel or the perpendicular direction. This comment is based on the smaller slope of the best line fitting the data on stress vs the number of cycles to failure (on a semi-logarithmic scale), which indicates a slower deterioration rate of the properties of the specimens. Thus, Weightman, using linear regression analysis, estimated an average value of the slope for all the femoral heads he tested and described the fatigue behaviour of cartilage with the linear regression function

\[ S = S_f - 1.65 \log_{10} N \]

where \( S \) is the operating tensile stress, \( N \) is the number of cycles to failure and \( S_f \) is the fracture stress of specimens harvested from a particular joint. In the present study, the values of the slope of the line fitting our data were \(-0.1\) and \(-0.3\) for testing in the parallel and perpendicular directions respectively, both of which are far less steep than the slope of the line in Weightman’s expression.

However, it is remarkable that in the present study it was not possible to apply to any of the test specimens loads similar to those that Weightman used in his test, even when testing in the parallel direction to the collagen fibres, i.e. the direction in which cartilage possesses its greatest strength. This is despite the fact that we used specimens of dimensions that were identical to those of the specimens used by Weightman. Moreover, the average value of fracture stress of 2.6 MPa, defined by the intercept of the regression lines with the ordinate (stress axis), is far smaller than the values calculated from the expressions of Weightman et al. [12, 15]. Using their relationship, an average value of 15.4 MPa was calculated for the 48-yr-old knee used in this study. Our predicted fracture tensile stress is thus some 17% of that predicted by Weightman for the femoral heads he tested. The fracture tensile stress estimated from his fatigue work is similar to the static tensile strength of cartilage specimens tested previously by Kempson [16]. Thus, there is a large discrepancy between our study and the three studies cited above in the properties of cartilage from donors of the same age. This needs to be clarified by further tests, which we are currently undertaking.

**Zonal variations in cartilage fatigue behaviour**

Our data indicate that the specimens from the middle layer seemed to have slightly worse fatigue resistance, breaking at a lower number of cycles than specimens from other layers. This is explicable by the much looser structure of the collagen in the middle zone. However, the scatter of the data between specimens from all zones is large and the slopes of the lines of regression do not differ significantly.

**Specimen elongation**

Specimen elongation measured at fracture ranged from 2 to 140% of the initial specimen length. The elongation was due entirely to stretching, as no slippage of the specimen out of the clamp was observed. This could be seen once the clamp had been removed; the ends of the specimen were still perfectly glued to the plastic blocks, which could only be separated from the
specimen with a scalpel. The large elongation range thus reflects the differences in cartilage structure within the different zones. In particular, the looser organization of collagen fibres and, perhaps, a weaker degree of cross-linking within this zone could explain the large elongation of specimens from the middle layer during the fatigue test. The superficial and deep layers, having more compact and more organized collagen, showed greater stiffness and much less flow. The changes in the shape of the load-elongation curve might well have been due to irreversible reorganization of the fibre bundles in addition to the straightening of macroscopic crimps in the collagen fibrils.

Fatigue life of cartilage
An attempt can be made to estimate the fatigue life of cartilage from the data obtained in this study. On the basis of the steeper of the two lines in the semi-logarithmic expressions of cartilage fatigue behaviour, we predict an extremely low number of cycles after which failure occurs: 100 000 cycles, equivalent to barely 1 month of level walking. Using the less steep slope of the second expression predicts an extremely long life: some ten thousand billion cycles! This prediction, though seemingly unrealistic, shows that the resistance of cartilage to fatigue in the direction parallel to the perpendicular direction.

Examination of the studies of Weightman et al. illustrates a particular difficulty encountered in such predictions, which is due to the sensitive nature of a semi-logarithmic relationship and its use for such predictions. Using Weightman's expressions for the fracture stress \( S_f \) and the age of the joint, \( a \), \( (S_f = 25.4 - 0.15a) \) and for the tensile stress applied, \( S \), and the number of cycles to failure, \( N \) \( (S = S_f - 1.65 \times \log_{10} N) \), a cartilage 70 yr old under a tensile stress of 3 MPa would have a predicted life of a further 16 yr, i.e. cartilage failure is predicted to occur at the age of 86 yr. If a slope of \(-1.60\) is assumed (instead of \(-1.65\), which is well within the confidence interval of 95\% for the slope, a predicted life of 27 yr is calculated, i.e. failure is predicted at the age of 97 yr. Hence, considering the error interval for each regression coefficient for a confidence level of 95\%, an error of 20–30 yr is reflected in the predicted age at which cartilage failure may occur.

Another difficulty (a perennial one) encountered in making realistic predictions of the fatigue life of cartilage from such data is ignorance of the previous loading history of a particular joint. The fatigue life of a joint can be greatly reduced if it has been subjected to extremely high stresses, such as those that may occur during trauma. Furthermore, it is not possible to estimate the effect of repair of the microdamage that occurs to cartilage as a result of prolonged cyclic loading. This possibility of repair leads to a great difference between the fatigue behaviour of an engineering component, in which the effect of fatigue is cumulative, and that of living tissue in which this effect is reduced, if not rectified, by the regenerative powers of the body.

Methodology
The methods used in this study to investigate the fatigue behaviour of cartilage, though labour-intensive, are by no means comprehensive or adequate to produce data that might better our understanding of the mechanism of cartilage fatigue. Although a large number of specimens were tested, these were from a single joint. This deficiency could be rectified in a future study by the use of multiple testing heads (which has cost implications), thus increasing the number of test specimens. It is essential to investigate simultaneously the regional variations in the compressive modulus of cartilage, in addition to the regional and zonal variations in collagen content, the degree of its cross-linking and the ultrastructure of cartilage. Results of such a study may help explain the scatter in the data we observed. In addition, cartilage specimens should be examined microscopically at different stages of stressing up to failure in order to understand the mechanism of failure.

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