

Impact testing to determine the mechanical properties of articular cartilage in isolation and on bone

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Received: 13 February 2007 / Accepted: 21 May 2007 / Published online: 10 July 2007
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Abstract The biomechanical response of cartilage to impact loads, both in isolation and in situ on its bone substrate, has been little studied despite the common occurrence of osteoarthritis subsequent to cartilage injury. An instrumented drop tower was used to apply controlled impact loads of different energies to explants of bovine articular cartilage. Results were compared with a conventional slow stress-strain test. The effects of the underlying bone were investigated by progressively shortening a core of bone removed with the cartilage, and by gluing cartilage samples to substrates of different moduli. The maximum dynamic modulus of isolated samples of bovine articular cartilage, at strain rates between 1100 and 1500 s⁻¹, was approximately two orders of magnitude larger than the quasistatic modulus and varied non-linearly with applied stress. When attached to a substrate of higher modulus, increasing the thickness of the substrate increased the effective modulus of the combination until a steady value was achieved. A lower modulus substrate reduced the effective modulus of the combination. Severe impacts resulted in damage to the bone rather than to the cartilage. The modulus of cartilage rises rapidly and non-linearly with strain rate, giving the tissue a remarkable ability to withstand impact loads. The presence of cartilage attenuated the peak force experienced by the bone and spread the impact loading period over a longer time.

Introduction

Articular cartilage provides a resilient and compliant articulating surface to the bones in diarthrodial joints. It protects the joint by distributing applied loads, so preventing potentially damaging stress concentrations, and provides a low-friction bearing surface to enable free movement of the joint. Trauma is well known for increasing the likelihood of articular cartilage degeneration and secondary osteoarthritis (OA). This may be either acute, such as might be experienced during an automobile accident, or chronic, due to occupational or sporting activities [1]. Elevated levels of proteoglycans, stromelysin-1 (MMP-3) and collagen fragments have been found in the synovial fluid at periods of up to 15 years following injury [2–4], though it is not clear if these are a consequence of primary damage to the cartilage that has not repaired or subsequent damage due to overloading because of instability caused by injury to other tissues. After injury or disease, functional tissue engineering faces the challenge of trying to repair or replace damaged tissues that serve a predominantly mechanical function. In such applications it has been noted that is essential to understand the normal and failure properties of the native tissues under all conditions likely to be experienced in vivo [5]; this includes high rates of loading.

Articular cartilage may be considered as a visco- or poro-elastic fibre-composite material and, as such, its mechanical properties depend strongly on the rate of loading. Most characterisation has been done using conventional materials testing machines and the modulus at low strain rates, and the equilibrium modulus, are well known [6–11]. Much less is known of its properties at high rates of loading. When walking, the ground reaction force typically rises to a peak during the first 100 ms after heel strike [12, 13] and during this phase loads across the knee

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joint have been calculated to be about three times body weight. This results in a loading rate of the order of 20 kN s^{-1} and strain rates of the order of 5 s^{-1} . To simulate trauma, it has been proposed that strain rates of $1,000 \text{ s}^{-1}$ (a deformation of 1,000 thicknesses per second) were needed to realistically model what happens to articular cartilage during an automobile accident [14]. Studies using drop towers to apply impacts to tissues have recorded strain rates in excess of 1000 s^{-1} [14–16] and loading rates of the order of $100\text{--}1,000 \text{ kN s}^{-1}$. A number of models have been developed to investigate the behaviour of articular cartilage at high strain rates, approximating to impact loads, both *in vivo* [17–21] and *in vitro* [22–27]. Only some of these, however, apply a true impact load, i.e. use a fast enough loading rate not just a large load [28].

Most commonly, tissues and materials are tested in isolation in order to understand their intrinsic properties. Only rarely, though, do they exist like this in real situations. Articular cartilage is firmly anchored to the underlying subchondral bone that not only provides a rigid support but also prevents lateral displacements at the base of the tissue. Interactions between the two tissues are important mechanically and biologically [29]. A reduction of the modulus of the bone is believed to spare the overlying cartilage from injury [30], while subchondral stiffening has been proposed as a cause for cartilage damage and OA, especially following impact loading [31]. In support of this, cartilage from patients with osteoporosis is often found to be in good condition with few signs of osteoarthritic changes and an inverse relationship between OA and OP has been reported [32, 33].

The aim of this study was to use impact loads of various energies and incident velocities to measure the mechanical properties of articular cartilage, and to determine how these are affected by the thickness and compliance of the underlying bone. Isolated bovine cartilage biopsies were subjected first to standard compression testing before being tested using a drop-tower. To investigate the interaction between cartilage and bone, other samples were tested *in situ* on bone and the depth of the bone was progressively reduced. In a different approach, cartilage samples were glued to substrates composed of cylinders of materials of different compliances and thicknesses. The cartilage was typically about 0.5 mm thick and cylindrical bone cores removed were about 10–12 mm long.

Materials and methods

Tissue preparation

Bovine forelimbs from animals under 24 months of age, cut above the carpal joint, were obtained from the abattoir

within 2 h of slaughter. Up to 17 full-depth articular cartilage samples were removed from each medial, plateau-like, metacarpal joint surface using a cork borer as described previously [34]. In this study, 108 samples of 5 mm diameter were used, and a further 10 of 9 mm diameter for comparison with cartilage-on-bone samples. After removal, each sample was placed immediately in an individual Eppendorf tube containing phosphate buffered saline (PBS) and allowed to equilibrate for at least 24 h as we have found this to be sufficient to achieve a stable mass [35]. The position of each sample on the joint surface was recorded [34]. Each sample was maintained immersed in PBS except during some of the measurement procedures. During these times care was taken to keep the sample moistened with PBS and the duration of any exposure to air was kept to a minimum. All samples were stored at -20°C until required and thawed to room temperature before any measurements were made.

Removing cores of bone with intact cartilage proved difficult due to the hardness of the bone. The method finally adopted was first to freeze the joint at -80°C . Cores were then drilled, to a depth of about 10 mm, using a specially designed 9 mm diameter coring bit (Bolton Surgical, Bolton) attached to a pillar drill, with constant irrigation with iced water and with the joints still frozen. The bone cores were then snapped at the base by applying pressure to the sides with a specially modified trephine [36] (design courtesy of Dr M. Hurtig). After removal, the base of each sample was trimmed parallel with the articular surface, using a mineralogical saw fitted with an aluminium oxide cut-off wheel (Accutom-2, Stuers, Solihull, UK), and placed in individual vials of PBS. Only two samples could be removed from the medial joint surface and a total of eight samples were tested. The position on the joint from where each sample was removed was noted. Bone cores with the cartilage removed were produced from similar samples.

The total thickness of each cartilage or cartilage-on-bone sample was measured optically using a Zeiss Stemi-2000 stereo microscope and AxioVision software (Zeiss Ltd, Welwyn Garden City). This system was calibrated using a graticule slide, marked with 0.01 mm divisions, for each magnification lens of the microscope. Any samples that were clearly wedge-shaped or had a non-uniform thickness were also identified and removed at this stage.

The wet weight of the isolated cartilage samples was measured three times to determine whether there was any swelling of the tissue post-impact; just prior to testing, immediately after the impact test and again 24 h later. Weighing was done, using a five-figure balance; each sample was lightly blotted between several layers of tissue paper soaked in PBS, to remove excess fluid from the

surface, before weighing it in a pre-weighed Eppendorf tube containing ~1 ml of PBS.

Slow compression testing

An Instron materials testing machine (model 5564, Instron Ltd, High Wycombe) fitted with steel anvils was used for unconfined compression tests on cartilage samples and bone plugs. A 10 N load cell was used for the isolated cartilage samples. The rate of compression was 100% strain min^{-1} (0.0167 s^{-1}). To ensure no damage was caused to the tissue samples prior to impact loading, the peak stress was limited to 0.15 MPa. A 3 mm thick steel plate was placed on top of the cartilage and loaded applied via a ball bearing to ensure uniform loading of the sample. After testing, samples were allowed to re-equilibrate in PBS for 30 min before impact testing. A 2 kN load cell was used for testing the bovine bone plugs. The rate of compression was chosen to be 20% strain min^{-1} (0.0033 s^{-1}) so that the cross-head speed was similar to that used for isolated cartilage samples. Testing was done to failure or the capacity of the load cell. The stress-strain relationship was non-linear and the modulus as a function of strain was found by differentiating the curve (Origin Software, Version 6.1; Aston Scientific Ltd, Stoke Mandeville). For the cartilage samples the modulus, E_{stat} , was calculated at a stress of 0.1 MPa. For the bone, E_{stat} was found at a stress of 10 MPa to enable comparisons to be made with impact test results.

Impact testing

The drop tower and instrumentation used for impact testing have been described in detail elsewhere and the data validated [37]. Briefly, a sample of known thickness was placed on a stainless steel plate on top of a piezoelectric force transducer. An impactor, fitted with an accelerometer, was released mechanically from a known height, taking account of the specimen thickness, and fell freely onto the sample. Two impactors, one weighing 100 g and the other 500 g were used in this study. An 8702B500 accelerometer (Kistler Instruments Ltd., Alton), capable of measuring accelerations up to 500 g, where g is the acceleration due to gravity, was used for testing isolated cartilage and an 8704B5000 accelerometer, with a higher acceleration limit of 5,000 g was found necessary for testing harder samples, such as bone and cartilage-on-bone samples. Data from both accelerometers and the force transducer were recorded at 50 kHz. The drop heights and impactor masses for the various tests are shown in Table 1.

Impact testing of cartilage-on-bone was performed by dropping a 100 g mass from a height of 50 mm onto each

Table 1 Drop heights and impactor masses used for impact testing of various samples

Tissue Sample	Sample diameter (mm)	Impactor mass (g)	Drop height (mm)
Cartilage	5	100	100
			80
			50
			25
			25
Cartilage on bone	9	100	50
			50
			25
			25
			25
Cartilage on substrate	9	100	50

9 mm diameter sample [37]. It was found that, in general, the test could be performed 3 times, with the length of the bone core bone being shortened by about 2 mm each time. The effective strain of the combination was found by dividing the measured displacement by the total sample length. Each core was of different starting length, to cover a range of lengths, and a slice of cancellous bone was removed first and used for slow and impact testing to determine the quasi-static and the dynamic moduli of the bone alone. Cartilage-on-bone and cartilage-on-substrate (see below) samples were held in place with a small drop of cyanoacrylate glue on the base to prevent them flying out during testing. Similar tests were also performed on the ten 9 mm diameter isolated cartilage samples.

To investigate the effects of the modulus of the underlying material on the impact response, full-depth cartilage samples, 9 mm in diameter, were glued to cylinders of various materials and different lengths using cyanoacrylate adhesive. This adhesive is easily peeled from the cartilage explants after removal from the substrate. The materials used were brass (modulus, $E = 100 \text{ GPa}$), Nylon ($E = 3.1 \text{ GPa}$) and cork ($E = 0.0035 \text{ GPa}$), chosen to cover a wide range of moduli. The cylinders were 9 mm in diameter and 3, 5, 7 and 10 mm in length and the effective strain in the combination was found as above. The same cartilage sample was tested at all four lengths on a given material using a 100 g mass dropped from a height of 50 mm.

Force data were converted to engineering stress by dividing by the original cross-sectional area of the sample. Accelerometer data were integrated twice, to find the displacement, and divided by the original thickness of the sample, as described above, to determine the engineering strain [37]. The stress-strain relationship was non-linear and the curve for each sample was differentiated to obtain the dynamic modulus. The maximum dynamic modulus, E_{max} ,

was found, and the modulus at stresses of 10 MPa and 5 MPa determined from the gradient at those points on the stress-strain curve to enable comparison of dynamic moduli at constant values of stress [35]. The mean stress rate and strain rate were calculated from the maximum stress or strain divided by the duration of the impact to that point.

The energy of the applied impact was calculated from the mass and drop height [35]. A force-displacement curve was produced for loading and unloading phases. The energy of deformation, W_c , was found from the area under the loading curve by integrating to the maximum strain (Origin Software, Version 6.1; Aston Scientific Ltd, Stoke Mandeville), and the energy released during restitution, W_r , by integrating the unloading curve from this point back to zero strain. The square of the energetic coefficient of restitution is then given by $e_*^2 = -W_r/W_c$ [38]. A perfectly elastic collision has $e_* = 1$ and a plastic collision in which all the kinetic energy is dissipated has $e_* = 0$.

Statistical tests

Data were tested for normality using the Kolmogorov-Smirnov test with the significance level set to $P = 0.05$. Normally distributed data are presented as mean \pm standard deviation, otherwise values are shown as median [25, 75%] confidence limits. Analysis of variance (ANOVA) was used to investigate site variation of properties. Association between parameters was calculated using Spearman's rank correlation. Best subsets regression was used to measure the contribution of various parameters to predicting a dependent variable, e.g. the maximum dynamic modulus. Linear regression was used to assess the linear dependency between the variables with the strongest association.

Results

Thickness and modulus of isolated cartilage

A typical force-displacement curve for an impact load on an isolated sample of bovine articular cartilage is shown

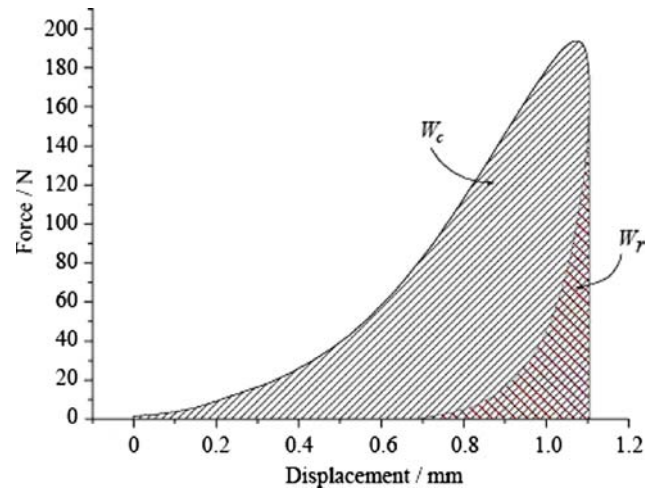


Fig. 1 Typical force-displacement curve for an impact test on articular cartilage. The areas corresponding to the work done in compression, W_c , and in restitution, W_r , are shown hatched

in Fig. 1. The maximum strain does not coincide with the maximum stress and the hatching shows the areas under the curve found by integration to calculate the coefficient of restitution. There were no significant (ANOVA, $P > 0.05$) topographical variations in cartilage thickness (0.56 ± 0.11 mm) or quasistatic modulus ($E_{stat} = 0.95 \pm 0.20$ MPa) over the medial metacarpal joint surface. The values for E_{max} , σ_{max} and e_{max} (Table 2) increased with drop height for both the 100 g and 500 g masses. However, the dynamic modulus calculated at a stress of 10 MPa, E_{10} , did not vary with location or drop height for the 100 g mass (73 ± 14 MPa), but showed a slight increase with drop height for the 500 g mass (Table 2). These more heavily impacted samples, however, were undoubtedly damaged; showing extensive fissuring and permanent elliptical deformation immediately after impact. For the 100 g impactor, the time from impactor contact to peak force was consistently about 0.45 ms for all drop heights. The duration of the loading with the 500 g impactor was longer: time to peak force for the 25 mm drop was about 0.7 ms and for the 50 mm drop was about 0.5 ms. Mean stress rates and strain rates for

Table 2 Mean values for maximum dynamic modulus, E_{max} , dynamic modulus at 10 MPa, E_{10} , maximum stress, σ_{max} , and maximum strain, e_{max} , calculated for each data set containing N samples for a given drop height and impactor mass. The mean stress rate, σ' , and strain rate, e' , are also given

Impactor	Drop height (mm)	N	E_{max} (Mpa)	E_{10} (MPa)	σ_{max} (MPa)	e_{max}	σ' (GPa s ⁻¹)	e' (s ⁻¹)
100 g	25	21	86 \pm 22	75 \pm 13	15.6 \pm 2.9	0.484 \pm 0.063	34.7	1100
100 g	50	23	100 \pm 32	71 \pm 16	24.5 \pm 3.5	0.60 \pm 0.13	54.5	1350
100 g	80	20	118 \pm 33	73 \pm 12	34.2 \pm 5.0	0.62 \pm 0.11	76.0	1370
100 g	100	19	128 \pm 28	72 \pm 13	40.5 \pm 4.6	0.677 \pm 0.085	90.0	1500
500 g	25	14	195 \pm 47	86 \pm 12	51.8 \pm 5.0	0.59 \pm 0.10	73.9	740
500 g	50	11	237 \pm 51	95 \pm 16	76.4 \pm 9.7	0.653 \pm 0.048	153.0	1090

Table 3 Mean values for energy of deformation per unit volume, W_c , the energetic coefficient of restitution, e_* and the median [25, 75%] values of percentage increase in mass $\Delta m\%$ of each sample after 24 h immersed in PBS following impact loading of isolated cartilage explants

Impactor mass (g)	Drop Height (mm)	W_c (mJ mm ⁻³)	e_*	$\Delta m\%$
100	25	0.67 ± 0.14	0.636 ± 0.080	2.24 [0.98, 3.44]
100	50	1.23 ± 0.26	0.46 ± 0.14	2.50 [1.61, 5.06]
100	80	2.00 ± 0.46	0.468 ± 0.050	5.70 [4.25, 7.40]
100	100	2.39 ± 0.37	0.405 ± 0.081	9.94 [5.92, 11.3]
500	25	10.1 ± 2.2	0.411 ± 0.086	22.9 [19.1, 29.3]
500	50	20.0 ± 2.3	0.265 ± 0.075	39.4 [33.9, 48.9]

each data set are shown in Table 2. Neither the maximum dynamic modulus nor the peak stress was affected by the cartilage thickness for tests with the 100 g impactor.

The mean values for the energy of deformation and the coefficient of restitution are shown in Table 3. For the 100 g impacts the energy of deformation corresponded to 94.1 ± 1.2%, and for the 500 g impacts 95.3 ± 2.6% of the theoretical impact energy calculated assuming zero friction. There was no correlation between energy of deformation per unit volume and cartilage thickness. Any mass lost during impact was not detectable with the balance available (resolution 0.01 mg). The increase in mass of the samples over the 24 h immersed in PBS immediately following the impact is also shown in Table 3. This was small for the lowest energy impacts, increased sharply following a further small increase in energy for the 100 g impactor, then continued to increase more gradually with energy for the 500 g impactor.

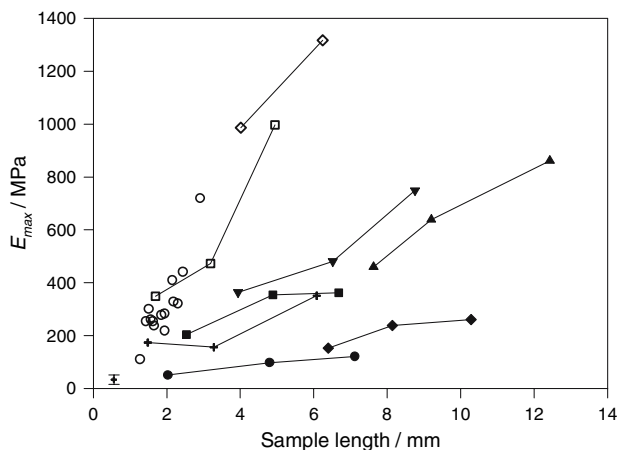


Fig. 2 The maximum dynamic modulus, E_{max} , for isolated bone cores (open symbols) and for cartilage-on-bone samples (closed symbols) decreases with decreasing bone length. Repeated tests on samples are shown by connecting lines, individual bone samples by open circles. The mean value and 95% confidence limits for isolated 9 mm diameter cartilage samples from separate tests are shown by the error bars to bottom left

Cartilage-on-substrate samples

The maximum dynamic modulus of isolated cancellous bone slices was $E_{max} = 302 \pm 66$ MPa, mean strain rate 390 ± 125 s⁻¹. The dynamic moduli at 10 MPa ($E_{10} = 295 \pm 60$ MPa) and at 5 MPa ($E_5 = 233 \pm 44$ MPa) were higher than the quasistatic modulus measured at 10 MPa ($E_{stat} = 214 \pm 51$ MPa, strain rate 0.003 s⁻¹). The dynamic moduli decreased with decreasing sample length (Fig. 2). Similarly, impact testing of bone with the cartilage in situ showed that the effective modulus of the combination, for simplicity also denoted by E_{max} in Fig. 2, also reduced as the length of the samples decreased in six out of the eight samples tested. The remaining two appeared to suffer earlier damage and showed no consistent trend. The maximum dynamic modulus for the 10 isolated 9 mm diameter cartilage cores was 32.8 ± 9.1 MPa, lower than that for the 5 mm diameter cores. For the same thickness of bone, the presence of cartilage reduced the value of the effective modulus of the combination. The energy of deformation of each cartilage-on-bone sample, and that for the isolated bone cores, was calculated from the area under the force-deflection curve. The coefficients of restitution did not differ between cores of bone with or without cartilage present. For bone alone, $e_* = 0.620 \pm 0.074$ ($N = 12$), while for cartilage-on-bone $e_* = 0.616 \pm 0.077$ ($N = 24$). Impact tests with the 500 g impactor from a drop height of 50 mm caused consistent failure of cartilage-on-bone cores. Samples either split vertically in two upon impact or damage was indicated by a sudden, marked reduction in effective modulus (Fig. 3). Damage was more obvious in the bone than the cartilage.

Attaching cartilage samples to a substrate with a greater modulus (brass, nylon) increased the effective modulus of the combination, E_{max} , whereas a lower substrate modulus (cork) resulted in a lower effective modulus of the combination. All these, however, were dependent on the length of the substrate (Fig. 4). For all materials a plateau appeared to be reached when the substrate thickness was about 10 times the thickness of the cartilage, after which changes were small.

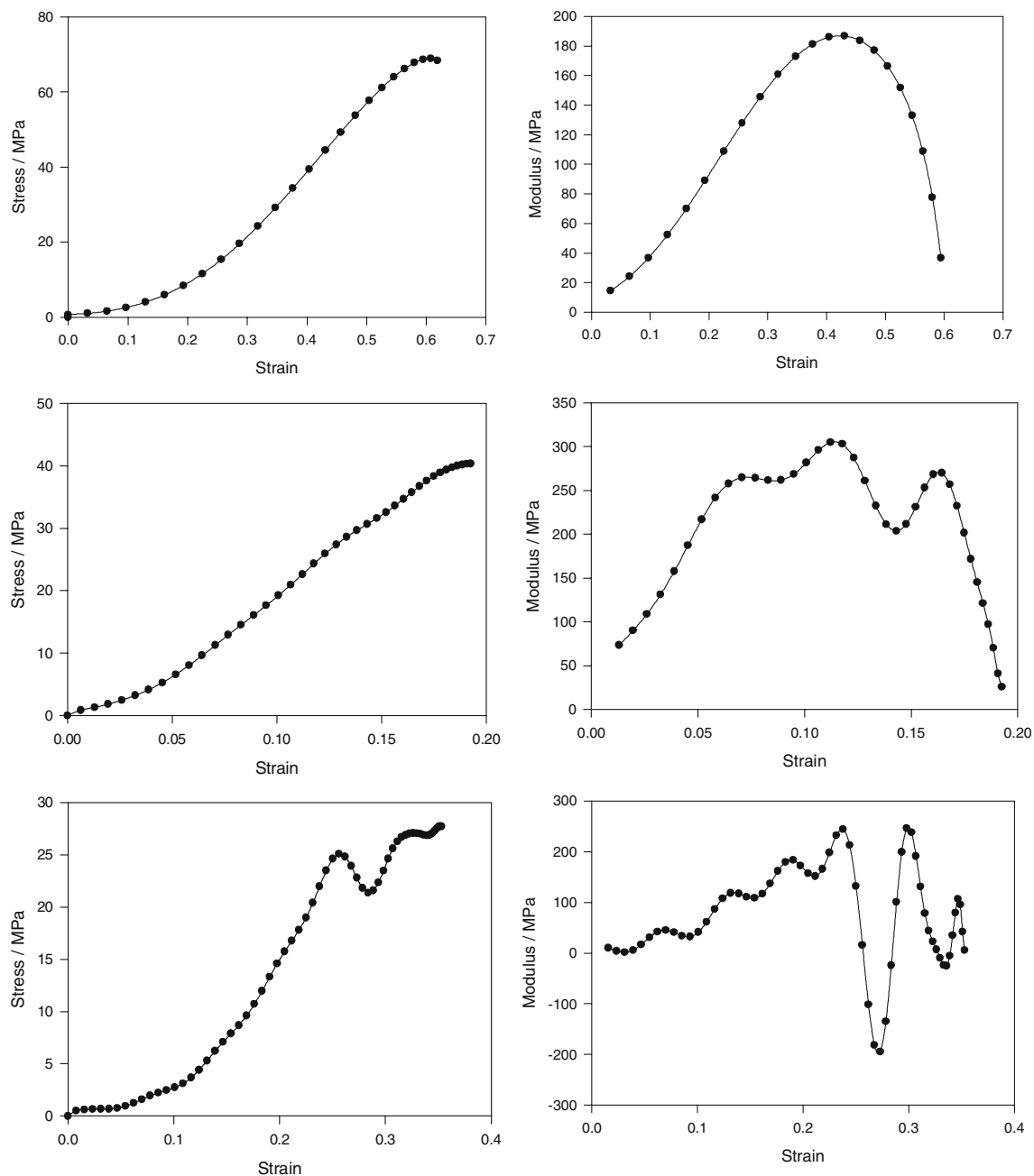


Fig. 3 Stress-strain curve to peak stress and the effective modulus, calculated by differentiating the stress-strain curve, for various 500 g, 50 mm impact tests of cartilage-on-bone samples to illustrate the types of damage observed. Top: Sample suffering no apparent

damage. Middle: In others, mild to moderate damage is shown by the sudden reduction in modulus. Bottom: Occasionally the sample split in two, with marked consequences for the measured curves

Discussion

The maximum dynamic modulus of isolated samples of bovine articular cartilage, at mean strain rates of between 1,100 and 1500 s^{-1} , varied non-linearly with applied stress and was approximately two orders of magnitude larger than the modulus values calculated from quasistatic compression tests. Increasing the drop height, and hence the energy

and speed of impact, for the 100 g mass impacts led to an increase in the maximum dynamic modulus. However, the dynamic modulus calculated at a constant stress of 10 MPa remained approximately constant despite an increasing strain rate. These data suggest that, at these large strain rates, the modulus of cartilage is determined by the stress and is insensitive to the strain rate. Similar conclusions were drawn by Park et al. from experiments using cyclical

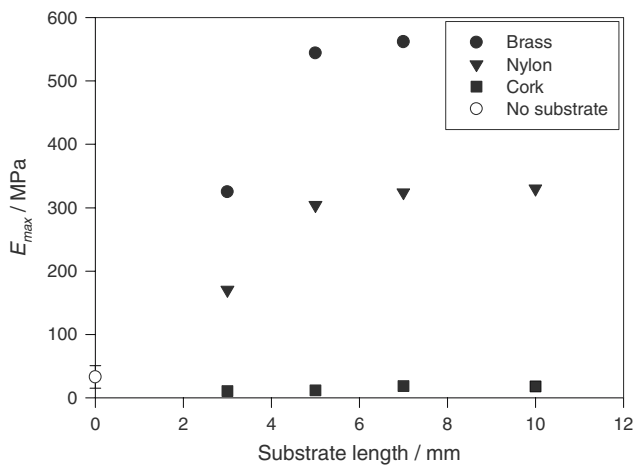


Fig. 4 The effective dynamic modulus, E_{max} , of the combination of cartilage attached to 9 mm diameter cylinders of different materials of various lengths. The mean value and 95% confidence limits for isolated 9 mm diameter cartilage samples from separate tests are shown at substrate length zero

loading up to 40 Hz [39] and by Oloyede et al. [40], who found the stiffness of cartilage to vary almost linearly with strain rate from 10^{-5} s^{-1} to 10^{-2} s^{-1} , but to change little from 10^{-2} s^{-1} to 10^3 s^{-1} , albeit at low stresses (0.5 MPa). This was attributed to there being two distinct regimes of mechanical behaviour for the cartilage governed by the rate of loading. At low rates the behaviour was dominated by fluid exudation, whereas at high loading rates the matrix behaved as an elastic solid and there was no contribution from fluid flow. Thus, the modulus tended towards a constant value as impact conditions were approached. The strain-rate independent modulus and the lack of fluid loss in the experiments reported here are consistent with that hypothesis. The coefficient of restitution, however, is considerably less than unity and does not indicate an elastic rebound. This could be because much higher stresses were produced by these impacts and tissue damage, even if not superficially apparent, could be occurring. For example, the formation of internal cracks would lead to energy dissipation and plastic behaviour. In contrast, the modulus of cancellous bone shows a strain-rate dependency.

Using the heavier, 500 g, mass the increase in impact energy and stress resulted in higher values for the dynamic modulus. However, these samples were badly damaged, shown by extensive fissuring and by a substantial increase in mass following replacement in buffer solution, due to swelling of the cartilage matrix. The damaged samples assumed an elliptical shape, because the cartilage has a greater tensile stiffness in the direction of the preferred alignment of collagen fibrils in the superficial layer and the tissue expands less in this direction [41]. Collagen orientation was not measured, but the fissures were also aligned

along the short axis of the ellipse and presumably reflect this collagen orientation. The time to reach peak stress and strain for these samples was longer than the 100 g impacts because of the greater inertia of the impactor. The maximum dynamic modulus was found to be less by approximately 100 MPa than that measured from equivalent impacts performed by Jeffrey et al. [35], who reported values at a stress of 50 MPa. Judging by the differential of the stress-strain curve, however, many of the samples in the current study were already damaged by this stage. The reasons for this may be that the earlier model of the drop tower did not include a force transducer, so forces were calculated assuming a frictionless drop, and the data acquisition rate was slower, possibly leading to inaccuracies in strain calculation. Together these could overestimate the stress and underestimate the strain resulting in an overestimate of the modulus.

The energy absorbed by the samples did not vary significantly with the small variation in thickness. With an increase in impact energy, the decreasing coefficient of restitution indicated that the impacts became more plastic in nature. This could be indicative of increasing amounts of damage in the samples as both the formation of cracks and permanent deformation might be expected to absorb greater amounts of energy. The extreme case was the 500 g impact from a height of 50 mm. This produced extensive damage and, compared with the 100 g impacts, approximately 5 times more energy per unit volume was absorbed by the samples.

There were no differences in cartilage thickness, quasistatic modulus or dynamic modulus values for samples removed from different sites over the bovine medial metacarpal joint surface. Similar results have been reported previously for thickness and quasistatic modulus, despite significant variations in collagen, glycosaminoglycan and water contents [34]. This study extends the range of strain rates and, though composition was not measured in this case, supports the hypothesis that interactions between matrix components are at least as important as their concentrations for determining the mechanical properties of the tissue, as might be expected for fibre-composite materials [34, 42].

All the cartilage samples were subjected to slow compression tests followed by an impact load after sample recovery. In this study, the sample thickness had no effect on the maximum dynamic modulus values for the bovine cartilage but did affect the quasistatic modulus. Unconfined compression testing is, in theory, a measure of the intrinsic properties of the material under test and should not be affected by sample dimensions. However, this assumes no friction between the sample and the platens used to compress it. Friction will restrict the lateral expansion of the sample, which in turn has the effect of making the sample

appear stiffer than it should since a higher compressive force is needed to deform a specimen by a given amount [43]. In addition the aspect ratio (height/ diameter) of the samples used for this study is of the order of 0.1. Ideally, this ratio should be about one, but this is not a practical possibility for specimens of cartilage. A small aspect ratio would make the specimen-platen considerations even more significant, and an indication of this problem may be indicated by the lower dynamic modulus found for the 9 mm diameter cores compared with the 5 mm cores. These issues, however, do not seriously impair the comparative nature of the tests in this study as all samples were subjected to the same testing regime. Despite these limitations, it is clear that the quasistatic modulus of the cartilage samples is considerably smaller than the modulus measured at higher strain rates and during an impact. This should be taken into consideration when building finite element models to simulate walking or injury and provides a challenging goal for tissue-engineered constructs to emulate these properties.

The behaviour of cartilage *in vivo* cannot be considered in isolation from the bone to which it is firmly attached. Mechanical testing of the combination, however, then becomes difficult. Ideally, we should like to know the strain in both the cartilage and the substrate and pilot studies using a high-speed camera indicate that, in future, it may be possible to measure these. In this study, measuring strain in the cartilage itself while on bone or a substrate was not attempted and hence the stiffness values can only be for the combination. In addition, because the properties of bone vary between individuals, and even between sites in one individual, we tried to do repeated tests on each core, varying the length between tests. It is possible that damage would accumulate before it became obvious but the trends shown were consistent in most samples, the aberrant ones we discarded on the basis that damage had likely occurred. During impact, though, the modulus of cartilage is of the same order of magnitude as that of the bone, so the mismatch between bone and cartilage is not as great as might be believed from quasi-static testing when there is a difference of two orders of magnitude. Not surprisingly, increasing the modulus of the substrate increases the measured dynamic stiffness of the combination. Similarly, as the length of the substrate increased the modulus of the combination increased but appears to reach a steady value which is lower than the modulus of the isolated material. Finite element modelling, albeit of quasi-static loading, has indicated that increasing the modulus of the substrate has little effect on stresses in the cartilage and that the modulus and thickness of the cartilage were the dominant factors determining the stresses in the subchondral bone [29]. The damage to the bone found for the most severe impacts is evidence in favour of this analysis. What the cartilage does

do is prevent very high local stresses occurring. Although it is thin and stiff it provides a cushioning surface over the bone, as shown by the attenuating and prolonging effect of the cartilage on the measured forces. It also appears to have better crack-stopping properties and is less prone to obvious damage.

Articular cartilage appears to behave as a quasi-elastic material at high strain rates providing the stress is not too high; i.e. it has a constant modulus at a given stress independent of strain rate. Its modulus, however, is non-linearly dependent on the applied stress and is approximately two orders of magnitude greater than traditionally reported, not much smaller than the modulus of the underlying cancellous bone. *In situ* on bone, the modulus of the combination reaches a constant value when the bone thickness exceeds about 10 times the cartilage thickness. Under these conditions, severe impacts produce damage in the bone rather than the cartilage.

Acknowledgments We thank the Arthritis Research Campaign for funding these studies (grant A0542) and the staff at Macintosh Donald, Portlethen, for kindly providing bovine forelimbs.

References

1. H. R. SCHUMACHER, JR. in "Osteoarthritis: diagnosis and surgical management", Edited by: R. W. Moskowitz, D. S. Howell, V. M. Goldberg and H. J. Mankin (2nd edn. W.B. Saunders Company, Philadelphia, 1993), p. 367
2. L. DAHLBERG, T. FRIDÉN, H. ROOS, M. W. LARK and L. S. LOHMANDER, *Brit. J. Rheumatol.* **33** (1994) 1107
3. L. S. LOHMANDER, L. A. HOERRNER, L. DAHLBERG, H. ROOS, S. BJÖRNSSON and M. W. LARK, *J. Rheumatol.* **20** (1993) 1362
4. L. S. LOHMANDER, Y. YOSHIHARA, H. ROOS, T. KOBAYASHI, H. YAMADA and M. SHINMEI, *J. Rheumatol.* **23** (1996) 1765
5. D. L. BUTLER, S. A. GOLDSTEIN and F. GUILAK, *J. Biomech. Eng. Trans. Asme* **122** (2000) 570
6. G. A. ATESHIAN, W. H. WARDEN, J. J. KIM, R. P. GREL-SAMER and V. C. MOW, *J. Biomech.* **30** (1997) 1157
7. M. FORTIN, J. SOULHAT, A. SHIRAZI-ADL, E. B. HUNZIKER and M. D. BUSCHMANN, *J. Biomech. Eng.* **122** (2000) 189
8. J. S. JURVELIN, M. D. BUSCHMANN and E. B. HUNZIKER, *Proc. Inst. Mech. Eng [H]* **217** (2003) 215
9. M. K. BARKER and B. B. SEEDHOM, *Rheumatology (Oxford)* **40** (2001) 274
10. K. A. ATHANASIOU, A. AGARWAL and F. J. DZIDA, *J Orthop. Res* **12** (1994) 340
11. G. E. KEMPSON, M. A. FREEMAN and S. A. SWANSON, *J Biomech.* **4** (1971) 239
12. M. K. BARKER. "Aspects of articular cartilage response to physiological cyclic loading with special reference to cumulative deformation" (PhD Thesis, University of Leeds, 1997)
13. G. BERGMANN, G. DEURETZBACHER, M. HELLER, F. GRAICHEN, A. ROHLMANN, J. STRAUSS and G. N. DUDA, *J. Biomech.* **34** (2001) 859

14. R. U. REPO and J. B. FINLAY, *J. Bone Joint Surg.* **59-A** (1977) 1068
15. L. V. BURGİN and R. M. ASPDEN, *J. Biomech.* **34** (2001) S39
16. B. M. NİGG and W. HERZOG, in “*Biomechanics of the Musculo-Skeletal System*” (2 edn. John Wiley & Sons Ltd., Chichester, England, 1998)
17. E. L. RADIN, H. G. PARKER, J. W. PUGH, R. S. STEINBERG, I. L. PAUL and R. M. ROSE, *J. Biomech.* **6** (1973) 51
18. R. C. THOMPSON, T. R. OEGEMA, J. L. LEWIS and L. WALLACE, *J. Bone Joint Surg.* **73A** (1991) 990
19. M. T. SERINK, A. NACHEMSON and G. HANSSON, *Acta Orthop. Scand.* **48** (1977) 250
20. J. M. DONOHUE, D. BUSS, T. R. OEGEMA and R. C. THOMPSON, *J. Bone Joint Surg.* **65-A** (1983) 948
21. R. C. HAUT, T. M. IDE and C. E. DE CAMP, *J. Biomech. Eng.* **117** (1995) 402
22. O. D. CHRISMAN, I. M. LADENBAUER-BELLIS, M. PANJABI and S. GOELTZ, *Clin. Orthop.* (1981) 275
23. J. B. FINLAY and R. U. REPO, *IEEE Trans. Biomed. Eng.* **25** (1978) 34
24. J. E. JEFFREY, L. A. THOMPSON and R. M. ASPDEN, *Biochim. Biophys. Acta* **1334** (1997) 223
25. G. N. DUDA, M. EILERS, L. LOH, J. E. HOFFMAN, M. KAAB and K. SCHASER, *Clin. Orthop.* (2001) 302
26. C. T. CHEN, N. BURTON-WURSTER, G. LUST, R. A. BANK and J. M. TEKOPPELE, *J. Orthop. Res.* **17** (1999) 870
27. T. FARQUHAR, Y. XIA, K. MANN, J. BERTRAM, N. BURTON-WURSTER, L. W. JELINSKI and G. LUST, *J. Orthop. Res.* **14** (1996) 417
28. R. M. ASPDEN, J. E. JEFFREY and L. V. BURGİN, *Osteoarthritis Cart.* **10** (2002) 588
29. F. H. DAR and R. M. ASPDEN, *Proc. Instn. Mech. Engrs. [H], J. Eng. Med.* **217** (2003) 341
30. E. L. RADIN and I. L. PAUL, *Clin. Orthop.* **78** (1971) 342
31. E. L. RADIN and R. M. ROSE, *Clin. Orthop.* **213** (1986) 34
32. C. COOPER, P. L. COOK, C. OSMOND and M. I. D. CAWLEY, *Ann. Rheum. Dis.* **50** (1991) 540
33. J. DEQUEKER, J. AERSSENS and F. P. LUYTEN, *Aging Clin. Exp. Res.* **15** (2003) 426
34. R. J. LEWIS, A. K. MACFARLAND, S. ANANDAVIJAYAN and R. M. ASPDEN, *Osteoarthritis Cart.* **6** (1998) 383
35. J. E. JEFFREY, D. W. GREGORY and R. M. ASPDEN, *Arch. Biochem. Biophys.* **322** (1995) 87
36. R. M. ASPDEN, in “*Bone Research Protocols*”, Edited by: M. H. Helfrich and S. H. Ralston (Human Press Inc, Totowa, New Jersey, 2003), p. 369
37. L. V. BURGİN and R. M. ASPDEN, *Med Eng Phys.* **29** (2007) 525
38. W. J. STRONGE, in “*Impact Mechanics*” (Cambridge University Press, Cambridge, 2000)
39. S. PARK, C. T. HUNG and G. A. ATESHIAN, *Osteoarthritis Cart.* **12** (2004) 65
40. A. OLOYEDE, R. FLACHSMANN and N. D. BROOM, *Connect. Tissue Res.* **27** (1992) 211
41. R. M. ASPDEN and D. W. L. HUKINS, *Proc. R Soc. Lond.* **B212** (1981) 299
42. R. M. ASPDEN, *Proc. R. Soc. Lond.* **B-258** (1994) 195
43. A. ODGAARD and F. LINDE, *J. Biomech.* **24** (1991) 691