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# The mechanical and material properties of elderly human articular cartilage subject to impact and slow loading

L.V. Burgin [1], L. Edelsten, R.M. Aspden *

*Musculoskeletal Research Programme, School of Medicine & Dentistry, University of Aberdeen, Aberdeen, UK*

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ABSTRACT

The mechanical properties of articular cartilage vary enormously with loading rate, and how these properties derive from the composition and structure of the tissue is still unclear. This study investigates the mechanical properties of human articular cartilage at rapid rates of loading, compares these with measurements at slow rates of loading and explores how they relate to the gross composition of the tissue. Full-depth femoral head cartilage biopsies were subjected to a slow, unconfined compression test followed by an impact at an energy of 78.5 mJ and velocity 1.25 m $s^{-1}$. The modulus was calculated from the slope of the loading curve and the coefficient of restitution from the areas under the loading and unloading curves.

Tissue composition was measured as water, collagen and glycosaminoglycan contents. The maximum dynamic modulus ranged from 25 to 150 MPa. These values compared with 1–3 MPa measured during quasi-static loading. The coefficient of restitution was 0.502 (0.066) (mean (standard deviation)) and showed no site variation. Water loss was not detectable. Composition was not strongly associated with modulus; water and collagen contents together predicted about 25% of the variance in modulus.



## 1. Introduction

Articular cartilage provides a resilient, low-friction bearing surface to bones where they articulate in diarthrodial joints. It is a remarkably smooth and tough material and protects the joint by distributing applied loads. Mechanical damage caused, for instance, by trauma may lead to osteoarthritis (OA) and studies *in vivo* have suggested that cell damage, oxidative stress and apoptosis may play a significant role in this process [1,2]. The chondrocytes are protected by being embedded in an extensive matrix that has a complicated structure. They can regulate their biosynthesis of matrix molecules, and thereby control the composition of the matrix, in order to match the mechanical properties of the tissue to the prevailing loading environment, but their regenerative capacity is limited. Relationships between the composition and structure of the tissue and its mechanical properties are still poorly understood and the advent and rapid expansion of attempts to engineer replacement articular cartilage make understanding this relationship increasingly urgent as efforts are made to match the repair tissue to the natural tissue [3]. Measurements of the mechanical properties depend strongly on the rate of loading [4] and the nature of the test performed, for example, confined or unconfined. In addition, many studies have used animal, especially bovine, tissue but it is becoming apparent that bovine tissue is not a good model for human tissue, metabolically, structurally or mechanically [5–9].

We have hypothesized that cartilage is a biological example of a fibre-composite material in which the strong and stiff collagen fibrils reinforce a mechanically weak proteoglycan gel [10]. In such materials interactions between components play an important role [11]. In a previous study of bovine cartilage, in which we found the site variation of composition over the tibial plateau to be similar to previous reports from other joints, we found no clear relationship between gross composition and modulus measured at slow rates of loading [12]. There are few studies relating tissue composition to mechanical properties at high rates of loading. Confined compression tests on bovine tissue using a porous impactor [13,14] reported moduli of over 1000 MPa, dependent on stress rate and proteoglycan and water contents. Water loss increased with stress but decreased with stress rate and the relationship between proteoglycan content and dynamic modulus was reported to be weak [14]. In our previous studies, however, no significant water loss was detected from bovine cartilage subjected to impact loads [15].

Early studies of the mechanical properties of cartilage subjected to impact loads reported that, per unit thickness, cartilage attenuated peak forces ten-times more effectively than bone [16,17].

* Corresponding author at: Musculoskeletal Research Programme, School of Medicine & Dentistry, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK. Tel.: +44 1224 437445.
*E-mail addresses:* L.V.Burgin@leeds.ac.uk (L.V. Burgin), Lorna.ramsay@education.gsi.gov.uk (L. Edelsten), r.aspden@abdn.ac.uk (R.M. Aspden).
[1] Current address: Medical Technologies Innovation and Knowledge Centre, X102 Medical and Biological Engineering – University of Leeds, Leeds LS2 9JT, UK.

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Using a drop tower to apply an impact load to achieve strains of 10–50% at strain rates of 500 $s^{-1}$ or 1000 $s^{-1}$. Finlay and Repo [18] found stresses of 25 MPa were required to produce structural damage or chondrocyte death in human tissue. These stresses corresponded to strains of approximately 20–30% and involved energy absorption of 1 MJ $m^{-3}$. No difference was found between the moduli at the two strain rates [19]. In subsequent studies, however, using a wider range of strain-rates in unconfined compression testing, moduli have been reported up to several hundred megaPascals with a dependency both on stress- or strain-rate and stress magnitude (summarized by Natoli and Athanasiou [20]). Oloyede et al. [21], using a pendulum device and bovine cartilage, showed that the compressive modulus at an applied stress of 0.5 MPa increased linearly as the strain rate increased from $5 \times 10^{-5}$ to $5 \times 10^{-3}$ $s^{-1}$ before reaching a limit of approximately 20 MPa. The applied stress was much less than typical physiological stresses and the J-shaped stress–strain curve would lead to a correspondingly lower measured modulus. At greater stresses, in unconfined compression testing of bovine cartilage using a drop tower, we found values for the maximum modulus of up to approximately 300 MPa, occurring at stresses of the order of 80 MPa. Thresholds for applied stress, stain-rate and energy that resulted in permanent damage to the tissue were identified by Verteramo and Seedhom [22] by measuring the viscoelastic storage and loss moduli in bovine cartilage following an impact load. They found changes in the mechanical properties following stresses above 25 MPa, similar to those found previously, with the energy absorbed per unit volume of 12.79 MJ $m^{-3}$. Differences have been reported, however, between human and bovine tissue subjected to impacts of equal energy using both an impactor, in which the whole tissue is loaded, and an indenter in which only a central core is subjected to direct loading. Higher modulus values were found in bovine tissue than human [5]. Some of the differences may be due to different thicknesses of tissue but differences in failure behaviour and modulus were more difficult to explain. Others found differences between human and bovine, albeit at slower loading rates, with moduli reported of about 1.6 MPa for human tissue and no attempt to relate mechanical measurements to composition [9].

The aim of this study was to measure the mechanical properties of elderly articular cartilage during impact loading, compare these data with similar measurements recorded from the same tissue under slow loading and investigate how these are related to collagen, glycosaminoglycan (GAG), and water contents of the tissue. To do this, full-depth cores of cartilage were taken from defined sites over the human femoral head. These data will provide a framework for comparison with biosynthetic constructs.

## 2. Materials and methods

### 2.1. Tissue preparation

Human femoral heads were obtained following a hemiarthroplasty for a fracture of the neck of the femur (eight male, six female, average age 79 years, age range 63–89 years). Approval was obtained for the use of this material from the Local Research Ethics Committee. Only femoral heads with visual evidence of minimal cartilage fibrillation were used. Full-depth cartilage samples with no bone, 5 mm in diameter, were removed from eleven sites over the femoral head using a cork borer and scalpel [12]. To ensure repeatability of sample sites, a master template was created (Fig. 1) and copied to ultrasound probe covers (SSL International plc, Knutsford) that are stretchy and semi-transparent. The template markings were then transferred to the cartilage by injecting a small spot of bromophenol blue through the probe cover into the femoral head. The supero-anterior (SA) and supero-posterior (SP) samples were at 30° from the most superior site (S) to encompass the region of peak loading [23]. Samples were maintained in phosphate-buffered saline (PBS) except during the testing procedures.

The thickness of each sample was measured optically using a Zeiss Stemi-2000 stereo-microscope and AxioVision software (Zeiss Ltd., Welwyn Garden City) calibrated with a graticule slide, marked with 0.01 mm divisions. Samples were placed on their side and the thickness measured perpendicular to the articular surface at several locations around the perimeter. Any samples that were measurably wedge-shaped, more than 0.05 mm difference across a sample, were removed at this stage. Excess fluid was removed from the surface by gentle blotting between moistened paper tissues and each sample was then weighed in a pre-weighed Eppendorf tube containing ~1 mL of PBS. Each biopsy was weighed three times: just prior to testing (one day after sample removal), immediately after the impact test and a third time 24 h later in order to measure how much water was lost or gained. In previous studies we have found that the tissue mass equilibrates within 24 h of removal from the joint [24]. Each sample was tested first in slow compression, equilibrated for at least 30 min in PBS and then subjected to an impact test as described below.

### 2.2. Slow compression testing

Unconfined compression testing was done using an Instron materials testing machine, model 5564, fitted with a 10 N load cell (Instron Ltd., High Wycombe). The cross-head speed was 100% strain $min^{-1}$ (0.0167 $s^{-1}$). The peak stress was limited to 0.15 MPa in order not to damage the tissue samples prior to impact loading. This was tested in pilot studies. A small metal plate and ball bearing between the cartilage and load cell ensured uniform loading of the sample. A second order polynomial was fitted to the stress–strain curve and the modulus, $E_{slow}$, found by differentiating the curve (Origin Software, Version 6.1; Aston Scientific Ltd., Stoke Mandeville) at a stress of 0.1 MPa.

### 2.3. Impact testing

The drop tower and instrumentation used for impact testing have been described in detail elsewhere [25]. Briefly, each sample was placed on a stainless steel plate on top of a piezoelectric force transducer (Kistler Instruments Ltd., Alton). An impactor, with a mass of 100 g, was released mechanically from a height of 80 mm above the articular surface and fell freely onto the sample. This corresponded to a calculated impact energy of 78.5 mJ and a contact velocity of 1.25 m $s^{-1}$. The impactor was fitted with an accelerometer (Kistler Instruments Ltd., Alton), capable of measuring accelerations up to 500*g*, where *g* is the acceleration due to gravity. These values were chosen based on our previous studies in order to produce minimal damage to the tissue.

Data from the force transducer and the accelerometer were low-pass filtered and the resulting curve overlaid on the original to check that no distortion had occurred. 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 this was divided by the original thickness of the sample to determine the engineering strain. The stress–strain curve for each sample was then differentiated to obtain the modulus. The peak dynamic modulus, $E_{dyn}$, was found as the maximum gradient and, for comparison with slow loading, the modulus at 0.1 MPa ($E_{0.1}$) from the gradient at this stress. Mean stress- and strain-rates were calculated from the peak value divided by the duration of impact. Peak strain-rate was calculated from the quotient of impact velocity and sample thickness, peak stress-rate from the stress-time data.