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# Viscoelasticity of articular cartilage: Analysing the effect of induced stress and the restraint of bone in a dynamic environment

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ABSTRACT

The aim of this study was to determine the effect of the induced stress and restraint provided by the underlying bone on the frequency-dependent storage and loss stiffness (for bone restraint) or modulus (for induced stress) of articular cartilage, which characterise its viscoelasticity. Dynamic mechanical analysis has been used to determine the frequency-dependent viscoelastic properties of bovine femoral and humeral head articular cartilage. A sinusoidal load was applied to the specimens and out-of-phase displacement response was measured to determine the phase angle, the storage and loss stiffness or modulus. As induced stress increased, the storage modulus significantly increased ($p < 0.05$). The phase angle decreased significantly ($p < 0.05$) as the induced stress increased; reducing from 13.1° to 3.5°. The median storage stiffness ranged from 548 N/mm to 707 N/mm for cartilage tested on-bone and 544 N/mm to 732 N/mm for cartilage tested off-bone. On-bone articular cartilage loss stiffness was frequency independent ($p > 0.05$); however, off-bone, articular cartilage loss stiffness demonstrated a logarithmic frequency-dependency ($p < 0.05$). In conclusion, the frequency-dependent trends of storage and loss moduli of articular cartilage are dependent on the induced stress, while the restraint provided by the underlying bone removes the frequency-dependency of the loss stiffness.

## 1. Introduction

Articular cartilage is a load bearing material found at the articulating ends of bones within joints of the body. Smooth joint motion is a result of the low friction at joints of the body, aided by a surface roughness of ~100 nm for articular cartilage (Ghosh et al., 2013). Osteoarthritis (OA) includes the degeneration of cartilage, leading to poor joint motion which is typically painful (Felson et al., 2000). Rapid heel-strike rise times, during gait, have been implicated in the onset of OA (Radin et al., 1991, 1986). These rapid heel-strike rise times were as low as 5–25 ms for the subset of the population potentially predisposed to OA (Radin et al., 1991, 1986). This is in contrast to estimated typical rise times of around 100–150 ms for otherwise healthy gait during walking (Fulcher et al., 2009). This rate of loading is important to the mechanical behaviour of cartilage, because its mechanical properties are rate dependent (Shepherd and Seedhom, 1997): cartilage is viscoelastic (Fulcher et al., 2009; Temple et al., 2016).

Viscoelastic materials can be characterised in terms of a storage, $E'$, and a loss, $E''$, modulus (Hukins et al., 1999) while a viscoelastic structure can be characterised in terms of a storage, $k'$, and a loss, $k''$, stiffness (Lawless et al., 2016). $E'$ characterises the ability of the material to store energy for subsequent elastic recoil; whereas, $E''$ characterises the ability of the material to dissipate energy (Menard, 2008). The viscoelastic properties of cartilage have been characterised over frequencies ranging from typical gait frequencies (≥ 1 Hz) and up to frequencies representative of rapid heel-strike rise times (90 Hz) (Fulcher et al., 2009). The implication was that cartilage, on-bone, undergoes a glass transition at around 10–20 Hz, with a frequency-independent loss modulus but a storage modulus which increases with frequency. Subsequently, Sadeghi et al. (2015b) determined that frequency, independent of load, was significantly correlated to increased failure of articular cartilage. The mechanism proposed, consistent with the hypothesis provided by Fulcher et al. (2009), was that at higher frequencies, the storage modulus increases but the loss modulus remains constant. Thus, the ability of the tissue to store energy is greater at higher frequencies. This increased energy, past a certain point, predisposes the tissue to undergo failure; thereby, dissipating energy within the tissue. Frequencies above a proposed glass transition (Sadeghi et al., 2017a, 2017b; Sadeghi et al., 2015b) appear to be of particular concern regarding failure.

Induced stresses in articular cartilage have been estimated to range from 1 to 6 MPa for moderate activities, such as walking (Ahmed and

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Burke, 1983; Brown and Shaw, 1983; Hodge et al., 1989) with peak stresses estimated to reach up to 10.7 MPa, for stair-climbing, and 18 MPa for rising from a chair (Hodge et al., 1989). This is in comparison to induced stresses of around 1–1.7 MPa estimated for hip and knee joints during 'ambulatory' activities, i.e. walking (Yao and Seedhom, 1993). The material properties of cartilage have previously been found to be strain-dependent (Barker and Seedhom, 2001). However, the relationship was not linear, instead resembling a U-shaped relationship. Different stress levels imply different strain, and potentially different mechanical response to loading (Barker and Seedhom, 2001). The relevance, though, of stress to the dynamic viscoelasticity of cartilage is currently unknown.

The juxtaposition of cartilage and bone will mean that a change in one will lead to a change on stress generated with the other (Dar and Aspden, 2003); hence, the relevance of understanding the interactions between articular cartilage and bone. The underlying subchondral bone to which cartilage is attached, has a restrictive effect (Aspden, 1990) on cartilage and prevents lateral displacement at the base of the tissue (Burgin and Aspden, 2008). For example, it has been suggested that the underlying bone would attenuate the increased energy dissipation with loading velocity observed off bone (Edelsten et al., 2010). The extrapolated implication being that cartilage on- and off-bone have different frequency-dependent loss moduli. This inference appears to be consistent with the finding that cartilage off-bone has a frequency-dependent loss modulus (Aspden, 1991; Temple et al., 2016), as opposed to a frequency-independent when on-bone. However, differences between testing procedures could make this inference invalid. For example, testing of cartilage samples in air as opposed to within a hydrating solution (e.g. Ringer's solution); since hydration alters the viscoelastic properties (Pearson and Espino, 2013) and predisposition to failure of articular cartilage (Fick and Espino, 2012).

The aim of this study was to determine the effect of the induced stress and restraint provided by the underlying bone on the frequency-dependent viscoelastic properties of articular cartilage. Some tests were performed on cartilage in a hydrating fluid (Ringer's solution) and others in air, in order to understand the limitations of comparing published studies performed under these different conditions. Except for bone restraint, viscoelasticity has been analysed in terms of $E'$ and $E''$. Bone restraint, has been analysed in terms of $k'$ and $k''$, since the combination of cartilage and bone is a structure and not a material.

## 2. Materials and methods

### 2.1. Specimens

Three bovine femoral heads and eight bovine humeral heads, of approximately between 18 and 30 months old, were obtained from a supplier (Dissect Supplies, Birmingham, UK); bovine cartilage is a suitable model for the dynamic viscoelasticity of human cartilage (Temple et al., 2016). Specimens were wrapped in tissue paper, and saturated in Ringer's solution, on arrival in the laboratory. Specimens were then stored in a freezer at −40 °C. Specimens were thawed for 12 h before testing. Freeze-thaw treatment does not alter the dynamic mechanical properties of articular cartilage (Szarko et al., 2010). Large scale damage of the cartilage on joints was not evident. However, India Ink (Loxley Art Materials, Sheffield, UK) was used to ensure that only intact surfaces were used for testing (Aspden, 2011; Meachim, 1972) because surface cracks alter the mechanical properties of articular cartilage (Burgin and Aspden, 2008).

Sixteen cylindrical test specimens (see Table 1) were obtained using a cork borer with a medical scalpel used to isolate the cartilage from the subchondral bone (Burgin and Aspden, 2008; Edelsten et al., 2010; Lewis et al., 1998; Temple et al., 2016). The specimens were 5.2 mm in diameter, but varied in thickness (see Table 1).

### 2.2. DMA frequency sweep

A Bose ElectroForce 3200 testing machine running WinTest 4.1 Dynamic Mechanical Analysis (DMA) software (Bose Corporation, Minnesota, USA; now, TA Instruments, New Castle, DE, USA) was used to quantify the viscoelastic properties. This approach has been used to characterise the viscoelastic properties of natural tissues (Barnes et al., 2016, 2015; Burton et al., 2017; Fulcher et al., 2009; Temple et al., 2016) and orthopaedic implants (Lawless et al., 2017, 2016). Each test specimen underwent a frequency sweep (1, 8, 10, 12, 29, 49, 71, and 88 Hz), following preloading at 25 and 50 Hz (1500 and 3000 cycles, respectively, with a 60 s rest period). For each frequency, the DMA software calculated a storage ($k'$) and loss ($k''$) stiffness as shown in Eqs. (1)–(3); where $k^*$, $F^*$ and $d^*$ are the magnitude of the complex stiffness, the magnitude of the force (from the Fast Fourier Transform, FFT, of the sinusoidal force wave) and the magnitude of the displacement (from the FFT of the sinusoidal displacement response wave), respectively. Further details can be found elsewhere (Lawless et al., 2016).

$$k^* = \frac{F^*}{d^*} \tag{1}$$

$$k' = k^* \cos\delta \tag{2}$$

$$k'' = k^* \sin\delta \tag{3}$$

The angle $\delta$ is the phase difference between the applied compressive force and the displacement.

A 20 mm diameter compression plate was used to compress articular cartilage specimens. This DMA frequency sweep was used for three different testing procedures described in Section 2.3.

### 2.3. Testing protocols

The DMA frequency-sweep was applied under three distinct testing protocols which focused on test specimens: (1) in air and in Ringer's solution; (2) loaded under different levels of sinusoidal loading to vary the induced stress; and (3) on- and off-bone.

For testing protocol-1, 8 test specimens (all from the femoral head; see Table 1) were tested following the DMA procedure (Section 2.2) in air or in Ringer's solution. To enable a paired comparison, each individual test specimen was tested under both conditions with half the test specimens tested first in air and the other half first in Ringer's solution. Between tests, each specimen was allowed to rest/recover whilst saturated in Ringer's solution for 30 min; this ensured cartilage returned to a hydrated state prior to the subsequent test, consistent with literature (Barker and Seedhom, 2001; Park et al., 2004). A sinusoidally compressive force was applied between 16 and 36 N (Fulcher et al., 2009; Temple et al., 2016). Peak loading induced maximal stresses of 1.7 MPa, estimated physiological for lower limb cartilage during walking (Yao and Seedhom, 1993).

For testing protocol-2, 8 test specimens, from the humeral head, were tested in air following the DMA procedure (Section 2.2) with a variety of three different sinusoidal loading ranges: (a) 2–22 N; (b) 16–36 N and (c) 65–85 N. This induced three different ranges of dynamic stress (Table 2). To enable paired comparisons, each specimen was tested under the three loading ranges with the order of testing varied with Excel Random Function (Redmond, Washington, USA).

For testing protocol-3, 8 test specimens were obtained from humeral heads and tested on-bone and then off-bone. These samples were not cut using a cork borer (discussed above) but by using a hollow drill-head attached to a drill (Burgin and Aspden, 2008). Cylindrical cartilage on bone specimens were obtained, 4.1 mm in diameter. These specimens underwent the DMA procedure outlined, above in the Section 2.2, first on-bone and subsequently after using a medical scalpel to isolate the cartilage from the bone. For both cartilage specimens on- and off-bone, a sinusoidally compressive force was applied between 10 and 24 N. This loading range induced a maximal stress of 1.8 MPa,