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Effect of the variation of loading frequency on surface failure of bovine articular cartilage

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Background: Mechanical loading of synovial joints can damage the articular cartilage surface and may lead to osteoarthritis. It is unknown if, independent of load, frequency alone can cause failure in cartilage. This study investigated the variation of articular cartilage surface damage under frequencies associated with normal, above normal and traumatic loading frequencies.

Method: Cartilage on bone, obtained from bovine shoulder joints, was tested. Damage was created on the cartilage surface through an indenter being sinusoidally loaded against it at loading frequencies of 1, 10 and 100 Hz (i.e., relevant to normal, above normal and up to rapid heel-strike rise times, respectively). The frequencies were applied with a maximum load in the range 60–160 N. Surface cracks were marked with India ink, photographed and their length measured using image analysis software.

Results: Surface damage increased significantly ($P < 0.0001$) with frequency throughout all load ranges investigated. The dependence of crack length, c, on frequency, f, could be represented by, $c = A(\log_{10}(f))^2 + B(\log_{10}(f)) + D$ where $A = 0.006 \pm 0.23$, $B = 0.62 \pm 0.23$ and $D = 0.38 \pm 0.51$ mm (mean ± standard deviation).

Conclusion: The increase in crack length with loading frequency indicated that, increased loading frequency can result in cartilage becoming damaged. The results of this study have implications in the early stages of osteoarthritis.

Introduction

In this study, experimental damage of the articular cartilage surface was produced by applying five sinusoidally varying compressive force ranges over three magnitudes of loading frequency. Articular cartilage can become damaged when subjected to repetitive mechanical loading[1–3]. However, the mechanism by which the surface of cartilage becomes damaged under loading frequencies associated with normal, above normal and rapid heel-strike rise time is unknown.

Articular cartilage is a compliant layer covering the much stiffer bone ends of a joint, preventing high contact stresses which could ultimately damage the bone in a joint. Cartilage needs, therefore, to be able to deform in order to increase the total surface area for contact, thereby reducing the overall stress[2,4]. Cartilage also provides smooth bearing surfaces, with surface roughness of 80–170 nm, in freely moving synovial joints[5].

The most recognized feature of osteoarthritis is the progressive damage of articular cartilage, resulting in impaired joint motion, severe pain, and disability[6]. The articular cartilage surface begins to change from a smooth to a rough or fibrillated appearance in early osteoarthritis[7]. Once damaged, it has a very limited ability to repair itself[8,9].

Rapid heel strike rise times during gait have been associated with the early onset of osteoarthritis[10,11]. Heel strike rise times in the normal population have been previously determined to be typically 100–150 ms. However, a subset of the population with heel-strike rise times from 5 to 25 ms have been identified and are fast enough to create impulsive loadings[12]. Repetitive impulsive/traumatic loading was found to provoke osteoarthritis in animal experiments[11]. In general, heel-strike rise times of 500 ms corresponds to a loading frequency of 1 Hz, whereas a rapid heel-strike rise time such as 5.4 ms is equivalent to a loading frequency of 92 Hz[12]. Cyclic compressive loading has been used to subject the surface of cartilage to damage[1,2]. However, little is known about the role of loading frequency in the initiation and progression of damage in cartilage.

Previous studies[12–14] which investigated changes in cartilage viscoelastic properties with frequency suggested that the

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likelihood of cartilage failure increases with loading frequency. This was suggested, because at higher frequencies the ability of the tissue to store energy, for elastic recoil, increased. It was suggested that if the energy available for storage exceeded a certain level it might induce damage to the cartilage. Damage caused by increasing the loading frequency has been suggested to be different to the damage caused by increasing load only following comparisons between failure patterns from static loading tests[15,16].

Another factor associated with the failure of articular cartilage is the mechanical overload of the joint[17]. Cartilage fissures have typically been formed in the regions exposed to high loads in the joint[18,19]. Therefore, the aim of this study was to determine the variation of articular cartilage surface damage with frequencies relevant to normal, above normal and rapid heel-strike loading, and how this relationship is altered by the maximum stresses which are induced in cartilage.

Method

Specimen preparation

Fresh bovine shoulders, from animals less than 30 months old, were obtained from a supplier (Johnston's Butcher, King's Heath, Birmingham, UK). On arrival in the laboratory they were wrapped in tissue paper and soaked in Ringer's solution. The bovine shoulders were then sealed in plastic bags, stored in a freezer at −40°C until they were required for testing[2,12–14]. Freeze-thaw treatment has not been found to alter the mechanical properties of articular cartilage[20–22] or bone[23]. Prior to testing, the specimens were thawed at room temperature for approximately 2 h[12,13]. Two 50 × 50 mm cartilage-on-bone specimens were obtained from the humeral head of a shoulder joint. Typically specimens had a total thickness of 20 mm (cartilage and subchondral bone) to allow sufficient bone for secure fixation in the test rig[12,14]. The specimens were secured into a custom-made test rig using acrylic cement (WHW plastics, Hull, UK). Each specimen was then bathed in Ringer's solution at room temperature throughout the whole test, as in previous studies[2,12].

India ink (Loxley Art Materials, Sheffield, UK) was used to confirm that each cartilage surface was initially free from defects. Large scale damage was not observed on the surface of any specimen before mechanical testing; this was expected because joints were not osteoarthritic[13,24].

Mechanical testing

An experimental protocol was developed to investigate the role of frequency of loading on damage to the articular cartilage surface. Damage was created using a sinusoidally varying compressive load with a solid cylindrical indenter. The indenter had a 5.2 mm diameter flat circular face with a 0.5 mm radius bevel in order to avoid high stresses around its edge. A Bose ElectroForce ELF3200 material testing machine, operated under the control of WinTest software (Bose Corporation, ElectroForce systems Group, Minnesota, USA) was used to perform indentation on the articular cartilage specimens. A total of 40 specimens were obtained from 20 bovine shoulder joints; it is unknown if any of the joints were from the same animal, so in the calculations of the 95% confidence intervals it was assumed that the joints came from 10 animals to avoid any possibility of dependant observations. Three sites, free from pre-existing lesions, were chosen for testing on each tissue sample over three magnitudes of loading frequency (i.e., one frequency per test site). Therefore, a total of 120 distinct test sites have been analysed for crack measurements. Frequencies tested ranged from normal, above healthy-gait and corresponding to rapid heel-strike rise times, using 1 Hz, 10 Hz and 100 Hz respectively[12,13]. Each test consisted of the cartilage tissue samples being loaded for 10,000 cycles[3] for each loading frequency individually. The number of cycles was kept constant for all tests over all three magnitudes of frequency, in order to be able to observe the change in damage when only frequency was altered. Surface effects may extend up to 1 mm from the loaded site in healthy cartilage[25]; therefore, an average distance of 5 mm was kept between the test sites. Five sinusoidally varying compressive force ranges were used for testing: 6–60 N, 9–90 N, 10–100 N, 12–120 N and 16–160 N. The maximum applied loads induced a nominal compressive stress of 2.8 MPa, 4.2 MPa, 4.7 MPa, 5.6 MPa and 7.5 MPa, respectively. Eight samples were tested for each loading range at 1 Hz, 10 Hz and 100 Hz. The loading ranges were chosen to determine in which loading range and frequency the surface damage was initiated and how it changes with load or frequency. These values were chosen based on preliminary tests and the stresses induced by the maximum load.

The thickness of each site tested was measured after testing, using an established technique which has been described previously[12,13,26]. Briefly, a sharp needle was pushed through the cartilage layer up to the underlying bone using the testing machine described above. The thickness of the cartilage was determined from the difference in displacement readings at two points where the needle comes into contact with the cartilage surface and the point at which the needle contacts the cartilage/bone interface.

Analysis of cartilage surface damage

India ink was applied to the cartilage surface to highlight any signs of failure following each test[1,24]. The cartilage surface was then photographed using a DSC-R1 Cyber-shot© digital camera (10 MP, 5 × Optical Zoom) 2.0″ (Sony Corporation, 6-7-35 Kitashinagawa, Shinagawa-ku, Tokyo, Japan) after each test. A scale-bar was included in each image, positioned in the field of view. Digital images were analysed using ImageJ (version 1.48, Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA). Surface damage was observed on the surface of cartilage as cracks and fissures. Lines were drawn manually along the length of all the cracks and fissures. The software was used to calculate their total length (in mm) with a 0.1 mm precision. The length of cracks and fissures were added together for each test site[2]. Image analysis measurements were repeated twice by one individual to ensure the measurements were repeatable.

Data analysis

The mean total crack length against the three frequencies (1 Hz, 10 Hz and 100 Hz) was analysed for all load ranges using Sigmaplot Version 11.0 (Systat Software Inc., London, UK). 95% confidence intervals were calculated with the n values shown in Fig. 1, so that independent observations could be assumed[27]. The relationship between total crack lengths against maximum loads was analysed in order to determine the variation of crack length when the maximum load range was altered. A polynomial or linear regression was used to fit a curve or line to the data. A P value <0.05 indicates that the curve or line fit was significant.

Results

A sample image taken from five cartilage samples at the five loading ranges is shown in Fig. 2. At lower loading frequencies such as 1 Hz and 10 Hz, cracks and fissures on the surface of articular cartilage following cyclic tests appeared to be single or parallel lines. However, at 100 Hz, the loaded region contained a greater