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# Effect of the Variation of Loading Frequency on Surface Failure of Bovine Articular Cartilage

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# Summary

*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 ± 0.23, $B$ = 0.62 ± 0.23 and $D$ = 0.38 ± 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.

*Keywords:* Articular cartilage, Crack, Failure, Frequency, Mechanical loading, Osteoarthritis.

## 1. 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 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.

## 2. 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 hours[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 (10MP, 5 x 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 Figure 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.

## 3. Results

A sample image taken from five cartilage samples at the five loading ranges is shown in Figure 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 number of branches. Cracks and fissures also increased in length with frequency for all samples, except for the lowest loading range of 6-60 N where there were no cracks at 1 or 10 Hz. There also appeared to be more crack branches at higher frequencies from qualitative observations (Figure 2).

The crack length was found to increase with loading frequency. This was true for individual tissue samples (Figure 3) and also when the mean values from 8 tissues samples were used (Figure 4). The mean cartilage thickness of specimens was 2.14 mm (range: 1.8–3.6 mm). Figure 4, shows the mean total crack length against frequency of 1, 10 and 100 Hz over five load ranges. The first signs of failure were observed in the loading range of 6-60 N (maximum peak nominal stress of 2.8 MPa) at 100 Hz. Maximum damage was observed in the loading range of 16-160 N (maximum peak nominal stress of 7.5 MPa) at 100 Hz. Signs of damage at a loading frequency of 1 Hz and 10 Hz were first observed in 10-100 N (maximum

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peak nominal stress of 4.7 MPa) and 9-90 N (maximum peak nominal stress of 4.23 MPa), respectively.

The crack length increased significantly (P<0.05) with increasing frequency for all loading ranges. Second order polynomial curves (equation 1) were found to fit the data well for all sites tested in the form:

$$c = A(log_{10}(f))^2 + B(log_{10}(f)) + D \qquad\qquad \text{for} \quad f \geq 1 \qquad (1)$$

where $c$ is the mean total crack length, $f$ represents frequency and A, B and D are constants. (Table I).

The crack length increased with increasing the maximum load (Figure 5). Mean total crack length increased by 1.1, 2.4 and 5.1 mm at loading frequencies of 1, 10 and 100 Hz, respectively. A linear relationship was found to fit the experimental data in the form:

$$c = EL + G \qquad\qquad \text{for} \quad 60 \leq L \leq 160 \qquad (2)$$

where $c$ is the mean total crack length, $L$ is the maximum load and $E$ and $G$ are constants. Mean values of the constants $E$ and $G$ were 0.02 mm.N$^{-1}$ (SD 0.019, range 0.013–0.051 mm.N$^{-1}$) and -1.74 mm (SD 0.77, range -2.54 – -1 mm), respectively.

## 4. Discussion

The results from this study show that surface cracks can form purely by mechanical means, and this progression is frequency dependant. This study has investigated the dependency of surface damage on loading frequency. Frequencies of 1, 10 and 100 Hz were used as they correspond to normal gait, above healthy gait heel-strikes and similar to traumatic heel-strike rates, respectively[1-14,20,28]. The results show that repetitive mechanical loading of

articular cartilage causes damage in the form of crack and fissures to its surface. Crack length was found to increase with increasing loading frequency; this increase was also found to be greater at higher maximum loads. An increase in cartilage damage with increasing the loading frequency was predicted in previous studies[12,14,28]. This prediction was made because of the change in viscoelastic properties of articular cartilage with frequency. At frequencies of up to 92 Hz, there was an increase in storage to loss modulus, i.e. the ability of the material to store energy rather than dissipate it increased[12]. The main mechanism for releasing the excess energy was suggested to be by the formation of cracks. The proposed mechanism was consistent with increased cartilage failure which occurs with increasing energy during impact loading *in vitro*[29,30].

Increase in the length of cracks and fissures during mechanical cyclic loading have been reported in previous studies, but only up to frequencies of 0.1 Hz [1] or 0.5 Hz [2] and 1 Hz [31]. In the current study damage was created on cartilage samples by applying 10,000 cycles over five load ranges with peak stresses from 2.8 to 7.5 MPa. The change in damage was observed when frequency alone was altered. Ewers *et al*. (2001)[32] who investigated the propagation of damage in rabbit cartilage after blunt impact *in vivo* also reported that the total length of fissures increased during the first 4 months following impact tests.

Articular cartilage is subjected to various ranges of peak stresses under light to moderate activities which typically range from 1 to 6 MPa[33,34]. Peak stress amplitudes, under more vigorous activities in natural joints have been estimated up to 12 MPa[35] or even 18 MPa[36]. The maximum peak stresses used in this study were from 2.8 to 7.5 MPa, which is similar to peak stresses acting on cartilage during light to moderate activities.

During the normal gait cycle for walking there is a rapid rise in foot-to floor reaction force from zero to a peak load of over body weight during the first 100 ms after heel strike[37,38]. These forces results in the deformation of articular cartilage. Thus, ground reaction forces at heel-strikes provide indirect information about internal joint loading. This is because a peak ground reaction force corresponds with the timing of peak loads in joints[38-41]. Therefore the increase in the loading rate of the ground reaction force (shorter rise times) will be in correlation with the changes in rise times of the reaction forces acting on articular cartilage in lower limb joints of the body[37].

It has been suggested that impulsive/traumatic rise times during heel strike could be implicated in the onset of osteoarthritis[11]. Repetitive mechanical loading can create fissures in the surface of cartilage[1]. Significant increase in crack length with frequency in peak stresses which resembles peak stresses acting on cartilage during light and moderate activities shows the important role of loading frequency in the initiation and propagation of damage on the surface of articular cartilage in this study. Other studies[12,13,28] have reported that the ability of cartilage to store and dissipate energy is altered with loading frequency. This may change the stress transfer to the underlying bone which leads to bone stiffening and ultimately failure of articular cartilage[13]. However, energy transfer between cartilage and subchondral bone has not been investigated in this study.

The effect of increasing the loading frequency was investigated in a comparable study[42] when shear force was used to create fractures on the surface of articular cartilage experimentally. Results from this study showed that with increasing the speed of the

applied load (increasing the frequency), surface cracks appeared on the surface of cartilage whereas low speed loads with the same energy, did not create cracks on the surface of cartilage.

The effect of slowly applied loads and suddenly applied loads on articular cartilage was suggested to differ considerably in previous studies[42-45]. This was associated with the deformation of cartilage under different loading frequencies with displacement of water inside and outside of the collagen network.  These studies assumed cartilage as a biphasic material composed of a solid matrix phase (20% of the total mass by weight) and an interstitial fluid phase (80%)[44]. It is not clear why the cartilage surface shows signs of cracking following cyclic tests, a behaviour mostly seen in brittle materials[46,47]. However, using a theoretical model[48], it has been shown that increasing the loading frequency, also increases the flow of interstitial fluid inside the cartilage tissue. This fluid flow in and out of the collagen matrix is eventually inhibited together at higher frequencies; therefore cartilage acts as a brittle, largely incompressible elastic solid. The biphasic mechanism was explained by assuming the solid matrix, to be a porous, permeable elastic solid and the interstitial fluid to be movable within the tissue[49]. Both phases were modelled to be intrinsically incompressible and the diffusive drag forces between the two phases resulted in the viscoelastic behaviour of the tissue[50,51]. However, this mechanism was not supported, when viscoelastic properties of cartilage were measured up to 10 Hz[14] or 90 Hz[12] as almost no variation was observed in loss modulus/stiffness with increasing the loading frequency. This might be due to the constraining effect of the underlying bone[14,52]. The underlying bone has previously been predicted to prevent an increase in loss modulus[53].

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Fluid flow is not the only possible mechanism which is involved in cartilage deformation and ultimately failure. Another alternative is to treat cartilage as a hydrated gel[54,55], undergoing glassy transition (transition from soft to glassy material), under high loading frequencies. This has been discussed in several studies[12,56,57] because of the existence of hydrated proteoglycans in the cartilage matrix which act as a natural hydrogel under deformation. This is consistent with increased injury observed with increased stiffness under increased loading frequencies[42].

Articular cartilage is a composite material in which collagen fibrils provide tensile reinforcement. The alignment of collagen is different in every layer of cartilage[52,56,58]. One of the mechanisms which has been suggested as being involved in crack growth under repetitive loading on the cartilage surface is the tension developed in the superficial layer of the cartilage surface (10-20 % of cartilage thickness)[59]. The investigation of failure propagation through the depth of articular cartilage will be the subject of a future study.

## 5. Conclusion

It can be concluded from this study that surface damage to cartilage following sinusoidal loading increased with frequency throughout all load ranges investigated. Cracks appeared on the surface of articular cartilage in lower load ranges (below a maximum peak stress of 4.7 MPa) only at loading frequencies which are associated with impulsive/traumatic and above healthy gait heel-strike rise times, 100 Hz and 10 Hz, respectively. However, cracks appeared at all loading frequencies in higher maximum load ranges (above maximum peak stress of 4.7 MPa). Variation of damage with different loading frequencies has implications in the early stages of osteoarthritis.

## 6. Acknowledgements

## 7. Contributions

HS carried out the experimental work, study design, data analysis and drafting the manuscript. DETS and DME participated in the study design, data analysis and revising the manuscript. All authors have read and approved the final manuscript.

## 8. Role of the funding source

## 9. Competing interests

The authors declare they have no competing interests.

## 10. References

1. Weightman BO, Freeman MAR, Swanson SAV. Fatigue of articular cartilage. Nature 1973;244:303–4.

2. Kerin AJ, Coleman A, Winsom MR, Adams MA. Propagation of surface fissures in articular cartilage in response to cyclic loading in vitro. Clin Biomech (Bristol Avon) 2003;18:960–8.

3. Zimmerman NB, Smith DG., Pottenger LA, Cooperman DR. Mechanical disruption of human patellar cartilage by repetitive loading in vitro Clin Orthop Relat Res 1988; 229:302–7.

4. Radin EL. Who gets Osteoarthritis and why? J Rheumatol Suppl 2004;70:10–5.

5. Ghosh S, Bowen J, Jiang K, Espino DM, Shepherd DET. Investigation of techniques for the measurement of articular cartilage surface roughness. Micron 2013;44:179–84.

6. Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997;350(9076):503–8.

7. Chiang EH, Laing TJ, Meyer CR, Boes JL, Rubin JM, Adler RS. Ultrasonic characterization of *in vitro* osteoarthritic articular cartilage with validation by confocal microscopy. Ultrasound Med Biol 1997;23:205–13.

8. Adams MA, Merrill AK, Kerin AJ. Mechanicaly-induced proteoglycan loss from damaged articular cartilage in-vitro. Trans Orthop Res Soc 1997;43:409.

9. Mankin HJ. The response of articular cartilage to mechanical injury. J Bone Joint Surg Am 1982;64:460–6.

10. Radin EL, Whittle MW, Yang KH, Jefferson R, Rodgers MM, Kish VL, OConnor JJ: The heelstrike transient, its relationship with the angular velocity of the shank, and effects of quadriceps paralysis. In Advances in Bioengineering. Edited by Lantz SA, King AI. New York: American Society of Mechanical Engineering; 1986:121–3.

11. Radin EL, Yang KH, Riegger C, Kish VL, O'Connor JJ. Relationship between lower-limb dynamics and knee-joint pain. J Orthopaed Res 1991;9:398–405.

12. Fulcher GR, Hukins DWL, Shepherd DET. Viscoelastic properties of bovine articular cartilage attached to subchondral bone at high frequencies. BMC Musculoskelet Disord 2009;10:61.

13. Espino DM, Shepherd DET, Hukins DWL. Viscoelastic properties of bovine knee joint articular cartilage: dependency on thickness and loading frequency BMC Musculoskelet Disord 2014;15:205.

14. Sadeghi H, Espino DM, Shepherd DET. Variation in viscoelastic properties of bovine articular cartilage below, up to and above healthy-gait relevant loading frequencies. 2014. Proc Inst Mech Eng H: Journal of Engineering in Medicine 2015;229:115–23.

15. Fick JM, Espino DM. Articular cartilage surface failure: an investigation of the rupture rate and morphology in relation to tissue health and hydration. Proc Inst Mech Eng H 2012;226:389–96.

16. Fick JM, Espino DM. Articular cartilage surface rupture during compression: investigating the effects of tissue hydration in relation to matrix health. J Mech Behav Biomed Mat 2011;4:1311–7.

17. Cooper C, McAlindon T, Coggon D, Egger P, Dieppe P. Occupational activity and osteoarthritis of the knee. Ann Rheum Dis 1994;53:90–3.

18. Byers PD, Hoaglund FT, Purewal GS, Yau AC. Articular cartilage changes in Caucasian and Asian hip joints. Ann Rheum Dis 1974;33:157–61.

19. Murray RC, Birch, HL, Lakhani K, Goodship AE. Biochemical composition of equine carpal articular cartilage is influenced by short-term exercise in a site-specific manner. Osteoarthritis Cartilage 2001;9:625–32.

20. Szarko M, Muldrew K, Bertram JEA. Freeze-thaw treatment effects on the dynamic mechanical properties of articular cartilage. BMC Musculoskelet Disord 2010;11: 231.

21. Kiefer GN, Sundby K, McAllister D, Shrive NG, Frank CB, Lam T, Schachar NS. The effect of cryopreservation on the biomechanical behaviour of articular cartilage. J Orthop Res 1989;7:494–501.

22. Swann, AC. The effect of mechanical stress on the stiffness articular cartilage and its role in the aetiology of osteoarthrosis. In *PhD* thesis University of Leeds;1988.

23. Seldin ED, Hirsch C. Factors affecting the determination of physical the properties of femoral cortical bone. Acta Orthop Scand 1966;37:29–48.

24. Meachim G. Light microscopy of indian ink preparation of fibrillated cartilage. Ann Rheum Dis 1972;31:457–64.

25. Fick JM. How the structural integrity of the matrix can influence the microstructural response of articular cartilage to compression. Connect Tissue Res 2013;54(2):83–93.

26. Shepherd DET, Seedom BB. Thickness of human articular cartilage in joints of the lower limb.  Ann Rheum Dis 1999;58:27–34.

27. Ranstam J. Repeated measurements, bilateral observations and pseudoreplicates, why does it matter? Osteoarthritis Cartilage 2012;20:473-475.

28. Pearson B, Espino DM. The effect of hydration on the frequency-dependant viscoelastic properties of articular cartilage Proc Inst Mech Eng H 2013;227:1246–52.

29. Jeffrey JE, Aspden RM. the biophysical effects of a single impact load on human and bovine articular cartilage. Proc Inst Mech Eng H 2006;220:677–86.

30. Verteramo A, Seedhom BB. Effect of a single impact loading on the structure and mechanical properties of articular cartilage. J Biomech 2007;40:3580–9.

31. Radin EL, Martin RB, Burr DB, Caterson B, Boyd RD, Goodwin C. Effect of mechanical loading on the tissue the rabbit knee. J Orthop Res 1984;2:221–34.

32. Ewers BJ, Dvoracek-Driksna D, Orth MW, Haut RC. The extent of matrix damage and chondrocyte death in mechanically traumatized articular cartilage explants depends on rate of loading. J Orthop Res 2001;19:779–84.

33. Ahmed AM, Burke DL. In-vitro measurement of static pressure distribution in synovial joints part I: tibial surface of the knee. J Biomech Eng 1983,105:216–25.

34. Brown TD, Shaw DT.  In vitro contact stress distributions in the natural human. hip J Biomech 1983;16:373–84.

35. Mathews MB, Decker L. Comparative studies of water sorption of hyaline cartilage. Biochim Biophys Acta 1977;497:151–9.

36. Hodge WA, Carlson KL, Fijan RS, Mann RW. Contact pressures from an instrumented hip endprosthesis J Bone Joint Surg 1989;71:1378–86.

37. Barker MK, Seedom BB. Articular cartilage deformation under physiological cyclic loading apparatus and measurement technique. J Biomech 1997; 30:377–81.

38. Bergmann G, Deuretzbacher G, Heller M, Graichen F, Rohlmann A, Strauss J, Duda GN. Hip contact forces and gait patterns from routine activities. J Biomech 2001;34:859–71.

39. Bassey EJ, Littlewood JJ, Taylor JG. Relations between compressive axial forces in an instrumented massive femoral implant, ground reaction forces, and integrated electromyographs from vastus lateralis during various 'osteogenic' exercises. J Biomech 1997; 30:213–23.

40. Park S, Kerbs DE, Mann RW. Hip muscle co-contraction: evidence from concurrent in vivo pressure measurement and force estimation. Gait Posture 1999;10:211–22.

41. Paul JP. Forces transmitted by joints in the human body. Proc Instn Mech Engrs -67 1966; 181(pt 37):8–15.

42. Tomatsu T, Imai N, Takeuchi N. Experimentally produced fractures of articular cartilage and bone: the effect of shear forces on the pig knee J Bone and Joint Surg 1992;74:457–62.

43. Buckwalter JA. Sports, joint injury, and posttraumatic osteoarthritis, J Orthop Sports Phys Ther 2003;33:578–88.

44. Kafka V. Surface fissures in articular cartilage: new concepts, hypotheses and modeling. Clin Biomech (Bristol, Avon) 2002;17:73–80.

45. Mow V, Rosenwasser M. Articular cartilage: bio-mechanics. In: Woo SLY, Buckwalter JA, Ed. Injury and Repair of the Musculoskeletal Soft Tissues. Park Ridge, IL: American Academy of Orthopaedic Surgeons 1988:427–63.

46. Silyn-roberts H, Broom ND. Fracture behaviour of cartilage-on-bone in response to repeated impact loading Conn Tiss Res 1990;24:143–56.

47. Kelly PA, J J O'Connor. Transmission of rapidly applied loads through articular cartilage Part 1: uncracked cartilage, Proc Inst Mech Eng 1996; 210:27–37.

48. Armstrong CG. An analysis of the stresses in a thin layer of articular cartilage in a synovial joint. Eng Med 1986;15:55–61.

49. Lu X, Mow VC. Biomechanics of articular cartilage and determination of material properties. Med Sci Sports Exerc 2008;40:193–9.

50. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic Creep and Stress Relaxation of Articular Cartilage in Compression: Theory and Experiments. J Biomech Eng 1980;102:73–84.

51. Martin JA. and Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology 2002;3:257–64.

52. Aspden RM. Constraining the lateral dimensions of uniaxially loaded materials increases the calculated strength and stiffness: application to muscle and bone. J Mater Sci Mater Med 1990;1:100–4.

53. Edelsten L, Jeffrey JE, Burgin LV. and Aspden RM.  Viscoelastic deformation of articular cartilage during impact loading. Soft Matter 2010;6:5206–12.

54. Kovach IS. A molecular theory of cartilage viscoelasticity. Biophys Chem 1996;59:61–73.

55. Kovach IS. The importance of polysaccharide configurational entropy in determining the osmotic swelling pressure of concentrated proteoglycan solution and the bulk compressive modulus of articular cartilage Biophys Chem 1995;53:181–7.

56. Hukins DWL, Aspden RM. Composition and properties of connective tissues. Trends Biochem Sci 1985;10:260–4.

57. Meakin JR, Hukins DWL, Aspden RM, Imrie CT. Rheological properties of poly(2-hydroxy methacrylate) (pHEMA) as a function of water content and deformation frequency. J Mater Sci Mater Med 2003;14:783–7.

58. Aspden RM, Hukins DWL. Collagen organization in articular cartilage, determined by x-ray diffraction, and its relationship to tissue function. Proc R Soc Lond Ser B 1981;212:299–304.

59. Korhonen RK, Wong M, Arokoski J, Lindgren R, Helminen HJ, Hunziker EB. Importance of the superficial tissue layer for the indentation stiffness of articular cartilage. Med Eng Phys 2002;24:99–108.

## Figure legends

**Figure 1** –Number of independent observations (n) used for calculating the 95% confidence

intervals in Figures 4 and 5. Each load range was tested at three frequencies on distinct test

sites (120 tests).

**Figure 2** –Representative images taken from cartilage samples following cyclic loading. The

black circles show the loaded region. Loading ranges included were (a) 6-60 N, (b) 9-90 N, (c)

10-100 N, (d) 12-120 N and (e) 16-160 N. The formation and increase in crack length can be

generally observed with increasing load and frequency. India ink was applied to the cartilage

surface to easily distinguish the surface cracks. A scale bar is included in every image in

order to measure the surface features.

**Figure 3** – Total crack length plotted against the logarithm (with base 10) of the loading

frequency for testing on individual tissue samples.  The three represent the load ranges of 6-

60 N (●), 9-90 N (○), 10-100 N (▼), 12-120 N (△) and 16-160 N (■).

**Figure 4** –Mean total crack length plotted against the logarithm (with base 10) of the

loading frequency for the load ranges of 6-60 N (●), 9-90 N (○), 10-100 N (▼), 12-120 N (△)

and 16-160 N (■). Second order polynomials (see equation 1) fitted the data well. Error bars

represent 95% confidence intervals, for clarity only positive error bars have been included.

When error bars are not visible they are smaller than the symbols used to represent the

data point.

**Figure 5** –Mean total crack length plotted against the maximum load for loading frequencies of 1 Hz (●), 10 Hz (○) and 100 Hz (▼).Each point in this graph represents the mean crack length of 8 measurements. The crack length is described by linear curve fits (see equation 2). *P*-value for all lines were *P*<0.05 which indicates that lines are statistically significant. $R^2$ is the squared correlation coefficients and shows how well the lines fit the data point. $R^2$ value for loading frequencies of 1, 10 and 100 Hz are 0.87, 0.97 and 0.99, respectively. Uncertainty of estimates is presented in the form of 95% confidence intervals for clarity only positive error bars have been included. When error bars are not visible it is because they are smaller than the symbols used to represent the data point.

# Figures and Tables

**Table I**
**Details of the constants from the mean total crack length against frequency curve fits.**

| Load range (N) | Total crack length ($c$) curve fit | | | | |
|---|---|---|---|---|---|
| | A | B | D | $R^2$ | *P*-Value |
| 6-60 | -0.32 | 0.32 | 0 | 1 | 0 |
| 9-90 | 0.03 | 0.51 | 0 | 1 | 0 |
| 10-100 | 0.12 | 0.56 | 0.1 | 1 | 0 |
| 12-120 | -0.1 | 0.75 | 0.63 | 1 | 0 |
| 16-160 | 0.3 | 0.99 | 1.18 | 1 | 0 |

The total crack length is described by lines of the quadratic form for each load range. They are characterized by constants A, B and D. $R^2$ is the squared correlation coeffi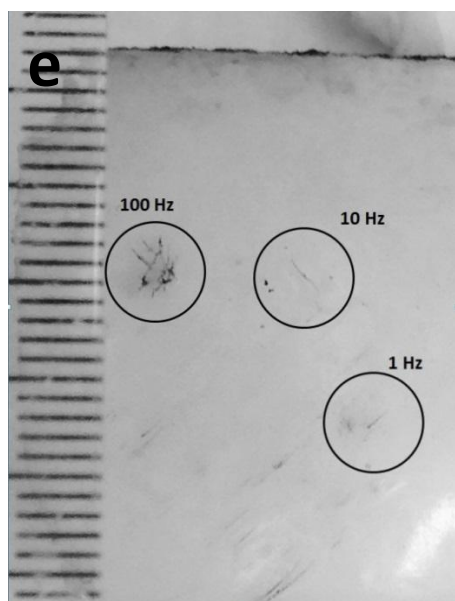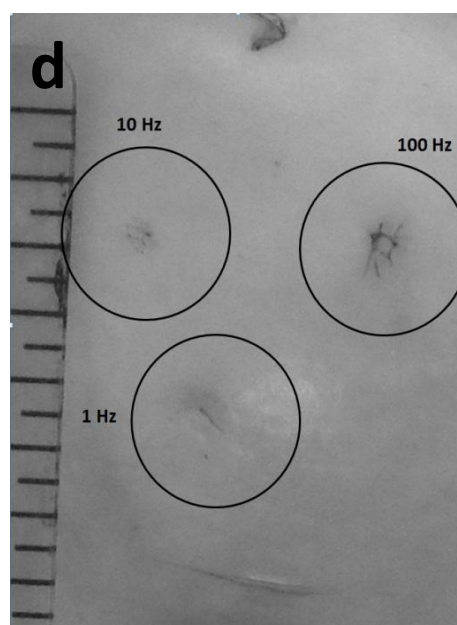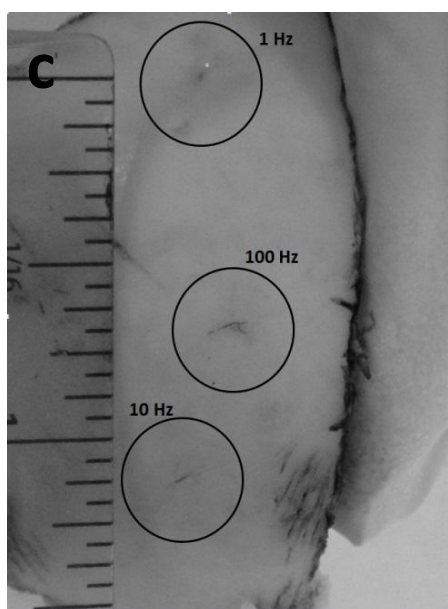cient and shows how well the line fits the data points. If P<0.05 it indicates that the lines are statistically significant.

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┌─────────────────────────────┐
│  - 10 animals               │
│  - 20 shoulder joints       │
│  -  40 tissue samples       │
└─────────────────────────────┘
```

| 6-60 N | 9-90 N | 10-100 N | 12-120 N | 16-160 N |

| - 8 tissue samples<br>- 5 joints<br>n=3 | - 8 tissue samples<br>- 6 joints<br>n=3 | - 8 tissue samples<br>- 4 joints<br>n=2 | - 8 tissue samples<br>- 5 joints<br>n=3 | - 8 tissue samples<br>- 5 joints<br>n =3 |
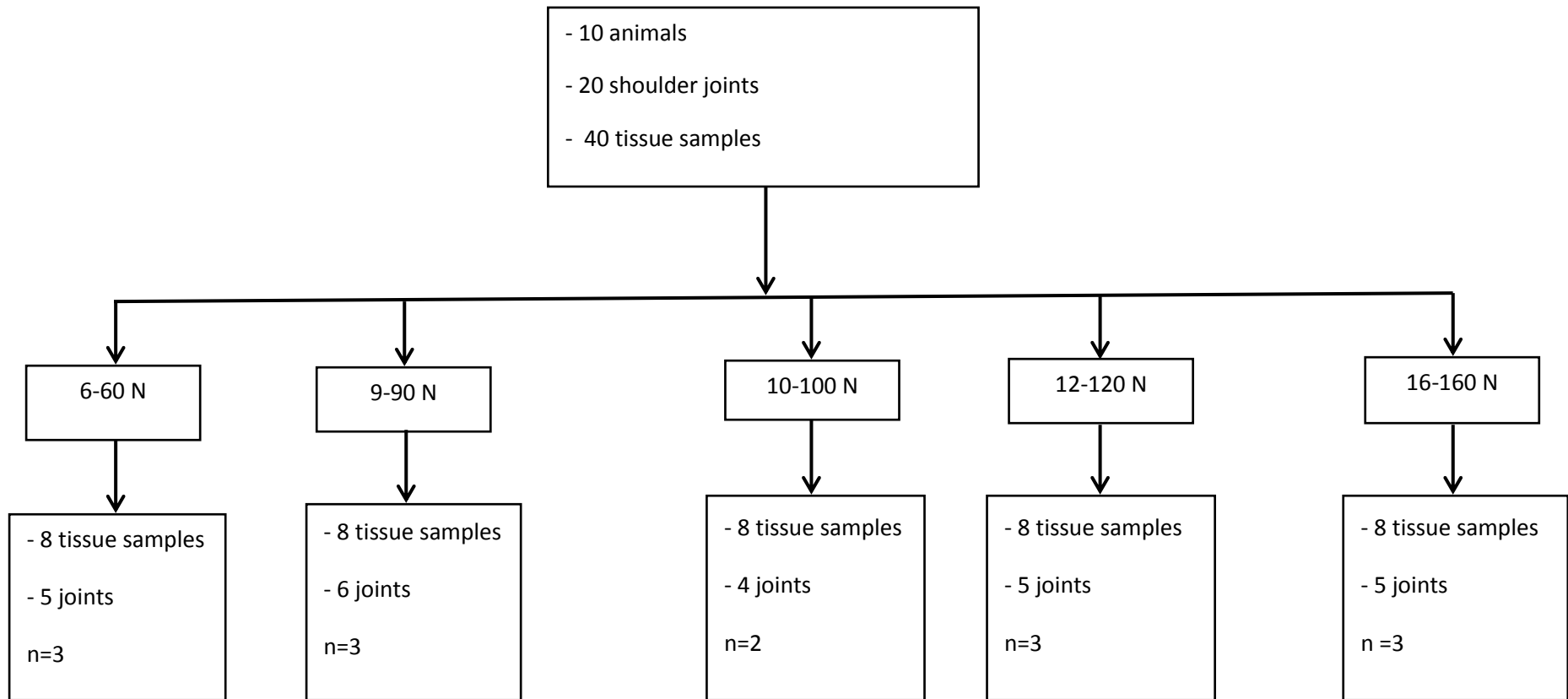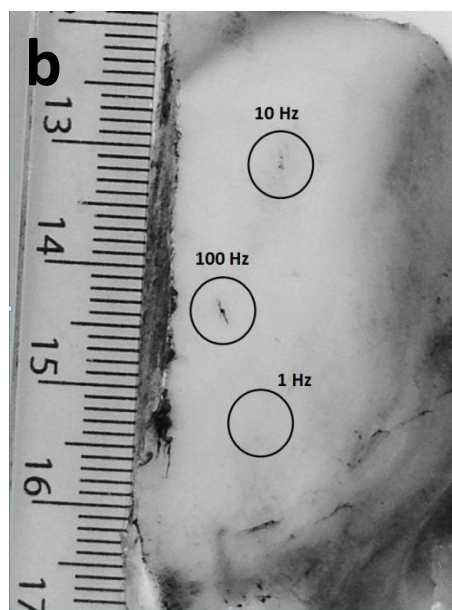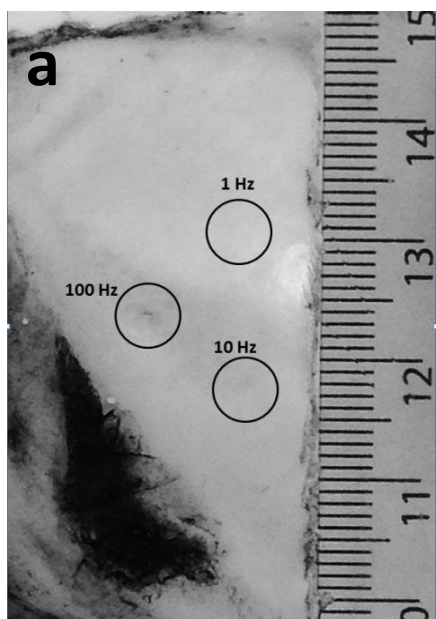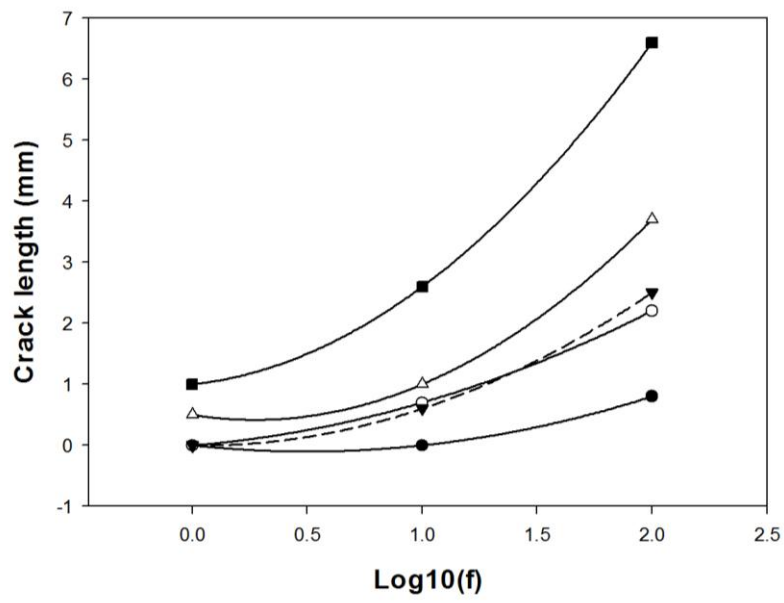
**Figure 1**

**Figure 2**

**Figure 3**

**Figure 4**

**Figure 5**