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# EFFECT OF INDENTER VELOCITY ON THE STIFFNESS OF ARTICULAR CARTILAGE

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## Introduction

Indentation is commonly used for non-destructive mechanical analysis of articular cartilage in healthy and diseased states. However, whilst mechanical property variation arising from indenter size has been investigated (Bae et al., 2003; Franke et al., 2007; Simha et al., 2007) a systematic analysis of the effect of other experimental factors has not been performed. Therefore, this study assessed the effect of conducting multiple indentations in one place and indentation velocity on derived stiffness and Young's modulus values.

## Methods

Eight bovine patellars were obtained from the local abattoir. From each patellar, three 5mm square osteochondral plugs were removed from the central region. The samples were washed in phosphate-buffered saline (PBS) overnight to remove surface synovial fluid. Samples were subsequently mounted in Plaster of Paris in a Petri dish whilst ensuring the surface was horizontal. PBS was added to the Petri dish to the level of the articulating surface to maintain sample hydration.

A MFP Nanoindenter (Asylum Research, Santa Barbara, CA) fitted with a Berkovich tip indented the articular cartilage surface to a depth of 5  $\mu\text{m}$  at loading and unloading velocities of 0.5  $\mu\text{m/s}$ , 5 $\mu\text{m/s}$  and 50  $\mu\text{m/s}$  using a triangular loading pattern. Ten indentations were performed in three positions on each sample, at least 1mm away from the sample edge. Ten seconds separated each indentation with a further 3 minutes gap between groups of 3. The slope of the unloading curve was evaluated yielding the contact stiffness ( $S$ ) at the given load and Young's modulus ( $E$ ) was calculated using standard methods.

Data were analysed using ANOVA evaluating differences in  $S$  and  $E$  due to indentation number, sample position, and animal. Significance was taken at  $p < 0.05$ . Data are reported mean  $\pm$  S.E.M.

## Results

Bovine patellar cartilage had an overall mean stiffness of  $337.34 \pm 9.547$  N/m and a Young's

modulus of  $9.6 \pm 0.29$  MPa ( $N = 2120$ ).  $S$  and  $E$  varied with animal, sample position, indentation number and indentation velocity ( $p < 0.01$ ). There was no consistent trend associated with indentation number, whilst the centrally located sample on the patellar was less stiff than proximal and distal samples. Post hoc analysis suggested 50  $\mu\text{m/s}$  elicited lower  $S$  and  $E$  compared to 0.5  $\mu\text{m/s}$  and 5 $\mu\text{m/s}$  ( $p < 0.001$ ).

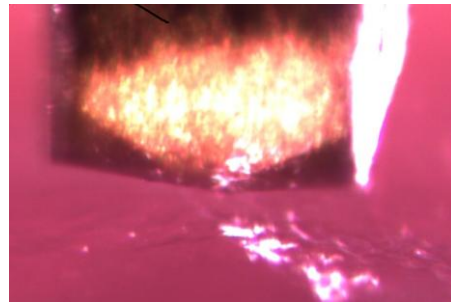


Figure 1: Cartilage surface being indented

## Discussion & Conclusion

Our overall mean value for  $E$  of bovine patellar cartilage is consistent with literature values for our indenter size (Franke et al., 2007, Simha et al., 2007). Since there was no consistent effect due to multiple testing in a single place, this suggests that a repeated measures protocol is appropriate for this tissue. The largest variation in  $S$  and  $E$  values were observed between animals implying that whilst our method was sufficiently powered, natural biological variation overshadowed experimental factors. The variation due to indenter velocity suggests that time-dependent surface relaxation affects the measured stiffness.

In conclusion, our data suggests that a slow retraction velocity should be employed to characterise the elastic response of the articular cartilage surface, thereby avoiding time-dependent effects.

## References

- Bae *et al*, Arthritis & Rheumatism, 48:3382-3394, 2006.
- Frank *et al*, Acta Biomaterialia, 3:873-881, 2007.
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