

CARTILAGE CELL VIABILITY AFTER IN VIVO IMPACT LOADING

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INTRODUCTION

Injury to articular cartilage is thought to be one of the initiators of osteoarthritis. One possible mechanism of cartilage degeneration is that cells are killed due to impact. The resulting metabolic load on the remaining cells prevents them from properly maintaining the extracellular matrix, leading to matrix degeneration that further impairs the ability of the cartilage to support normal loads leading to further degeneration. Most impact models have used cartilage explants. The validity of taking cartilage out of its natural environment and testing isolated sections is suspect. The only other in vivo impact model in an intact joint has looked at mechanical properties of the cartilage after impact and not biological markers such as cell viability (Haut et al. 1995). The purpose of this study was to look at cartilage viability at different impactation energies in an in vivo rabbit model.

METHODS

14 New Zealand white rabbits were used for this study. Rabbits were injected with acepromazine and then anaesthetized with isoflurane. They were mounted in a custom stereotaxic frame where their hips were pinned and experimental knee supported at approximately 90°. In 13 rabbits, the patella was subjected to either 0J, 2.5J, 5J or 7.5J impacts by dropping a weight from a specific distance. One rabbit knee was subjected to 10 >5J impacts. Cartilage from the patella and the loaded portion of the femoral groove was harvested 24 hours later and 50 µm full thickness sections were prepared with a vibratome. Slices were stained with Syto 13 and Ethidium Bromide and were then viewed under a fluorescent microscope and photographed with a digital camera. Live (green) and dead (red) cells were counted with a custom cell counting program.



Figure 1: Impact set-up showing impactor in approximation to the rabbit's patella.

RESULTS AND DISCUSSION

Cell viability in the first 13 rabbits ranged from 72 -86%. Ten impacts of the patella caused cell viability to drop to 57%. There was a trend that cell death was occurring more at the intermediate and middle layers than the superficial layer.

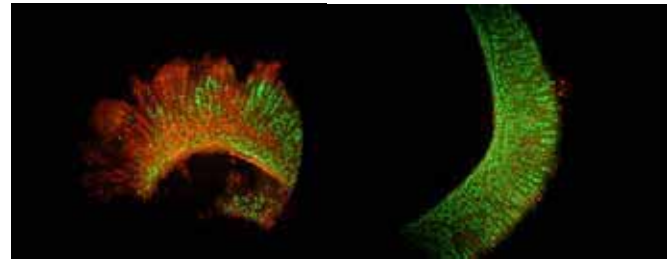


Figure 2: Two sample patella sections. The left section was subject to 10 impacts while the right section was a control (0J).

The cell viability protocol used was not sensitive enough to detect differences between the single impact conditions. This was due to high baseline cell death and variability in cell death in all samples including controls. Impact of the cartilage of one knee 10 times resulted in a much more consistent level of cell death throughout the samples.

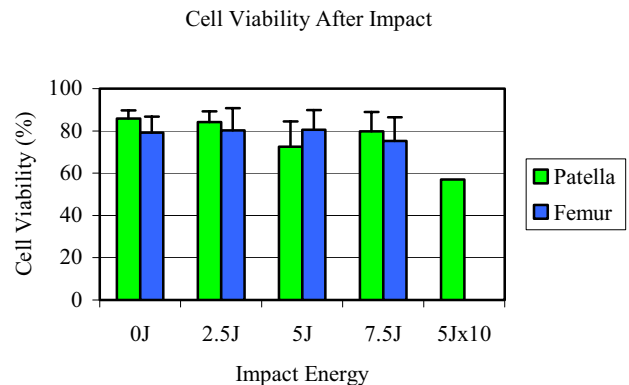


Figure 3: Cell viability for each impact energy.

CONCLUSIONS

Cell viability testing needs to be refined to reduce variability. Future studies will look at cell viability using a confocal microscope to minimize artefacts from cutting the tissue. Despite problems with variability, it appears that cartilage is much more robust in vivo than in vitro explants tests have suggested.

REFERENCES

Haut, R.C., Ide, T.M. and De Camp, C.E. (1995) *J Biomech Eng* **117**, 402-8.

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