

Direct current influences Ca^{2+} signalling in chondrocytes

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INTRODUCTION: It is well established that there exists a coupling of the electric fields detected within cartilage tissue to the mechanical deformation to which the tissue is subjected [1,2]. The mechanism by which this coupling occurs is yet undetermined. In neural and muscle cells electric fields instigate the opening of voltage operated channels causing Ca^{2+} ion influx into the cytosol. The present study investigates the effect of electric current on Ca^{2+} signalling in chondrocytes.

METHODS: *Cell Preparation:* Full depth bovine articular chondrocytes were isolated from the metacarpophalangeal joint and seeded at 10^7 cells/mL in agarose^[3]. Following a 24 h equilibration period, the constructs were labelled with 5 μM Fluo-4 AM in bicarbonate free (BF) medium for 1 h at 37°C. Thereafter, constructs were washed for 10 min in BF medium. Selected constructs were treated with 10 μM verapamil in BF medium during the 10 min wash period. *Electric stimulation:* Each construct was mounted in a Perspex chamber containing its corresponding wash medium and connected by agarose saltbridges to platinum electrodes in dilute KCl solutions (Figure 1). A current density of 4 mA/cm^2 , supplied by an electrophoresis power pack, was passed through the construct for 10 min. Cell viability was maintained at > 95%. *Calcium imaging:* Images of 10-30 cells per field of view per construct were visualised using a confocal microscope associated with an inverted microscope and captured with a x 20 plan apo lens every 4 s for the 10 min imaging period.

RESULTS: The application of (direct current) dc did not alter the percentage of cells exhibiting a Ca^{2+} response. For cells that were not exposed to dc, verapamil significantly reduced the percentage of cells showing a response. By contrast, verapamil did not influence the response when cells were exposed to dc.

DISCUSSION & CONCLUSIONS: These data suggest that dc did not affect overall Ca^{2+} response. However, the application of dc did appear to alter the mechanism of the response. In the absence of dc the response could be markedly reduced by verapamil suggesting a mechanism dependent on voltage operated channels. During the application of dc, verapamil did not affect the response,

implying that Ca^{2+} influx is independent of voltage operated channels. This suggests that some mechanism other than the opening of voltage operated Ca^{2+} channels may influence chondrocyte response to electrical stimulation.

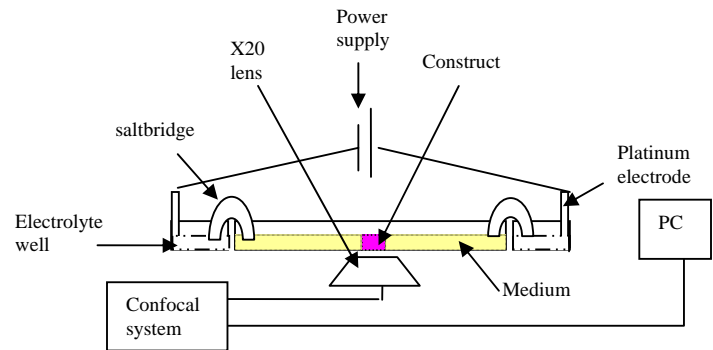


Fig. 1: Schematic of the electrical stimulation chamber

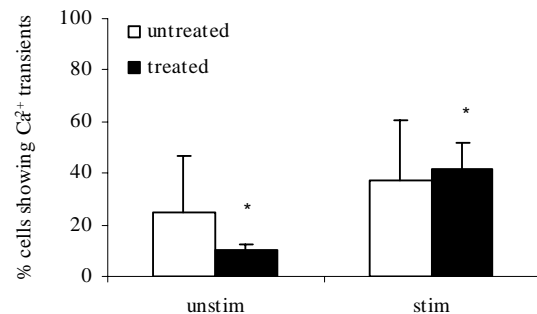


Fig. 2: The effect of dc and/or verapamil on Ca^{2+} response in chondrocytes. Data represents the mean \pm SD of 2 cell isolations

REFERENCES: [1] Biosynthetic response to mechanical and electrical forces in *The Biology of Tooth Movements* (eds L.A. Norton and C.J. Burstone) CRC Press, Inc pp 335-347. [2] N. Szasz et al., (2003) *Meet Orthop. Res Soc.* [3] D.A. Lee and D.L. Bader (1995) *In vitro Cell Dev Biol Anim* 31(11):828-835.

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