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**Introduction:** Chondrocytes are known to respond to their mechanical environment, and alter their metabolism accordingly, through the process of mechanotransduction. This process is poorly understood and may involve numerous intracellular mediators including calcium. Intracellular calcium signalling is involved in processes as diverse as cell division and apoptosis. Moreover, calcium transients in chondrocytes have been observed following stimulation by fluid flow and micromanipulation [1, 2]. Sources of increased intracellular calcium may include entry across the plasma membrane via the emptying of intracellular stores. However, calcium signalling in chondrocytes following a more physiological mechanical stimulus such as compression has not been investigated. In the present study, a well established and phenotypically stable model system has been used to test the hypothesis that mechanical compression induces an increase in intracellular calcium in chondrocytes.

**Materials and Methods:** Chondrocytes were isolated from the metacarpalphalangeal joint of 18 month old steers by sequential enzyme digestion and seeded in 4% agarose at a cell density of 10 x 10^6 cells.ml^-1. Agarose constructs were produced (4mm x 3mm x 3mm) and cultured in DMEM + 20% FCS for up to three days. Constructs were labelled with the calcium-sensitive ratiometric fluorescent stain, Indo-1/AM (10 µM) in EBSS (Earl’s Balanced Salt Solution (EBSS), (1.8mM-Ca²⁺; 20mM-Hepes; pH 7.4). Cells were visualised using a real-time confocal laser scanning microscope (Noran Instruments, Milton Keynes, UK). Fluorescent light from calcium bound (405 nm) and unbound (495 nm) Indo-1 was detected simultaneously and intensity values divided (405/495) to provide a ratio value proportional to and indicative of changes in calcium concentration.

On days 1 and 3 of culture, basal calcium ratio measurements were calculated for samples of at least 200 cells in unstrained agarose constructs. In a separate study, individual chondrocytes were visualised in an unstrained construct at intervals of 3 seconds for a 2 minute period to establish calcium baseline values. 20% compressive strain was applied to the construct and the same cells were monitored for up to 5 minutes in the compressed state. The process was repeated for a total of 98 and 68 cells on days 1 and 3 respectively.

**Results:** Basal unstrained calcium levels showed no difference with time in culture with mean values of 1.02 ± 0.29 and 1.03 ± 0.29 on days 1 and 3 respectively. Cells subjected to 20% strain deformed from a spherical to an oblate ellipsoid morphology. A representative chondrocyte image in an unstrained and compressed agarose construct is shown in figure 1a with resulting calcium ratio values. On day 1, 51% of deformed cells exhibited a calcium response compared to 8% of control cells (Fig. 2a). By day 3, 34% of strained cells responded when compared to 7% in the control group. The majority of responses observed were transient in nature lasting between 30 and 90 seconds following a delay of approximately 150 seconds after the application of strain. Cells compressed on day 1 in the presence of EGTA showed a decrease in the number of calcium responses compared to the number of calcium responses observed in deformed cells in unsupplemented EBSS (Fig. 2b). In addition, the presence of Gd⁴⁺ also reduced the number of responses in compressed cells. Unstrained measurements in all groups showed similar results.

**Discussion:** This study demonstrates that cell deformation leads to an increase in intracellular calcium on days 1 and 3. Differences observed between days 1 and 3 may be due to a decrease in the degree of cell deformation as a result of the elaboration of the pericellular matrix [3]. Re-organisation of cytoskeletal components has been observed over the first 3 days in culture [4] which may alter the action of stretch-sensitive ion channels and therefore reduce the number of responses. The presence of EGTA and Gd⁴⁺ reduced the number of responses observed suggesting a requirement of extracellular Ca²⁺ (or influx) across the plasma membrane possibly through stretch-sensitive ion channels. These results constitute an important initial stage in the understanding of the physiological role of intracellular calcium in chondrocytes under a mechanical influence.

**References:**

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