**TEMPORAL CHANGES IN CELL AND NUCLEUS DEFORMATION IN ISOLATED CHONDROCYTES COMPRESSED IN ALGINATE SCAFFOLDS**

**Introduction** The health of articular cartilage *in vivo* is dependent on physiological loading, which results in compression of the tissue and cell deformation [1]. Consequently, there is an increasing interest in the use of mechanical conditioning applied to isolated cells in 3D scaffolds in order to achieve optimal tissue engineered cartilage repair. However, little is known of the mechanotransduction pathways within either cartilage or cell seeded scaffolds. Alginate gel has been proposed as a suitable scaffold for cartilage repair [2]. The present study examines the influence of compressive strain on the potential signalling pathways involving cell and nucleus deformation within chondrocytes seeded in alginate scaffolds.

**Methods** Chondrocytes were isolated from bovine metacarpalpalphangeal joint cartilage using pronase and collagenase and seeded in either 1.2% or 2% GMB low viscosity alginate (Kelco). The cell-alginate was crosslinked in 100 mM CaCl2 for 15 minutes and then cut into half cores, 5mm in height. The half cores were secondarily crosslinked in 100 mM CaCl2 for 15 minutes and then cultured overnight in DMEM + 20% FCS at 37°C. Samples of cells (n=100) in both 1.2% and 2% alginate specimens were visualised over a 60 minute period of 20% compression. Cell diameters were measured. Alginate (X) and perpendicular (Y) were considered in a plane orthogonal to the compression and the deformation index (X/Y) calculated. Analysis of nucleus deformation was conducted using only the 2% alginate constructs. Individual cells and their nuclei were imaged first in the unstrained state and twice in the compressed state at 0-10 minutes and 15-30 minutes following compression. The compression induced percentage changes in cell and nucleus diameters were recorded.

**Results** The relaxation moduli measured 60 minutes after compression for 1.2% and 2% alginate were 1.8 KPa and 2.8 KPa respectively. In both alginites 20% compression resulted in immediate cell deformation from a spherical (X/Y=1.0) to an oblate ellipsoid morphology (X/Y=0.7). In 2% alginate this level of cell deformation was maintained over a 60 minute period. By contrast in 1.2% alginate there was a statistically significant increase in deformation index (Fig. 1) indicating a relaxation in cell deformation towards a spherical morphology. Measurement of individual cells in 2% alginate indicated that compression resulted in a reduction in X diameter and corresponding increase in Y diameter for both cell and nucleus as shown in Figure 2. There was no significant change in the level of cell diameter strains between the 2 time points but a significant relaxation in the nucleus deformation, particularly in the Y axis.

This study indicates that compression of cell seeded alginate scaffold resulted in cell deformation characterised by a reduction in X diameter and an increase in Y diameter, consistent with conservation of cell volume. The level of cell deformation in 1.2% alginate reduced in line with the viscoelastic stress relaxation exhibited by the alginate, such that after 60 minutes the cells were almost spherical. This suggests that at this time point the modulus of the cells was greater than the relaxation modulus of 1.2% alginate but less than that of 2% alginate, in agreement with previous studies [4]. Cell deformation in 2% alginate resulted in deformation of the nucleus, although to a lesser extent than that experienced by the cell indicating that the nucleus is stiffer than the cytoplasm. The relaxation in nucleus deformation, despite the constant cell deformation, may be attributed to cytoskeletal reorganisation. Alternatively, the decrease in nucleus Poisson’s ratio from 0.41 to 0.17 suggests a reduction in nucleus volume.

**Discussion** Compression of cell seeded alginate scaffold resulted in cell deformation characterised by a reduction in X diameter and an increase in Y diameter, consistent with conservation of cell volume. The level of cell deformation in 1.2% alginate reduced in line with the viscoelastic stress relaxation exhibited by the alginate, such that after 60 minutes the cells were almost spherical. This suggests that at this time point the modulus of the cells was greater than the relaxation modulus of 1.2% alginate but less than that of 2% alginate, in agreement with previous studies [4]. Cell deformation in 2% alginate resulted in deformation of the nucleus, although to a lesser extent than that experienced by the cell indicating that the nucleus is stiffer than the cytoplasm. The relaxation in nucleus deformation, despite the constant cell deformation, may be attributed to cytoskeletal reorganisation. Alternatively, the decrease in nucleus Poisson’s ratio from 0.41 to 0.17 suggests a reduction in nucleus volume.

This study indicates that compression of cell seeded alginate scaffolds results in potential mechanotransduction events involving cell and nucleus deformation. The relaxation of nucleus deformation with possible volume reduction indicates temporal changes in these signalling pathways. Furthermore, the mechanical properties of the scaffold relative to those of the cell clearly influence the level of cell deformation and hence the feasibility of mechanical conditioning.

**References**


**Fig. 1** Deformation indices for cells in 1.2% (A) and 2% (A) alginate compressed to 20% strain for a period of 60 minutes. A statistically significant positive correlation was observed for 1.2% but not 2% alginate.

**Fig. 2** Mean cell and nucleus diameter strains in 2% alginate measured at two time points following compression. Error bars indicate SEM, n=20-30. Statistically significant differences between the two time points have been indicated based on a paired Student t test (*p<0.05, ***p<0.01).