

Connexin43 expression in cartilage progenitor cells and its possible role in cell differentiation.

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INTRODUCTION: Recent research has identified the presence of a progenitor cell population in the surface zone of articular cartilage¹. These cells have been shown to possess an extended cell cycle, be capable of forming large colonies from a single cell and can engraft functionally into a variety of connective tissues. It is thought these cells are required for the appositional growth of the tissue. During normal skeletal development, one of the earliest events seen is the formation of a dense cell mesenchymal cell condensation. It is thought that an increase in gap junction formation within this cell condensation is responsible for passing signals between cells and directing differentiation². A novel non-invasive method for observing cell interactions *in vitro* has recently been developed using an ultrasound wave. When placed in the ultrasound wave trap cells have been shown to form 2D aggregates without altering the cells in any way³. In this study, the ability of the progenitor cells to express connexin43 and form functional gap junctions was examined.

METHODS: Initially cells were suspended in an ultrasound trap to create the formation of cell aggregates which were immunolabelled for connexin43. To determine if these connexin molecules were capable of forming functional gap junctions, cells were labelled with the gap junction permeable cell tracker CMFDA. Three groups of cells were used: surface zone cells (containing approximately 0.5% progenitors and 99.5% differentiated chondrocytes), a clonal progenitor population and surface/progenitor mix. In each case a 1:3 ratio of labelled:unlabelled cells was used.

RESULTS: Although the progenitor cells do express connexin on their surface, they are incapable of transferring dye between cells. Non-progenitor cells isolated from the surface zone of articular cartilage are able to form functional gap junctions almost immediately after aggregate formation. When these cells are mixed with unlabelled progenitor cells, dye transfer also occurs from the non-progenitor surface zone cells toward the progenitor cells

DISCUSSION & CONCLUSIONS: It has already been demonstrated that connexin expression and gap junction formation are vital for articular cartilage development. This study shows that populations of progenitor cells isolated from the surface of bovine articular cartilage express connexin43 in culture. After aggregate formation, connexin43 expression occurs predominantly at regions of cell-cell contact. Despite this the cells are incapable of forming functional gap junctions with other progenitor cell populations after one hour in the ultrasound trap. Mixing the progenitor cells with terminally differentiated cells does however result in dye transfer, suggesting that they are capable of communicating with more differentiated cells types. It is possible that signals transferred by mature cells within the surface of cartilage could be a signal for the progenitor cells to undergo differentiation.

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