

ARTICULAR CARTILAGE PROGENITOR CELLS: CHONDROGENIC POTENTIAL DURING EXPANSION IN MONOLAYER CULTURE

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INTRODUCTION: Current strategies in cartilage repair are based on the transplantation of cells from a variety of sources into the defect in question in order to generate a functional repair tissue. Difficulties arise in the use of chondrocytes due to a limited mitotic ability of mature chondrocytes *in vitro* and rapid loss of phenotype, known as dedifferentiation, in monolayer culture [1]. Previous research has identified a population of articular cartilage progenitor cells in the superficial zone of articular cartilage with an extended cell cycle time, a high affinity for fibronectin in cell adhesion assays, ability to form colonies *in vitro* from a low seeding density [2]. Here we report on the isolation and extensive subculture of these cells together with an evaluation of their chondrogenic ability at various passages by transfer into pellet culture.

METHODS: *Cell isolation:* Superficial zone cells were isolated by sequential digestion in pronase and collagenase of superficial zone cartilage from the articular cartilage of 2-3 week old bovine metatarsophalangeal joints. After isolation, 4000 superficial zone cells in serum free DMEM were seeded into wells of fibronectin coated 6-well plates and incubated at 37°C for 20 minutes. After 20 minutes, the media was removed and discarded. Fresh DMEM containing 10% FCS was added to each well. *Expansion in Culture:* Cells were subsequently transferred into 75cm² and 175cm² culture flasks. Growth was maintained by continual passaging at a ratio of 1:3. At various passages aliquots of cells were removed for pellet culture and Real-time PCR (Taqman). Controls consisted of normal chondrocytes isolated from the full thickness of bovine articular cartilage. *Pellet Culture:* Aliquots of 250,000 cells were resuspended in a serum free chondrogenic media containing TGFβ-1 and centrifuged to form a pellet. Pellets were incubated for 14 days and then paraffin embedded and sections stained with Safranin O/haematoxylin and immunolabelled with antibodies to collagen I and II. Taqman was used to quantify expression of collagen I, II, aggrecan and versican mRNA in the pellets.

RESULTS: *Growth:* Articular cartilage progenitor cells underwent approximately 61 population doublings over 162 days in culture. At P0 the cells had a doubling time of approximately 24 hours where after the cells maintained a moderately constant rate of growth with cells requiring subculture every 3-4 days. This was maintained until P28 when the rate of growth declined with subculture being required every 7-8 days. After P35 cell growth had virtually stopped. *Pellet Culture:* Articular cartilage progenitor cells expanded up to P22 and grown in pelleted micromasses synthesised a hyaline-like cartilage matrix that stained strongly with Safranin O. P1, P3, P6, P9 and P12 (25 pop doublings) pellets were rich in collagen II protein and mRNA whereas pellets derived from P22 (42 population doublings) cells contained low levels of collagen II. Pellet cultures of freshly isolated normal chondrocytes also stained strongly with Safranin O and contained abundant collagen II. Dedifferentiated normal chondrocytes at P8 contained negligible collagen II.

DISCUSSION & CONCLUSIONS: Although this study has illustrated the high expansion potential of articular cartilage progenitor cells, more importantly, the cells retain the ability to synthesise a cartilage-like hyaline matrix rich in collagen II even after 12 passages (25 pop doublings). In contrast, normal bovine articular chondrocytes rapidly dedifferentiated in monolayer and completely lost the ability to redifferentiate in pellet culture at P8 (13 pop. doublings). This important property to retain the ability to form cartilage after extensive expansion in culture may enable the generation of large cell banks for use in allogeneic tissue engineering applications. Investigations are currently focusing on developing human articular cartilage progenitor cells isolation and expansion protocols and employing these cells in cartilage repair strategies.

REFERENCES: ¹K. Von Der Mark, V. Gauss, H. Von Der Mark, P. Muller. (1977) *Nature* 267, 531-2.
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