

Equine Cartilage Repair: An *in vitro* comparison between articular cartilage progenitor cells and bone marrow derived stromal cells.

H. E. McCarthy¹, J Bara², S. Singhrao¹, C.Archer¹

¹Connective Tissue Biology Labs, School of Biosciences, Cardiff University, Wales, GB.

²Spinal Studies & ISTM, Keele University, Shropshire, UK.

INTRODUCTION: Acute articular cartilage injuries are a major concern for the athletic horse. Partial thickness defects are non-healing and although full thickness defects may elicit an intrinsic healing response, the repair tissue formed is fibrocartilaginous and functionally inferior to the native tissue.

Chondrocytes and mesenchymal stem cells are the two main candidate cell sources for cell based therapies, however, it is still unclear as to which is most appropriate. A chondrocyte progenitor population isolated from the surface zone of bovine and human articular cartilage may present a novel cell source for cell-based articular cartilage repair. Here, equine articular cartilage progenitor cells (ACPC) and equine bone marrow-derived stromal cells (BMSC) are compared as potential cell sources for equine cartilage repair.

METHODS: Distal forelimbs of five horses aged between 2 and 8 years were obtained from an equine abattoir. ACPC were isolated from the surface zone of cartilage from the metacarpophalangeal joint by adhesion onto fibronectin¹ whilst BMSC were isolated from bone marrow stroma from the cannon bone using the classic plastic adhesion method. Monolayer cultures of both cell types were expanded in the presence of bFGF. Cells were subsequently fixed and immunolabelled for Notch-1, CD166 and CD90.

The differentiation potential of ACPC and BMSC was compared. Both chondrogenic and osteogenic differentiation was performed in 3D pellet cultures whilst adipogenic differentiation was induced in monolayer culture. Evidence for differentiation was made via histological evaluations.

RESULTS: Clonally derived BMSC and ACPC were expanded through a minimum of 20 population doublings in 21 days. Immunolabelling showed both ACPC and BMSC expressed the cell fate selector gene, Notch-1, and the putative stem cell markers CD90 and CD166.

Chondrogenic induction in both cell types produced pellets that were smooth and

iridescent in appearance and stained positively for toluidine blue and safranin O. Immunohistochemistry revealed positive labelling for type II collagen and aggrecan. Type X collagen was not detected in ACPC pellets but was detected in all BMSC pellets indicating a hypertrophic cartilage phenotype.

Osteogenic induction of ACPC and BMSC produced pellets that were irregular and matt in appearance and were positive upon von Kossa staining. Immunohistochemistry revealed positive labelling for osteocalcin suggesting bone formation. Adipogenic induction in both cell types revealed a positive result via oil red O staining.

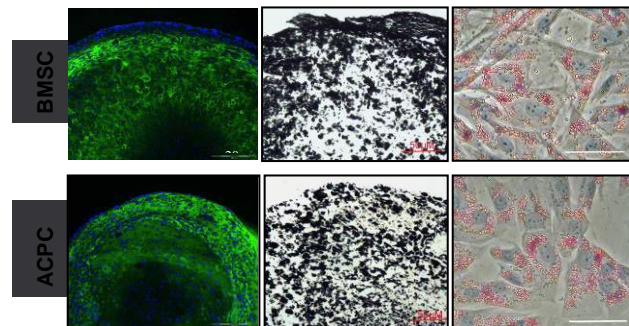


Fig. 1: Type II collagen labelling in chondrogenic induced pellet cultures (left), von Kossa staining in osteogenic induced pellet cultures (middle) and oil red O staining in adipogenic induced monolayer cultures (left).

DISCUSSION & CONCLUSIONS: ACPC and BMSC have demonstrated functional equivalence in their differentiation capacity. However, ACPC may be considered superior to BMSC in producing cartilage capable of functional repair. BMSC became hypertrophic; this is considered disadvantageous to any repair process as cells may undergo terminal differentiation and differentiate into osteoblasts. Additionally, ACPC hold an advantage over normal chondrocytes as they can divide to produce more cells at a faster rate and maintain their phenotype when cultured extensively.

REFERENCE: ¹ Dowthwaite et al (2004). J. Cell Sci. 117, 889-897.