

Survival of Bone Marrow Stromal Cells within Hydrogels: A comparison to Nucleus Pulposus Cells and Articular Chondrocytes

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INTRODUCTION: Bone marrow stromal cells (BMSC) are good candidates for cell based tissue regeneration strategies (e.g. for the Intervertebral disc) due to their proliferation and differentiation capacity. Most often BMSC are cultured in a 3D hydrogel matrix (e.g. alginate and agarose). For substantial tissue regeneration, survival of these cells inside these scaffolds is essential. Up to date, little is known about the fate of BMSC within these gels. We hypothesize that ACs and NP cells will thrive within these gels, in contrast to undifferentiated BMSCs, but that chondrogenic differentiation of the latter is expected to increase their survival.

METHODS: Bone marrow was harvested from five calves. BMSCs were isolated based on their capability to attach to the culture flasks. NP cells and ACs from the same animals were isolated by standard sequential pronase and collagenase digestion. Primary NP cells and ACs and passaged BMSCs (P2) were cast in 1.2% alginate or 2% ultra low gelling agarose (4 dia. x 2 mm discs) and cultured for 7 days in DMEM supplemented with 10% FCS, 50µg/ml ascorbic acid-2-phosphate, 1% non-essential amino acids (NEAA) and 1% Penicillin/Streptomycin. Half the BMSCs were cultured in chondrogenic medium (DMEM supplemented with 1% ITS+, 50µg/ml ascorbic acid-2-phosphate, 1% NEAA, 1% Penicillin/Streptomycin, 10ng/ml TGFβ₁ and 10⁻⁷ M Dexamethasone). At days 0 and 7, number of living cells, DNA, and chondrogenic gene expression was assessed. Cryosections were stained for collagen type II.

RESULTS: In 7 days, the amount of living NP cells and ACs increased in both scaffold types compared to day 0. In contrast, number of living BMSC decreased in both hydrogels, which was ameliorated to some extent by the addition of TGFβ₁. DNA confirmed this trend (figure 1). NP cells and ACs both expressed the 'chondrogenic' genes and were stained positively for collagen type II. TGFβ₁ pushed

the BMSCs towards chondrogenic lineage by up-regulating their mRNA expression of collagen II and aggrecan. Furthermore, in samples cultured in the presence of TGFβ₁ collagen type II was demonstrated.

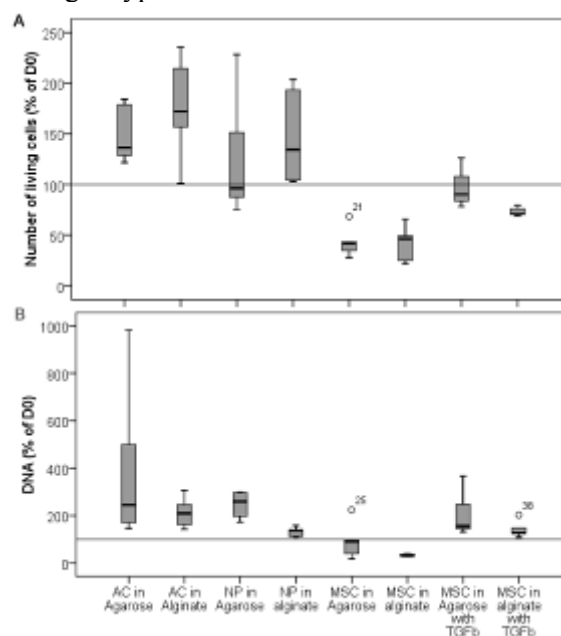


Figure 1: BMSC, NP cell and AC survival in agarose (Ag) and alginate (Al) at day 7, assessed by Live/dead (A) and DNA quantification (B)

DISCUSSION & CONCLUSIONS: Under the chosen conditions, these hydrogels appear to provide an appropriate environment for NP cells and ACs as indicated by their proliferation in culture and maintenance of phenotype. However, only a small fraction of the initially seeded undifferentiated BMSC could thrive in the same hydrogels under the same conditions. Since cell number and hence cell survival is a key factor for tissue regeneration, cell survival of BMSC within hydrogels should be improved. Addition of TGFβ₁ to the cell culture resulted in chondrogenic induction and improved culture survival, but only to a limited extent. Hence, pre-conditioning of BMSCs may be a worthwhile strategy.