

## Synovial Fluid Stem Cells: A Potential Cell Source for Cartilage Tissue Engineering

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**INTRODUCTION:** Adult mesenchymal stem/progenitor cells (MSCs) are potentially useful for engineering cartilage tissue because of their ease of proliferation and good chondrogenic capacity given the appropriate differentiation signals. However, chondrogenic cells derived from differentiating MSCs isolated from bone marrow have been reported to undergo hypertrophy and mineralization *in vivo*<sup>1</sup>. Recently Jones et al<sup>2</sup> have demonstrated the presence of mesenchymal stem cells (MSCs) synovial fluid of patients with arthritis and normal SF fluid. The aim of this study was to isolate SF-derived MSCs from normal SF and human osteoarthritic SF and investigate their utility for cartilage tissue engineering using PGA scaffolds.

**METHODS:** MSC cultures were isolated from synovial fluids and cultured for at least 24 population doublings prior to conventional *in vitro* functional assays of chondrogenesis, adipogenesis and osteogenesis. Cultures of bovine chondrocytes were used as for a comparator. Scaffolds of PGA (Cellon, 5mm diameter, 2mm thick) were seeded with 2 x 10<sup>6</sup> MSCs or chondrocytes<sup>3</sup>. Some constructs were cultured in Dulbeccos Modified Eagle's medium, 3% FCS, 10 mM HEPES, non-essential amino acids, 1 mg/ml BSA, insulin/transferrin/selenium, 10<sup>-7</sup>M dexamethasone, penicillin and streptomycin, 10 ng/ml transforming growth factor  $\beta_1$  (TGF  $\beta_1$ ) for the complete incubation period. Others were incubated with TGF  $\beta_3$  and TGF  $\beta_1$ . Polyclonal MSC cultures were also pre-cultured in alginate gel for 14 d in medium supplemented with TGF  $\beta_1$  prior to seeding on PGA. Constructs were mounted in OCT, and frozen sections taken for analysis of the extracellular matrix. Collagen I, and II were detected immunochemically. Proteoglycans were detected as glycosaminoglycans (GAGs) and localized using Alcian Blue or Toluidine Blue and quantified using dimethylmethylene blue.

**RESULTS:** SF-derived MSCs readily seeded onto the PGA scaffolds (96%). Immunohistochemical staining indicated all constructs produced some extracellular matrix with deposition of collagen II and proteoglycan. However, the SF-MSC constructs yielded less extracellular matrix (ECM) than chondrocyte constructs using cells of a similar passage number (571-801  $\mu$ g sGAG/construct, n=4 vs 80-130  $\mu$ g sGAG/construct, n=7). This matrix also had a relatively immature morphology compared to constructs of primary chondrocytes cultured under the same conditions. No mineralization was detected in any constructs. Initial experiments of pre-incubation of polyclonal MSCs in alginate gels before seeding on the PGS yielded larger constructs (28 mg, vs 12 mg) containing higher levels of GAGs (173 $\mu$ g sGAG/construct vs 111 $\mu$ g sGAG/construct). Treatment of constructs with TGF  $\beta_3$  in addition to TGF  $\beta_1$  also enhanced the amount of ECM produced by clonal SF-MSC constructs.

**DISCUSSION & CONCLUSIONS:** SF-MSCs formed immature chondrogenic constructs on PGA. The chondrogenic capacity of these stem cells was enhanced by treating the constructs with TGF  $\beta_3$  in addition to TGF  $\beta_1$  or by a short pre-incubation in alginate gel and exposure to TGF  $\beta_1$  prior to seeding onto PGA. The results suggest that synovial fluid represents a potentially attractive source of MSCs which may have utility for cartilage repair therapies in trauma and arthritis.

**REFERENCES:** <sup>1</sup>Pelttari K et al. Arthritis and Rheum 2006;54:324-3266. <sup>2</sup>Jones EA et al. Arthritis And Rheumatism 2004;50:817-827. <sup>3</sup>Crawford A. and Dickinson S.C. Methods Mol. Biol. 238:147-157, 2004.

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