

Functional Properties of Cartilaginous Tissues Generated from Mesenchymal Stem Cells Isolated from Different Tissue Sources

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INTRODUCTION: Mesenchymal stem cells (MSCs) possess the ability to proliferate extensively *ex vivo* while maintaining their multipotent differentiation capabilities making them an attractive cell type for cell-based cartilage repair strategies. MSCs can be isolated from a number of sources, including bone marrow, adipose tissue, synovial tissue and infra-patellar fat pad among others¹⁻³. The objective of this study is to determine the functional properties of cartilaginous tissues generated from porcine MSCs isolated from different tissue sources, and to compare these properties to those derived from donor matched chondrocytes.

METHODS: Porcine MSCs were isolated from the bone marrow (BM) of the femur, subcutaneous fat (SF) and infrapatellar fat pad (FP). Donor matched chondrocytes (CC) were harvested from the articular surface of the femoro-patellar joint. Culture-expanded chondrocytes (CC) and MSCs (P3) were encapsulated in agarose (final concentration of 2%) at a cell density of 15×10^6 cells/mL. Agarose hydrogel constructs were maintained in a chemically defined chondrogenic medium (CM) consisting of DMEM GlutaMAX™ supplemented with penicillin (100 U/mL)-streptomycin (100 µg/mL), 100 µg/ml sodium pyruvate, 40 µg/ml L-proline, 50 µg/ml L-ascorbic acid-2-phosphate, 1 mg/ml BSA, $1 \times$ insulin-transferrin-selenium, 100 nM dexamethasone and 10 ng/ml recombinant human TGF-β3. To assess construct functionality, samples were analysed biomechanically (equilibrium and dynamic modulus), biochemically (DNA, sGAG and collagen content) and histologically (alcian blue, picro-sirius red) at week 0, 3 and 6.

RESULTS: After 6 weeks in free swelling culture, mean sGAG content was highest in CC seeded constructs (1.48 % w/w), compared to 0.95 % w/w in FP, 0.09 % in BM and 0.01 % in SF seeded constructs, see Fig. 1. Negligible chondrogenesis was observed in SF seeded

hydrogels, as evidenced by weak alcian blue staining for sGAG (data not shown). Robust chondrogenesis was observed in FP seeded hydrogels. This translated into superior mechanical properties for FP seeded hydrogels compared to other MSC sources, although still lower than that derived for CC, see Fig. 2.

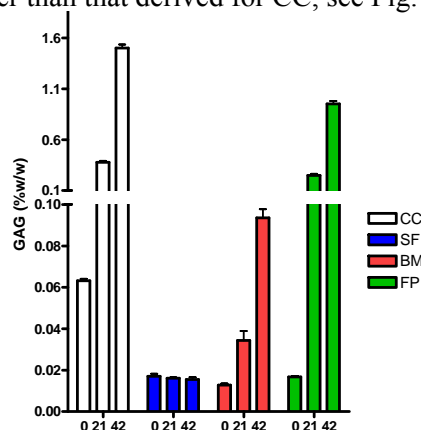


Fig. 1: sGAG content in engineered tissue at weeks 0, 3 and 6.

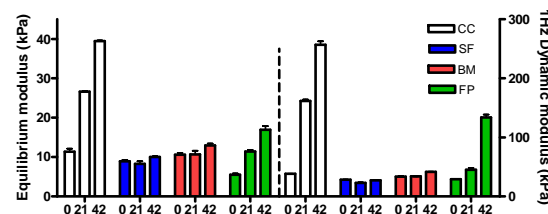


Fig. 2: Equilibrium and dynamic modulus of engineered tissues at weeks 0, 3 and 6.

DISCUSSION & CONCLUSIONS: MSCs isolated from within the synovial joint (e.g. the FP) would appear to possess a superior potential to generate functional cartilaginous tissues compared to other MSC sources. This study confirms the potential of FP derived MSCs for cartilage repair.

REFERENCES: ¹ Sakaguchi, Y. et al. *Arthritis Rheum* 52, 2521, 2005. ² English, A. et al *Rheumatology*, 46, 1676, 2007. De Bari, C. et al. *Arthritis Rheum* 44, 1928, 2001.

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