

Intra-articular Injection of Allogeneic Immunoselected STRO-3+ Mesenchymal Precursor Stem Cells into Ovine Joints with Pre-existing Osteoarthritis Improves Articular Cartilage Integrity 6 Months Post Administration.

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INTRODUCTION: Our previous studies had shown that bilateral meniscectomy in Merino ewes resulted in pathological changes in articular cartilage, subchondral bone and synovial tissues that simulated the development of early human osteoarthritis (OA)[1]. Mesenchymal stem cells (MSC) + bio-scaffolds have been widely studied for their ability to repair chondral/osteochondral defects, but their therapeutic effects when injected directly into OA joints has received limited attention. In this study we investigated the ability of immuno-selected Stro-3+ mesenchymal precursor cells (MPC) to preserve cartilage in an ovine model of established OA.

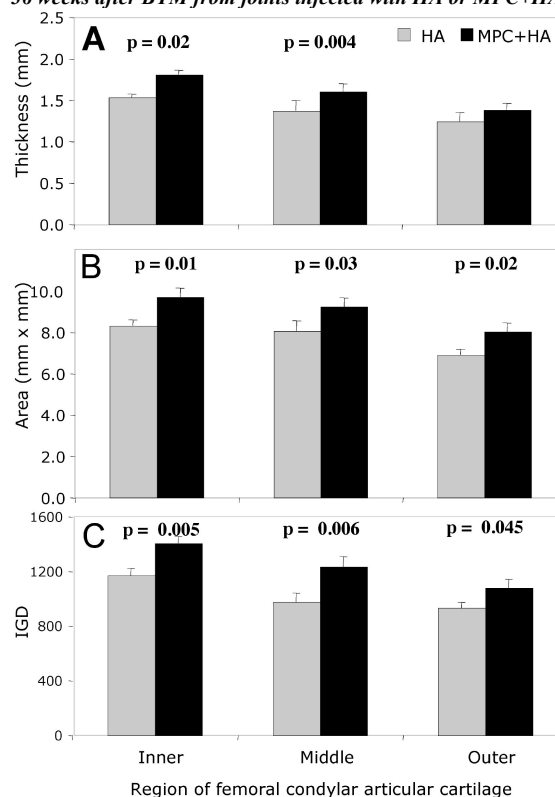
METHODS: Open bilateral total medial meniscectomy (BTM) was undertaken in 18 adult Merino ewes that had undergone ovariectomy 3 months earlier. Twelve weeks post BTM, 6 ewes were sacrificed while both stifle (knee) joints of the remaining 12 meniscectomised ewes were randomly injected with either 2mL high MW HA or 100 million MPC suspended in 2mL Profreeze plus 2mL HA. The injected ewes were divided into two groups of 6 that were sacrificed 24 and 36 weeks post-BTM, i.e. 12 and 24 weeks post HA or MPC+HA injection. At necropsy, joints were opened, menisci removed and the medial femoral and tibial plateaux photographed. The recorded images were scored by 2 blinded observers for gross morphological changes to cartilage using a 0 - 4 scale. Synovium from the suprapatellar fold and a 5mm wide coronal osteochondral slice were removed from the mid-line of the femur and tibia of each joint and processed for histological examination. Cartilage and synovial histopathology were assessed by two blinded observers using a modified Mankin Scoring (MMS) system [2] and a published synovial scoring system [3]. Osteochondral sections were also examined histomorphometrically [1] using computer-assisted image analysis to generate quantitative data on the cartilage dimensions and an index of the proteoglycan (PG) content of Toluidine Blue (TB) stained sections. Intact patellae from all joints were removed within 1 hour of sacrifice and immediately frozen and stored prior to topographical biomechanical indentation studies to determine the stiffness and phase lag of the articular cartilage [4]. Differences in treatment outcomes were assessed using Kruskal-Wallis ANOVA, Mann Whitney U or Wilcoxon tests for nonparametric (scoring) data and Student t-test for parametric data with $p < 0.05$ considered statistically significant (SS).

RESULTS: The gross morphological assessment of joints from the untreated ewes 12 weeks post-BTM confirmed that OA was well developed as identified by the mean cartilage morphological scores which were 75-87% of the maximum score. The mean cartilage morphological scores for HA or 100 million MPC+ HA injected joints were not different 24 or 36 weeks post BTM, but both afforded lower scores than the untreated 12 week post-BTM ewes. The total mean MMS for the femoral cartilages at 36 weeks for the MPC+HA treatments was lower than the corresponding score for the joints that received only HA and showed a significantly lower cell number ($p = 0.01$) and a trend ($p = 0.06$) for stronger inter-territorial TB staining. The histomorphometric analysis of cartilage thickness, area and intensity of TB staining as an index of PG content for the 3 regions (inner, middle and outer) of the femoral condyles from the injected joints at 36 weeks post-BTM showed SS between treatments groups. Femoral cartilages from the MPC+HA injected joints were thicker (Fig 1A) and occupied a larger area (Fig 1B) than the corresponding cartilages of HA injected joints. This was accompanied by a higher content of PGs as assessed from the TB stained sections (Fig 1C). The MPC+HA injected joints at 36 weeks post-BTM also afforded thicker ($p = 0.005$) and a larger area ($p = 0.002$) of inner femoral cartilage than the 12 week post-BTM untreated controls, while HA injected joints were not different. No significant differences were observed between the synovial pathology scores for the intra-articular treatments. The results of the indentation studies on the patella cartilages from the injected joints failed to demonstrate any difference in the biomechanical properties of the cartilages for the two treatments but changes were identified with

respect to time elapsed post-BTM and the untreated 12 week post-BTM group. The stiffness of the patella cartilages from the MPC+HA at 24 weeks post-meniscectomy was significantly higher than at 12 weeks ($P = 0.05$) and 36 weeks ($P < 0.01$) while HA alone showed no SS difference from the 12 week control mean values.

DISCUSSION: The results of this study demonstrated that a single intra-articular injection of 100 million MPC + HA into joints with established early OA can, over an intervening period of 24 weeks, slow the progression of joint pathology to a greater extent than HA alone. Surprisingly, the chondroprotective effect mediated by the MPC was observed to be more significant 24 weeks after administration than after 12 weeks. The reasons for this finding are presently unclear however, it is possible that growth factors such as members of the TGF-beta superfamily, eg BMPs, released by the MPC [5][6] were supportive of the delayed anabolic (compensatory) phase of cartilage to the altered mechanical stresses imposed across the joint by BTM. This view was reinforced by the histomorphometric findings of higher cartilage volumes and staining for PGs in the MPC injected groups than at the commencement of treatment at 12 weeks post-BTM.

Figure 1. Regional thickness (A), cross-sectional area (B) and integrated grey-scale density (IGD)(C) of femoral articular cartilage 36 weeks after BTM from joints injected with HA or MPC+HA.



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