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TGF-β3 and BFGF Induction of in vitro Chondrogenesis in Human Bone Marrow and Blood-Derived Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) offer great promise for cell-based therapies. However, the mechanism of MSCs to respond to local differentiation signals *in vivo* has yet to be clarified. Furthermore, MSCs possess the ability to differentiate into multiple mesenchymal lineages. Thus, the use of MSCs as a source for *in vitro* tissue engineering to repair articular cartilage defects requires the development of methodology in the initiation, promotion and maintenance of chondrogenesis. Chondrogenesis occurs when MSCs are grown under conditions that include three-dimensional culture substrate, serum-free nutrient medium and certain growth factors. MSCs were isolated and cultured from bone marrow and peripheral blood. Mononuclear cells were separated from both types of samples using Ficoll–Paque PLUS by centrifugation, suspended in cell culture medium with antibiotics and were plated onto tissue culture flasks. Suspended cells were subsequently removed after 5 days of culture, and adherent cells left to grow. Cells were detached using trypsin–EDTA after reaching 70–90% confluence. Cells were sub-cultured (2–5 passages) prior to further immunophenotyping analyses and differentiation experiments. To induce chondrogenesis, MSCs were cultured in alginate 3-dimensional scaffolds with chondrogenic medium containing 10 ng/ml transforming growth factor-β3 (TGF-β3) and fibroblast growth factor-basic (BFGF). Resultant chondrogenic-MSCs were harvested after 3-4 weeks in culture. To assess chondrogenesis, alcian-PAS and safranin-O were used to determine cartilage matrix proteoglycan expression. Chondrogenesis was also quantitated by sulphated glycosaminoglycan (S-GAG) production measured by 1,9-dimethylmethylene blue (DMMB) assay. The isolated MSCs exhibited positivity for the markers CD44, CD105 and CD166, and negativity for hematopoetic stem cell marker CD34. The results showed a significant cellular

expression of proteoglycans and glycosaminoglycans in chondrogenic-MSCs. This study proves that successful induction of *in vitro* chondrogenesis is possible from both bone marrow and peripheral blood-derived MSCs which may provide an alternative, viable option for stem cell therapies.