

EFFECT OF BETA-GLYCEROPHOSPHATE ON CHONDROGENESIS OF MURINE ADIPOSE-DERIVED MESENCHYMAL CELLS IN A BIPO TENT MEDIUM

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Introduction Due to their abundance and easy accessibility, pluripotent adipose-derived mesenchymal cells (AMCs) offer vast therapeutic potential in orthopaedic tissue engineering. No current tissue engineering strategy has yet succeeded in developing osteochondral plugs that are composed of two integrated tissues in a single bipotent medium. Recently in our lab, bone morphogenetic protein 6 (BMP-6) was used to develop a bipotent medium that is capable of promoting both chondrogenesis and osteogenesis in murine AMCs. The bipotent medium is composed of DMEM with 10% FBS and 1% penicillin-streptomycin supplemented with ascorbic acid (AA), β -glycerophosphate (β GP) and BMP-6. β GP is a critical component of the bipotent medium due its important role in osteogenesis and mineralization. Since the effect of β GP on chondrogenesis of AMCs is unknown, a study was conducted to investigate how β GP affects AMC chondrogenesis in vitro.

Methods AMCs were harvested from inguinal fat pads of 25-30 days old FVB mice and expanded in growth medium consisting of DMEM with 10% FBS and 1% penicillin-streptomycin. After expansion, AMCs were pelleted by centrifugation at 200,000 cells/well in sterile polypropylene round-bottom 96-well plates. Growth medium supplemented with 100 μ g/ml AA, 100 ng/ml BMP-6 and 0, 2 or 10mM β GP was applied to the cells. For each of the following experiments, three replicates were performed. At day 7, mRNA expression of Aggrecan, Sox9, Collagen type II, TGF β 1 and 18s were assessed via RT-PCR. 10 pellets were pooled together for gene expression analysis in each condition and replicate. At day 12, sulfated glycosaminoglycans (sGAG) accumulation of cell pellets was quantified using a commercially available sGAG kit. DNA content of the same pellets was analyzed using the Picogreen dsDNA quantification method. The sGAG quantities of pellets were then normalized to DNA content. 12 pellets were pooled together in sGAG and dsDNA assays for each sample.

Results No significant difference in expression of any of the chondrogenic-relevant genes was found between the groups. Similarly, there were no significant differences in sGAG/DNA ratio or in DNA content between the three conditions.

Conclusion The effect of β GP as a component of a bipotent medium on chondrogenesis of murine AMCs was investigated in this study. The addition of β GP to murine AMCs in chondrogenic cultures did not show any reduction in expression of chondrogenic-relevant genes, DNA content, or sGAG production at the timepoints that we investigated. These results suggest the feasibility of an osteochondral medium that simultaneously induces bone-like mineralization and cartilage matrix accumulation. Further research into the effect of β GP on chondrogenesis and hypertrophy of AMCs is currently being conducted in our lab.