Introduction: Adipose-derived mesenchymal cells (AMCs) are a promising cell source for orthopaedic tissue engineering applications due to their accessibility and multi-lineage potential1. However, future use in bone and cartilage regeneration requires development of successful strategies for driving AMCs to osteogenic and chondrogenic lineages. Recent work has demonstrated that bone morphogenetic protein 6 (BMP-6) has both osteogenic and chondrogenic effects on marrow-derived stem cells, depending on the context. Although studies have indicated that BMP-6 has strong chondrogenic effects on AMCs, its osteogenic effect has not yet been examined. The first objective in this study was to determine the osteogenic effect of BMP-6 on AMCs cultured in monolayer. We also hypothesized that a single medium containing BMP-6 could induce both osteogenesis and chondrogenesis in AMCs depending on the cell culture environment. Therefore, the second objective was to determine the bipotent effect of BMP-6 on differentiation of AMCs grown in monolayer compared to pellet culture.

Materials and Methods: AMCs were isolated from 25-30 day old FVB mice and expanded in growth medium (DMEM, 10% FBS and 1% penicillin-streptomycin). Cells were seeded in monolayer at 5,000 cells/cm² or pelleted by centrifugation at 200,000 cells/well in sterile polystyrene round-bottom 96-well plates. Growth medium supplemented with 100 μg/mL ascorbic acid, 10 mM β-glycerophosphate and 0 or 100 ng BMP-6 (R&D Systems) was applied to the cells. Quantitative real-time PCR was used to assess mRNA expression of Runx2, Osteocalcin (OCN), and Aggrecan (AGC1) at 7d. Expression was normalized to 18S. Alkaline phosphatase (ALP1) activity at 7d was determined using Fast Blue stain and Sensolite pNPP colorimetric kit (Anaspec) then normalized to total protein via BCA assay (Pierce). To quantify mineralization, Alizarin staining at 14d was used in monolayer. Alizarin staining at 14d was measured in 560 nm. Protaglycan expression in pellets was assessed by Western blotting (12d, ALPCO). Student’s t-test was used to determine statistical significance (p<0.05).

Results: Addition of BMP-6 to culture medium strongly enhanced markers of osteogenesis and chondrogenesis depending on culture conditions. In AMCs cultured in monolayer, BMP-6 upregulated Runx2 and OCN gene expression (Fig 1A and B, n=3), ALP1 activity and mineralization (Fig 2A and B; n=3) in comparison to control. In pellet culture, Runx2 and OCN gene expression was unchanged by BMP-6 in comparison to control. However, AGC1 gene expression (Fig 1C, n=3) and protaglycan accumulation, as observed by histology and sGAG quantification (Fig 3, n=3) were increased by BMP-6. AGC1 gene expression was unchanged by BMP-6 in AMCs cultured in monolayer (Fig 1C). Comparing monolayer to pellet culture, BMP-6 exerted an osteogenic effect only on cells in monolayer and a chondrogenic effect only on cells in pellet culture, as determined by gene expression (Fig 1).

Discussion: Previous work has identified BMP-6 as a strong inducer of chondrogenesis in AMCs5, but its osteogenic effect on these cells had yet to be studied. Our work indicates that BMP-6 promotes both osteogenesis and chondrogenesis in AMCs, conditional upon the cell culture environment. We have also shown that the bipotent effect of BMP-6 on differentiation can be demonstrated with a single medium formulation. Addition of BMP-6 to cells grown in monolayer resulted in potent upregulation of osteogenic gene expression and mineralization but exerted no osteogenic effect on AMCs differentiated via pellet culture. Rather, BMP-6 increased chondrogenic markers in pellet culture but not in monolayer, suggesting chondrogenesis via BMP-6 prevails over osteogenesis in pellet culture conditions. These data demonstrate that BMP-6 promotes osteogenesis in monolayer while enhancing chondrogenesis in pellet culture. The bipotent effect of this single medium containing BMP-6 has significant potential for osteochondral tissue engineering applications with AMCs. Future studies have been designed to fully characterize gene expression over time, and elucidate the differentiation mechanisms involved in our culture conditions.

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References:

Figure 1: Osteogenic and chondrogenic gene expression depends on culture conditions. A) Runx2, B) Osteocalcin and C) Aggrecan. Control set to 1 and data expressed as fold change from control. * indicates significant difference between monolayer and pellet culture; *** indicates significant difference from respective control, p<0.05.

Figure 2: Alkaline Phosphatase activity and mineralization was enhanced by BMP-6 in AMCs cultured in monolayer. A) Quantified Alkaline Phosphatase activity (top) and representative Fast Blue stain (bottom). B) Quantified mineralization (top) and representative Alizarin stain (bottom). * indicates significant difference from control, p<0.05.

Figure 3: Proteoglycan expression was increased by BMP-6 in AMCs grown in pellet culture. A) Histological staining of A) control and B) BMP-6 pellets. C) sGAG quantification. * indicates significant difference from control, p<0.05.