Osteogenic Differentiation of Human Adipose Stromal Vascular Cells in BMP-2 Presenting Gelatin Hydrogels

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Statement of Purpose: Stromal vascular fraction (SVF) cells from human lipoaspirate tissue are easy to obtain and demonstrate great potential for use in bone tissue engineering. Our group and others have confirmed the effect of BMP-2-supplemented media on osteogenic differentiation of these cells when encapsulated in biomaterial hydrogels, which have the advantage of being injectable and fitting any defect shape and size [1]. Here, the role of BMP-2 presentation method, exogenous vs. presentation from a hydrogel, on SVF cells in methacrylated gelatin (gelMA) hydrogels was investigated. By removing the need for repeated BMP-2 dosing, this work lays the foundation for a clinically-relevant injectable bone tissue engineering system using autologous cells.

Methods: SVF cells were obtained from lipoaspirate tissue as previously described [2]. GelMA was made by reacting methacrylic anhydride with gelatin type B [3]. Fresh SVF cells were mixed with 10% gelMA solution in DMEM-F12 containing Irgacure 2959 photoinitiator and photocrosslinked with UV light (3.5 mW/cm2, 320-500 nm). For growth factor loaded groups, these solutions also contained 25 or 50 µg BMP-2/mL. Hydrogels were cultured in DMEM-F12 containing 10% serum, ascorbic acid and β-glycerophosphate. To compare growth factor presentation from the hydrogels to exogenous BMP-2, one group of hydrogels was supplemented with 100 ng/mL BMP-2 in the media. Over four weeks of culture these hydrogels were presented with 700 ng BMP-2 in total, compared with 500 ng or 1,000 ng in the BMP-2 loaded hydrogels. After two weeks, DNA and alkaline phosphatase (ALP) production were measured using Picogreen and p-Nitrophenyl Phosphate (pNPP) assays, respectively. After four weeks, hydrogels were frozen in OCT and cryosectioned. They were then fixed in acetone before staining with Alizarin Red-S for calcium. Statistical analysis was performed using ANOVA with Tukey post-hoc tests. Data is expressed as mean ± standard deviation.

Results: BMP-2, presented exogenously in cell culture media or from a biomaterial hydrogel, led to increased expression of ALP, an osteogenic marker, compared to samples cultured without growth factor at two weeks (Figure 1). Changing levels of loaded BMP-2 supports a concentration-dependent growth factor effect; there may be a trend of enhanced ALP production with increasing amount of BMP-2. Notably, a similar ALP response was achieved using less loaded BMP-2 (25 µg/mL) than that supplied exogenously. Next, it was important to look at matrix mineralization, a marker of late stage osteogenic differentiation. Alizarin Red S staining for calcium indicates that the 50 µg/mL BMP-2 loaded group was the only condition to show significant mineral nucleation after four weeks (Figure 2). This further supports the hypothesis that BMP-2 presented from a biomaterial hydrogel leads to a dose-dependent enhanced osteogenic differentiation as compared to exogenously delivered growth factor.

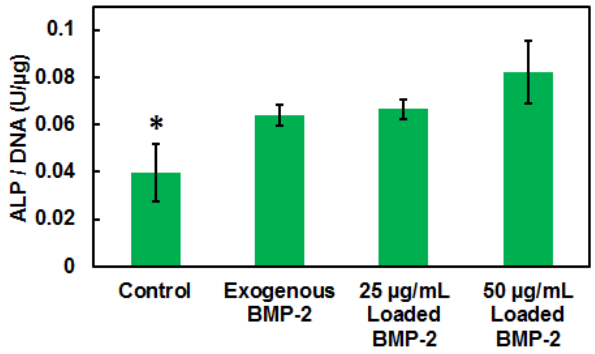

Figure 1: Alkaline phosphatase expression after two weeks of culture, * indicates $p<0.05$ compared to all other groups.

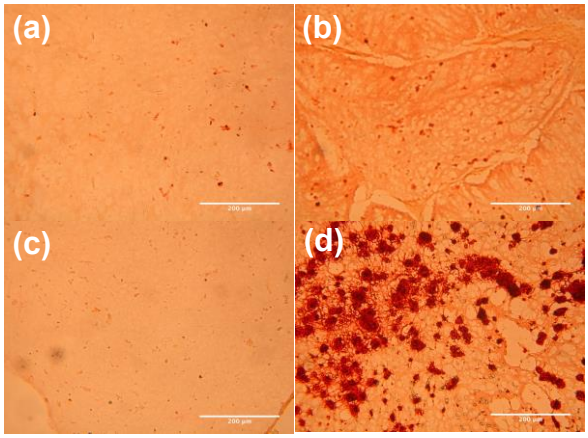

Figure 2: Photomicrographs of Alizarin Red stained histologic sections of (a) control hydrogels, (b) hydrogels with BMP-2 delivered exogenously, (c) hydrogels loaded with 25 µg BMP-2/mL and (d) hydrogels loaded with 50 µg BMP-2/mL. Scale bars are 200 µm.

It is important to note that the SVF cells were not frozen before use, and were cultured in the absence of dexamethasone, a common component of osteogenic media.

Conclusions: Localized growth factor presentation exhibits advantages over exogenous supplementation during culture for driving SVF osteogenesis. BMP-2 delivery from gelMA may be beneficial for matrix mineralization by SVF cells compared to a similar amount of exogenously delivered growth factor. Future work will include tailoring BMP-2 spatiotemporal presentation, better understanding the time course of osteogenesis, and evaluating this system with cells from multiple donors.

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