Enhanced Osteogenic Differentiation of Adipose-Derived Stem Cells in Growth Factor Presenting Gelatin Hydrogels

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Statement of Purpose: Human adipose-derived stem cells (hASCs) show great potential for use in bone tissue engineering. These cells are easily obtained and undergo osteogenic differentiation *in vitro* and *in vivo* in response to bone morphogenetic protein-2 (BMP-2) when cultured on ceramic scaffolds [1]. However, the role of BMP-2 on hASC osteogenesis in hydrogels, which have the advantages of being injectable and fitting any defect shape, is unknown. Here, the effect of exogenous BMP-2 on hASCs in methacrylated gelatin (gelMA) hydrogels was investigated. Release profiles of BMP-2 from gelMA and the influence of retained growth factor on osteogenic differentiation of encapsulated hASCs were also measured. This work lays the foundation for an injectable bone tissue engineering system using autologous cells.

Methods: hASCs were obtained from lipoaspirate and expanded to passage 3 [2]. GelMA was made by reacting methacrylic anhydride with gelatin type B [3]. GelMA solutions (10% w/v) were made in DMEM-F12 containing Irgacure 2959 photoinitiator and 10 million hASCs/mL For growth factor loaded hydrogels, 50 µg BMP-2/mL gelMA was added to the solution or to dry, crosslinked gelatin microspheres mixed into the gelMA [4]. After UV crosslinking, hydrogels were cultured for 2 weeks in DMEM-F12 containing 10% serum, ascorbic acid and β-glycerophosphate. Total or intracellular alkaline phosphatase (ALP) expression was measured by pNPP assay. DNA content was measured by PicoGreen assay. Growth factor release in PBS was measured with ^{125}I-labeled BMP-2 and a scintillation counter. Statistical analysis was performed using ANOVA with Tukey post-hoc tests. All graphs show mean ± standard deviation.

Results: To study the effect of BMP-2 on hASCs in gelMA, hydrogels were cultured with varying levels of exogenously supplied BMP-2. A statistically significant and BMP-2 concentration-dependent increase in hASC expression of ALP, an early marker of osteogenic differentiation, was found (Fig 1). Notably, this occurred in the absence of dexamethasone, a component of most osteogenic media. These results motivated the development of a controlled release system for BMP-2 from gelMA hydrogels. While BMP-2 loaded in gelMA alone displayed a strong burst release, BMP-2 loaded into gelatin microspheres mixed into the gelMA prior to crosslinking resulted in a smaller burst and more than

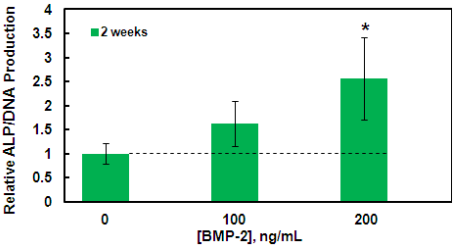

Figure 1. BMP-2 effect on ALP production by hASCs in gelMA p<0.01 compared to 0 ng/mL BMP-2 (*)

50% was retained in the hydrogels after one week, likely due to electrostatic interactions between the BMP-2 and the gelatin (Fig 2). The amount of growth factor loaded in each hydrogel is half of what would be used for three weeks in culture with 200 ng/mL BMP-2, a considerable reduction. To evaluate bioactivity of BMP-2 in this

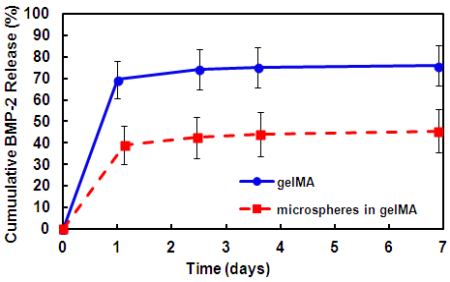

Figure 2. BMP-2 release from gelMA hydrogels in PBS

system, BMP-2 loaded, hASC-laden hydrogels were cultured in the absence of exogenous growth factors. BMP-2 loaded into the gelMA itself, and especially into gelatin microspheres within the gelMA hydrogels, led to significantly enhanced ALP expression (Fig 3). This increase occurred as early as one week, indicating accelerated osteogenesis compared to conditions with gelMA alone or with empty microspheres.

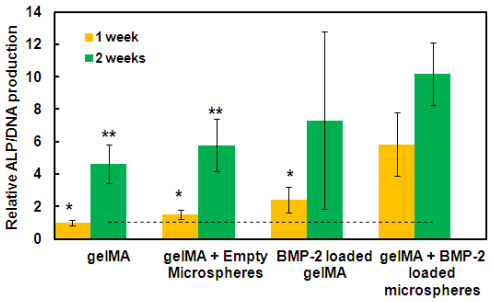

Figure 3. ALP expression by hASCs in BMP-2 loaded gelMA p<0.01 compared to gelMA + BMP-2 microspheres condition at one (*) or two (**) weeks

Conclusions: BMP-2, delivered exogenously or from a hydrogel, is a strong osteogenic signal to hASCs in gelMA. GelMA with BMP-2 loaded gelatin microspheres is a promising system to deliver BMP-2 to hASCs: it is injectable, achieves the same magnitude effect with much less growth factor than exogenous delivery, and maintains growth factor bioactivity. Future work will include tailoring BMP-2 spatiotemporal presentation and evaluating construct mineralization.

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