Eliminating Dexamethasone from Osteogenic Media Leads to the Production of Angiogenic Factors by Microencapsulated Adipose Stem Cells

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Statement of Purpose: Cell based therapies using adipose-derived stem cells (ASCs) provide one possible form of treatment for fractures or non-unions because they are known to be multipotent (1), easily isolated, and readily available, but they tend to disperse from the treatment site (2). It is important that a method of delivery is developed to retain cells at the injury site and maintain their ability to generate appropriate soluble factors to enhance tissue specific healing. We previously demonstrated that ASCs encapsulated in injectable alginate microbeads produce angiogenic factors *in vitro* and *in vivo* (3); pretreatment of the microencapsulated ASCs with chondrogenic medium reduced production of angiogenic factors but induced production of chondrogenic factors (3). The goal of this study was to determine whether pretreatment with osteogenic medium would result in production of osteogenic factors and if so, could the medium be modified to optimize both osteogenic and angiogenic factors.

Methods: ASCs were isolated from inguinal fat pads of Sprague-Dawley rats under an Institutional Animal Care and Use Committee (IACUC) approved protocol. First passage rASCs were plated at 5000 cells/cm^2 or microencapsulated in low viscosity, high mannuronate (LVM) alginate in 50mM calcium crosslinker solution containing glucose at 1x10^7 cells/ml alginate using a 6kV electrostatic potential (3,4). rASCs alone or microencapsulated rASCs were treated with mesenchymal stem cell growth medium (GM) for 5 days, then GM, GM + dexamethasone (GM+d), osteogenic medium (OM), OM-ascorbic acid (OM-aa), OM-dexamethasone (OM-d), or OM-dexamethasone and ascorbic acid (OM-aa-d) for an additional 5 days. ELISAs were used to analyze soluble factors in the conditioned media. Cells were released from the microbeads and alkaline phosphatase specific activity of cell lysates was determined. mRNA levels were analyzed for Vegfa, Fgf2, Tgfβ-1, Bmp2, Gremlin 1 (Grem1), and Noggin (Nog). Statistical significance was determined by multi-way ANOVA with post hoc analysis by Bonferroni correction to Student's t-test (n=6, per variable).

Results: Microencapsulating rASCs increased the local factor production compared to cells grown in a monolayer. OM, OM-aa and GM+d reduced the production of FGF (Figs. 1A-1C) and VEGF in comparison to GM. Once dexamethasone was removed from OM or OM-aa, the FGF and VEGF production was regained to similar levels obtained with GM (Figs. 1B and 1C). Similar results were reflected in the mRNA levels of Fgf2 (Fig. 1D-1F) and Vegfa. Dexamethasone is a glucocorticoid and is known to reduce the production of angiogenic factors, as was seen in cultures treated with GM+d. Upon removing dexamethasone from the medium, similar FGF and VEGF levels were obtained as microencapsulated rASCs treated with GM. mRNA levels for Bmp2, Nog, and Grem1 decreased with OM treatment, and returned to a similar level as expressed in GM once dexamethasone was removed. There is no direct relationship between ascorbic acid and angiogenic factor production, however it is known to affect collagen levels (5). Removing ascorbic acid from the medium had no effect on cell response within the microbeads. These data may suggest that microencapsulated ASCs treated with OM-d will enhance bone formation during the regeneration process.

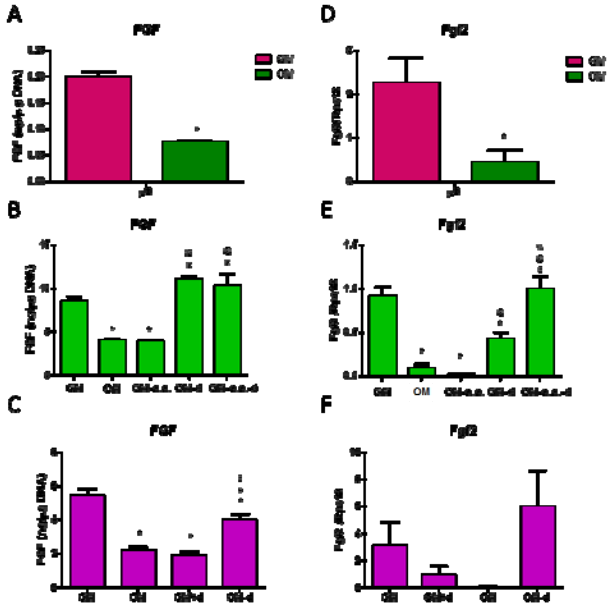

Figure 1. (A-C) FGF retention within microbeads incorporating rASCs (μB), (D-F) mRNA levels of Fgf2 in microencapsulated rASCs treated with GM, GM+d, OM, OM-a.a, OM-d, OM-a.a-d for 5 days * $p < 0.05$ v. GM, $ vs. OM/GM+d, @ vs. OM-a.a, # vs. OM/TCPS

Conclusions: These microencapsulated cells can be induced to produce local factors that are required for bone regeneration once they are treated with the appropriate media.

References:

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