2011 AAID Annual Meeting – 3rd Place – Poster Presentations

Identification of Osteogenic Gene Families Enhanced by Bioactive Glass Ions

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Objectives: Previously, we showed that bioactive glass dissolution products (ions) enhanced collagen expression by MC3T3-E1 cells. In this study, we tested the hypothesis that these ions enhance other key osteogenic gene families associated with mineralized tissue formation for future application in Ti implant osseointegration.

Methods: Bioactive coating glass (6P53-b, 53 wt.%SiO2) and commercial Bioglass (45S5, 45 wt.%SiO2) were soaked in α-MEM (72 h, 0.016 gm L–1), liberating ion extracts into the media. These media were collected, measured (inductively coupled mass spectrometry) for their respective ion concentrations, and supplemented (10%FBS, 1%pen-strep, 50 mg L –1 AA, 10 mM βGP) to make glass conditioned medium (GCM). MC3T3-E1 subclone 4 osteoblast-like cells were seeded (50,000 cells cm –2) and cultured in either GCM or control media (supplemented α-MEM, as described above) to study the effects on cellular differentiation and mineralization for 30 days. Cells were lysed (time points: 2, 6, 10, 14, and 20 d) for total RNA, converted to cDNA and measured for gene expression (osteogenesis qPCR arrays). qPCR amplification was analyzed (sigmoidal curve fitting, ΔΔCT, Two-way ANOVA with Tukey's post-hoc for statistical significance, $p<0.05$). Visualization of ECM collagen type 1 (Picrosirius stain) and mineralized tissue formation (Alizarin stain) was also performed.

Results: Prepared GCM had significantly increased concentrations of Si ($p<0.05$, 45S5 and 6P53-b) and Ca ($p<0.05$, 45S5). These higher ion concentrations led to increased gene expression of several key osteogenic markers including Col1α1, Col1α2, ALP, LOX, Runx2, and OCN genes . ECM collagen type 1 and mineralized tissue formation was enhanced in the presence of GCM treatments as compared to control treatments. Results of mineralized tissue formation were visually confirmed by histological Alizarin-stained samples.

Conclusion: GCM enhanced the expression of several key osteogenic genes resulting in enhanced mineralized tissue formation relative to control treated cells.