Cellular Evaluation of Bone Morphogenetic Protein-Derived Oligo-peptides as Candidate Biomolecules for Surface-Modified Scaffolds in Bone Tissue Engineering

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Statement of Purpose: Synthetic materials used for tissue engineering often suffer from a lack of bioactivity, which can limit the extent of tissue integration at the interface between the host and implanted biomaterial. The addition of cell-binding signals in the form of short-chain oligo-peptides, can endow materials with the biological cues needed to mimic native cell-matrix protein interactions **[1]**. Oligo-peptides derived from bone morphogenetic proteins (BMPs) can serve as candidate biomolecules in the fabrication of surface-modified, osteoinductive biomaterials for tissue engineering. Evaluating the osteoinductivity of BMP-derived oligo-peptides is crucial towards understanding their potential use in tissue-engineered constructs for human bone graft replacements. In this study, oligo-peptides derived from human BMP-2 and BMP-7 were evaluated for short-term, *in vitro* osteoinductive potential using human, bone marrow-derived mesenchymal stem cells (hMSCs).

Methods: Oligo-peptides derived from human BMP-2 and BMP-7 proteins, were custom synthesized by AnaSpec (San Jose, CA). BMP-2 fragment was derived from amino acid residues 73-92 of the mature human BMP-2 protein [**2**]. BMP-7 fragment was derived from amino acid residues 111-130 of the mature human BMP-7 protein [**3**]. Passage 4 hMSCs were cultured at 37°C, 5% CO_2 in well-plates containing basal growth medium (Cambrex, East Rutherford, NJ). After 3 days, cells were fed mineralization medium supplemented with or without BMP-fragments (200 ng/mL) for 14 days. Cells cultured in medium supplemented with 200 ng/mL of recombinant human BMP-2 (rhBMP-2, Cell Sciences, Canton, MA) served as a positive control, while cells cultured in un-supplemented medium served as a negative control. BMP fragments were evaluated for short-term, osteoinductive potential by assessing hMSCs for: 1) cellular proliferation by tetrazolium reduction (Promega, Madison, WI); 2) alkaline phosphatase (ALP) activity by colorimetric assay (BioRad, Hercules, CA); and 3) mineral deposition by Alizarin Red and von Kossa staining.

Results/Discussion: By day 10, hMSCs cultured in medium supplemented with BMP-fragments demonstrated a percentage increase in cell number comparable to that of rhBMP-2 controls (*data not shown*). Cells cultured with BMP-fragments increased significantly in ALP expression over the course of the study, with BMP-7 fragment producing the greatest increase change (*Figure 1*). Cells cultured in the presence of BMP-fragments also demonstrated an increase in mineralized nodule formation as detected by both Alizarin Red and von Kossa staining (*Figure 2*).

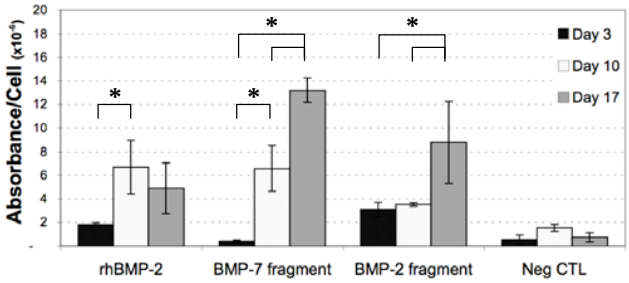

Figure 0: Normalized ALP activity expressed as absorbance (492 nm) per cell (*$p < 0.05$). BMP-7 fragment induced the greatest increase in ALP activity.

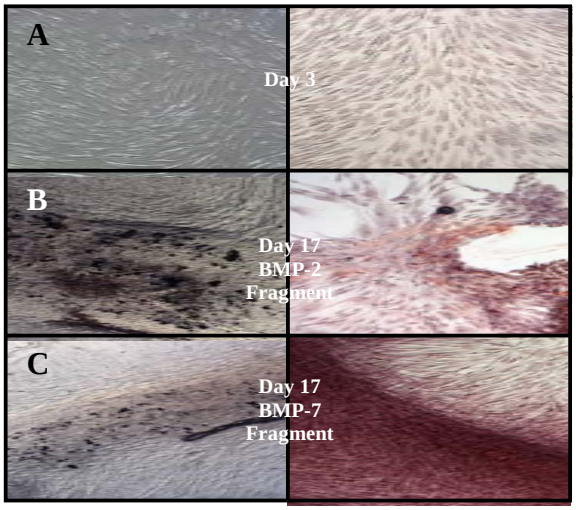

Figure 2: Von Kossa (left) and Alizarin Red (right) staining of *in vitro* mineralized nodule formation: A) No mineralized deposits detected at day 3; B & C) mineralized deposit staining at day 17.

Conclusions: BMP-derived oligopeptides successfully promoted the *in vitro* osteoinduction of hMSCs as characterized by increased ALP expression and mineralized extra-cellular matrix deposition. The mitogenic and morphogenic activity of these oligo-peptides can be utilized in the fabrication of materials that mimic the native biological environment and encourage directed bone tissue regeneration. Currently, we are exploring the use of short-chain, BMP-derived oligo-peptides for the fabrication of surface-modified polymer scaffolds for bone tissue engineering.

References: [1] *Yang XB, et al. Bone. 2001;29: 523-531.* **[2]** *Saito A, et al. Biochim Biophys Acta. 2003;1651:60-67.* **[3]** *Kirkwood K, et al. J Oral Implant;2003;29:57-65.*