Amelogenin Peptide (Fraction C) Induces Osteogenesis and Cementogenesis in hMSCs

Olivares-Navarrete R[1], Vesper K[1], Dard M[2], Mauth C[2], Ranevski R[2], Schwartz Z[1], and Boyan BD[1]
[1]Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia, USA;
[2]Institut Straumann AG, Basel, Switzerland

Statement of Purpose: Amelogenin is the principal constituent of enamel matrix derivative (EMD), an extracellular matrix protein complex derived from fetal porcine tooth germ. In its commercial form (Emdogain®, Institut Straumann AG, Basel, Switzerland), EMD is used to promote regeneration of the periodontal ligament, including new bone and cementum formation. Previously we showed that a 5 kDa peptide present in EMD (Fraction C), is the N-terminus of amelogenin and increases osteoblast differentiation markers in osteoblast-like cell cultures. In the present study, we examined whether human mesenchymal stem cells (hMSCs) are also sensitive to Fraction C and if it differentiate hMSCs to an osteogenic or cementogenic phenotype.

Methods: Human mesenchymal stem cells (hMSCs) were seeded at 5000 cells/cm2 and cultured until confluence. At confluence, cells were treated with 1µg/ml or 10ug/ml of either EMD, recombinant human amelogenin (Amel) or Fraction C for 12 hours for gene expression and 24 hours for alkaline phosphatase specific activity and soluble factor quantification. Effects on gene expression were assessed by real-time PCR for markers of osteogenesis [alkaline phosphatase (ALP), osteocalcin (OCN), osteoprotegerin (OPG), collagen I (COLI)], bone morphogenetic protein-2 (BMP2), and RUNX2 as well as cementogenesis [cementum attachment protein (PTPLA) and cementum protein 1 (CEMP1)]. After 24 hours of treatment, cell number and alkaline phosphatase specific activity were measured in cell lysates and levels of OCN, OPG, TGF-β1, and VEGF-A quantified in the conditioned media.

Results: Treatment with EMD, Amel, and Fraction C induced expression of osteogenic markers in hMSCs. Fraction C caused the greatest expression of ALP, OCN, OPG, and COLI in comparison with the other treatments. Both Fraction C and EMD increased expression of BMP2 to comparable levels. Amel and Fraction C significantly increased levels of RUNX2 (Fig 1A) (C>Amel). Expression of the cementogenic marker PTPLA was induced in cells treated with 1µg/ml of EMD and with both 1µg/ml and 10ug/ml of Fraction C. However, CEMP1 increased only with the Fraction C treatment at both concentrations. Fraction C increased alkaline phosphatase specific activity (Fig 1B) with both concentrations tested and increased levels of OCN and OPG in the conditioned media. Amel, EMD, and Fraction C increased levels of TGF-β1 and VEGF-A in comparison to control groups.

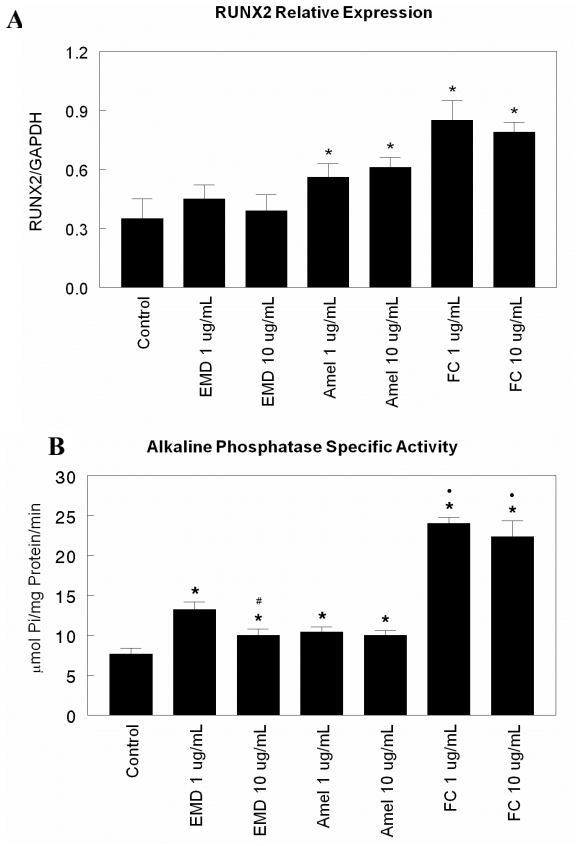

Figure 1: (A) The effect of Fraction C on RUNX2 expression. Both concentrations of Amel and Fraction C increased expression when compared to control. EMD had no effect on RUNX2 expression. (B) The effect of Fraction C on alkaline phosphatase specific activity. All treatments increased alkaline phosphatase specific activity with respect to control, with greatest increases in Fraction C treated cells. *p<0.05, Treatment v. control; #p<0.05, 10µg/ml v. 1 µg/ml; •p<0.05, FC v. EMD and Amel.

Conclusion: These results indicate that the Fraction C component of EMD acts on hMSCs, and contributes to create an optimal osteogenic and cementogenic environment. These results suggest that Fraction C may be the active component of EMD and amelogenin with respect to bone and cementum regeneration observed in *in vivo* studies and clinically.

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