Differential Gene Expression of Matrix Metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are Modulated During Osteogenic/Odontogenic Differentiation from Human Dental Pulp Stem Cells (DPSCs) by BMP-7

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ABSTRACT

Constant remodeling of extracellular matrix is a hallmark during physiological conditions, such as stem cell differentiation, embryogenesis and tissue repair. Matrix metalloproteinases (MMP) play a key role in these processes. Mesenchymal Stem Cells derived from dental pulp are multipotent and have the capacity to differentiate into mesenchymal lineages. Bone morphogenetic proteins (BMP) are a family of signaling molecules critically involved at various stages in the formation of a variety of tissues and organs including bones and teeth. Recently, BMP-7 has been described to induct DPSC differentiation into odontoblastic-like cells. However, it is unknown the gene expression profile of MMPs and TIMPs during osteogenic/odontogenic differentiation induction by BMP-7. In this study, we evaluated differential gene expression of MMPs and TIMPs during osteogenic/osteogenic differentiation induction from DPSCs in vitro by qPCR. DPSCs isolated from extracted human third molars (collagenase/dispase digestion at 37°C) were grown in α-MEM medium + 10% FBS and differentiation induction in presence of osteogenic medium (10 mM β-glycerophosphate, 1 µM dexamethasone and 50 µg/ml ascorbate) and odontogenic medium (10 mM β-glycerophosphate, 1 µM dexamethasone and 50 µg/ml ascorbate + 50 ng/mL BMP-7) for 21-days. During osteogenic differentiation, MMP-2, MMP-3, MMP-25 and TIMP-4 were downregulated upregulated throughout 21-days in relation to DPSC. During odontogenic differentiation, MMP-2, MMP-25 and TIMP-4 were upregulated from 7 to 21-days, whereas, MMP-3 was upregulated from 14 to 21-days. Our results suggest that BMP-7 may regulate MMP and TIMP gene expression during osteogenic/odontogenic differentiation in vitro from DPSCs. Keywords: Dental pulp stem cells, MMP, TIMP, BMP-7, and Osteoblast/Odontoblast Differentiation. Financial Support: FAPESP

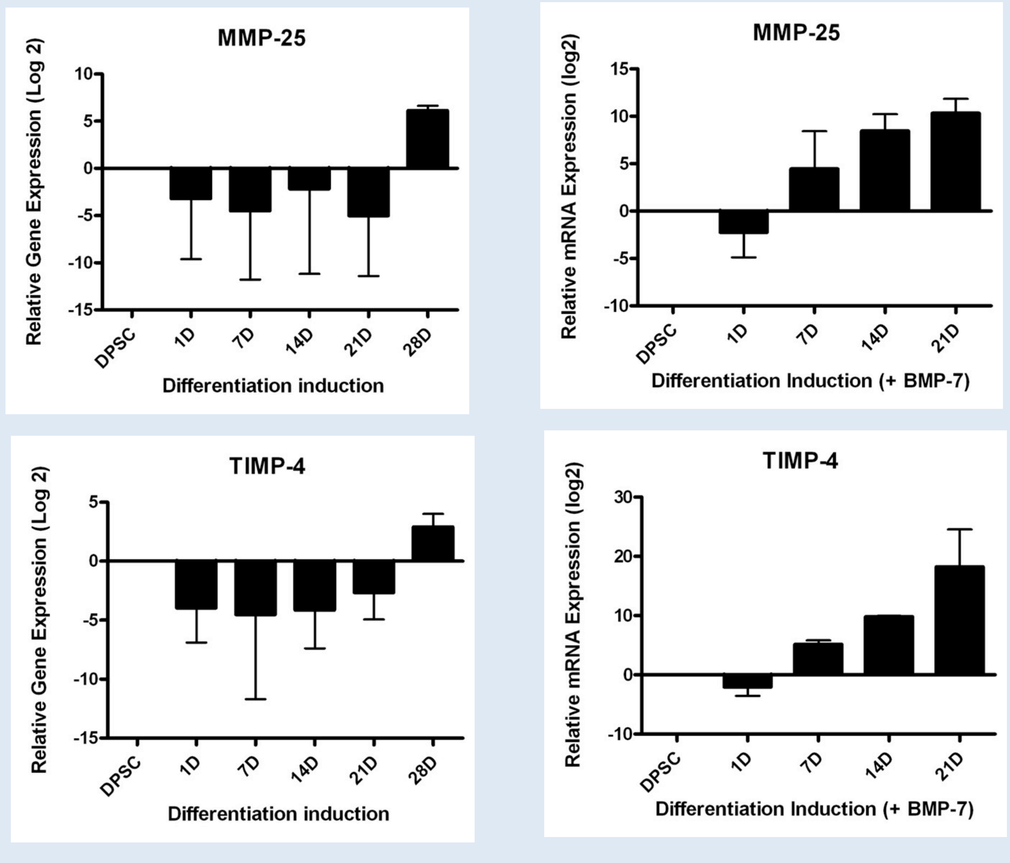


Figure 1. *Differential gene expression of MMPs and TIMPs after osteogenic and odontogenic (+BMP-7) differentiation induction from Dental Pulp Stem Cells evaluated by relative real-time PCR analysis (qRT-PCR). Results are relative to the normalized expression of housekeeping gene (GAPDH) and reference sample (DPSC – undifferentiated cell).*

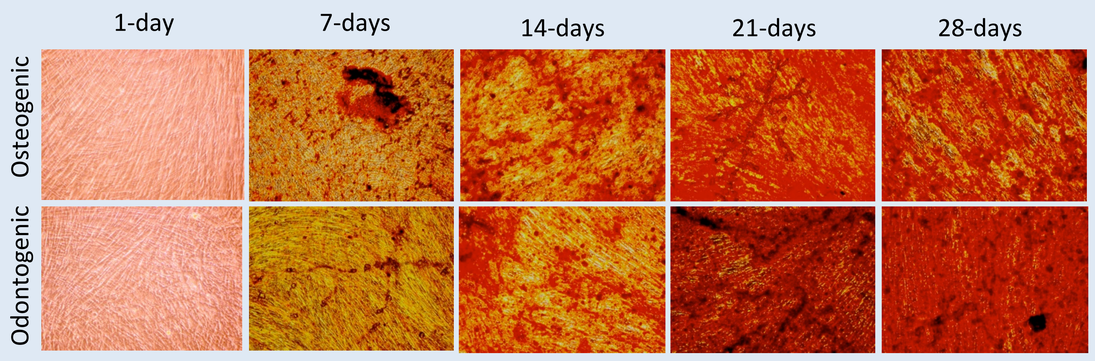
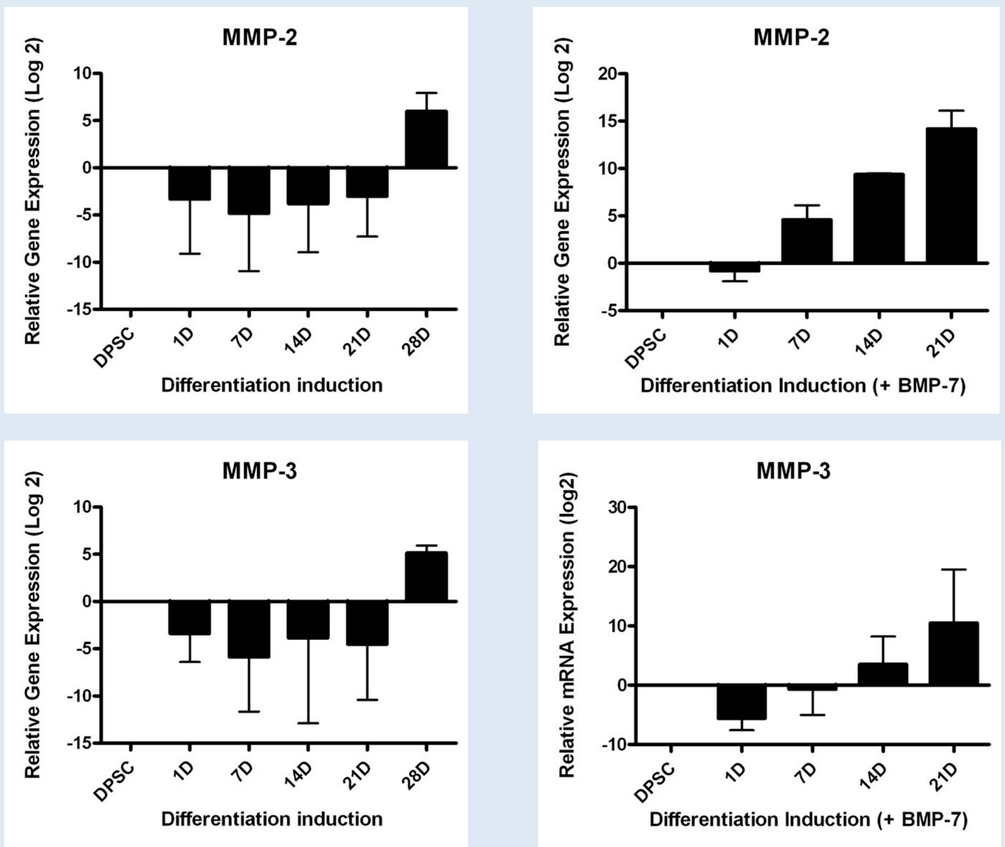


Figure 2. *Alizarin red staining for calcification nodules during osteogenic and odontogenic differetiation from DPSCs.*

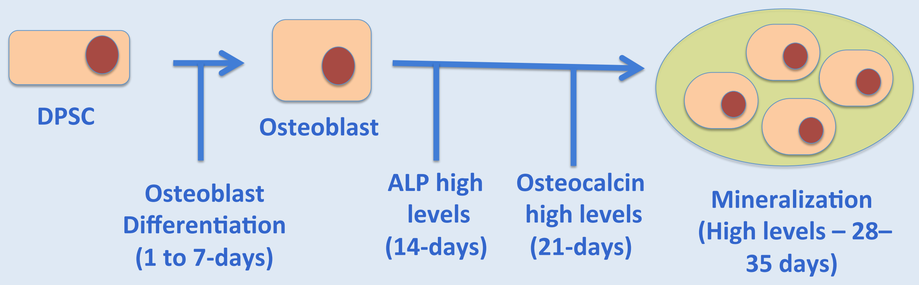
MATERIALS AND METHODS

- Cell Culture: Normal human impacted third molar was collected from 2 adult (between 18 and 32-years old) at the Dental Clinic of University Hospital at University of São Paulo under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. The pulp tissue was gently separated from the crown and root and then digested in a solution of 6 mg/ml collagenase type I (Gibco) and 8 mg/ml dispase (Gibco) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-µm strainer. Cells were cultured in α-MEM + 10% SFB + 100 µM L-ascorbic acid 2-phosphate (clonogenic medium), and then incubated at 37°C in 5% CO_2 (adapted from Gronthos et al 2000; 2011).
- Differentiation induction: for osteogenic and odontogenic (addiction of BMP-7) for 28 and 21-days, respectively. Osteogenic medium was constituting by α-MEM + 10% SFB + 1 µM dexamethasone + 10 mM β-glicerofhosfate + 50 µg/mL L-ascorbic acid 2-phosphate and odontogenic medium by adding 50 ng/mL BMP-7Verification of calcification nodule was assessed by alizarin red stain.
- qRT-PCR: Total RNA was used as template for cDNA synthesis in a RT-PCR reaction. qRT-PCR reaction was performed by SYBR® Green Dye I (Applied Biosystems - 40 cycles at 60°C). Relative quantification was performed by Pfaffil method (2001). GAPDH was used as housekeeping gene and undifferetiated cell as reference sample (DPSCs).

RESULTS



DISCUSSION AND CONCLUSION



- BMP-7 can drives osteogenic and odontogenic differentiation from human tooth germ stem cells (Neslihan et al 2014);
- BMP-7 upregulates MMP-2 via Smad-1 (Gordon et al 2009);
- BMP-7 may be a signaling molecule for tooth and bone bioengineering.

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