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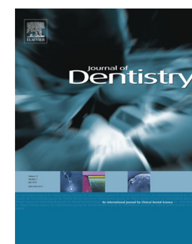

# Effects of the enamel matrix derivative on the proliferation and odontogenic differentiation of human dental pulp cells

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ABSTRACT

*Objective:* The enamel matrix derivative (EMD) has a positive effect on the proliferation of human periodontal ligament cells and the healing of periodontal tissues. The aim of this study was to evaluate the effects of EMD on the proliferation and differentiation of human dental pulp cells (hDPCs) in vitro.

*Methods:* hDPCs were isolated from human impacted third molars and cultured in vitro. After treatment with100 μg/mL EMD, the proliferation of hDPCs was determined by a cell counting kit 8 (CCK8) assay. After incubation in EMD osteogenic induction medium for 14 days, the osteogenic differentiation of hDPCs was evaluated by alkaline phosphatase (ALP) activity, alizarin staining and the expression of osteogenesis-related genes.

*Results:* The EMD osteogenic induction medium enhanced the proliferation of hDPCs. After osteogenic induction, EMD increased the osteogenic potential of hDPCs, as measured by alkaline phosphatase activity and calcium accumulation; the expression levels of osteogenesis-related genes, such as ALP, DSPP, BMP, and OPN were also upregulated. In addition, the expression levels of odontogenesis-related transcription factors Osterix and Runx2 were upregulated.

*Conclusions:* EMD could enhance the mineralization of hDPSCs upregulated the expression of markers for odontoblast/osteoblast-like cells. Further studies are required to determine if EMD can improve pulp tissue repair and regeneration.

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## 1. Introduction

The health of teeth depends on the integrity of the hard tissue and the activity of the pulp and periodontal tissues, which are responsible for supplying nutrition to the teeth. Lack of nourishment provided by the pulp tissue can increase the risk of tooth fracture. In immature permanent teeth, impaired root development is another outcome associated with lack of nutritional support of the pulp. Maintaining the viability of the residual pulp tissue as far as possible is the main consideration in the procedure of pulp therapy. Direct pulp capping and pulpotomy are therapeutic approaches for exposed vital pulp, in which the formation of reparative dentine is facilitated by sealing the pulpal wound with a dental material.[1] After direct pulp capping and pulpotomy, the differentiation and proliferation of dental pulp cells (DPCs) are influenced by the activity of dental materials.[2,3]

Emdogain (Straumann AG, Basel, Switzerland) is a commercial enamel matrix derivative (EMD), derived from porcine developing enamel matrix. The main component of EMD is amelogenins. In addition, EMD contains low concentrations of

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matrix metalloproteinases and growth factors, including transforming growth factor β1 (TGF-β1), BMP-2 and BMP-4.[4] Several studies have shown that EMD influences the migration, attachment, proliferative capacity and biosynthetic activity of periodontal ligament cells.[5,6] Thus, it is considered effective in improving the healing process of replanted teeth and teeth with periodontal diseases.[7,8] Recently, it was suggested that EMD could also be used for pulp regeneration. Previous studies showed that amelogenin participates in the maturation and growth of dental pulp cells during tooth formation.[9] Animal experiments and clinical studies showed that EMD promotes reparative processes in the dental pulp.[10]

The addition of EMD can promote periodontal cell proliferation[11]; therefore, it was hypothesized that EMD exerts its therapeutic effect by providing an extracellular matrix that forms a more natural microenvironment for cells, stimulating cell attachment and differentiation.[12] EMD is also reported to induce a process mimicking normal odontogenesis and can thereby serve as a biologically active pulp dressing agent, which specifically induces pulpal wound healing and hard tissue formation without affecting healthy pulp.[13,14] It has been demonstrated that when mineral trioxide aggregate (MTA) and EMD were applied to human DPCs together, the cells differentiated into odontoblast-like cells, suggesting a synergistic effect of these two materials.[15] A recent study reported that Emdogain combined bismuth oxide containing Portland cement could improve cell growth and differentiation of human DPCs (hDPCs).[16] However, the direct effects of EMD on dental pulp, and the underlying mechanisms remain unclear. The aim of this study was to evaluate the effects of EMD on the proliferation and differentiation of hDPCs in vitro.

## 2. Materials and methods

EMD gel (30 mg/mL and 0.7 mL) (Emdogain; Biora AB, Malmö, Sweden) was diluted with Eagle's medium (α-MEM, GIBCO/BRL, Grand Island, NY, USA) to a final concentration of 100 μg/mL.

### 2.1. Cell culture

Human impacted third molars were collected from an adult (22 years, male) at the clinic of the Peking University School of Stomatology and used to culture hDPCs. The patient provided written informed consent, and the ethical committee of the Medical School of Peking University approved the protocol to obtain extracted teeth. After the teeth surfaces had been cleaned, the teeth were cut around the cementum-enamel junction with sterilized dental fissure burs to expose the pulp chamber. The pulp tissue was gently separated from the crown and root, and subsequently digested in a solution of 3 mg/mL collagenase type I (Sigma, St. Louis, MO, USA) and 4 mg/mL dispase (Sigma) for 1 h at 37 °C. Single-cell suspensions were obtained by passing the cells through a 70-mm strainer (Falcon, BD Biosciences, San Jose, CA, USA).

Single-cell suspensions ($0.5–1.0 \times 10^3$/well) of hDPCs were seeded into 6-well plates (Costar, Corning Life Sciences, Tewksbury, MA, USA) containing α-MEM supplemented with 15% foetal bovine serum (FBS; Hyclone Thermo Scientific, Logan, UT, USA), 100 μg/mL penicillin, 100 mg/mL streptomycin (Sigma, St. Louis, USA), 100 μg/mL EMD and incubated at 37 °C in 5% $CO_2$. The control medium contained α-MEM, antibiotics and 15% FBS.

To induce differentiation, cells were cultured in a control medium with osteogenic induction media (OSTEO), comprising 50 m g/mL ascorbic acid (Sigma), 10 mmol/L β-glycerophosphate (Sigma), and 0.1 m mol/L dexamethasone (Sigma), as described previously.[6] EMD (100 μg/mL was added to the OSTEO as the experimental group (EMD).

### 2.2. Determination of EMD concentration

hDPCs ($1 \times 10^3$/well) expended ex vivo were seeded into 96-well plates, cultured with EMD (0, 1, 10, 100 μg/mL) for 24 h in 37 °C. A cells counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay was then carried out and repeated five times for each sample to evaluate the number of viable cells, according to the manufacturer's instructions. Untreated cells were used as the control group. 10 μL of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt was added to each well before the culture plate was incubated at 37 °C for 4 h. Absorbance was measured at 450 nm in a microplate reader. The mean values of the optical density were calculated and analyzed statistically for cell number at each dilution of the samples (version 13.0; SPSS, Chicago, IL, USA). The dilution of EMD at which hDPCs had the highest cell viability was chosen for subsequent studies.

### 2.3. Growth tendency study

According to the above experiment, 100 μg/mL EMD was chosen for the growth tendency study, with the untreated hDPCs as the control group. hDPCs ($1 \times 10^3$/well; expanded ex vivo) were seeded into five 96-well plates separately. At 1, 3, 5 and 7 days after cell seeding, a CCK-8 assay was carried out with eight replications to evaluate the number of viable cells, following the same procedure as described in ''determination of EMD concentration''. The growth curves of the two groups were analyzed using SPSS software (version 13.0; SPSS, Chicago, IL, USA).

### 2.4. Alkaline phosphatase activity

After 7 and 14 days culture in the OSTEO, EMD and normal control medium, cells were rinsed three times in phosphate-buffered saline (PBS) and then lysed for 10 min in 100 mM Tris base with 1% Triton-X100. Alkaline phosphatase activity (AKP activity) was determined in the lysate by measuring the release of p-nitrophenol using SIGMAFAST™ p-nitrophenyl phosphate (Sigma) as a substrate after 45 min at 37 °C. Absorbance was measured at 420 nm using a Microplate Reader (ELx808IU, BioTek, Winooski, VT, USA).

### 2.5. Alizarin Red staining

After 7 and 14 days of culture in the EMD, OSTEO and normal control medium, the hDPCs were fixed in 4% paraformaldehyde for 30 min and washed in PBS, and the mineralization of the extracellular matrix was detected by staining with 1% Alizarin

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