Research Article

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Long-Term Release Profiles of Growth Factors From DPSCs on Demineralized Dentin Under Osteogenic Environment and Their Effects on Odontogenic Differentation

Osteojenik Ortam ile Demineralize Dentinde Kültür Edilen DPKH'lerden Büyüme Faktörlerinin Uzun Süreli Salım Profili ve Odontojenik Farklılaşma Üzerine Etkisi

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ABSTRACT

It is known that bone morphogenetic protein (BMP-2) and vascular endothelial growth factor (VEGF) are released from dentin and stem cells, concomitantly to participate in the odontogenic differentiation of dental pulp stem cells (DPSCs). However, long-term release profiles of VEGF and BMP-2 from DPSCs on demineralized dentin have not yet been investigated under osteogenic differentiation condition. In this study, after demineralization of human dentin by 15% ethylenediaminetetraacetic acid (EDTA) for 15 min, isolated human DPSCs were cultured on demineralized dentin for 28 days and the release amount of VEGF and BMP-2 and their effects on odontogenic differentiation of cells were investigated. Results showed that cells well attached to demineralized dentin surfaces and proliferated. At the end of day 28, BMP-2 and VEGF release from demineralized dentin was determined as 0.45 and 18.5 ng/mL respectively in the presence of cells. The amount of released growth factors resulted in 10.7-fold increase in Dentin Matrix Protein 1 (DMP-1) expression whereas Bone Sialoprotein (BSP) expression was not upregulated. In an attempt to increase the odontogenic related gene expressions, 10 ng/mL of each recombinant growth factors (rhBMP-2 and rhVEGF) were added into osteogenic medium and DMP-1 and BSP expression were evaluated. As compared to the control group, in terms of DMP-1, rhBMP-2 and rhVEGF resulted in 32.9-fold and 26.4-fold increase, respectively ($p<0.05$). On the other hand, rhBMP-2 and rhVEGF caused a 4.7-fold and 3.6-fold increase of BSP expression, respectively ($p<0.05$). As a result, it has been concluded that to increase the odontogenic differentiation of DPSCs, the growth factors can be exogeneously used in the environment.

Keywords: pulp regeneration, dental pulp stem cells, vascular endothelial growth factor, bone morphogenetic protein, odontogenic differentiation

ÖZET

Kemik morfogenetik proteini (BMP-2) ve vasküler endotel büyüme faktörünün (VEGF) dentin ve kök hücrelerden salınarak, diş pulpa kök hücrelerinin (DPKH) odontogenik faklılaşmasına katıldıkları bilinmektedir. Bununla birlikte, demineralize dentin üzerindeki DPKH'lerden VEGF ve BMP-2'nin uzun süreli salım profilleri henüz osteojenik farklılaşma ortamında araştırılmamıştır. Bu çalışmada,

insan diş dentini % 15'lik etilendiamintetraasetik asit (EDTA) ile 15 dakika demineralize edildikten sonra insan diş pulpasından izole edilen DPKH'ler demineralize dentin üzerinde 28 gün kültür edilmiş ve VEGF ve BMP-2'nin salım miktarları ve bunların odontojenik farklılaşması üzerindeki etkileri araştırılmıştır. Sonuçlar, hücrelerin demineralize dentin yüzeyine oldukça iyi tutunduklarını ve çoğaldıklarını göstermiştir. 28 gün sonunda DPKH'lerin varlığında, demineralize dentinden 0.45 ng/mL BMP-2 salımı gözlenirken, hücre varlığında 18.5 ng/mL VEGF salımı olmuştur. Büyüme faktörlerinin bu miktarları Dentin Matriks Protein-1 (DMP-1) ifadesinde 10.7 katlık artış sağlarken, Kemik Sialoprotein (BSP) ifadesinde bir artış olmamıştır. Hücre farklılaşmasını artırmak için osteojenik ortama rekombinat büyüme faktörlerinden (rhBMP-2 ve rhVEGF) 10 ng/mL eklenmiş ve DMP-1 ve BSP ifadeleri değerlendirilmiştir. Kontrol grubu ile kıyaslandığında, rhBMP-2 ve rhVEGF eklenmesi, DMP-1 ifadesinde sırasıyla 32.9 ve 26.4 kat artışa neden olmuştur ($p<0.05$). Diğer taraftan, rhBMP-2 ve rhVEGF, BSP ifadesinde sırasıyla 4.7 ve 3.6 katlık artış sağlamıştır ($p<0.05$). DPKH'lerin odontojenik farklılaşmasının artırılmasında ortama eksojen VEGF ve BMP-2'nin eklenmesinin yararlı olacağı sonucuna varılmıştır.

Anahtar Kelimeler: pulpa rejenerasyonu, dental pulpa kök hücresi, vasküler endotel büyüme faktörü, kemik morfogenetik proteini, odontogenik farklılaşma

1. INTRODUCTION

Pulp necrosis in immature permanent teeth leads to cessation of root development and increases the risk of root fracture [1]. In these cases, pulp revascularization allows thickening and lengthening of root canal walls and maturation of the roots [2]. This biologically based procedure aims to regenerate pulp tissue inside the root canals, promote root development and restore the structure and function of the pulp-dentin tissue [3]. Although this treatment can allow the root development, treatment outcomes are mostly unpredictable including poor root development that can jeopardize the long-term retention of the natural tooth structure [4]. Thus, the regeneration of pulp-like tissue in the root canal is still a major issue in regenerative endodontics to be addressed.

Dental pulp tissue is a soft connective tissue that has dentinal tubules on the dentin side lined with odontoblasts and fibrous tissue containing blood vessels. This tissue also contains dental pulp stem cells (DPSCs) that are responsible for dentin-pulp regeneration [5]. Pulp regeneration initiates with the formation of new vascular tissue inside the pulp space and odontoblasts lining against the existing dentin. DPSCs can differentiate to odontoblasts and regenerate a dentin-pulp complex [6]. At this stage, some growth factors which are released from dentin, participate in the differentiation of DPSCs to odontoblasts [6]. Among them, bone morphogenetic proteins such as BMP-2, BMP-4, BMP-7 play important role in dentin-pulp regeneration [7]. In this respect, BMP-2 is required for odontogenic/osteogenic differentiation of DPSCs and the blockage of this signal molecule was shown to inhibit the differentiation of the cells [8]. Recently, apart from the angiogenic effect of vascular endothelial growth factor (VEGF), it has been shown that VEGF is released from DPSCs at undifferentiated conditions and triggers the odontogenic differentiation of DPSCs [9, 10].

To date, several studies have investigated the release of these growth factors from the dentin matrix to understand the mechanism of dentin-induced odonto/osteogenesis [11-14]. These bioactive molecules can be released by caries [15], application of dental materials [16], acids [17] or chelating agents [18] with the demineralization of dentin discs. Galler and co-workers investigated VEGF release from human dentin discs following 10% EDTA treatment for 5, 10 or 20 min and reported that 32 pg/mL of VEGF released after 20 min exposure [19]. In the other previous study, human dentin samples were conditioned with descending concentrations of the EDTA (17, 10 and 5%) for 5 min for each solution and BMP-2 release was investigated. Researchers reported that the amount of released BMP-2 was 123 pg/mL after 3 days and it was gradually decreasing to 4 pg/mL after 15 days [20]. The released amount of growth factors from dentin is very low and the demineralization process can further result in degradation of the growth factors [18]. Considering the clinical importance of the released amount of growth factors for the differentiation of DPSCs into odontoblast, it is crucial to know long-term release profiles of VEGF and BMP-2 from DPSCs on demineralized dentin, and whether these released amounts of growth factors result in a high level of odontogenic differentiation of DPSCs. For this reason, this study aimed to

investigate the long-term release profiles of VEGF and BMP-2 during odontogenic differentiation of DPSCs on dentin discs, and determine the odontogenic effect of endogenously released and exogenously added recombinant growth factors on the cell differentiation on the conditioned dentin disc *in vitro*.

2. MATERIALS AND METHODS

2.1 CELL ISOLATION AND CULTURE

Previously isolated and characterized human DPSCs were used in this study [21]. Briefly, pulp tissue was removed from the teeth, minced, and digested in a solution of 3 mg/mL collagenase type I (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at 37°C. Cells were cultured with α-Minimum Essential Medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gemini Bio-Products, Woodland, CA, USA), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 1% antibiotic-antimycotic (Life Technologies, Grand Island, NY, USA) and maintained in 5% CO_2 at 37°C. The cells were then passaged at 80% confluence until third passage and were used for the further experiments.

2.2 PREPARATION OF HUMAN DEMINERALIZED DENTIN

Forty-five freshly extracted human third molars were collected from healthy patients (aged 18-25 yr). The teeth were then transported to the laboratory in a sterile transport medium. Soft tissues and outer cementum were removed from the tooth surfaces with a periodontal curette. Dentin discs (1.5-mm thick and 6 mm width tooth slice per tooth) were prepared by making two parallel transverse cuts at the cervical region using a slow-speed 0.3 mm microtome saw (Isomet Buehler, IL, USA) under cooling with sterile phosphate buffered saline (Cellgro, Herndon, VA, USA) as described previously [22]. The obtained dentin discs were treated with betadine for 30 min and 1.5% sodium hypochlorite (Werax, Izmir, Turkey) for 10 min to remove the organic component and the debris originating from pulp tissue and microorganisms. The samples were soaked in 15% ethylenediaminetetraacetic acid (EDTA, Werax, Izmir, Turkey) for 15 min at room temperature for the demineralization and release of growth factors [23]. The samples were then incubated in the cell culture medium at 37°C for 7 days to remove the residual disinfection agents and were observed for the microbial contamination [23]. These dentin discs were then used in the following experiments.

2.3 EVALUATION OF CELL VIABILITY AND MORPHOLOGY OF DPSCs

Cell viability on dentin discs was analyzed after 1, 3, 5, 7 and 14 days using the MTT assay. For this purpose, fifteen demineralized dentin discs as prepared previously were placed in a 24-well plate according to the time periods each containing three dentin discs (n=3). DPSCs ($5x10^4$ cells/disc) were then seeded onto each demineralized dentin discs and incubated in the culture medium. DPSCs ($5x10^4$ cells/well) in the monolayer culture were used as a control group (n=3). At each time point, the culture medium was removed and the cells were incubated with 5 mg/mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] reagent (Sigma-Aldrich, St. Louis, Missouri, USA) for 4 h. Following that, dimethyl sulfoxide (Sigma-Aldrich, St. Louis, Missouri, USA) was added to dissolve the formazan crystals and the optical density was measured at 570 nm using a micro plate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA).

The morphology of DPSCs on demineralized dentin discs was observed using a scanning electron microscope (SEM, EVO, 50; Carl Zeiss NTS GmbH, Oberkochen, Germany) after 1 d of incubation. For this purpose, DPSCs ($5x10^4$ cells/disc) on dentin discs (n=3) were fixed in 2.5% gluteraldehyde for 30 min and dehydrated in a graded series of ethanol. They were further fixed in hexamethyldisilazane (Sigma-Aldrich, St. Louis, Missouri, USA) and air-dried. After gold coating, the samples were visualized using SEM.

2.4 DETERMINATION OF RELEASE PROFILES OF VEGF AND BMP-2

The release profiles of VEGF and BMP-2 from DPSCs on demineralized dentin discs were determined using an enzyme-linked immunosorbent assay (ELISA, R&D Quantikine, Minneapolis, MN, USA). For this purpose, six dentin discs for VEGF release and six dentin discs for BMP-2 release were placed in a 24-well plate. Following that, DPSCs ($5x10^4$ cells/disc) were seeded onto demineralized dentin discs (n=3) and incubated in odontogenic medium for 28 days. Three dentin discs without DPSCs were used

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as the control group. The released media was exchanged with 1 mL of fresh medium at specific time points (1, 4, 7, 14 and 28 days). The collected supernatants were frozen at -20°C until analysis. A standard curve was created using VEGF and BMP-2 standards and the quantification was performed based on the obtained calibration curve. The obtained release profiles of VEGF and BMP-2 were further used for the addition of exogenous growth factor at specific time intervals.

2.5 GENE EXPRESSION ANALYSIS

To determine the effects of exogenous growth factors on odontogenic differentiation of DPSCs on demineralized dentin, DPSCs ($5x10^4$ cells/disc) were incubated on demineralized dentin using odontogenic medium and rhVEGF containing odontogenic medium or rhBMP-2 containing odontogenic medium for 28 days (n=3) by refreshing the differentiation medium twice a week. DPSCs ($5x10^4$ cells/well) on monolayer culture were used as a control (n=3). For the addition of rhVEGF and rhBMP-2 (R&D Quantikine, Minneapolis, MN, USA) into odontogenic medium, the growth factors were prepared according to manufacturer instructions and 10 ng/mL of each growth factor was added to odontogenic medium. rhVEGF was added into odontogenic medium for the first 14 days. After that, for further 14 days, the cells were incubated with odontogenic medium without addition of growth factor. On the other hand, rhBMP-2 was continuously added into odontogenic medium during 28 days of incubation of DPSCs on dentin discs. Following the incubation of the cells, the odontogenic differentiation of DPSCs was identified by the expressions of two odontogenic markers [dentin matrix protein (DMP-1) and bone sialoprotein (BSP)] using qPCR. For the analysis, total RNA was extracted with the GeneMatrix Universal RNA Purification Kit (Eurx, Poland). cDNA was synthesized using a high-capacity M-MLV Reverse Transcriptase RNase H- (Solis BioDyne, Tartu, Estonia) and qPCR was carried out with HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia).

Primer sequences used in the study for DMP-1 (F:ATGCCTATCACAACAAACC), R:CTCCTTTATGTGACAACTGC); for BSP (F:AAAGTGAGAACGGGGAACCT, R:GATGCAAAGCCAGAATGGAT). The comparative Ct method, $2^{-(\Delta\Delta Ct)}$, was used to quantify the relative gene expression compared to the level of housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase, GAPDH). The analysis was performed in triplicate from three different samples.

2.6 STATISTICAL ANALYSIS

All data were analyzed using the SPSS statistical software package (version 21.0, Chicago, IL, USA) and expressed as mean ± SD. The differences between the groups for MTT and ELISA analysis were determined using Student t-test. The data for the gene expressions among the experimental groups were analyzed using one-way ANOVA and post-hoc Tukey HSD test. Values are considered statistically significant when $p < 0.05$.

2.7 ETHICAL STATEMENT

This study was approved by Hacettepe University Ethics Committee (GO17/195-03).

3. RESULTS

3.1 VIABILITY AND MORPHOLOGY OF DPSCs ON DEMINERALIZED DENTIN DISCS

DPSCs grew exponentially for 7 days and the viability was doubled at 14 days. The viability rate of cells on demineralized dentin disc was similar to the control group ($p>0.05$) (Fig. 1A). Results showed that demineralized dentin discs had open dentinal tubules without any erosion and exposed fiber bundles of intertubular and peritubular dentin (Fig. 1B-a). The cells stretched out and attached onto dentin surface by cytoplasmic extensions through dentinal tubules (Fig. 1B-b to d).

3.2 RELEASE PROFILES OF VEGF AND BMP-2 FROM DPSCs ON DEMINERALIZED DENTIN

The amount of VEGF and BMP-2 release was shown in Fig. 2. Regarding the release of VEGF, the amount of growth factor from DPSCs on dentin discs were significantly higher than the demineralized dentin alone at all culture periods ($p<0.05$). 0.45 ng/mL of VEGF was released from demineralized dentin alone while 2.3 ng/mL was released in the presence of DPSCs at 7 days. Then, VEGF release from DPSCs on

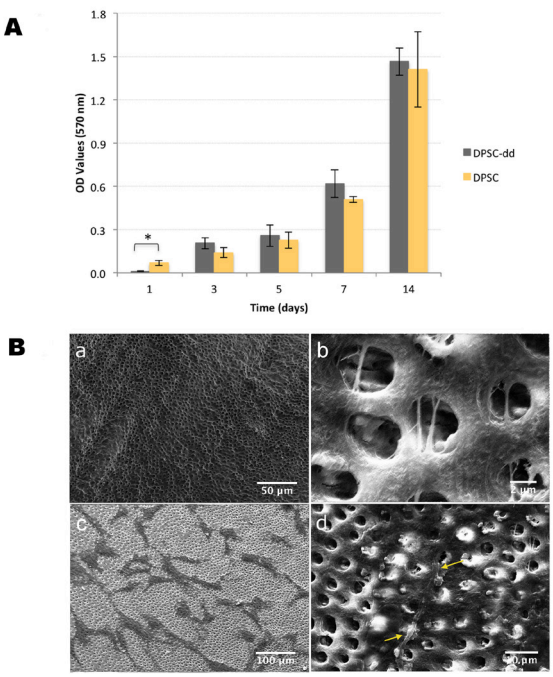

Figure 1. Proliferation and morphology of DPSCs on demineralized dentin (dd) (n=3). **A)** The viability of DPSCs on demineralized dentin (DPSC-dd), **B)** Scanning electron microscope images of dentin and DPSCs ($5x10^4$ cells/disc) on demineralized dentin at 1 day. (a) SEM images showed that demineralized dentin has open dentinal tubules and exposed fiber bundles of intertubular and peritubular dentin. Cell attachment onto the dentin surface at lower (c) and higher (b-d) magnification. Arrows indicate the cell cytoplasmic extensions on dentin discs.

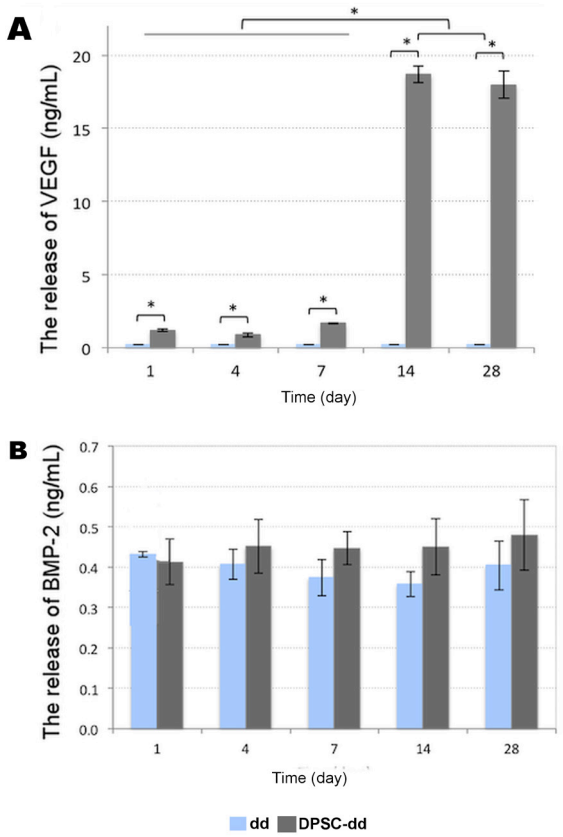

Figure 2. The release of (**A)** VEGF and (**B)** BMP-2 from demineralized dentin (dd) and DPSCs on demineralied dentin (DPSC-dd) (n=3). *significant difference = $p<0.05$.

dentin discs were increased substantially to 18.5 ng/mL at 14 days ($p<0.05$) and continued as constant until 28 days. For BMP-2, the released amount was similar in the presence or absence of DPSCs, and continued at a low rate (0.45 ng/mL) constantly up to 28 days ($p>0.05$).

3.3 THE EFFECTS OF GROWTH FACTORS ON ODONTOGENIC DIFFERENTIATION OF DPSCs

The effect of endogenously released and exogenously added recombinant growth factors on odontogenic gene expressions (DMP-1 and BSP) of DPSCs on demineralized dentin was investigated and shown in Fig. 3. Accordingly, DPSCs on demineralized dentin discs in osteogenic medium (DPSC-dd) showed a 10.7-fold increase in DMP-1 expression as compared to the control group (DPSCs on monolayer culture) ($p<0.05$) (Fig 3A). However, BSP expression was not upregulated in both conditions ($p>0.05$). Therefore, in the attempt to increase cellular differentiation, we added 10 ng/mL of rhVEGF and rhBMP separately into osteogenic medium according to previously identified released profiles. Due to the lower amounts of VEGF release from demineralized dentin discs in the presence of DPSCs within 14 days, rhVEGF was added into odontogenic medium for the first 14 days, while rhBMP-2 was continuously added into odontogenic medium for 28 days for the determination of their effects on odontogenic differentiation of DPSCs on dentin discs.

The addition of rhVEGF caused a 2.5-fold increase in DMP-1 and a 4.0-fold increase in BSP expression as compared with the DPSC-dd. Also, it showed a 26.4-fold increase in DMP-1 and 3.6-fold increase in BSP expression than the control group ($p<0.05$).

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The addition of rhBMP-2 showed 3.1-fold and 5.3-fold increase of DMP-1 and BSP expressions, respectively, than the DPSCs on dentin discs, while 32.9-fold increase in DMP-1 and 4.7-fold increase of BSP expressions were observed as compared to the control group ($p<0.05$). Regarding the effect of rhVEGF and rhBMP-2 on the gene expressions, rhBMP-2 containing odontogenic medium resulted in 1.2-fold higher DMP-1 and 1.3-fold higher BSP expressions as compared to the effect of rhVEGF containing odontogenic medium.

4. DISCUSSION

The release of specific growth factors from cells and microenvironments is an inductive mechanism for cell growth and differentiation during the regenerative process. During pulp regeneration, the release of growth factors from dentin disc is required for the differentiation of DPSCs into odontoblasts [24]. This study investigated long-term release profiles of two essential growth factors (VEGF and BMP-2) during odontogenic differentiation of DPSCs on demineralized dentin. Furthermore, we investigated the effect of endogenously released and exogenously added rhVEGF and rhBMP-2 factors on the odontogenic differentiation of DPSCs on demineralized dentin.

In agreement with previously reported studies, we showed that dentin discs demineralized with EDTA showed biocompatibility for DPSCs [12, 25]. SEM observation revealed a dense, stretched and anchored DPSCs on demineralized dentin surfaces. The viability of DPSCs on demineralized dentin was markedly increased after 14 days of incubation. This increase could stem from the increased release of VEGF from DPSCs as shown in the present study (Fig 2). Based on these results, VEGF seems to be more associated with the proliferation of DPSCs rather than BMP-2 as reported in a previous study [9, 10].

Several studies have reported the released amount of growth factors from dentin disc after dentin conditioning. A previous study demonstrated that dentin releases transforming growth factor-β1, fibroblast growth factor and VEGF after conditioning with EDTA [19]. However, dentin disc (8 mm diameter and 200 μm thickness) has been shown to release as low as 32 pg/mL VEGF by 20 min EDTA conditioning [19]. Another recent study did not show detectable release of VEGF or BMP-2 from dentin disc after EDTA conditioning [18]. In these studies, the limited amount of growth factors might stem from the detection of growth factors directly in the test solutions (EDTA or citric acid) that can damage the stability of growth factors [19]. In our study, after conditioning of dentin disc with EDTA solution for 15 min, the growth factors were allowed to release into odontogenic medium for 28 days and the amount of released VEGF and BMP-2 was found higher than those reported in the literature. Presumably, this can be attributed to osteogenic medium which ensures the stability of growth factors.

In order to clarify the source of growth factors, firstly, we analyzed the amount of VEGF and BMP-2 in odontogenic medium alone. However, none of these were detected in the osteogenic medium. On

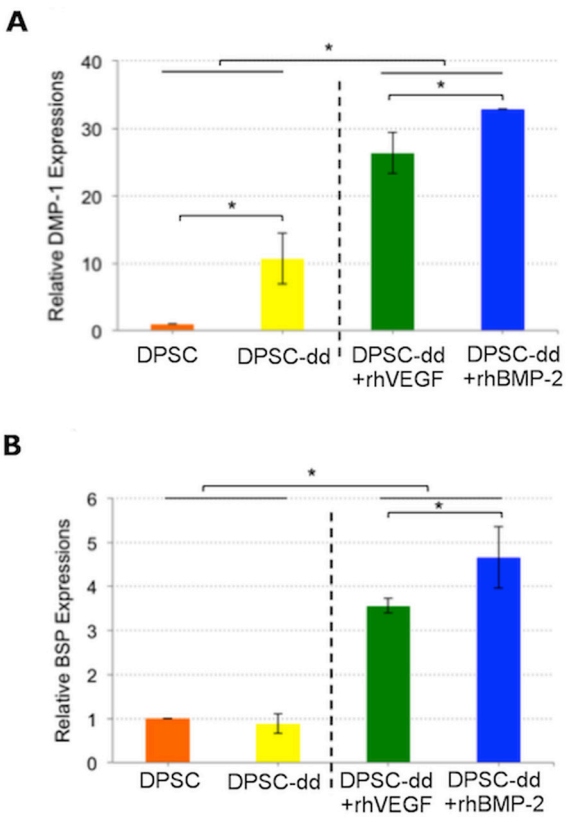

Figure 3. The effect of released growth factors from DPSCs, DPSCs on demineralized dentin (DPSC-dd) and added recombinant growth factors on (**A)** Dentin Matrix Protein 1 (DMP-1) and (**B)** Bone Sialoprotein (BSP) expressions. The results represent the mean ± SD of three samples performed in triplicate (*significant difference= $p<0.05$).

the other hand, ELISA results showed that the presence of DPSCs on demineralized dentin markedly increased (about 40-fold) the VEGF release after 14 days as compared with the demineralized dentin alone. Since it is known that DPSCs produce VEGF spontaneously [10, 26], this release of VEGF can be attributed to the presence of DPSCs. In the previous study, amount of released VEGF from DPSCs in the cell culture medium was reported as about 2.4±0.3 ng/mL after 2 days of culture. In our study, the amount of released VEGF was found about 8-fold higher than reported value and measured as 18.5 ng/mL after 14 days of culture. This difference might be attributed to the using of cell culture conditions and culture duration. As mentioned before, we cultured DPSCs on demineralized dentin discs in osteogenic medium for 28 days and investigated growth factor releases in a time depended manner. So, based on our results and previously reported data, we may conclude that when DPSCs differentiate into odontoblasts, they produce much more VEGF as compare to undifferentiation conditions. On the other hand, BMP-2 release was similar regardless of the presence of DPSCs. Asgary and coworkers have reported that, about 26 pg/mL of BMP-2 released from DPSCs under differentiation or undifferentiation conditions [27]. In our study, we found a higher amount of BMP-2 release that was measured as 0.45 ng/mL (Fig.2). These differences may be attributed to the presence of demineralized dentin disc.

In the present study, the odontogenic effect of the endogenous released and exogenous growth factor addition on DPSCs in the presence of dentin disc was evaluated. qPCR results showed that released VEGF and BMP-2 successfully induced DMP-1 expression whereas BSP expression was not induced. Therefore, to increase both DMP-1 and BSP expressions, we added growth factors into the osteogenic medium [9, 28]. A previous *in vitro* study showed that the addition of rhVEGF during the early periods of culture increased the viability of mouse-derived bone marrow-mesenchymal stem cells, while a constant addition for 28 days did not affect the proliferation [29]. Meanwhile, the continuous addition of rhBMP-2 increased the osteogenic gene expressions [29]. In our study, the endogenous release profile of VEGF and BMP-2 was previously determined for the addition of exogenous growth factors at a specific time of cell differentiation. According to the endogenous release profile of growth factors, the release of VEGF was lower for the first 7 days and then it showed a prominent increase at 14 days. The release of BMP-2, however, was low in the presence or absence of DPSCs during all culture periods. By following the release profiles of VEGF and BMP-2, DPSCs on demineralized dentin were incubated in odontogenic medium that contained rhVEGF for the first 14 days while rhBMP-2 was continually added into the odontogenic medium during all culture periods. Results clearly showed that the exogenously added VEGF and BMP-2 enhanced DMP-1 and BSP expression much more than endogenously released growth factors (Fig 3). Based on these results we can conclude that endogenously released amount of VEGF and BMP-2 into osteogenic medium does not result in a high level of odontogenic genes expressions. To increase odontogenic differentiation of DPSCs on dentin disc, exogenously growth factors need to be added into the osteogenic medium. In agreement with our results, a previous study also showed that the addition of rhBMP-2 into demineralized dentin granules resulted in 27.9-fold increase in bone formation after 4 weeks of subcutaneous mouse implantation [30]. One of the most used growth factor in clinical applications is BMP-2 and it is approved by Food and Drug Administration (FDA) for the treatment of bone or dental tissue related health problems [31]. However, some complications like ectopic bone formation, osteoclast activation, osteolysis, and subsidence are reported due to BMP-2 treatment in clinic. But, researchers stated that, these adverse effects can be suppressed by using some alternative delivery systems, appropriate dosing or addition of VEGF, bisphosphonates and anti-inflammatory drugs in treatment protocols [32, 33]. Therefore, we should recommend that, loading of growth factors into appropriate carries to delivery or using both growth factors at the same time can be more effective strategies than the direct applications as a treatment strategy.

5. CONCLUSIONS

Our findings show that VEGF and BMP-2 are continuously released from DPSCs on demineralized dentin for up to 28 days, but their amount does not ensure a high degree of odontogenic differentiation of cells, even if the osteogenic induction medium is used. Therefore, we concluded that the addition of growth factors exogenously to the environment ensures higher odontogenic differentiation of DPSCs

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on dentin. Therefore, it should be emphasized that growth factors can be added to treatment protocols via novel strategies like polymeric delivery systems to increase the clinical outcome in terms of dental pulp tissue regeneration in clinical endodontic applications.

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7. CONFLICT OF INTEREST

The authors deny any conflicts of interest, any financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials used in this manuscript, nor have any such arrangements existed in the past three years.

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