Towards osteogenic differentiation of human dental pulp stem cells on PCL-PEG-PCL/zeolite nanofibrous scaffolds

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ABSTRACT
Presently, tissue engineering has been developed as an effective option in the restoration and repair of tissue defects. One of the tissue engineering strategies is to use both biodegradable scaffolds and stimulating factors for enhancing cell responses. In this study, the effect of zeolite was assessed on cell viability, proliferation, osteo/odontogenic differentiation, and mineralization of human dental pulp stem cells (hDPSCs) cultured on poly (e-caprolactone) – poly (ethylene glycol)-poly (e-caprolactone) (PCL-PEG-PCL) nanofibers. For this purpose, PCL-PEG-PCL nanofibrous scaffolds incorporated with zeolite were prepared via electrospinning. Both PCL-PEG-PCL and PCL-PEG-PCL/Zeolite nanofibrous scaffolds revealed bead-less constructions with average diameters of 430 nm and 437 nm, respectively. HDPSCs were transferred to PCL-PEG-PCL nanofibrous scaffolds containing zeolite nanoparticles. Cell adhesion and proliferation of hDPSCs and their osteo/odontogenic differentiation on these scaffolds were evaluated using MTT assay, Alizarin red S staining, and qRT-PCR assay. The results revealed that PCL-PEG-PCL/Zeolite nanofibrous scaffolds could support better cell adhesion, proliferation and osteogenic differentiation of hDPSCs and as such is expected to be a promising scaffold for bone tissue engineering applications.

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Introduction
Nowadays, nanobiomaterials have become popular in drug delivery systems and regenerative medicine [1,2]. These materials are widely applied in cancer therapy [3,4], photodynamic therapy [5] and tissue engineering including cartilage and bone regeneration [6,7].

Treatment of critical-sized bone defects due to trauma, tumor resection and congenital reasons is a major challenge for maxillofacial surgeons. The autografts are usually considered in these defects. However, the morbidity of the donor site and limited availability compromise the application of this method [8]. On the other hand, bone tissue engineering is a promising approach for bone reconstruction with fewer complications compared to autografts [9–11]. Tissue engineering is a combination of scaffolds, cells, and bioactive substance. The scaffold in bone tissue engineering should have biodegradability, biocompatibility and mechanical strength [12]. The nanoscale substrate in bone tissue engineering promotes cell migration, cell adhesion and proliferation [9]. The nanofibrous PCL-PEG-PCL scaffolds fabricated by electrospinning method have shown these properties which could replicate the natural extracellular matrix (ECM) [13,14]. These biodegradable scaffolds provide a suitable three-dimensional structure required for cell adhesion and cell delivery to specific sites [15]. Meanwhile, multipotent stem cells could differentiate into specific cell lineages [16]. Human dental pulp stem cells with a potential of odontogenic and osteogenic differentiation are one of the sufficient and available sources for tissue engineering [17,18]. These cells have proved to be more available and shown higher cell proliferation compared to bone marrow mesenchymal stem cells (MSC) [19].

The nanoparticles loaded on scaffolds as bioactive molecules could enhance the cell adhesion, cell function, cell interaction and biomineralization [1,6,9,15]. Zeolite has a microporous structure and is widely used in biomedical research and tissue engineering field [20,21].

Zeolite molecules have been used as an antibacterial agent, drug carrier and anti-cancer agent [22]. The surface of zeolite nanoparticles in contact with physiological fluid could be covered by a layer of biomaterials such as calcium and phosphate
ions, which could enhance the cell adhesion, collagen formation and apatite crystallization. Therefore, zeolite nanoparticles could be considered as osteoinductive materials [23]. Zeolite framework contains aluminum, silicon and oxygen. Silicon has an important role in the formation of hard tissue, especially in the early stage of bone calcification [24,25].

The present study was conducted to evaluate the effect of adding zeolite nanoparticles to PCL-PEG-PCL scaffolds on cell adhesion, proliferation and osteo/odontogenic differentiation of hDPSCs. The PCL-PEG-PCL scaffolds were fabricated by electrospinning method as with our previous study [13].

Materials and methods

Synthesis and characterization of PCL-PEG-PCL polymer

The synthesis and characterization of PCL-PEG-PCL polymer were described in our previous study in detail [9]. Briefly, ring-opening polymerization of ε-caprolactone (20.0 g, 175 mmol) (Sigma-Aldrich Co., Steinem, Germany) and PEG (2.0 g, 0.5 mmol) (Sigma-Aldrich Co., Steinem, Germany) was catalyzed by Sn (Oct)2 (0.5 wt%) (Sigma-Aldrich Co., Steinem, Germany). The polymerization was performed at 130 °C under stirring in a nitrogen atmosphere for 12 h. The unreacted species were removed by dissolving the product in methylene chloride (Merck Chemical Co, Darmstadt, Germany) and precipitated in an excess of cold hexane (Sigma-Aldrich Co., Steinem, Germany). The molecular weight of the product was 45454 g/mol as described in our previous study [9].

Electrospinning of PCL-PEG-PCL/zeolite nanofibers

To prepare the 15 wt% PCL-PEG-PCL solution, PCL-PEG-PCL was dissolved in chloroform/methanol (Merck Chemical Co.) (3:1 v/v) solvent mixture. The solution was magnetically kept stirring at room temperature for 24 h. A polymeric solution containing synthesized zeolite nanoparticles was prepared by mixing PCL-PEG-PCL solution and a certain amount of zeolite nanoparticles (15 wt%) of the initial polymer weight. This solution was sonicated for 20 min to completely disperse the zeolite nanoparticles in the PCL-PEG-PCL solution.

For electrospinning, PCL-PEG-PCL and PCL-PEG-PCL/Zeolite solutions were separately transferred into a 5 ml plastic syringe with a 16 G needle. The flow rate of spinning solutions was adjusted at 1–1.2 ml/h, a high voltage of 15–17 kV and a 10–12 cm syringe-collector distance set through electrospinning process. All nanofibers were produced at room temperature and then stored in vacuum until usage.

Isolation and characterization of hDPSCs

We used hDPSCs which were isolated in our previous study [26]. All experimental protocols were approved by the Ethics Committee of Tabriz University of Medical Sciences (TUOMS) which was in compliance with Helsinki declaration and all participants signed the informed consent (Approval No. IRTBZMED.REC.1395.1203).

Before seeding hDPSCs on scaffolds, 70% ethanol and ultraviolet light were used to sterilize the scaffolds for 60 min and 20 min, respectively. Then, the scaffolds were washed with sterile PBS (Gibco, Singapore) three times. The sterile scaffolds were incubated at 37 °C for 24 h. HDPSCs were trypsinized and seeded on scaffolds with 100,000 cells in each well of 24-well and 96-well plates. The control group contained hDPSCs cultured without scaffolds on tissue culture plates (TCPs). The medium of the plates contained DMEM (Gibco, Singapore) supplemented with 0.01 fetal bovine serum (Gibco, Singapore), 100 U/mL penicillin, 100 μg mL⁻¹ streptomycin, and 1× amphotericin B. The plates were kept in an incubator at 37 °C with 5% CO₂. The cell culture medium was refreshed every 3 days during this period.

Proliferation, viability, and differentiation of hDPSCs cultured on nanofibers

Cell proliferation analysis by the MTT test

The cell survival rate and proliferation of hDPSCs on nanofibrous scaffolds were assessed by MTT assay. The cells were harvested by 0.05% trypsin containing 1 mM EDTA (Gibco, Singapore), after which 500 μL (3–4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Carlsbad, CA, USA) was added to the wells after 3, 7 and 12 days to perform the MTT assay. After 4 h of incubation, DMSO (Merck Chemical Co.) was added. The color change and amount of dissolved blue furazan crystals were examined by spectrophotometry at 570 nm. The process of MTT assay was repeated three times and the data were reported as mean ± SD.

Cell morphology

After 14 days, the morphologies of the hDPSCs on mentioned scaffolds were assessed by FE-SEM. Briefly, PCL-PEG-PCL and PCL-PEG-PCL/Zeolite scaffolds were washed with PBS three times and then fixed with 3.5% glutaraldehyde for 1 h at 25 °C. Then, the scaffolds were rinsed with PBS and dehydrated with ethanol at different concentrations (50, 70, 90 and 100%). Finally, the samples were air-dried overnight and were then analyzed by FE-SEM.

Alizarin red S activity

After 21 days, mineral deposition produced by hDPSCs on scaffolds was assayed by Alizarin Red S staining protocols [27]. For this purpose, after washing the cells by water, ice-cold 70% ethanol was used to fix them. After 1 h, the ethanol was washed by water after which 40 mm Alizarin red S solution (pH 4.2) was added and left for 15 min. Excess Alizarin red S solution was washed several times with water. Afterward, 10% (W/V) cetylpyridinium chloride in 10 mm sodium phosphate was applied for 15 min. Alizarin red S levels were assessed at 570 nm by absorbance measurement.

qRT–PCR

Twenty-one days after seeding of hDPSCs on scaffolds, total RNA of the cells was isolated using Trizol (YektaTajhizAzma, Tehran, Iran) reagent according to the manufacturer’s instructions. The extracted RNA yield and value were assessed by Gel-electrophoresis and Nanodrop (Thermo Scientific,
Waltham, MA, USA). cDNA synthesis kit (YektaTahijAzma, Iran) used 1 μg extracted total RNA for cDNA synthesis. The synthesized CDNA, Syber green Master Mix, and Primers of BMP2, RUNX2, DSPP and BGLAP genes were mixed and the PCR data were analyzed by ΔΔCT method. The primer sequences are offered in Table 1.

**Statistical analysis**

ANOVA and T-test were used for statistical comparison of groups and p values < .05 was considered significant. All the experiments were carried out in triplicate.

**Results and discussion**

**Fabrication of PCL-PEG-PCL/zeolite nanofibers**

PCL-PEG-PCL nanofibers are extensively studied in bone regeneration applications. Both PCL and PEG are acknowledged as biocompatible and biodegradable polymers allowing for successful culturing of cell types. Also, PCL and PEG have shown good results in forming cell scaffold complexes [28,29]. Zeolite nanoparticles were added to the PCL-PEG-PCL polymer solution before electrospinning to improve the characteristic of osteogenic induction. Several electrospinning parameters including electric voltage, concentration, feed injection rate, and distance of source electrode were modified to achieve bead-less nano-fibrous scaffolds with relatively similar surface morphology. Figure 1 demonstrates the FE-SEM images of PCL-PEG-PCL nanofibers with or without zeolite nanoparticles along with their diameter distribution. Both PCL-PEG-PCL and PCL-PEG-PCL/Zeolite nanofibrous scaffolds revealed bead-less constructions with smooth surface morphologies and high interconnected pores. Upon the incorporation of zeolite nanoparticles to PCL-PEG-PCL nanofibers, the average diameter of fibers increased. Specifically, the average diameter of the PCL-PEG-PCL fibers was 430.31 ± 180.8 nm, while that of PCL-PEG-PCL/Zeolite fibers was 437.9 ± 176.1 nm. Highly porous and interconnected pore constructions are the requirements for tissue engineering applications since they can provide an appropriate 3D space for cell growth and proliferation and would be helpful for water, nutrient and growth factor transportations as well [11].

**Proliferation, viability, and differentiation of hDPSCs cultured on nanofibers**

**MTT assay**

The cell viability and proliferation of hDPSCs over the PCL-PEG-PCL/Zeolite nanofibrous scaffolds as well as on PCL-PEG-PCL scaffolds without zeolite incorporation were investigated using MTT assay on days 3, 7 and 12 (Figure 2). hDPSCs cultured on TCPs containing DMEM without nanofibers was taken as a control. The proliferation of hDPSCs was not suppressed as being seeded on PCL-PEG-PCL and zeolite coated PCL-PEG-PCL nanofibrous scaffolds. These results indicated that zeolite nanoparticles on PCL-PEG-PCL scaffolds did not have toxic effects on hDPSCs. According to MTT results, the viability and proliferation of stem cells increased over time. The PCL-PEG-PCL/Zeolite scaffolds revealed significantly higher cell viability compared to the PCL-PEG-PCL group at the same time periods. Zeolite membranes also improved cell viability in the study of Tavolaro et al. [30]. The proliferation of viable hDPSCs over the PCL-PEG-PCL/Zeolite scaffolds was particularly considerable on the 12th day. These results indicate the positive effects of the combination of zeolite with PCL-PEG-PCL scaffolds on the cell viability and proliferation revealing their biocompatibility.

**FE-SEM analysis**

Figure 3 reveals the adhesion and proliferation of hDPSCs on the PCL-PEG-PCL nanofibers with or without zeolite after 14 days of culture. As shown in the FESEM images, the hDPSCs could adhere and uniformly disperse on the surface of PCL-PEG-PCL and PCL-PEG-PCL/Zeolite nanofibrous scaffolds and exhibited cortical cell morphologies. The production of long cytoplasmic prolongations on nanofibers evidenced good cytocompatibility and close interaction between stem cells and nanofibrous scaffolds. The hDPSCs penetrated into interconnected pores, secreted a large amount of extracellular matrix, covered almost the entire area of nanofibers and finally produced multilayer cell sheets such that the underlying nanofibrous samples would not be detectable. The cell density was high on all nanofibers. However, on the PCL-PEG-PCL/Zeolite nanofibers, hDPSCs grew far better. These observations seem to be evidence of the capacity of these improved nanofibrous scaffolds for adhesion and proliferation of hDPSCs and established the remarkable potential of these samples for bone regeneration applications.

**Alizarin red S staining**

Osteoblasts release a large amount of extracellular deposits on the surface of substrates. This process is known as mineralization which represents successful in vitro bone forma-

| Name | Forward | Reverse |
|------|---------|---------|
| BMP2 | GAAAGGGAGGCAAGAAGAAG | GAAGCAGCAAGCTAAGGAC |
| BGLAP | ATTTGCTACCTCCATCA | AGGGCATTTGGGCTATC |
| DSPP | CTTGTCATGAGGTGATAAG | TCTACTTCGCCCCACTTAG |
| RUNX2 | ACCCTGACCTAACCCTGCTTC | GGGCGTCAGGAACAAACTA |
| GAPDH | CAAGATCAGCAATGCCTCC | GCCACATCGCCACAGTTCC |

Table 1. Sequences of primers used for qPCR.

Using Alizarin red S staining on PCL-PEG-PCL/Zeolite nanofibrous scaffolds, the formation of calcium nodules was observed and quantified. The results revealed that Alizarin red S staining was significantly higher on all nanofibers. However, on the PCL-PEG-PCL/Zeolite nanofibers, hDPSCs grew far better. These observations seem to be evidence of the capacity of these improved nanofibrous scaffolds for adhesion and proliferation of hDPSCs and established the remarkable potential of these samples for bone regeneration applications.

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**Alizarin red S staining**

Osteoblasts release a large amount of extracellular deposits on the surface of substrates. This process is known as mineralization which represents successful in vitro bone forma-
positive effect in simulating mineralization, making them appropriate candidates for bone tissue engineering.

**qRT-PCR analysis**

The qRT-PCR analysis was performed to evaluate the effect of zeolite on the expression of key genes in bone and dentin formation such as osteocalcin, BMP2, RUNX2, and DSPP after seeding on nanofibrous scaffolds (Figure 5). According to the results, the expression of BMP2 and BGLAP genes in the PCL-PEG-PCL/Zeolite nanofibers was significantly higher than that in other groups ($p < .05$ and $p < .01$). BGLAP gene-encoded osteocalcin protein which is an important component of non-collagenic part of bone extracellular matrix [32]. BMP2 plays an important role in the formation of calcified tissues and osteoblastic differentiation [33]. Also, RUNX2 is known as an early osteoblastic differentiation factor [34] which was significantly higher in PCL-PEG-PCL/Zeolite nanofibers group ($p < .05$). On the other hand, DSPP gene as a classic odontogenic differentiation marker [16] did not show significant up-regulation compared with nanofibrous scaffolds. In summary, the results imply that incorporation of zeolite in PCL-PEG-PCL nanofibers up-regulated the expression of genes responsible for osteogenic differentiation of hDPSCs, but in odontogenic differentiation, it did not show significant differences.

**Conclusion**

The cell viability, cell adhesion and proliferation of hDPSCs on PCL-PEG-PCL/Zeolite nanofibrous scaffolds were significantly higher than in other groups. Alizarin red S staining and qRT-PCR analysis confirmed the osteogenic differentiation of hDPSCs on PCL-PEG-PCL incorporated with zeolite nanoparticles. These results suggest that zeolite nanoparticles
on PCL-PEG-PCL scaffolds could have a key role in

Figure 3. FE-SEM images of hDPSCs morphology on the (A,B) PCL-PEG-PCL, (C,D) PCL-PEG-PCL/Zeolite scaffolds.

Figure 4. (A) hDPSCs osteogenic differentiation on TCPs (control), PCL-PEG-PCL and PCL-PEG-PCL/Zeolite scaffolds after 21 days of cell seeding. *p < .01; (B) hDPSCs were stained with Alizarin red S staining at 21th day to envisage mineralized bone matrix.
osteoblastic physiology and could be a promising means in bone tissue engineering.

**Ethical approval**

All experimental protocols were approved by the Ethics Committee of Tabriz University of Medical Sciences (TUOMS) which was in compliance with Helsinki declaration (Approval No. IRTBZMED.REC.1395.1203). All participants signed the informed consent and no animal experiments were carried out for this article.

**Disclosure statement**

The authors report no conflict of interest.

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**References**

[1] Nasr FH, Khoee S, Dehghan MM, et al. Preparation and evaluation of contact lenses embedded with polycaprolactone-based nanoparticles for ocular drug delivery. Biomacromolecules. 2016; 17:485–495.

[2] Tarasi R, Khoobi M, Niknejad H, et al. β-cyclodextrin functionalized poly (5-amidoisophthalicacid) grafted Fe3O4 magnetic nanoparticles: A novel biocompatible nanocomposite for targeted doxetaxel delivery. J Magn Magn Mater. 2016;417:451–459.

[3] Hosseini Sadr S, Davaran S, Alizadeh E, et al. Enhanced anticancer potency by thermo/pH-responsive PCL-based magnetic nanoparticles. J Biomater Sci Polym Ed. 2018;29:277–308.

[4] Sadr SH, Davaran S, Alizadeh E, et al. PLA-based magnetic nanoparticles armed with thermo/pH responsive polymers for combination cancer chemotherapy. J Drug Deliv Sci Technol. 2018;45:240–254.

[5] Gholibegloo E, Karbasi A, Pourhajibagher M, et al. Carnosine-grafted graphene oxide conjugates decorated with hydroxyapatite as promising nanocarrier for ICG loading with enhanced antibacterial effects in photodynamic therapy against Streptococcus mutans. J Photochem Photobiol B. 2018;181:14–22.

[6] Hokmabad VR, Davaran S, Aghazadeh M, et al. Fabrication and characterization of novel ethyl cellulose-grafted-poly (ε-caprolactone)/alginate nanofibrous/macroporous scaffolds incorporated with nano-hydroxyapatite for bone tissue engineering. J Biomater Appl. 2019;33:1128.

[7] Saghebasl S, Davaran S, Rahbarghazi R, et al. Synthesis and in vitro evaluation of thermosensitive hydrogel scaffolds based on (PNIPAAm-PCL-PEG-PCL-PNIPAAm)/Gelatin and (PCL-PEG-PCL)/Gelatin for use in cartilage tissue engineering. J Biomater Sci Polym Ed. 2018;29:1185–1206.
[8] Ge S, Zhao N, Wang L, et al. Bone repair by periodontal ligament stem cell seeded nanohydroxyapatite-chitosan scaffold. Int J Nanomed. 2012;7:5405.

[9] Hoomabad VR, Davaran S, Aghazadeh M, et al. A comparison of the effects of silica and hydroxyapatite nanoparticles on poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone)/chitosan nanofibrous scaffolds for bone tissue engineering. Tissue Eng Regen Med. 2018;15:735–750.

[10] Li D, Sun H, Xu X, et al. Facile method to prepare PLGA/hydroxyapatite composite scaffold for bone tissue engineering. Mater Technol. 2013;28:316–323.

[11] Raiesdasteh Hoomabad V, Davaran S, Ramazani A, et al. Design and fabrication of porous biodegradable scaffolds: a strategy for tissue engineering. J Biomater Sci Polym Ed. 2017;28:1797–1825.

[12] Kouhi M, Morsheid M, Varshosaz J, et al. Poly (ε-caprolactone) incorporated bioactive glass nanoparticles and simvastatin nano-composite nanofibers: preparation, characterization and in vitro drug release for bone regeneration applications. Chem Eng J. 2013;228:1057–1065.

[13] Valizadeh A, Bakhtiary M, Akbarzadeh A, et al. Preparation and characterization of novel electrospun poly (ε-caprolactone)-based nanofibrous scaffolds. Artif Cell Nanomed Biotechnol. 2016;44:504–509.

[14] Venugopal JR, Low S, Choon AT, et al. Nanobioengineered electrospun composite nanofibers and osteoblasts for bone regeneration. Artif Organs. 2008;32:388–397.

[15] Liao F, Chen Y, Li Z, et al. A novel bioactive three-dimensional β-tricalcium phosphate/chitosan scaffold for periodontal tissue engineering. J Mater Sci Mater Med. 2010;21:489–496.

[16] Asghari F, Salehi R, Aghazadeh M, et al. The odontogenic differentiation of human dental pulp stem cells on hydroxyapatite-coated biodegradable nanofibrous scaffolds. Int J Polym Mat Polym Biomater. 2016;65:707–720.

[17] Samiei M, Agazadeh M, Alizadeh E, et al. Osteogenic/odontogenic bioengineering with co-administration of simvastatin and hydroxyapatite on poly caprolactone based nanofibrous scaffold. Adv Pharm Bull. 2016;6:353.

[18] Chen Z, Song Y, Zhang J, et al. Laminated electrospun nHA/PHB-composite scaffolds mimicking bone extracellular matrix for bone tissue engineering. Mater Sci Eng C. 2017;72:341–351.

[19] Yamaza T, Kentaro A, Chen C, et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem cell Res Ther. 2010;1:5.

[20] Li Y, Jiao Y, Li X, et al. Improving the osteointegration of Ti6Al4V by zeolite MFI coating. Biochem Biophys Res Commun. 2013;460:151–156.

[21] Shameli K, Ahmad MB, Zargar M, et al. Fabrication of silver nanoparticles doped in the zeolite framework and antibacterial activity. Int J Nanomed. 2011;6:331.

[22] Ninan N, Grohens Y, Elain A, et al. Synthesis and characterisation of gelatin/zeolite porous scaffold. Eur Polym J. 2013;49:2433–2445.

[23] Derakhshankhah H, Hosseini A, Taghavi F, et al. Molecular interaction of fibrinogen with zeolite nanoparticles. Sci Rep. 2019;9:1558.

[24] Kavya K, Dixit R, Jayakumar R, et al. Synthesis and characterization of chitosan/chondroitin sulfate/nano-SiO2 composite scaffold for bone tissue engineering. J Biomed Nanotechnol. 2012;8:149–160.

[25] Kavya K, Jayakumar R, Nair S, et al. Fabrication and characterization of chitosan/gelatin/nSiO2 composite scaffold for bone tissue engineering. Int J Biol Macromol. 2013;59:255–263.

[26] Samiei M, Aghazadeh M, Movassaghpour AA, et al. Isolation and characterization of dental pulp stem cells from primary and permanent teeth. J Am Sci. 2013;9:153–157.

[27] Stanford CM, Jacobson PA, Eanes ED, et al. Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 10601 B5P). J Biol Chem. 1995;270:9420–9428.

[28] Xie J, MacEwan MR, Schwartz AG, et al. Electrospun nanofibers for neural tissue engineering. Nanoscale. 2010;2:35–44.

[29] Fu N, Liao J, Lin S, et al. PCL-PEG-PCL film promotes cartilage regeneration in vivo. Cell Prolif. 2016;49:729–739.

[30] Tavolaro P, Catalano S, Martino G, et al. Zeolite inorganic scaffolds for novel biomedical application: Effect of physicochemical characteristic of zeolite membranes on cell adhesion and viability. Appl Surf Sci. 2016;380:135–140.

[31] Boskey AL. Biominaleralization: conflicts, challenges, and opportunities. J Cell Biochem. 1998;72:83–91.

[32] Papagerakis P, Berdal A, Mesbah M, et al. Investigation of osteocalcin, osteonectin, and dentin sialophosphoprotein in developing human teeth. Bone. 2002;30:377–385.

[33] Matsubara T, Kida K, Yamaguchi A, et al. BMP2 regulates ostein through Msx2 and Runx2 during osteoblast differentiation. J Biol Chem. 2008;283:29119–29125.

[34] Aghazadeh M, Samiei M, Alizadeh E, et al. Towards osteogenic bioengineering of dental pulp stem induced by sodium fluoride on hydroxyapatite based biodegradable polymeric scaffold. Fibers Polym. 2017;18:1468–1477.