Ionic Extraction of a Novel Nano-sized Bioactive Glass Enhances Differentiation and Mineralization of Human Dental Pulp Cells

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Abstract

Introduction: This study aimed to investigate the effects of a novel nano-sized 58S bioactive glass (nano-58S BG) on the odontogenic differentiation and mineralization of human dental pulp cells (hDPCs) *in vitro*. **Methods:** Extractions were prepared by incubating nano-58S BG, 45S5 BG, or 58S BG particulates in Dulbecco modified Eagle medium at 1% w/v for 24 hours and were filtrated through 0.22-μm filters. The supernatants were used as BG extractions. The hDPCs were cultured in nano-58S BG, 45S5 BG, and 58S BG extractions. The proliferation of hDPCs was evaluated using the methylthiazol tetrazolium assay. Odontogenic differentiation was evaluated based on the real-time polymerase chain reaction of differentiation- and mineralization-related genes, namely, alkaline phosphatase (ALP), collagen type I, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1. The gene expressions were verified using ALP activity assessment, immunocytochemistry staining of osteocalcin and DSPP, and mineralization assay using alizarin red S stain. **Results:** All BG extractions up-regulated the expression of odontogenic genes, and the most significant enhancement was in the nano-58S BG group. All BG extractions, especially nano-58S, increased ALP activity, osteocalcin and DSPP protein production, and mineralized nodules formation. **Conclusions:** Compared with regular BG, the novel nano-58S BG can induce the differentiation and mineralization of hDPCs more efficiently and might be a better potential candidate for dentin-pulp complex regeneration. *(J Endod 2013;■:1–6)*

Key Words

Differentiation, human dental pulp cells, mineralization, nano-sized bioactive glass

Vital pulp therapy is aimed at treating injuries by pulp capping to seal the pulp, stimulating the formation of tertiary dentin, and maintaining pulp vitality. During tertiary dentin formation, progenitor cells recruit, differentiate into odontoblast-like cells, further secrete extracellular matrix, and induce the matrix mineralization into new dentin (1–3).

For pulp-capping materials, the potential to stimulate tertiary dentinogenesis is one of the key issues to the success of pulp-capping therapy. Calcium hydroxide has been considered as a long-standing conventional material for pulp capping (4). However, Horsted et al (5) found that the newly secreted dentin under calcium hydroxide is generally porous and that gaps can be observed. In addition, the poor physical properties and solubility of calcium hydroxide may lead to the failure of pulp capping (6–8). Recently, mineral trioxide aggregate (MTA) has been proven to possess better effects as a pulp-capping material compared with calcium hydroxide (9, 10). The exposed pulp capped with MTA shows less inflammation, less necrosis, and thicker dentin bridge formation. The randomized clinical study of Nair et al (11) confirmed a better prognosis when MTA was used as the pulp-capping agent. However, MTA also has several disadvantages, such as its long setting time and the potential to discolor dental tissues. Therefore, new pulp-capping materials that possess better properties and bioactivity should still be identified.

Bioactive glass (BG) is a kind of highly biocompatible, osteoinductive, and osteoconductive calcium silicate–based biomaterial (12). It has been applied in periodontitis treatment (13, 14), maxillary cystic bone defects treatment (15), and implantation (16). Studies have proven that BG regulates osteoblast behavior by altering the expression of several relative genes. These genes have an important function in cell proliferation (17), differentiation, and bone matrix formation (18–20). Moreover, the ionic products of BG dissolution have an indispensable function. In recent years, several investigators have found that nano-sized sol-gel BG particles possess a higher specific surface area, a more regular size, and better bioactivity compared with traditional micron-sized BG particles (21). Dentin and bone generation need many common components, such as tissue matrix proteins and cell signal transduction factors (22). However, little information is available regarding the effects and mechanisms of BG on the differentiation and mineralization of human dental pulp cells (hDPCs). The purpose of this study was to investigate and compare the effects of the ionic extraction of novel nano-sized 58S BG, traditional sol-gel 58S BG, and traditional melt 45S5 BG on the differentiation and mineralization of hDPCs.

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Supported by the National Natural Science Foundation of China (Project 51372005 and 50830101).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.08.018

Materials and Methods

BG Preparation

Nano-sized 58S BG (particle size between 10 and 100 nm, composed of 58% SiO_2, 33% CaO, and 9% P_2O_5 [w/w]), traditional sol-gel 58S BG (particle size between 2 and 20 μm, composed of 58% SiO_2, 33% CaO, and 9% P_2O_5 w/w]), and traditional melt 45S5 BG (particle size between 1 and 10 μm, composed of 45% SiO_2, 24.5% CaO, 6% P_2O_5, and 24.5% Na_2O [w/w]) were all prepared by the National Engineering Research Center for Human Tissue Restoration and Reconstruction, South China University of Technology, Guangzhou, China. The preparation methods of the novel nano-58S BG were described previously (23).

BG Extraction

The BG particulates were sterilized in a dry heat oven at 180°C for 4 hours. The BG extractions were prepared by incubating nano-58S BG, 58S BG, or 45S5 BG in Dulbecco modified Eagle medium (DMEM) (Gibco, Gaithersburg, MD) at a concentration of 1 mg/mL at 37°C for 24 hours. The mixtures were centrifuged at 14,000*g*, and the supernatant was filtrated through a 0.22-μm filter (Millipore, Billerica, MA). The concentration of calcium (Ca), silicon (Si), and phosphorus (P) in the solution was detected using inductively coupled plasma optical emission spectroscopy analysis (iCAP 6300; Thermo Electron Corporation, Waltham, MA). All BG extractions were supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 2 mmol/L L-glutamine, 100 mg/mL streptomycin, and 100 U/mL penicillin. The pH of the BG extraction medium was adjusted to 7.4 for cellular experiments.

Isolation of hDPCs and Cell Culture

The hDPCs were isolated from the pulp of the third molar or the first premolar (because of orthodontic reasons) of patients aged 14–28 years old at the Peking University Hospital of Stomatology, Beijing, China. Informed consent was obtained from all the patients. The pulp was separated from the crowns and roots, minced into small pieces, and digested in 3 mg/mL collagenase type I (Sigma-Aldrich, St Louis, MO) and 4 mg/mL dispase (Roche, Indianapolis, IN) at 37°C and shaken at 300 rpm (Thermomixer; Eppendorf, Hamburg, Germany) for 1 hour. The cell suspensions were collected and seeded onto a culture plate containing DMEM with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in 5% CO_2. The cells were detached with 0.25% trypsin-EDTA (Gibco, Gaithersburg, MD) and were expanded when 70%–80% confluence was reached. The hDPCs of the third and fourth passages were used in this study. The cells from the same passage were used in the same experiment.

Cell Proliferation Assay

The hDPCs were seeded at a density of 5,000 cells per well onto a 96-well plate and were cultured for 7 days with the extractions of nano-58S BG, 58S BG, 45S5 BG, and growth medium (control group). The medium was changed every 2 days. The proliferation of hDPCs was determined using the methylthiazol tetrazolium (MTT) assay (Amresco, Solon, OH) method. About 20 μL MTT at a concentration of 5 mg/mL was added to each well and incubated for 4 hours at 37°C. The medium was then removed. The purple formazan crystals were dissolved in 150 μL dimethyl sulfoxide (Sigma-Aldrich) and were lightly shaken for 15 minutes on a microplate shaker. The optical density was measured at a spectrophotometric absorbance of 490 nm by using an ELx808 absorbance microplate reader (BioTeK Instruments, Winooski, VT). Six data of parallel wells were obtained for each sample.

Real-time Polymerase Chain Reaction

The hDPCs were cultured in a 35-mm dish with an initial density of 5,000 cells/cm^2. At days 1, 3, and 7, the total RNA was extracted using a Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was reversely transcribed into complementary DNA by reverse transcriptase (Promega, Madison, WI) according to the manufacturer's instructions.

Real-time polymerase chain reaction (PCR) was performed using the FastStart Universal SYBR Green PCR Master mix (Roche) and the ABI 7500 thermal cycler (Applied Biosystems, Foster City, CA) according to the manufacturers' instructions. The expressions of the differentiation and mineralization markers in the hDPCs, namely, alkaline phosphatase (ALP), collagen type I (Col I), dentin sialophosphoprotein (DSPP), and dentin matrix protein 1 (DMP1), were monitored. The forward and reverse primers used for real-time PCR were designed by Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA) according to the complementary DNA sequences available in GenBank (Table 1). The thermal cycling conditions were as follows: 50°C for 2 minutes and 95°C for 10 minutes and 40 cycles of 94°C for 15 seconds and 60°C for 1 minute. Glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for the quantification of gene relative expression.

Two parallel samples were tested, and the threshold cycle (Ct) values were the corresponding mean. The experiment was triplicated. The fold change was defined as the relative expression compared with the value of the control group on day 1, which was calculated as $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct_{target} - Ct_{GAPDH}$ and $\Delta\Delta Ct = \Delta Ct_{target} - \Delta Ct_{target\ control\ day\ 1}$.

ALP Assay

The hDPCs were seeded at a density of 5,000 cells/cm^2 onto 12-well plates and were cultured for 7 or 14 days in the extractions of nano-58S BG, 58S BG, 45S5 BG, and growth medium (control

TABLE 1. The Primers Sequence used for Real-time Reverse Transcription-PCR

Target gene	Primer sequence (5′ to 3′)	Product size (bp)
GAPDH	Forward: 5′-CAACGGATTTGGTCGTATTGG-3′ Reverse: 5′-GCAACAATATCCACTTTACCAGAGTTAA-3′	72
DSPP	Forward: 5′-ATATTGAGGGCTGGAATGGGGA-3′ Reverse: 5′-TTTGTGGCTCCAGCATTGTCA-3′	136
DMP-1	Forward: 5′-TTTTAGGAAGTCTCGCATCT-3′ Reverse: 5′-TGGGACCATCTACGTTTT-3′	209
Col I	Forward: 5′-CGAAGACATCCCACCAATCAC-3′ Reverse: 5′-TGTCGCAGACGCAGAT-3′	98
ALP	Forward: 5′-AGCACTCCCACTTCATCTGGAA-3′ Reverse: 5′-GAGACCCAATAGGTAGTCCACATTG-3′	77

ALP, alkaline phosphatase; Col I, collagen type I; DMP-1, dentin matrix protein 1; DSPP, dentin sialophosphoprotein; GAPDH, glyceraldehydes 3-phosphate dehydrogenase.

group). ALP activity was determined using an Alkaline Phosphatase Assay Kit (Nanjing Jiancheng Technological Inc, Nanjing, China). The cells were rinsed with cold phosphate buffer solution (PBS), scraped in 1 mL 0.1% Triton X-100 (Sigma-Aldrich), homogenized by ultrosonication on ice, and centrifuged at 18,000*g* for 15 minutes. The supernatant (30 μL) was added with the proper reagents of the kit and incubated for 30 minutes according to the manufacturer's instructions. The absorbance at 490 nm was measured spectrophotometrically using an ELx808 absorbance microplate reader (BioTeK Instruments). A standard curve of known concentrations was generated concurrently, and the protein concentrations were determined using the BCA protein assay (Piece, Rockford, IL). ALP activity was normalized to per gram of protein.

Matrix Mineralization Assay

Alizarin red S staining was used to assess matrix mineralization. hDPCs were seeded at 5,000 cells/cm^2 in the extractions of nano-58S BG, 58S BG, and 45S5 BG with osteo-/dentinogenic medium (containing 10 mmol/L β-glycerophosphate, 10 nmol/L dexamethasone, and 50 mg/L ascorbic acid). Control cells cultured in only the growth medium were used as the negative control samples (non–osteo-/dentinogenic control group); those cultured in the growth medium with osteo-/dentinogenic medium were assessed as the positive control samples (osteo-/dentinogenic control group). After being cultured for 21 days, the cells were rinsed 3 times in PBS and were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 minutes. The cells were washed using deionized water 3 times, stained with 40 mmol/L alizarin red S solution (pH = 4.2) (Sigma-Aldrich) for 15 minutes at room temperature, and then washed 3 times. The cells were observed for mineralized nodules under an inverted microscope (TE2000-U; Nikon Corporation, Tokyo, Japan).

For the semiquantification of matrix mineralization, 1 mL of 100 mmol/L cetylpyridinium (Sigma-Aldrich) was used to dissolve the dried alizarin red S–stained cellular matrix. The optical density was detected by absorbance at 562 nm using the ELx808 absorbance microplate reader.

Immunocytochemical Assay of OCN and DSPP

hDPCs were seeded at 5,000/well onto sterilized coverslips in 24-well plates for the immunocytochemical detection of DSPP expression on day 7 and OCN expression on day 14. The cells were washed 3 times with PBS and were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 30 minutes at room temperature. After being punched with 0.1% Triton X-100 and immersed in 3% H_2O_2 for 20 minutes at room temperature, the cells were blocked with 0.5% goat serum in PBS. Afterward, rabbit antihuman OCN and DSPP primary antibodies (dilution 1:300; Santa Cruz Biotechnology Inc, Santa Cruz, CA) were added overnight at 4°C. Subsequently, the cells were incubated with goat antirabbit secondary antibody for 1 hour at 37°C and were enzymatic immunohistochemically stained using a broad-spectrum immunoperoxidase diaminobenzidine kit (Zhongshan Bioengineering, Beijing, China) according to the manufacturer's instructions. Finally, the cells were counterstained with hematoxylin-eosin and were visualized and photographed under an inverted microscope (TE2000-U).

Statistical Analysis

Each experiment was done in triplicate, and each result was averaged by at least 3 parallel samples and measurements. All data are expressed as mean ± standard deviation. Variance and statistical significance were determined with the Student *t* test using the SPSS Statistics 17.0 software (SPSS Inc, Chicago, IL). Differences were considered significant at $P < .05$.

Results

Ionic Concentrations in BG Extractions

The ionic concentrations of Si, Ca, and P in the 3 BG extractions (1 mg/mL) are shown in Table 2. Among the 3 groups, the highest concentrations of Ca and Si were detected in the nano-58S BG extraction. The concentration of P was very low in both the nano-58S BG and 58S BG extractions.

Effect of BG Extractions on hDPCs Proliferation

The effect of BG extractions on hDPC proliferation was evaluated using the MTT assay (Fig. 1*A*). The optical density values at 490 nm of the 3 BG groups were all lower than that of the control group ($P < .05$). No statistical difference was found among the 3 BG groups ($P > .05$).

Effects of BG Extractions on the Expressions of Genes Related to hDPC Differentiation

The messenger RNA (mRNA) expression of ALP, Col I, DSPP, and DMP1 genes of hDPCs cultured in BG extractions on days 1, 3, and 7 are shown in Figure 1*B–E*. Compared with the control group, the mRNA expression of ALP in hDPCs was up-regulated by all 3 BG extractions on days 1 and 3 and was more significantly up-regulated by the extraction of nano-58S BG on day 1. On day 7, the up-regulation of ALP was significant in the nano-58S and 58S groups, with no difference between the 45S5 and control groups. Compared with the control group, the mRNA expression of Col I was up-regulated by all 3 BG extractions and was more significantly up-regulated by the extraction of nano-58S BG on day 1; however, it was not affected on days 3 and 7 by the 3 BG extractions. Similarly, the mRNA expressions of DSPP and DMP1 were also up-regulated by all 3 extractions on day 1. However, nano-58S BG extraction more significantly enhanced DSPP and DMP1 gene expression on day 3 compared with the extractions of 58S BG, 45S5 BG, and the control group.

The ALP activity assay results showed that ALP activity was significantly elevated in nano-58S, 58S, and 45S5 groups compared with the control group on day 7 of culture ($P < .05$). No difference was found among the BG groups. On day 14, ALP activity was lower than on day 7. Nevertheless, no statistical difference was found among the BG and control groups (Fig. 1*F*).

The immunocytochemical assay of DSPP on day 7 and OCN on day 14 showed positive staining of both proteins in the cytoplasm of the hDPCs in the BG groups. The most intense staining was found in the nano-58S group; meanwhile, moderate, light, and negative staining were found in the 58S, 45S5 group, and control groups, respectively (Fig. 2).

Effect of BG Extractions on hDPC Mineralization

The mineralization assay results indicated that nano-58S significantly promoted mineralized nodules formation after 21 days of culture in the osteo-/dentinogenic medium. On day 21, no red staining was observed in the non–osteo-/dentinogenic control group, and moderate positive red nodules staining was observed in the osteo-/dentinogenic control group. For the BG groups, the density of red mineralized nodules was significantly higher in the nano-58S group than in the other groups. Numerous mineralized nodules were observed in the nano-58S

TABLE 2. Concentrations of Si, Ca, and P in the BG Extractions

	Si (μg/mL)	Ca (μg/mL)	P (μg/mL)
DMEM (control)	0.57	75.33	29.18
45S5	44.23	88.88	13.34
58S	64.98	123.60	0.22
Nano-58S	71.00	154.50	1.72

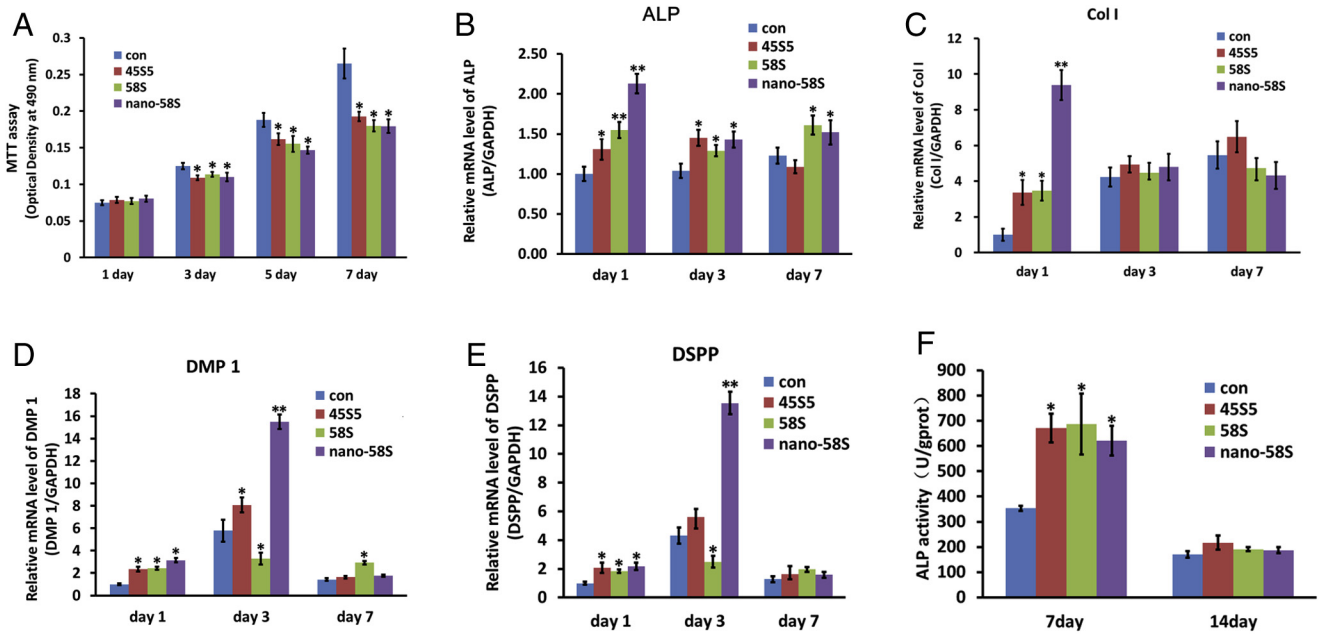

Figure 1. Effects of nano-58S, 45S5, and 58S BG extractions on hDPCs proliferation (*A*) and differentiation (*B–E*). (*B–E*) Real-time PCR results of differentiation and mineralization gene expression. The data are relative expression levels shown as a fold difference compared with the control group on day 1 and are normalized to the expression level of the housekeeping gene (ie, GAPDH). The data are the mean values ± standard deviation of 3 individual experiments. Real-time PCR was run twice for each sample. (*F*) The effects of nano-58S, 45S5, and 58S BG extractions on ALP activity in hDPCs (analysis of variance, $*P < .05$; $**P < .01$ versus the control group).

group. The 58S group showed moderate red nodule staining similar to the positive control group with many mineralized nodules. The 45S5 group showed slightly positive results with many Ca depositions but had less mineralized nodules than the osteo-/dentinogenic control group (Fig. 3*A–E*). The results of the semiquantification of Ca deposition were consistent with those of the alizarin red S staining (Fig. 3*F*). The OD_{562} of the nano-58S group was significantly higher than that of the osteo-/dentinogenic control group. No statistical difference in OD_{562} values was found between the 58S and osteo-/dentinogenic control groups. The OD_{562} value of the 45S5 group was significantly lower than that of the osteo-/dentinogenic control group.

Discussion

This study assessed the effects of BG extractions on the differentiation and mineralization of hDPCs from gene expression level to protein production level. The investigated genes, namely, ALP, Col I, DSPP, and DMP1, were identified as specific markers of odontogenic differentiation and dentin regeneration of hDPCs. ALP is the marker of early differentiation and extracellular matrix mineralization (24). Col I is the predominant collagen in the dentin as a matrix structural framework for inorganic deposition (25). Compared with traditional BG groups, nano-58S BG enhanced the ALP and Col I expression of hDPCs more significantly and at an earlier time. The gene expression of DSPP and DMP1 of the hDPCs treated by nano-58S BG were also considerably higher than those of the traditional BG and control groups on day 3. After the expression and translation of the DSPP gene, the polypeptide is modified into dentin sialoprotein and dentin phosphoprotein, which are the most major noncollagenous proteins in the dentin extracellular matrix. Dentin sialoprotein has an important function in the initiation of dentin mineralization, whereas dentin phosphoprotein is involved in the maturation of mineralized dentin (26). DMP1 has a significant function

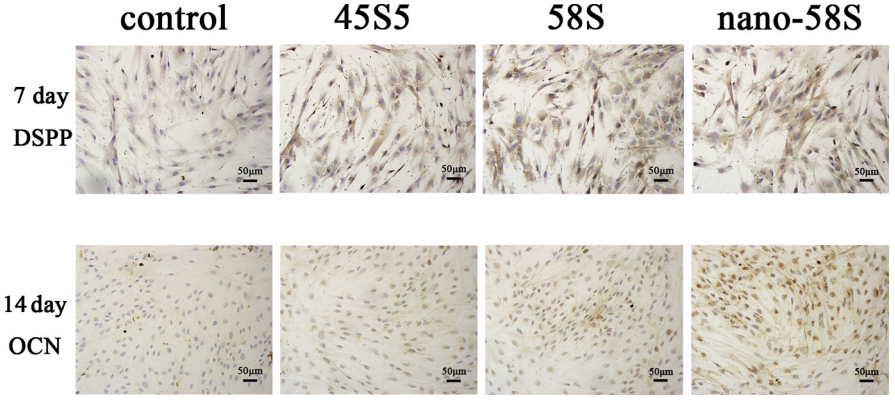

Figure 2. Immunocytochemical staining of DSPP on day 7 and OCN on day 14 in hDPCs cultured in nano-58S, 45S5, 58S BG extractions, and control medium.

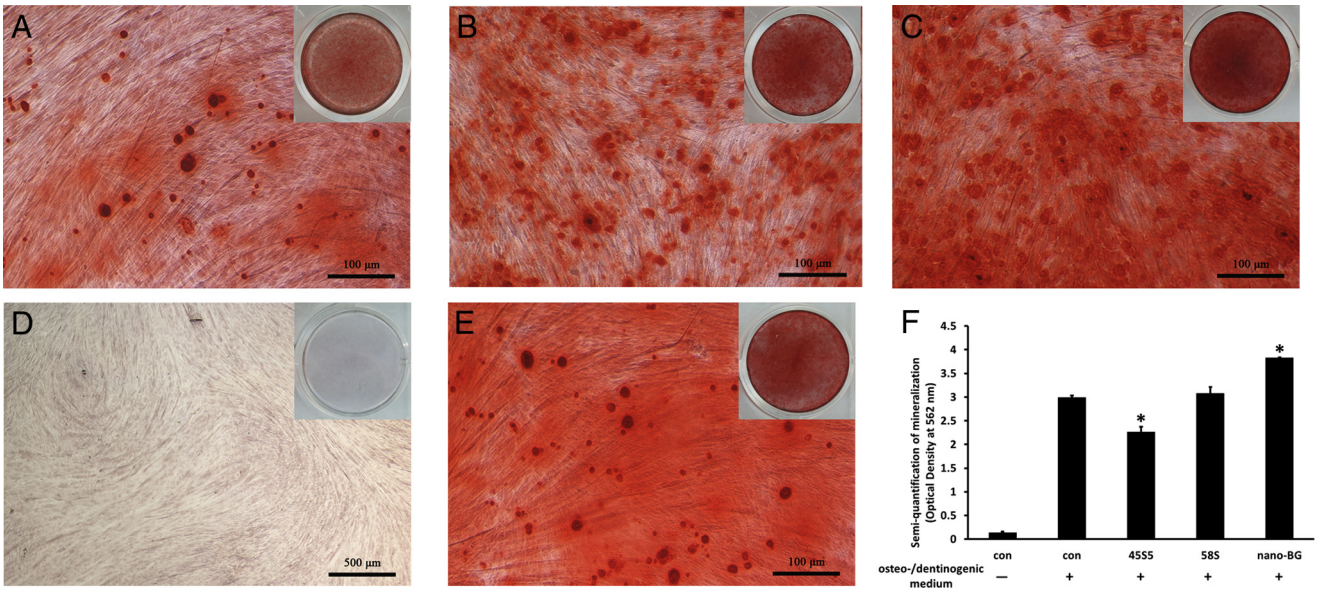

Figure 3. Mineralization assay of hDPCs cultured in BG extractions. Alizarin S staining of mineralized nodules of hDPCs cultured in (*A–C*) 45S5, 58S, nano-58S BG extractions and (*D* and *E*) control medium on day 21. (*A*) Alizarin S staining of hDPCs cultured in 45S5 extraction with osteo-/dentinogenic medium. (*B*) Alizarin S staining of hDPCs cultured in 58S extraction with osteo-/dentinogenic medium. (*C*) Alizarin S staining of hDPCs cultured in nano-58S extraction with osteo-/dentinogenic medium. (*D*) Alizarin S staining of hDPCs cultured in growth medium as a negative control. (*E*) Alizarin S staining of hDPCs cultured in growth medium with osteo-/dentinogenic medium as a positive control. (*F*) Semiquantification of calcium deposits of hDPCs cultured in nano-58S, 45S5, and 58S BG extractions and control medium on day 21 (analysis of variance, $*P < .05$ versus the osteo-/dentinogenic control group).

in mineralization regulation and can bind specifically with the DSPP promoter and activate its transcription (27). In addition, DMP1 can induce the differentiation of mesenchymal stem cells into odontoblasts (28) and promote matrix mineralization (29). The higher and earlier up-regulation of these genes suggest that the novel nano-58S BG could more effectively promote hDPCs to odontogenic differentiation.

ALP, DSPP, and OCN production were detected at the protein level to verify the changes in gene expression. The ALP activity was consistent with the mRNA expression of ALP. The expression pattern of the ALP activity in this study was similar to that in a previous study in which the ALP activity of the dental pulp cells of rats reached its peak on day 5 during the cell proliferation phase and was reduced on day 10 when the cells began to differentiate (30). The present results of ALP activity indicate that BG extractions could induce earlier differentiation (ie, expeditious beginning of odontogenesis in hDPCs).

The immunocytochemical staining results revealed that hDPCs cultured with nano-58S extraction expressed the most DSPP on day 7 and OCN on day 14. Meanwhile, moderate protein expression was found in the cells of 58S and 45S5 groups, and minimal DSPP or no OCN was produced in the cells of the control group. These results are consistent with those of mRNA expression of the genes examined. These findings also suggest a time span from gene activation to protein production. The results of DSPP indicate that BG could effectively induce hDPCs to differentiate into odontoblast-like cells, and nano-58S is the most effective among the BG groups.

The start of mineralization is the marker of the late stage of odontoblast differentiation (31). The alizarin red S staining result shows that mineralized nodules exist both in the osteo-/dentinogenic control and osteo-/dentinogenic BG extraction groups. However, the cells in the osteo-/dentinogenic nano-58S extraction group formed more mineralized nodules compared with the cells in the other groups. The semiquantitative assay results were also in agreement with those of the staining assay. Among the BG groups, the nano-58S extraction most effectively promoted the differentiation and mineralized nodule formation in hDPCs. The results are also consistent with the enhanced gene expression and protein production of ALP, Col I, DSPP, and DPM 1 in hDPCs treated with nano-58S extraction.

Recently, several kinds of inorganic Ca silicate–based material, such as Biodentine (Septodont, Lancaster, PA) (composed of tricalcium/dicalcium silicate, Ca carbonate, and zirconium dioxide), alpha-tricalcium phosphate, hydroxyapatite/tricalcium phosphate, and MTA (mainly composed of tricalcium aluminate, tricalcium silicate, Ca oxide, and Si oxide) have been studied for pulp capping. Most of these materials showed a favorable effect and bioactivity (32–35). Given the same composition, Ca and Si from BG also have an active function in their bioactivity. Compared with traditional sol-gel 58S BG and melt 45S5 BG, the novel nano-sized 58S BG possesses smaller particulates and larger specific surface area (21). These characteristics enable nano-58S to release more ions and induce higher concentrations of Si and Ca than traditional BG, which may have an important effect in bioactivity increase and gene expression activation. During the preparation of the BG extractions, the nano-sized property induced the particulate aggregate into a cluster of grains. The mixture of BG particulate and DMEM was centrifuged at 14,000*g* to isolate the particulates from the supernatant fluid. The mixture was then filtrated through a 0.22-μm filter to ensure the asepsis of the extractions.

hDPCs in the BG groups proliferated throughout the culture period; however, the process was slower than that in the control group. This phenomenon may be caused by the higher Si and Ca ionic concentrations in BG extractions, which have an inhibitory effect on cell proliferation. Zhou et al (32) also found that fibroblasts exposed to high concentrations of extraction of Biodentine, a Ca silicate–based material, showed lower viability than those exposed to low concentrations (32). Meanwhile, the proliferation rate of cells slows down when the cells begin to differentiate (25). Therefore, the lower rates of proliferation in the BG groups could also be caused by the higher number of cells in the BG groups that entered the differentiation phase compared with the control group. This result was supported by the higher

expressions of differentiation-related genes in the BG groups compared with the control group.

The different effects of BG extractions on proliferation, differentiation, and mineralization may be because of their different ionic releasing rates and abilities. Given the higher specific area, nano-58S released more Si and Ca ions into the medium compared with 58S and 45S5 in a short time. Higher concentrations of Si and Ca induced higher gene expression up-regulation and lower proliferation. The balance of ionic concentration between cell viability and differentiation needs further investigation.

In summary, the current study showed that the expression of genes and proteins related to the differentiation and mineralization of hDPCs, namely, ALP, Col I, OCN, and DSPP, were significantly enhanced when the hDPCs were treated with BG extractions, especially the novel nano-58S extraction. Based on the results, the ionic extraction of the novel nano-sized BG could have a more favorable effect on inducing the differentiation and mineralization of hDPCs. Therefore, the novel nano-sized BG might be a promising alternative for pulp-dentin complex regeneration.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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