The Ionic Products from Mineral Trioxide Aggregate–induced Odontogenic Differentiation of Dental Pulp Cells via Activation of the Wnt/β-catenin Signaling Pathway

Yi-Wen Chen, PhD,*† Chia-Che Ho, PhD,* Tsui-Hsien Huang, DDS, PhD,‡§ Tuan-Ti Hsu, PhD,* and Ming-You Shie, PhD*‡

Abstract

Introduction: Mineral trioxide aggregate (MTA) has been successfully used in clinical applications in endodontics. However, little is known about the involvement of Wnt/β-catenin signaling in human dental pulp cells (hDPC) differentiation with the interaction of MTA in hard tissue regeneration, especially in odontogenesis. Therefore, the aim of this study was to explore odontogenic/osteogenic gene expression and the protein secretion of hDPCs cultured with consecutive concentrations of MTA extracts and carefully examine the particular molecular mechanism that occurs during this process. Methods: MTA extracts were prepared by immersing MTA powders into Dulbecco modified Eagle medium at a concentration of 200 mg/mL. hDPCs were cultured with various concentrations of MTA extracts, and the resulting changes in the cells, such as proliferation and odontogenic differentiation, were measured. Results: The results indicate that hDPC proliferation increases remarkably in a time-dependent manner in most treatment groups, except for the highest concentration group (200 mg/mL). The Wnt/β-catenin signaling pathway–related genes and proteins are significantly raised when hDPCs are cultured in a wide concentration range of MTA extracts compared with a control, except for the highest concentration group (100 mg/mL), on days 3 and 7 (P < .05). Conclusions: These variations indicate that Wnt/β-catenin signaling is involved in MTA extract–induced odontogenic differentiation of hDPCs. (J Endod 2016;42:1062–1069)

Key Words
Dental pulp cell, ion concentration, mineral trioxide aggregate, odontogenic, Wnt/β-catenin

Since its introduction in 1993, mineral trioxide aggregate (MTA), a type of calcium silicate (CS)–based cement, has come to be used worldwide in endodontic treatment, not only as a root-end filling material but also for direct pulp capping and apexification and in regenerative endodontic procedures (1, 2). MTA is a type of Portland cement consisting of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum, and bismuth oxide (3). CS-based materials have been proven to exhibit excellent in vitro bioactivity and lead to the precipitation of a bonelike apatite layer (4, 5), which may indicate the material’s ability to integrate into hard tissue (2, 6). The bioactivity of CS materials has led to their use in constructing scaffolds with human mesenchymal stem cells for treating bone defects (7, 8). Human dental pulp cells (hDPCs) express increased proliferation and odontogenesis differentiation as shown by a higher expression of several odontogenesis markers contained in dentin matrix protein 1 (DMP-1) (9).

Our previous studies (10–14) have shown that CS-based materials can be used for hard tissue regeneration and have the ability to stimulate osteogenesis differentiation in various stem cells, such as bone marrow stromal cells, adipose-derived stem cells, hDPCs, and periodontal ligament cells. However, the interactions of MTA and hDPCs in processes including adhesion, proliferation, and odontogenesis differentiation (gene and protein expression) and the possible molecular mechanism are completely unknown. Ions released from CS-based materials have been shown to stimulate cell activity in osteoblasts and promote mineral deposition at the material-dentin interface (15, 16). The released concentration of Si ions from CS-based materials has distinct effects on different cell types. For example, it inhibits osteoclastogenesis in macrophages (6) and promotes angiogenesis in hDPCs (13). Therefore, CS-based cements

Significance
Ca- and Si-containing MTA extracts significantly promote the proliferation of hDPCs and show a positive odontogenic effect. We consider the most important mechanism for the markedly stimulatory effect of MTA extracts on the odontogenesis differentiation of hDPCs is related to the activation of the Wnt/β-catenin signaling pathway. In addition, we think that MTA cement has great potential for improving hard tissue regeneration because of their excellent osteogenesis and odontogenesis stimulation.
have been formulated into dentin replacement restorative materials in dentistry (2, 10, 17). Zhou et al (18) have shown that Ca-Mg-Si–based ceramic extracts significantly promote the adhesion and proliferation of human periodontal ligament cells and exhibit a strong pro-osteogenic effect. The possible mechanism for the markedly stimulatory effect of extracts on the osteogenic differentiation of human periodontal ligament cells is related to the activation of the Wnt/β-catenin signaling pathway. However, as described by Bohner (19) in a leading opinion article, until now, no clear link has been made between the enhanced biological performance of Si-based materials and Si release.

Wingless proteins, termed Wnt, are a family of cysteine-rich glycoproteins. Nineteen types of these have been found in mammals and are reported to regulate functions related to cell fate (20, 21). Wnt signals are transmitted through at least 3 different distinct intracellular signaling pathways: the canonical Wnt/β-catenin pathway and 2 noncanonical pathways (22). The canonical Wnt signaling pathway is a signaling cascade transduced by Wnt proteins involving stabilization and the nuclear accumulation of β-catenin and the activation of Wnt target genes (23). Furthermore, Wnt/β-catenin’s role in signaling pathways in bone formation and tooth morphogenesis in adult tissues has been well established (24, 25). However, little is known about the involvement of Wnt/β-catenin signaling in hDPC differentiation when interacting with MTA in hard tissue regeneration, especially in odontogenesis. Therefore, the aim of this study was to explore the odontogenic/osteogenic gene expression and protein secretion of hDPCs cultured with various concentrations of MTA extracts and to analyze the potential molecular mechanisms that are active during this process.

Materials and Methods

Preparation of Specimens

The MTA (ProRoot MTA; Dentsply, Tulsa, OK) used in this study was prepared following the manufacturer’s instructions. MTA powder mixed with deionized water in a liquid/powder ratio of 0.3 mL/g was used to mix the cement. After mixing with water, the cement was shaped in Teflon molds (diameter: 6 mm, height: 2 mm; Tainan, Taiwan). These specimens were then stored in an incubator at 37°C and 100% relative humidity for 1 day to set.

Morphology

To evaluate the in vitro bioactivity of MTA, the produced samples were immersed in 10 mL normal Dulbecco modified Eagle medium (DMEM) at 37°C. Before soaking in DMEM, all the specimens were sterilized by immersion in 75% ethanol followed by exposure to an ultraviolet light for 1 hour. After soaking for 1 day, the specimens were removed from the solution, and the morphology of the specimens was examined under a scanning electron microscope (SEM; JSM-6700F, JEOL, Tokyo, Japan) operated in the lower secondary electron image mode at 3-kV acceleration voltage.

hDPC Isolation and Culture

The hDPCs used were freshly derived from caries-free, intact premolars that had been extracted for orthodontic treatment purposes as described previously (26). The patients gave informed consent, and approval from the Ethics Committee of Chung Shan Medicine University Hospital, Taichung, Taiwan, was obtained (CSMUH no. CS14117). The patients were immersed in 10 mL normal Dulbecco modified Eagle medium (DMEM) at 37°C. Before soaking in DMEM, all the specimens were sterilized by immersion in 75% ethanol followed by exposure to an ultraviolet light for 1 hour. After soaking for 1 day, the specimens were removed from the solution, and the morphology of the specimens was examined under a scanning electron microscope (SEM; JSM-6700F, JEOL, Tokyo, Japan) operated in the lower secondary electron image mode at 3-kV acceleration voltage.

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Cell Morphology

After the cells had been seeded for 1 and 7 days, the samples were washed 3 times with cold PBS and fixed in 1.5% glutaraldehyde (Sigma-Aldrich) for 2 hours. The specimens were then dehydrated using a graded ethanol series for 20 minutes at each concentration and dried with liquid CO2 using a critical point dryer device (LADD 28000; LADD, Williston, VT). The fixed specimens were mounted on stubs, coated with gold, and viewed using an SEM. For immunofluorescent staining, cells were seeded on MTA for the same time period. Cells were fixed using 4% paraformaldehyde for 30 minutes and permeabilized using 0.1% Triton X-100 (Sigma-Aldrich) for 15 minutes; after which, the specimens were incubated with AlexaFluor-594–conjugated phalloidin (Invitrogen, Grand Island, NY) (F-actin, red) for 1 hour at room temperature. The nuclei were then stained with 4′,6-diamidino-2-phenylindole dilactate for 1 hour at room temperature. Finally, the samples were washed with PBS 3 times and photographed under indirect immunofluorescence using a Zeiss Axioskop 2 microscope (Carl Zeiss, Thornwood, NY).

Preparation and Characterization of the Extracts of MTA

The extracts were prepared by immersion MTA setting into DMEM at a concentration of 200 mg/mL (sample size: 0.1 g, diameter = 6 mm, height = 3 mm) according to ISO/EN 10993-5. After immersion at 37°C for 24 hours, the mixture was sterilized using a 0.2-mm filter. Serial dilutions of extracts (100, 50, 25, 12.5, and 6.25 mg/mL) were prepared using DMEM, and then 10% fetal bovine serum and 1% penicillin/streptomycin were added. The Ca and Si ion concentrations of the extract were analyzed using an inductively coupled plasma-atomic emission spectrometer (Optima 7000DV; PerkinElmer, Waltham, MA). In addition, the pH meter was used to measure the pH value of the extracts.

Proliferation of hDPCs Cultured with MTA Extracts

The proliferation of hDPCs in the MTA powder extract was examined using a PrestoBlue assay (Invitrogen). The cells were cultured in a 96-well tray at an initial density of 5 × 10^4 cells per well; after the cell adherence for 24 hours, the appropriate extracts were added. After being cultured for various predetermined lengths of time (1, 3, and 7 days), each well was filled with a 1:9 ratio of PrestoBlue in fresh DMEM and incubated at 37°C for 60 minutes. The solution in each well was then transferred to a new 96-well plate and read using a Tecan Infinite 200 PRO microplate reader (Tecan, Männedorf, Switzerland) at 570 nm with a reference wavelength of 600 nm. The results were obtained in triplicate from 3 separate experiments for each test. Cells cultured on tissue culture plates with DMEM were used as a control (Ctl).

Alkaline Phosphatase Activity

The level of alkaline phosphatase (ALP) activity was determined after cell seeding for 3 and 7 days (27). The process was as follows: the cells were lysed from 96 wells using 0.2% NP-40 (Sigma-Aldrich) and centrifuged for 10 minutes at 2000 rpm after washing with PBS. ALP activity was determined using p-nitrophenyl phosphate (Sigma-Aldrich) as the substrate. Each sample was mixed with p-nitrophenyl phosphate in 1 M diethanolamine buffer for 15 minutes, after which the reaction was stopped by the addition of 5 M NaOH and quantified by absorbance at 405 nm. All experiments were performed in triplicate.
Western Blot

Western blot analysis was performed using cell lysates of hDPCs that had been cultured for 7 days. The hDPCs were lysed in NP-40 buffer on ice for 1 hour, and the suspensions were centrifuged at 14,000g. The cell lysates were separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. The membrane was blocked in 2% bovine serum albumin for 30 minutes and then immunoblotted with the primary anti–dentin sialophosphoprotein (DSPP), anti–DMP-1, and β–actin (GeneTex, San Antonio, TX) for 3 hours; afterward, it was washed 3 times with a tris-base saline buffer containing 0.05% Tween-20 (Sigma-Aldrich). A horseradish peroxidase–conjugated secondary antibody was subsequently added, and the proteins were visually examined using enhanced chemiluminescent detection kits (Invitrogen). The stained band was scanned and quantified using a densitometer (Syngene, Frederick, MD) and ImageJ software (National Institutes of Health, Bethesda, MD). The protein expression level was normalized to the β–actin for each group.

Mineralization

The accumulated calcium deposition was analyzed after 14 days using alizarin red S stain as described in a previous study (28). In brief, the cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 15 minutes and then incubated in 0.5% alizarin red S (Sigma-Aldrich) at a pH of 4.0 for 15 minutes at room temperature in an orbital shaker (25 rpm). After the cells were washed with PBS, photographs were taken using an optical microscope (BH2-UMA; Olympus, Tokyo, Japan) equipped with a digital camera (Nikon, Tokyo, Japan) at 200× magnification.

Wnt/β-catenin Pathway Assay

The involvement of the Wnt/β-catenin signaling pathway was also investigated, and the gene expression of axis inhibition protein 2 (Axin2), beta-catenin (β-catenin), and Wingless-3a (Wnt3a) were analyzed using real-time polymerase chain reaction (PCR). Total RNA was extracted from hDPCs cultured with different concentrations of the MTA extracts with TRIzol Reagent (Invitrogen). The real-time reverse transcription polymerase chain reaction (RT-qPCR) primers were selected from the NCBI Sequence database and are listed in Table 1. SYBR Green qPCR Mixes were used for target messenger RNA and analyzed using the ABI Step One Plus System (Applied Biosystems, Foster City, CA). In addition, the Axin2, β-catenin, and Wnt3a protein expressions were also confirmed using an enzyme-linked immunosorbent assay (ELISA) (Abbexa, Cambridge, UK). We used the assay following the manufacturer’s procedure, which has higher sensitivity. The reaction was terminated by the addition of a stop solution and read at 450 nm using a Tecan Infinite 200 Pro plate reader (Tecan, Mannedorf, Switzerland).

The Effect of the Wnt/β-catenin Pathway on the Odontogenic Differentiation of hDPCs

The effect of the Wnt/β-catenin pathway on hDPCs cultured with MTA extracts was further investigated both with and without 10 mmol/L cardamonin (ie, a commercial Wnt/β-catenin inhibitor

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward sequences</th>
<th>Reverse sequences</th>
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<tr>
<td>β–actin</td>
<td>5′-TCAGCAATGCCTCCTGCAC</td>
<td>5′-TCTGGGTTGGCATGATGTC</td>
</tr>
<tr>
<td>Wnt3a</td>
<td>5′-TGGACAAAGCTACCAGGGAGT</td>
<td>5′-CCCACAAACTCGATGTCCTC</td>
</tr>
<tr>
<td>β–catenin</td>
<td>5′-GCTACTTGTGGATTGATCGGATC</td>
<td>5′-CCCTGTCACGCAAAAGGT</td>
</tr>
<tr>
<td>Axin2</td>
<td>5′-CCCCAACGACGCGGTG</td>
<td>5′-CGGTGGACACCTGCCAG</td>
</tr>
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Figure 1. The surface morphology of MTA (A) before and (B) after immersion in DMEM. The electron microscope and immunofluorescence images of the hDPCs were cultured on MTA for (C and E) 1 day and (D and F) 3 days.
After being cultured for 7 days, gene expression and alizarin red S staining were performed.

**Odontogenic Assay**

The production of DSPP and DMP-1 released from cells cultured on different specimens for 7 and 14 days were quantified using an ELISA (Abcam, Cambridge, MA). Following the manufacturer’s procedure, we used the assay, which has higher sensitivity than other similar measuring products. The reaction was terminated by the addition of stop solution and read at 450 nm using the Tecan Infinite 200 Pro plate reader. The concentrations of DSPP and DMP-1 were determined from each standard curve of pure protein. Each experiment was performed 3 times.

**Statistical Analysis**

One-way analysis of variance statistical analysis was used to evaluate the significance of the differences between the mean values in comparison with those of the controls. Scheffé multiple comparison testing was used to determine the significance of deviations in the data for each specimen. In all cases, the results were considered statistically significant with a P value <.05.

**Results**

**Microstructure of MTA Specimens**

The bioactivity of MTA cement was examined to determine changes in surface structure. Figure 1A shows the representative morphology of MTA cement. In general, they exhibit a dense and smooth surface containing particle entanglement and micropores. After immersion in DMEM for 1 day, the surface of MTA cement underwent important changes in that a layer of apatite with tiny spherical shapes on the surface was formed (Fig. 1B).

**Cell Morphology**

The facilitation of cell adhesion on MTA using different DMEMs was confirmed and observed using an SEM and immunofluorescence images. When the hDPCs were seeded onto MTA materials with DMEM for 3 hours (Fig. 1C) and 3 days (Fig. 1D), the cells clearly exhibited adhesive tendencies. Rhodamine phalloidin/4',6-diamidino-2-phenylindole staining shows that at the same time point hDPCs cultured on MTA display the F-actin stress fiber morphologies of the cells (Fig. 1E). Fluorescence staining of hDPCs in Figure 1F indicates
a homogenous distribution of F-actin fibers and filament elongation of cells on the MTA.

The Effect of MTA Extracts on hDPC Proliferation and Differentiation

There was no Si ion in the Ctrl, but there was an increase of Si ions in MTA extracts because the extract concentrations increased from 6.25 to 200 mg/mL (Table 2). The Ca level in the MTA extracts was confined in the range of 1.3–1.5 mmol/L. The cytotoxicity of hDPCs cultivated on MTA extracts have been calculated using a PrestoBlue assay (Fig. 2A). Variation in cell viability was not significant \((P > .05)\) for all concentrations over a 1-day period. Interestingly, the proliferation of hDPCs increased remarkably in a time-dependent manner in most treatment groups, except the highest concentration group (200 mg/mL). A significant increase of cell proliferation was found in hDPCs grown in the MTA extract concentrations of 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL compared with the Ctrl after 3 and 7 days \((P < .05)\). However, the proliferation of the concentration of 100 mg/mL was slightly lower than 50 mg/mL after the cells had been cultured for 3 and 7 days. The relative ALP activity increased significantly after hDPCs were cultured with different concentrations of MTA extracts compared with the Ctrl. MTA extracts at concentrations of 12.5–100 mg/mL significantly elevated the ALP activity in hDPCs compared with the Ctrl after 3 and 7 days \((P < .05)\). However, ALP activity showed a significant decrease in 100-mg/mL extracts \((P < .05)\).

Calcium silicate–based material has previously been shown to stimulate odontogenesis differentiation in hDPCs. To analyze the potential of the hDPCs for odontoblast differentiation after being cultured with MTA extracts, the cells were cultured with different concentrations of extracts for up to 7 days to estimate the protein expression levels of differentiation markers (DSPP and DMP-1) using the Western blot method of measurement (Fig. 2C). Results of Western blot analysis show that DSPP and DMP-1 proteins increase progressively as the concentration of the ionic products from the MTA extracts are increased, whereas the protein secretion in the presence of 100 mg/mL significantly decreases \((P > .05)\). The DSPP and DMP-1 protein expression in hDPCs in the MTA extracts (12.5–100 mg/mL) for 7 days was also observed \((P < .05)\). However, ALP activity showed a significant decrease in 100-mg/mL extracts \((P < .05)\).

Activation of the Wnt/\(\beta\)-catenin Signaling Pathway in hDPCs Stimulated by MTA Extracts

Expression of the Wnt/\(\beta\)-catenin signaling–related genes was measured by real-time PCR after culturing hDPCs in different concentrations of MTA extracts for 3 and 7 days (Fig. 3). The expression of Wnt3a (Fig. 3A), \(\beta\)-catenin (Fig. 3B), and Axin2 (Fig. 3C) was significantly higher in the hDPCs cultured in a wide concentration range of MTA extracts compared with the Ctrl, except for in the highest concentration group (100 mg/mL), on both day 3 and 7 \((P < .05)\).

The Effect of Inhibiting the Wnt/\(\beta\)-catenin Signaling Pathway on the Odontogenic/osteogenic Differentiation in hDPCs Stimulated by MTA Extracts

The 12.5 mg/mL and 50 mg/mL MTA extracts were the most effective in promoting odontogenesis differentiation in hDPCs. The following experiments were performed according to this condition with 10 mmol/L cardamonin, and the results show that cardamonin significantly inhibits the expression of odontogenesis in genes found in hDPCs cultured in MTA extracts \((P < .05)\). ELISA results confirm that 10 mmol/L cardamonin inhibits Wnt3a (Fig. 4A) and \(\beta\)-catenin (Fig. 4B) gene expression. Cardamonin significantly \((P < .05)\) (65.9% and 58.6%) inhibited...
gene expression of Wnt3a and β-catenin compared with samples without cardamonin or in 50 mg/mL MTA extracts, respectively. Interestingly, it was noted that cardamonin effectively abrogated the expression of ALP (Fig. 4C), DSPP (Fig. 4D), and DMP-1 (Fig. 4E) in hDPCs cultured in MTA extracts compared with the Ctl group. The effect of blackening the Wnt/β-catenin signaling had a negative effect on the calcium deposition in hDPCs culture with MTA extracts.

**Figure 4.** (A) Wnt3a, (B) β-catenin, (C) ALP, (D) DSPP, and (E) DMP-1 gene expression of hDPCs cultured with 12.5 mg/mL and 50 mg/mL MTA extracts contained 10 mmol/L cardamonin. *A significant difference (P < .05) compared with the Ctl without cardamonin. #A significant difference (P < .05) compared with the Ctl with cardamonin. (F) Alizarin red S staining showing that blocking the Wnt/β-catenin signaling had a negative effect on the calcium deposition of hDPCs culture with MTA extracts.

MTA has therapeutic properties and is useful for endodontic repairs. It has also been reported to help in regenerating good hard tissue. The bioactive properties of MTA may have a beneficial effect on unambiguously verify the activation of the Wnt/β-catenin pathway in hDPCs cultured in MTA extracts and that the supplementation of cardamonin inhibits the positive effect of MTA extracts on the odontogenesis differentiation of hDPCs.

**Discussion**

MTA has therapeutic properties and is useful for endodontic repairs. It has also been reported to help in regenerating good hard tissue. The bioactive properties of MTA may have a beneficial effect on
cells compared with other composite resins that possess no bioactivity, but the mechanisms remain unclear. The present results show that MTA significantly promotes adhesion, proliferation, mineralization nodule formation, and osteogenesis/odontogenesis-related protein expression of hDPCs. Moreover, the Wnt/β-catenin signaling inhibitor significantly decreases the odontogenesis of MTA extracts. Several studies have focused on Wnt family gene expression during tooth development (22).

The formation of the apatite precipitates on materials’ surface has proven to be useful in predicting the hard tissue—bonding ability of material in vitro (29, 30). The Si–OH functional groups of calcium silicate—based materials have been shown to act as nucleation centers for apatite precipitation (31, 32). The Ca ions released from MTA possibly originate from less-ordered hydration products and can significantly increase apatite growth by promoting local Ca supersaturation, as reported in a previous study (2). In addition, pulp cells have garnered considerable attention in pulp tissue regeneration research because of their differentiation potential ability to proliferate (33). Thus, it is of great importance to prepare suitable bioactive cements to promote the response of human periodontal ligament cells (hDPCs) for better pulp tissue regeneration. The adhesion and proliferation of hDPCs on MTA surfaces are spread out. In addition, the numbers of hPDLS adhering to cements grows with the increase of culture time, indicating good cytocompatibility of silicate-based materials (34).

To investigate the MTA extracts on the proliferation of hDPCs, cells were cultured in cell culture plates with various mediums prepared from cement extract for 1, 3, and 7 days. The results suggest that suitable concentrations of MTA extract significantly promote hDPC proliferation (16). It is already well established that MTA extracts play an important role in affecting cell behavior, which can promote DNA and protein synthesis and regulate Ca-conducting channels (35). A previous study clearly shows that bone incorporating various nutrients in the form of trace elements and Ca, Si, and Mg has been found to play central roles in promoting bone formation and regeneration. However, these trace elements are essential cofactors for enzymes involved in the synthesis of the mineral component of bone (36). In addition, the protein expression of DSPP and DMP-1 is promoted in hDPCs with MTA extracts on day 7. Alizarin red S also supplies further evidence that MTA extracts positively regulate the Ca deposition of hDPCs. We surmised that MTA extract would have an effect on odontoblastic differentiation in hDPCs, and after performing our experiments, we found the expression of odontogenic differentiation markers such as DSPP, DMP-1, and ALP. These results are similar to those of Wu et al (37), who reported that Si ions up-regulate the protein expression of ALP, Cementum protein 1 (CEMP1), and Runx-related transcription factor 2 (RUNX2) in hDPCs. In a recent study, we showed the positive effect of Si ions on odontogenesis differentiation of hPDLS (14). However, we also found that ionic products in MTA extracts at 100 mg/mL inhibit hDPC activity such as proliferation and differentiation. It has been clearly established that the cell death pathway triggered by high concentrations of MTA extracts (200 mg/mL) leads to cell death via apoptosis pathways, which may be explained by hyperosmolality (38).

Previous studies have verified that Wnt is an important signaling molecule in odontogenesis and dentin regeneration (20). During crown and root/periodontium formation, action in the Wnt/β-catenin pathway plays a key role (39). However, the overexpression of Wnt1 can inhibit ALP activity and the formation of a calcium deposit, as does the overexpression of β-catenin in hDPCs (40). This means that, to date, the biological effects on hDPCs have not been fully understood. Nemoto et al (41) showed that Wnt3a increases the proliferation of dental cells, such as cementoblasts, and also significantly improves proliferation and self-renewal capacity of hDPCs at 50 ng/mL. In the present study, we prepared various concentrations of MTA extracts to stimulate the osteogenesis differentiation of hDPCs and tried to determine whether the Wnt/β-catenin signaling pathways are involved in this process. The results show that the promotion of Wnt/β-catenin signaling pathways occurs in response to MTA extract dilutions. Our own results show the promotion of Wnt3a and β-catenin by treatment with MTA extracts at both the RNA and protein levels in hDPCs. Wnt3a has been proven to have a positive effect on odontogenesis-related gene and protein expression in hDPCs (20). Meanwhile, the Wnt/β-catenin–related protein inhibitors lead to the inhibition of odontogenesis protein expression of ALP, DSP, and DMP-1. These results suggest the intricate mechanism by which Wnt/β-catenin pathways affect hDPC behavior by MTA extracts and underline the need for comprehensive understanding of the roles of suitable concentrations of MTA.

In summary, the Ca- and Si-containing MTA extracts significantly promote the proliferation of hDPCs and show a positive odontogenic effect. We consider the most important mechanism for the markedly stimulatory effect of MTA extracts on the odontogenesis differentiation of hDPCs is related to the activation of the Wnt/β-catenin signaling pathway. In addition, we think that MTA cement has great potential for improving hard tissue regeneration because of their excellent osteogenesis and odontogenesis stimulation.

Acknowledgments

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