Original Article

Effect of Thai White Portland Cement Mixed with Bismuth Oxide and White ProRoot® MTA on Cementoblastic Differentiation in Human Cementoblast-Like Cell Line

Treesukhon Eakbannasingh
Graduate Student
Department of Operative Dentistry
Faculty of Dentistry,
Chulalongkorn University
Chootima Ratisoontorn
Assistant Professor
Department of Operative Dentistry
Faculty of Dentistry,
Chulalongkorn University

Correspondence to:
Assistant Professor Dr. Chootima Ratisoontorn
Department of Operative Dentistry
Faculty of Dentistry, Chulalongkorn University
Henri-Dunant Rd, Patumwan, Bangkok 10330
Tel./Fax: 02-2188795
E-mail: Chootima@gmail.com

Abstract

The objective was to investigate the effects of Thai white Portland cement mixed with bismuth oxide and white ProRoot® MTA on gene expression and alkaline phosphatase activity of human cementoblast-like cell line. Human cementoblast-like cell line was cultured with extracted media of white Portland cement mixed with bismuth oxide or white ProRoot® MTA at different extraction time points (days 1, 3 and 7). The expression of alkaline phosphatase, bone sialoprotein, type I collagen and osteocalcin were examined by quantitative real time polymerase chain reaction and alkaline phosphatase activity was also determined by enzymatic assay. Differences in relative expression ratio and alkaline phosphatase activity were analyzed by Kruskal-Wallis test. ($p < .05$) The results showed that Both white Portland cement mixed with bismuth oxide and white ProRoot® MTA statistically significantly upregulated alkaline phosphatase and bone sialoprotein gene expression at day 3. But only Thai white Portland cement mixed with bismuth oxide significantly increased type I collagen expression at day 1. However, gene expression of osteocalcin was significantly decreased by both Thai white Portland cement mixed with bismuth oxide and white ProRoot® MTA-treated cells at day 3. Alkaline phosphatase activity was statistically significantly increased at day 7 in Thai white Portland cement mixed with bismuth oxide-treated cells comparing to control, but there was no statistically significant difference between white ProRoot® MTA and control groups. In Conclusion, Thai white Portland cement mixed with bismuth oxide and white ProRoot® MTA could induce alkaline phosphatase and bone sialoprotein expression of human cementoblast-liked cell line.

Key words: alkaline phosphatase activity; cementoblast; gene expression; mineral trioxide aggregate; white Portland cement

Introduction

Root end-filling material is the key factor of endodontic periapical surgery to prevent the passage of microorganisms and their products from the root canal system to the periradicular tissues. Mineral trioxide aggregate is currently a favorable root end-filling material because of its sealing ability[1-3] insolubility in tissue fluid[3] biocompatibility[4] insusceptibility to the presence of moisture[3] antimicrobial effect[5] radiopacity and dimensional stability[3,6] as well as of its capability of promoting periradicular hard tissue formation.[7-11] However, mineral trioxide aggregate is very expensive, difficult to manipulate

and also has long setting time.[12] Portland cement has similar chemical composition to mineral trioxide aggregate (Tulsa Dental, OK, USA) except for bismuth oxide which is a constituent of mineral trioxide aggregate for radio-opacity.[12] Many studies indicated that Portland cement and mineral trioxide aggregate have similar physical properties, pH, setting time, solubility, dimensional change, compressive strength, *in vitro* and *in vivo* biocompatibility and antimicrobial effect.[6,13,14]

The deposition of cementum after endodontic periapical surgery is considered a desired healing response and a prerequisite for the reformation of a functional periodontal attachment.[15] Many histological studies have shown the presence of new cementum tissues in contact with the mineral trioxide aggregate retrofilling material on defective tooth roots in monkeys[9] and dogs.[7, 8, 16, 17] *In vitro* study revealed that mineral trioxide aggregate permitted murine cementoblastic cell (OCCM-30) expressing mRNA involved in cementoblastic differentiation, alkaline phosphatase (ALP), bone sialoprotein (BSP), collagen type I (COL I) and osteocalcin (OCN)[18,19] and induced a biomineralization of these cells.[19] Portland cement also allows the expression of mineralization related genes, such as osteonectin (ON) and dentin sialoprotein (DSPP) and induces ALP activity in early stage of mineralization on cultured human pulp cell.[20,21]

Portland cement, which is comparatively inexpensive material, has been proposed to use as an alternative retro-filling material. The previous studies showed that Thai white Portland cement mixed with bismuth oxide had similar chemical constituents and physical properties to white ProRoot® MTA[22,23] and was also biocompatible with primary human alveolar bone osteoblast.[24] However, the effects of Thai white Portland cement mixed with bismuth oxide on the expression of mineralization markers have not been investigated.

The purposes of present study were to investigate the effects of Thai white Portland cement mixed with bismuth oxide and white ProRoot® MTA on the mRNA expression of markers of cementoblastic differentiation in human cementoblast-like cell line, as well as to examine the effects of these materials on cellular ALP activity as a potential indicator of cementogenesis.

Materials and methods

Cell culture An immortalized human cementoblast cell line (HCEM) used in this study was a gift from Dr. Masae Kitagawa and Dr. Mutsumi Miyaichi, Hiroshima university. The study was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. These cells have been previously isolated and characterized.[25] Cells were cultured in minimum essential medium alpha (·-MEM, Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) plus penicillin G solution (10 U/ml) and streptomycin (10 mg/ml) in a humidified atmosphere of 5% CO_2 at 37°C.

Samples and extract media preparation Retrofilling materials used in this study were Thai white Portland cements mixed with bismuth oxide (PC) and white ProRoot® MTA (MTA) (Tulsa Dental, OK, USA). PC was Thai white Portland cements mixed with bismuth oxide (Fluka, Spain) and sterilized with ethylene oxide gas. MTA was prepared according to the manufacturer's instructions. For PC, one gram of powder was mixed with 0.3 ml of sterile distilled water.[22] Standard cylinder discs of 6 mm. in diameter and 1 mm. in height of each tested material were prepared using plastic molds under aseptic conditions, and then incubated for three hours in 95% humidity at 37°C. After removing the specimens from the molds, the samples of each material were placed into a 60-mm. tissue culture dish. Extract preparation was adapted from International Standard ISO 10993-12 (1999). Extracts of the retrofilling materials were prepared as follows: 220 µl/ sample of the cell culture media supplemented with 10% FBS and penicillin G solution (10 U/ml), and streptomycin was placed over the material discs, then incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The extract medium was pipette off the dish each day for usage. Subsequently, new medium was added to the dish for the following day extraction.

Real-time Polymerase chain reaction $3X10^5$ HCEM cells were seeded into 60-mm. tissue culture dish and cultured as previously described overnight. The culture media were replaced with extraction media every day. At days 1, 3 and 7, total RNA was extracted using the RNeasy Mini Kit (Qiagen K.K., Japan) and a reverse transcription was performontroled using Improm- II reverse transcriptase (Improm-II™, Promega Corp, USA) as follows: 1 µg of RNA was added to 5 µl of reverse transcription mixture (1 µl of Oligo DT and Rnase-free water). The mixture was denatured at 70°C for 5 min and chilled immediately on ice. Four microliters of ImProm-II™ 5X Reaction Buffer, 4.8 µl of $MgCl_2$, and 1.0µl of dNTP Mix (final concentration 0.5 mm. for each dNTP), 0.5 µl of Recombinant RNasin® Ribonuclease Inhibitor and 1.0 µl of ImProm-II™ Reverse

Transcriptase were added and incubated at 25 °C for 5 min followed by 42°C for 1 hour. The reactions were inactivated by heating at 70°C for 15 min. A reaction without reverse transcriptase was used as a negative control.

Real-time polymerase chain reaction (PCR) was performed on a mixture of 5 µl of complementary DNA and 15 µ L of master mix containing 10 µL SYBR Green PCR Master Mix (Invitrogen, USA), 1 mM of primers, and PCR-grade water using Lightcycler® 480 Real-Time PCR System (Roche Applied Science, Germany). The specific primers used in this study were ALP, BSP, COL-I and OCN. Beta-2-microglobulin (B2M) was used as a reference gene. Dnase free water was used as a negative control. The specific primer sequences were as follows: ALP (120 bp) forward 5' – GACAAGAAGCCCTTCACTGC-3', reverse 5' - AGACTGCGCCTGGTAGTTGT-3'[26]; BSP (208 bp) forward 5 '- GGGCAGTAGTGACTCATCCGAA-3', reverse 5- TCCATAGCCCAGTGTTGTAGCAG 3'[27]; COL I (114 bp) forward AAGGTCATGCTGGTCTTGCT 3', reverse 5' GACCCTGTTCACCTTTTCCA 3'[28]; OCN (70) forward 5' GAAGCCCAGCGGTGCA 3', reverse 5' CACTACCTCGCTGCCCTCC 3'[28]; B2M (114 bp) forward 5' GATGAGTATGCCTGCCGTGT 3', reverse 5' CAATCCAAATGCGGCATCT 3'[29]. A four-segment Light Cycler ™ PCR amplification and melting curve protocol were used. The program was set at 10 minutes denaturation step at 95°C followed by 45 amplification cycles at 95°C, 60°C and 72°C for 10 seconds. Melting curve analysis was performed at 95°C for 5 seconds, 65 °C for 1 minute and heating to 97°C using a ramp rate of 0.11°C/sec with continuous monitoring of fluorescence. The protocol was ended with a cooling segment (40 °C). Lightcycler® 480 software (Roche Applied Science, Germany) was used to estimate the threshold cycle numbers (crossing points; Cp). Data obtained by relative quantification with efficiency correction based on the crossing points calculated by the Light-Cycler™ software (Roche Applied Science, Germany). The relative quantification in gene expression was determine using 2^{-Ct} method described previously.[30] The quantification of each mRNA expression was evaluated by performing real-time PCR in triplicate.

Alkaline Phosphatase activity $1X10^5$ HCEM cells were plated per well in 24-well plates. After an initial attachment period of 24 hours, they were exposed to an extracted media. The extracted media was replaced every day. After 1, 3 and 7 days in culture, cells were lysed with 400 µl of cell lysis buffer and centrifuged at 300 X g for 3 minutes. The 100 µl of each cell lysate were added to 100 µl *p*-nitrophenylphosphate (pNPP) (Sigma- USA, product code. P7998) in 96-well plates. After 30 min of incubation at room temperature, the reaction was stopped by addition of 50 µl of 3 N NaOH solution. The absorbance was read at 405 nm using a microplate reader (Anthos Zenyth 200rt, UK). To determine the specific activity of ALP, protein concentrations in each lysate were determined using the DC protein assay (Bio-Rad, USA). 5 µl of each cell lysate were added into 96-well plates. After that, 25 µl of reagent A (an alkaline copper tartrate solution) mixed with 200 µl of reagent B (a dilute Folin Reagent) was added into each well. They were incubated for fifteen minutes at room temperature with gentle agitation. Absorbance was measured at 750 nm using the microplate reader. The ALP activity was expressed as µmol/µg of protein/second. The experiments were performed in triplicate.

Statistical analysis The data were expressed as the means of each group±standard deviations (s.d.). Group difference was evaluated using Kruskal-Wallis test followed by Conover-Inman test for post hoc multiple comparisons. Statistical significance of differences was defined as $p < .05$.

Results

Expression of cementoblastic differentiation marker genes by real-time quantitative RT-PCR

We examined the cementoblastic marker gene expression of HCEM at days 1, 3 and 7 in culture with regular medium (control), PC extracted medium and MTA extracted medium. The levels of ALP, BSP, COL I and OCN were determined by real-time quantitative RT-PCR using B2M as reference gene. The normalized relative quantification of each samples are shown in Fig. 1.

ALP ALP expression was significantly increased at day 3, then decreased at day 7 in all three groups. PC and MTA upregulated ALP expression at day 1 but there was no statistically significant difference comparing to control group. At day 3, the significant increase in ALP expression was observed in PC and MTA-treated cells when compared with the control group. (Fig. 1A)

BSP There was no significant difference in BSP expression among the three groups at day 1 and day 7. However, both PC and MTA significantly increased BSP gene expression compared with the control group at day 3. (Fig. 1B)

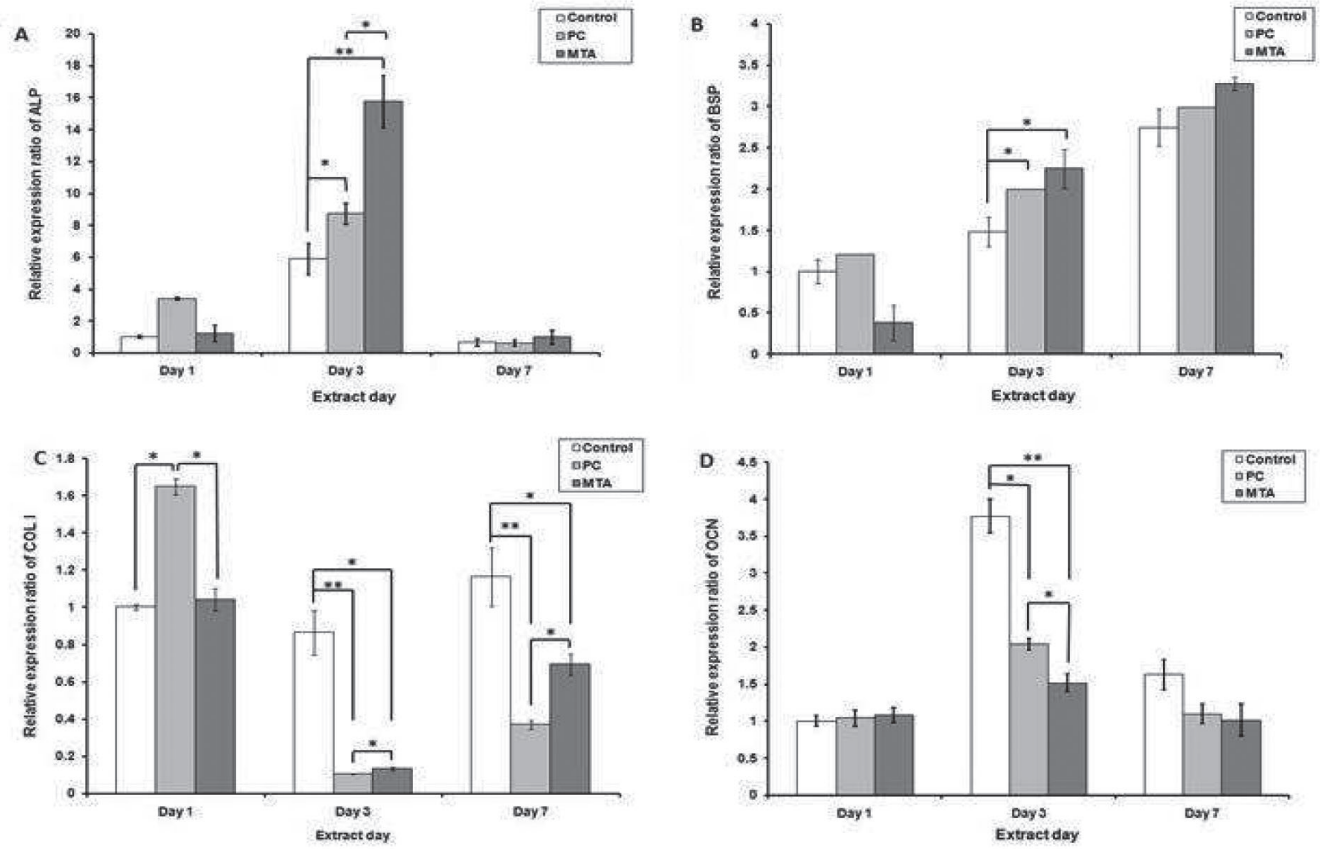

Fig. 1 *Relative expression ratio of ALP (A), BSP (B), COL I (C), OCN (D) of HCEM treated with PC or MTA extract at days 1, 3 and 7. The Relative quantification real-time PCR was performed as described in Materials and Methods. B2M was used as a reference gene. The bar represents the mean±standard deviation. An asterisk (*) indicates that p < .05, two asterisks (**) indicates that p < .001 in comparison with the control. ALP = alkaline phosphatase, BSP = bone sialoprotein, COL I = type I collagen, OCN = osteocalcin, PC = Thai white Portland cement mixed with bismuth oxide, MTA = white ProRoot® MTA.*

COL I PC significantly increased COL I expression compared with control and MTA groups at day 1. (Fig. 1C) However, there was a significant decrease expression in both PC and MTA groups compared with the control group at day 3 and day 7.

OCN There was no difference among three groups at day 1and day 7. However, PC and MTA groups decreased OCN expression compared with the control group at day 3. (Fig. 1D)

ALP activity ALP activity of three groups was observed at day 3 and increased at day 7. The ALP activity was statistically significantly higher in the PC group at day 7 comparing to MTA and control group. However, there was no significant difference between MTA and control group. (Fig. 2)

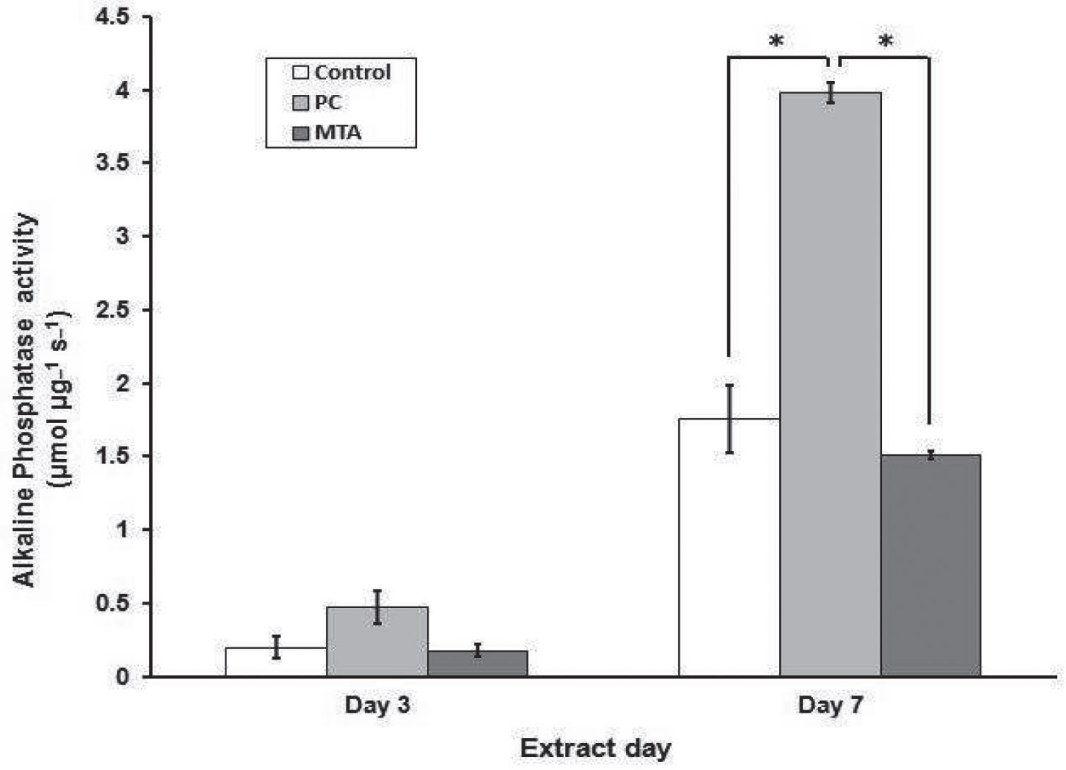

Fig. 2 *Alkaline phosphatase activity of HCEM treated with material extracts and untreated control media at days 3 and 7. The bar represents the mean±standard deviation. An asterisk (*) indicates that p < .05 in comparison with the control. PC = Thai white Portland cement mixed with bismuth oxide, MTA = white ProRoot® MTA.*

Discussion

Cementogenesis is a critical event for regeneration of periodontal tissue. This study utilizes the human cementoblast-like (HCEM) cell lines that have been established from human extracted tooth by tranfection of *hTERT* gene and shown to express specific marker of cementum, cementum-derived protein (CP-23). These cell lines were suggested to be useful cell models for investigating the mechanism of differentiation of human cementoblasts.[25]

This study used extract media method. Previous study showed that this concentration of extracted media was biocompatible in primary human alveolar bone osteoblast.[24] Under clinical conditions, interstitial tissue fluids circulate constantly through the vascular system. Therefore, this study changed the extracted solution daily to mimic the clinical situation.

ALP is a phosphate-releasing enzyme which is believed to participate in cementum mineralization.[31-35] Mineral trioxide aggregate induced ALP expression and activity in both PDL and gingival fibroblasts.[36] Although, there have not been fully clarified the detailed mechanism regulating cementoblastic differentiation but cementoblasts share many phenotypical features with osteoblast. Therefore, there is a possibility that expression profile of molecular factor regulated cementoblastic differentiation in similar manner as osteoblast. In this study, ALP mRNA expression of all groups showed the peak level at day 3 and decresed at day 7. This may be indicated that ALP gene expression elevated in a matrix maturation stage and declined in mineralization stage.

MTA induced the ALP expression in many cell types such as human periodontal ligament fibroblast[37] human dental pulp stromal cells (DPSCs)[38] and human osteosarcoma cell line (MG63)[39] and murine cementoblast cells (OCCM-30).[18] In this study, PC and MTA upregulated ALP expression compared to

control group at day 3. However, Nakayama et al's study[40] showed that there was no difference in expression of ALP mRNA in rat bone marrow cells (RBM) in the control group and MTA group at days 1, 2 and 3.

In this study, ALP activity was not detectable at day 1 because the amount of alkaline phosphatase protein may be too small to detect the activity or high pH during setting stage of the materials caused protein denature.[41] The result of this study showed no significant difference of ALP activity between MTA and control groups similar to the studies in rat bone marrow cells (RBM) and osteoblast-like cell line (MG-63) in which mineral trioxide aggregate maintained a level of ALP activity equivalent to unstimulated cells.[40,42] In addition, our results showed increased ALP activity at day 7 in PC group consistent with the study of Min et al[21] which discovered that Portland cement and Portland cement mixed with bismuth oxide increased ALP activity.

We also found that ALP gene expression at day 1 is in agreement with the level of ALP activity. On the contrary, ALP mRNA level in MTA group was higher than PC group at day 3 and day 7 but the enzyme activity was less than PC group. The inconsistent ALP gene expression and ALP activity in PDL and gingival fibroblasts has been previously reported.[36]

The possible mechanism that regulates ALP activity is a state of alkalinity. The high pH of calcium hydroxide stimulates the mineralization through activation of tissue enzymes like ALP[43] whereas changing in calcium ion does not appear to affect enzymatic activity.[44] Previous study revealed that Thai white Portland cements mixed with bismuth oxide and white ProRoot® MTA showed high pH level similar to calcium hydroxide.[22] However, the exact mechanism how PC or MTA regulate ALP activity is still unclear. Further studies should be carried out to investigate this phenomenon.

BSP is one of major noncollagenous glycoproteins that are specially expressed in mineralized connective tissues.[45-47] It is chemotactic to pre-cementoblasts and promotes their adhesion and differentiation.[48] High level of BSP expression is associated with initiating formation of the mineralizing cementum matrix[46,47] and this protein is a useful and reliable marker for cementoblast differentiation.[49] In the previous study, BSP expression by MC3T3-E1 cells from 24 to 72 hours was evident in MTA-treated and control cultures[50] and BSP expression was also found to be upregulated in MTA-treated human dental pulp cells.[51] These findings were consistent with our results that PC and MTA upregulated BSP expression when compared to control group at day 3.

COL I is an essential component of the extracellular matrix that is required to mineralize matrix formation.[52] In cementum, collagen promotes cellular attachment, functions to maintain the integrity of hard and soft tissue, and is active in their development, maturation, and repair.[35] Previous study revealed that mineral trioxide aggregate suppressed COL I expression in rat bone marrow osteoblast-like cells.[40] It is consistent with our current study that both white ProRoot® MTA and PC significantly decreased COLI expression at day 3 and day 7 but only PC upregulated COL I expression at day 1.

A study on murine immortalized cementoblast (OCCM-30), adding white ProRoot® MTA powder into cultured media at various concentrations found that 0.02 and 0.002 mg/ml concentrations could upregulate BSP and COL mRNA expression on days 3 and 5.[19] In this study, MTA also increased BSP expression in HCEM cell line at days 3 and 7. However, our result showed COL I expression decreased at days 3 and 7 which could be due to different methods of sample preparation.

An *in situ* hybridization study has shown that OCN expression was selective to root lining cells and was not present throughout the periodontal ligament in CD-1 mice. These findings indicated that OCN is marker selective to cementoblast.[53] The result of this study showed that OCN gene expression markly decrease in PC and MTA-treated groups compared with the untreated control group at day 3. This finding agrees with a report in OCCM-30 that OCN expression was not increased in the presence of white ProRoot® MTA.[19] However, our finding does not agree with Thomson et al's study which demonstrated that cementoblasts maintain expression of OCN in the presence of mineral trioxide aggregate by confocal microscopy[18] and Koh et al's study which used ELISA assays to found that OCN production was enhanced when osteoblasts (MG-63) were grown on mineral trioxide aggregate.[39] The differences between these studies and ours could be because of different detection methods.

One possible mechanism that PC and white ProRoot® MTA contribute to increased cementoblastic differentiation is the Si ion which is released from calcium silicate, one of the main constituents in both PC and white ProRoot® MTA. This Si ion has been previously reported to increase gene expression of the differentiation markers, COL I ,OCN, ALP, and ALP activity of MG-63 osteoblast-like cells.[54] Therefore, the ionic dissolution products releasing from PC and MTA are probably beneficial to the differentiation of HCEM. Further studies are necessary to be

performed to indicate the bioactive components releasing from PC and MTA.

Based on these results, PC and MTA could induce ALP and BSP expression and ALP activity which are known to be involved in differentiation of human cementoblast. Therefore, PC and MTA could be beneficial in cemental repair after apicoectomy and root-end filling with these materials.

Conclusion

Thai white Portland cement mixed with bismuth oxide and white ProRoot® MTA could induce cementoblastic differentiation of human cementoblast like cell line. Therefore, this study suggested that Thai white Portland cement mixed with bismuth oxide may be an alternative material for retrofilling procedure.

Acknowledgements

This study was supported by Fund, The Asia Research Center (The Korea Foundation for Advanced Studies) Chulalongkorn University. The authors would like to thank Dr. Masae Kitagawa and Dr. Mutsumi Miyaichi (Hiroshima university) for a gift of human cementoblast-like cell line. We also wish to thank Associate Professor Dr. Rangsini Mahanonda, Mr. Noppadol Sa-Ard-Iam and Ms. Pimprapa Rerkyen (Immunology laboratory Faculty of Dentistry, Chulalongkorn University) for their help and support.

References

1. Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling materials: effects of blood contamination. ***J Endod*** 1994;20:159-63.
2. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. ***J Endod*** 1995;21:109-12.
3. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. ***J Endod*** 1993;19:591-5.
4. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root end filling materials. ***J Endod*** 1995;21:489-92.
5. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. ***J Endod*** 1995;21:403-6.
6. Islam I, Chng HK, Yap AU. Comparison of the physical and mechanical properties of MTA and portland cement. ***J Endod*** 2006;32:193-7.
7. Baek SH, Plenk H Jr, Kim S. Periapical tissue responses and cementum regeneration with amalgam, SuperEBA, and MTA as root-end filling materials. ***J Endod*** 2005;31:444-9.
8. Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. ***J Endod*** 1995;21:603-8.
9. Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP. Histologic assessment of mineral trioxide aggregate as a root-end filling in monkeys. ***J Endod*** 1997;23:225-8.
10. Holland R, de Souza V, Nery MJ, Faraco Junior IM, Bernabe PF, Otoboni Filho JA, et al. Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. ***Braz Dent J*** 2001;12:3-8.
11. Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. ***J Endod*** 1999;25:161-6.
12. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. ***J Endod*** 1995;21:349-53.
13. Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. ***Braz Dent J*** 2000;11:3-9.
14. Saidon J, He J, Zhu Q, Safavi K, Spangberg LS. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod*** 2003;95:483-9.
15. Andreasen JO. Cementum repair after apicoectomy in humans. ***Acta Odontol Scand*** 1973 ;31:211-21.
16. Regan JD, Gutmann JL, Witherspoon DE. Comparison of Diaket and MTA when used as root-end filling materials to support regeneration of the periradicular tissues. ***Int Endod J*** 2002;35:840-7.
17. Shabahang S, Torabinejad M, Boyne PP, Abedi H, McMillan P. A comparative study of root-end induction using osteogenic protein-1, calcium hydroxide, and mineral trioxide aggregate in dogs. ***J Endod*** 1999;25:1-5.

18. Thomson TS, Berry JE, Somerman MJ, Kirkwood KL. Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. ***J Endod*** 2003;29:407-12.
19. Hakki SS, Bozkurt SB, Hakki EE, Belli S. Effects of mineral trioxide aggregate on cell survival, gene expression associated with mineralized tissues, and biomineralization of cementoblasts. ***J Endod*** 2009;35:513-9.
20. Min KS, Kim HI, Park HJ, Pi SH, Hong CU, Kim EC. Human pulp cells response to Portland cement in vitro. ***J Endod*** 2007;33:163-6.
21. Min KS, Lee SI, Lee Y, Kim EC. Effect of radiopaque Portland cement on mineralization in human dental pulp cells. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod*** 2009;108:e82-6.
22. Sirichaivongsakul S, Panichuttra A. Comparison of chemical composition and physical properties of two Thai White Portland cements with bismuth oxide versus White ProRoot$^{/E}$ MTA. ***CU Dent J***. 2008;31:145-58.
23. Werasopon P, Panichuttra A. Chemical composition and physical properties of Thai white Portland cements and bismuth oxide mixed with calcium chloride and methyl cellulose. ***CU Dent J***. 2010;33:207-20.
24. Jearanaiphaisarn T, Ratisoontorn C. Cytotoxicity of two Thai white portland cements mixed with bismuth oxide on primary human osteoblasts. ***CU Dent J***. 2009;32:179-90.
25. Kitagawa M, Tahara H, Kitagawa S, Oka H, Kudo Y, Sato S, et al. Characterization of established cementoblast-like cell lines from human cementum-lining cells in vitro and in vivo. ***Bone*** 2006;39:1035-42.
26. Shen B, Bhargav D, Wei A, Williams LA, Tao H, Ma DD, et al. BMP-13 emerges as a potential inhibitor of bone formation. ***Int J Biol Sci*** 2009;5:192-200.
27. Cordonnier T, Layrolle P, Gaillard J, Langonne A, Sensebe L, Rosset P, et al. 3D environment on human mesenchymal stem cells differentiation for bone tissue engineering. ***J Mater Sci Mater Med*** 2010;21:981-7.
28. Ratisoontorn C, Seto ML, Broughton KM, Cunningham ML. In vitro differentiation profile of osteoblasts derived from patients with Saethre-Chotzen syndrome. ***Bone*** 2005;36:627-34.
29. Wellmann S, Taube T, Paal K, Graf V Einsiedel H, Geilen W, Seifert G, et al. Specific reverse transcription-PCR quantification of vascular endothelial growth factor (VEGF) splice variants by LightCycler technology. ***Clin Chem*** 2001;47:654-60.
30. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. ***Methods***. 2001;25:402-8.
31. Beertsen W, Everts V. Formation of acellular root cementum in relation to dental and non-dental hard tissues in the rat. ***J Dent Res*** 1990;69:1669-73.
32. Groeneveld MC, Everts V, Beertsen W. Alkaline phosphatase activity in the periodontal ligament and gingiva of the rat molar: its relation to cementum formation. ***J Dent Res*** 1995;74:1374-81.
33. Groeneveld MC, Everts V, Beertsen W. Formation of afibrillar acellular cementum-like layers induced by alkaline phosphatase activity from periodontal ligament explants maintained in vitro. ***J Dent Res*** 1994;73:1588-92.
34. Groeneveld MC, Everts V, Beertsen W. A quantitative enzyme histochemical analysis of the distribution of alkaline phosphatase activity in the periodontal ligament of the rat incisor. ***J Dent Res*** 1993;72:1344-50.
35. Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. ***Periodontol 2000*** 2000;24:73-98.
36. Bonson S, Jeansonne BG, Lallier TE. Root-end filling materials alter fibroblast differentiation. ***J Dent Res*** 2004;83:408-13.
37. Yan P, Yuan Z, Jiang H, Peng B, Bian Z. Effect of bioaggregate on differentiation of human periodontal ligament fibroblasts. ***Int Endod J*** 2010;43:1116-21.
38. Paranjpe A, Zhang H, Johnson JD. Effects of mineral trioxide aggregate on human dental pulp cells after pulp-capping procedures. ***J Endod*** 2010;36:1042-7.
39. Chen CL, Huang TH, Ding SJ, Shie MY, Kao CT. Comparison of calcium and silicate cement and mineral trioxide aggregate biologic effects and bone markers expression in MG63 cells. ***J Endod*** 2009;35:682-5.
40. Nakayama A, Ogiso B, Tanabe N, Takeichi O, Matsuzaka K, Inoue T. Behaviour of bone marrow osteoblast-like cells on mineral trioxide aggregate: morphology and expression of type I collagen and bone-related protein mRNAs. ***Int Endod J*** 2005;38:203-10.
41. Haglund R, He J, Jarvis J, Safavi KE, Spangberg LS, Zhu Q. Effects of root-end filling materials on fibroblasts and macrophages in vitro. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***. 2003;95:739-45.

42. Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. ***J Biomed Mater Res*** 1997;37:432-9.
43. Estrela C, Sydney GB, Bammann LL, Felippe Junior O. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. ***Braz Dent J***. 1995;6: 85-90.
44. Gordon TM, Ranly DM, Boyan BD. The effects of calcium hydroxide on bovine pulp tissue: variations in pH and calcium concentration. ***J Endod*** 1985;11:156-60.
45. Somerman MJ, Sauk JJ, Foster RA, Norris K, Dickerson K, Argraves WS. Cell attachment activity of cementum: bone sialoprotein II identified in cementum. ***J Periodontal Res***. 1991;26:10-6.
46. Macneil RL, Sheng N, Strayhorn C, Fisher LW, Somerman MJ. Bone sialoprotein is localized to the root surface during cementogenesis. ***J Bone Miner Res***. 1994;9:1597-606.
47. MacNeil RL, Berry J, Strayhorn C, Somerman MJ. Expression of bone sialoprotein mRNA by cells lining the mouse tooth root during cementogenesis. ***Arch Oral Biol***. 1996;41:827-35.
48. Somerman MJ, Shroff B, Agraves WS, Morrison G, Craig AM, Denhardt DT, et al. Expression of attachment proteins during cementogenesis. ***J Biol Buccale*** 1990;18:207-14.
49. Ganss B, Kim RH, Sodek J. Bone sialoprotein. ***Crit Rev Oral Biol Med*** 1999;10:79-98.
50. Tani-Ishii N, Hamada N, Watanabe K, Tujimoto Y, Teranaka T, Umemoto T. Expression of bone extracellular matrix proteins on osteoblast cells in the presence of mineral trioxide. ***J Endod*** 2007;33:836-9.
51. Min KS, Yang SH, Kim EC. The combined effect of mineral trioxide aggregate and enamel matrix derivative on odontoblastic differentiation in human dental pulp cells. ***J Endod*** 2009;35:847-51.
52. Stein GS, Lian JB, Owen TA. Relationship of cell growth to the regulation of tissue-specific gene **expression** during osteoblast differentiation. ***FASEB J*** 1990;4:3111-23.
53. D'Errico JA, MacNeil RL, Takata T, Berry J, Strayhorn C, Somerman MJ. Expression of bone associated markers by tooth root lining cells, in situ and in vitro. ***Bone*** 1997;20:117-26.
54. Sun J, Wei L, Liu X, Li J, Li B, Wang G, et al. Influences of ionic dissolution products of dicalcium silicate coating on osteoblastic proliferation, differentiation and gene expression. ***Acta Biomater*** 2009;5:1284-93.

ผลของพอร์ตแลนด์ซีเมนต์ที่ผลิตในประเทศไทยที่ผสมบิสมัตออกไซด์และไวท์โปรรูทเอ็มทีเอต่อการเปลี่ยนสภาพของเซลล์สร้างเคลือบรากฟันชนิดซีเมนโตบลาสไลค์เซลล์ไลน์ของมนุษย์

ตรีสุคนธ์ เอกบรรณสิงห์
นิสิตบัณฑิตศึกษา ภาควิชาทันตกรรมหัตถการ
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ชุติมา ระติสุนทร
ผู้ช่วยศาสตราจารย์ ภาควิชาทันตกรรมหัตถการ
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ติดต่อเกี่ยวกับบทความ:
ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร. ชุติมา-ระติสุนทร
ภาควิชาทันตกรรมหัตถการ
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330
โทรศัพท์/โทรสาร: 02-2188795
E-mail: chootima@gmail.com

บทคัดย่อ

การศึกษามีวัตถุประสงค์เพื่อศึกษาผลของพอร์ตแลนด์ซีเมนต์สีขาวที่ผลิตในประเทศไทยที่ผสมบิสมัตออกไซด์และไวท์มิเนอรัลไตรออกไซด์แอกกริเกตต่อการแสดงออกของยีนและการทำงานของเอนไซม์อัลคาไลน์ฟอสฟาเตส ต่อเซลล์สร้างเคลือบรากฟันชนิดซีเมนโตบลาสไลค์เซลล์ไลน์ของมนุษย์ โดยมีวัสดุและวิธีการ คือเลี้ยงเซลล์สร้างเคลือบรากฟันชนิดซีเมนโตบลาสไลค์เซลล์ไลน์ของมนุษย์ด้วยอาหารเลี้ยงเซลล์ที่สกัดจากพอร์ตแลนด์ซีเมนต์สีขาวที่ผสมบิสมัตออกไซด์หรือไวท์โปรรูทเอ็มทีเอในช่วงเวลาที่แตกต่างกันคือ ณ วันที่ 1, 3 และ 7 วัดการแสดงออกของยีนอัลคาไลน์ฟอสฟาเตส โบนไซอะโลโปรตีน คอลลาเจนชนิดที่ 1 และออสทีโอแคลซินด้วยวิธีเรียลไทม์พอลิเมอเรสเชนรีแอกชันและวัดการทำงานของเอนไซม์อัลคาไลน์ฟอสฟาเตสด้วยวิธีวิเคราะห์เอนไซม์วิเคราะห์ความแตกต่างของสัดส่วนการแสดงออกของยีนและการทำงาน ของเอนไซม์อัลคาไลน์ฟอสฟาเตส ด้วยสถิติการทดสอบของครัสคัล-วอลลิสที่ระดับนัยสำคัญ 0.05 ผลการศึกษาพบว่าพอร์ตแลนด์ซีเมนต์สีขาวที่ผสมบิสมัตออกไซด์และไวท์โปรรูทเอ็มทีเอกระตุ้นการแสดงออกของยีนอัลคาไลน์ ฟอสฟาเตสและโบนไซอะโลโปรตีนอย่างมีนัยสำคัญทางสถิติที่วันที่ 3 แต่เฉพาะกลุ่มพอร์ตแลนด์ซีเมนต์สีขาวที่ผสมบิสมัตออกไซด์จะกระตุ้นการแสดงออกของยีนคอลลาเจนชนิดที่ 1 ในวันที่ 1 ส่วนการแสดงออกของยีน ออสทีโอแคลซินจะลดลงในทั้งสองกลุ่มทดลองในวันที่ 3 และเฉพาะพอร์ตแลนด์ซีเมนต์สีขาวที่ผสมบิสมัตออกไซด์จะกระตุ้นการทำงานของเอนไซม์อัลคาไลน์ฟอสฟาเตส อย่างมีนัยสำคัญทางสถิติในวันที่ 7 สรุปได้ว่าพอร์ตแลนด์ซีเมนต์สีขาวที่ผลิตในประเทศไทยที่ผสมบิสมัตออกไซด์และไวท์มิเนอรัลไตรออกไซด์แอกกริเกตกระตุ้นการแสดงออกของยีนอัลคาไลน์-ฟอสฟาเตสและโบนไซอะโลโปรตีนต่อเซลล์สร้างเคลือบรากฟันชนิดซีเมนโตบลาสไลค์เซลล์ไลน์ของมนุษย์