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Cytotoxicity of two Thai white portland cements mixed with bismuth oxide on primary human osteoblasts

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Abstract

Objective To compare the cytotoxicity of two Thai white portland cements mixed with bismuth oxide (PCA and PCB) with white mineral trioxide aggregate (white ProRoot® MTA) on primary human osteoblasts and to investigate cell morphology on the materials.

Materials and methods PCA, PCB and white ProRoot® MTA were mixed with distilled water. Standard cylinder discs of each tested material were prepared. Primary human osteoblasts were exposed to material extracts on different extraction time points (days 1, 3, 7 and 14). Cell viability was assessed by using methyltetrazolium assay. Differences in mean percentage of cell viability were analyzed by one-way analysis of variance ($p < 0.05$). Cell morphology was observed by scanning electron microscope after being seeded on the material discs and incubated for 24 and 72 hours.

Results For the day 1-extracts, PCB extract was more toxic than the others. However, for the day 3-extracts, percentages of cell viability in PCA and PCB extracts at 72 hours were significantly higher than that in white ProRoot® MTA extract ($p < 0.05$). Culture of cells in day 7-PCA extract showed significantly more cell viability at 72 hours compared to that of white ProRoot® MTA extract ($p < 0.001$). Cultures of cells in day 14-extracts showed no significant differences in cell viability among tested materials. Cells were able to attach and spread well on PCA and PCB within 24 and 72 hours.

Conclusion Days 3, 7 and 14-extracts of PCA and PCB were not toxic to primary human osteoblasts. However, day 1-PCB extract was more toxic than those of white ProRoot® MTA and PCA. The primary human osteoblasts could attach to PCA and PCB in a similar fashion to white ProRoot® MTA.

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Key words: *bismuth oxide; cytotoxicity; mineral trioxide aggregate; primary human osteoblasts; white portland cement*

Introduction

Several studies have reported the success rate of non-surgical root canal treatment to be between 45-98.7%.[1-3] Although the preferred treatment of endodontic failure is non-surgical retreatment, this may not be achieved because of the complexity of root canal systems or physical barriers such as post and core restoration and separated instruments. Surgical endodontic therapy becomes an indication when non-surgical retreatment is impractical or unlikely to improve the previous result.

Nowadays, mineral trioxide aggregate (MTA) is the most popular retro-filling material because it has many favorable properties: biocompatibility to periradicular tissue,[4-6] good sealing ability,[7-9] ability to induce periradicular tissue regeneration,[4,6,10] antimicrobial activity,[11] radiopacity and dimensional stability.[12] However, commercially available MTA is very expensive, difficult to handle and has long setting time.[13] Portland cement is a material that has major chemical components similar to MTA.[14] A number of previous studies that compared MTA with portland cement indicated that they were similar in chemical compositions except for the inclusion of bismuth oxide in MTA.[15-17] Portland cement and white portland cement were also found to be similar to MTA in physical properties, pH, radiopacity, setting time, solubility, dimensional change and compressive strength.[12] Moreover, there are several biocompatibility studies comparing portland cement with MTA both *in vitro* and *in vivo*.[18-23] For cytotoxicity testing, portland cement showed a comparable level of cell viability to MTA in mouse fibroblast, (L-929)[18] human osteoblast-like cell (SaOS-2),[19,20] human endothelial cell (ECV 304),[21] mouse lymphoma cell (L5178Y)[22] and Chinese hamster ovary cell (CHO K-1).[23] In addition, the characteristics of cells in the presence of MTA and portland cement were similar from the scanning electron microscope studies.[19,20]

Portland cement, which is much cheaper than MTA, has been proposed to be used as an alternative retro-filling material. Recently, a study showed that two Thai white portland cements mixed with bismuth oxide have chemical constituents and physical properties similar to white ProRoot® MTA.[24] These two Thai white portland cements were approved by Thai Industrial Standards Institute (TISI no. 133-1975).[24] However, there are no reports of cytotoxicity of these Thai white portland cements. Therefore, this study focused on the cytotoxicity of two Thai white portland cements mixed with bismuth oxide compared to white ProRoot® MTA.

The purposes of the present study were to compare the cytotoxic effect of two Thai white portland cements mixed with bismuth oxide and white ProRoot® MTA on primary human osteoblasts using the extract test and methyltetrazolium (MTT) assay, and to investigate and compare cell morphology of primary human osteoblasts in contact with these materials by scanning electron microscope.

Materials and methods

Cell culture

Primary human osteoblasts were established from the bone samples collected from patients who underwent impacted third molar removal at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University. The protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. Informed consents were obtained before surgery. Bone samples were immediately placed in culture medium, Dulbecco's modified Eagle medium with F12 nutrient mixture, (Gibco BRL, USA), supplemented with 2 mM L-glutamine, penicillin G (50 U/ml), streptomycin (50 µg/ml), amphotericin B (2.5 µg/ml) (Gibco BRL, USA) and 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, USA). The samples were washed twice

with phosphate buffer saline (PBS) and soft tissue was removed by scraping with a sterile scalpel. The samples were cut into small pieces, then transferred to a 35-mm tissue culture dish containing 2 ml of the culture medium and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells from the 3rd to 5th passage were used in the following experiments.

Osteogenic maker gene expression by reverse transcription polymerase chain reaction (RT-PCR) analysis

Cells were seeded into 60-mm culture dishes (1.8-2.5 x 10^5 cells/dish) and incubated overnight. Then, the culture medium were replaced with the culture medium supplemented with 50 µg/ml of ascorbic acid (AA) and 10 mM of β-glycerophosphate (β-GP). For the negative control group, it was cultured in the medium without AA and β-GP. Culture medium was changed twice weekly. On days 3, 7, 14, 21 and 28, RNA was extracted using the RNeasy Mini Kit (Qiagen, Chatworth, CA, USA) according to the manufacturer's instructions. The RNA samples were further purified by successive treatment with DNase I (Qiagen, Chatworth, CA, USA). These RNA extracts were stored at -80°C. One microgram of total RNA was used for reverse transcription with random hexamer and Improm-II reverse transcriptase (Improm-II™, Promega Corp, USA), following the manufacturer's instructions. The cDNA was used for detection of bone markers; collagen type I-alpha 2 (COLIA2), alkaline phosphatase (ALP), bone sialoprotein 2 (BSP2), osteopontin (OPN), osteocalcin (OCN), by polymerase chain reaction (PCR). PCR amplifications were performed using Taq DNA polymerase (Qiagen, Chatworth, CA, USA) by the Mastercycler gradient (Eppendorf, Germany) for 35 cycles at 95°C for 30 seconds (ALP, COLIA2, BSP2, OPN, GAPDH) or 1 minute (OCN), at 55°C for 1 minute (ALP, COLIA2, BSP2, OPN) or at 60°C for 1 minute (OCN, GAPDH), at 72°C for 1 minute, and then a final extension at 72°C for 2 minutes. Specific primer sequences were as follows: COLIA2 (461 bp) forward 5′-GGACACAATGGATTGCAAGG-3′, reverse 5′-TAACCACTGCTCCACTCTGG-3′[25]; ALP (475 bp) forward 5′-ACGTGGCTAAGAATGTCATC-3′, reverse 5′-CTGGTAGGCGATGTCCTTA-3′[26]; BSP2 (408 bp) forward 5′-TTAGCTGCAATCCAGCTTCC-3′, reverse 5′-CTCCCCCTCGTATTCAACG-3′[27]; OPN (522 bp) forward 5′-GCATCACCTGTGCCATACC-3′, reverse 5′-CATTCAACTCCTCGCTTTCC-3′[27]; OCN (297 bp) forward 5′-ATGAGAGCCCTCACACTCCTC-3′, reverse 5′-GCCGTAGAAGCGCCGATAGGC-3′[25]; GAPDH (438 bp) forward 5′-TCATCTCTGCCCCCTCTGCTG-3′, reverse 5′-GCCTGCTTCACCACCTTCTTG-3′[28]. PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide.

Alkaline phosphatase (ALP) activity assay

To determine the ALP activity, cells were seeded into 24-well tissue culture plates (Nunc, Denmark) (1.5 x 10^4 cells/well) and incubated overnight. Then, the culture medium was replaced with the medium supplemented with 50 µg/ml of AA and 10 mM of β-GP. For the negative control group, it was cultured in a medium without AA and β-GP. The culture medium was changed twice weekly. On days 3, 7, 14, 21 and 28, cells were washed 3 times with PBS and fixed with 70% ethanol for 10 minutes. One tablet of BCIP/NBT (Sigma-Aldrich, USA), dissolved in 10 ml of water, provides 10 ml substrate solution contains BCIP (0.15 mg/ml) and NBT (0.30 mg/ml). Five-hundred microliters of the substrate was added to the fixed cells for 15 minutes then washed 3 times with PBS. The blue stain on osteoblasts which indicated ALP activity was investigated by plain observation and under a light microscope. The experiment was performed in triplicate for each time point.

Alizarin red S staining of mineralized nodules

Cells were cultured and fixed on days 3, 7, 14, 21 and 28 as described in the method for ALP activity assay. Fixed cells were stained with 500 µl of 0.5% w/v Alizarin red S for 1 hour and then washed 3 times with 70% ethanol. The wells were air-dried after washing.[29] The experiment was performed in triplicate for each time point.

Sample preparation

Retrofilling materials used in this study were two Thai white portland cements mixed with bismuth oxide (PCA and PCB) and white ProRoot® MTA (Tulsa Dental Products, Tulsa, OK, USA). PCA and PCB were Thai white portland cements mixed with bismuth oxide (Fluka, Spain) at the ratio of 4:1 by weight[24] and sterilized with ethylene oxide gas. Intermediate Restorative Material (IRM®; Caulk, Dentsply, USA) was used as a positive control. Both white ProRoot® MTA and IRM® were prepared according to the manufacturer's instructions. For PCA and PCB, one gram of powder was mixed with 0.3 ml of sterile distilled water[24]. 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, then incubated for 3 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 for extract preparation.

Cytotoxicity test

Extracts of the retrofilling materials were prepared as follows: 220 µl/sample of the cell culture medium without 10% FBS was placed over the material discs, then incubated at 37° C in a humidified atmosphere of 95% air and 5% CO_2. The medium was daily replaced with a new medium until the time point of extraction was due (1, 3, 7 and 14 days after sample preparation). Twenty-four hours before the extraction time point, the medium was replaced by cell culture medium with 10% FBS.

Cells (1×10^4 per well) were seeded in 96-well plates and incubated in 95% humidity and 5% CO_2 at 37° C for 24 hours. The culture medium was replaced with 180 µl of the extracts of the materials on days 1, 3, 7 and 14. Cells were incubated in each extract for 24 and 72 hours. Eight replicates for each material extract at each time point were performed. Culture medium with 10% FBS was used as a negative control and the extract of IRM® on day 1 was used as a positive control.

Cell viability was determined by MTT assay. The extract medium was removed. The wells were washed twice with PBS, then replaced with 100 µl of DMEM without phenol red (Gibco BRL, USA) containing 0.5 mg/ml of MTT (3-(4,5,dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; Sigma Chemical Co., St. Louis, MO, USA). After 3-hour incubation at 37° C in a humidified atmosphere of 5% CO_2, MTT solution was removed and then 200 µl of dimethyl sulfoxide (DMSO) was added into each well to solubilize the formazan crystals. Plates were shaken for 5 minutes on a plate shaker to achieve a uniform color. Optical densities were measured at 570 nm wavelength in a multiwell spectrophotometer. Mean and standard deviation of the percentage of cell viability were calculated. Differences in mean cell viability between materials at each extraction time points were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc multiple comparison test at the 95% confidence interval ($p < 0.05$).

Cell morphology

Two samples of each material which were incubated in culture medium for 1 day were placed into a 96-well tissue culture plate. Primary human osteoblasts (1×10^4 cells/well) were seeded on the tested materials and incubated at 37° C in a humidified atmosphere of 5% CO_2 for 24 and 72 hours. Cells

cultured on cover slips were used as a control group. The discs of tested materials along with the cells grown on their surfaces were washed three times with a cacodylate-buffered solution, fixed with 200 µl of 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 2 hours at room temperature. After fixation, the tested material discs were washed again with a cacodylate-buffered solution, then dehydrated in ascending grades of ethanol, dried with hexamethyldisilazane (HMDS; Sigma-Aldrich, USA) for 5 minutes, mounted on copper stubs and sputter-coated with 15 nm gold palladium. Cell morphology on material discs were examined under scanning electron microscope (JSM-5410LV, JOEL, Japan) at 1,000X.

Results

Characteristics of the primary human osteoblasts

RT-PCR analysis of RNA extracts from primary human osteoblasts on days 3, 7, 14, 21 and 28 showed that these cells expressed osteogenic makers; COLIA2, ALP, BSP2, OPN and OCN (Fig. 1A). Alkaline phosphatase activity and mineralized nodule formation were first detected on day 14 and increased continuously until day 28 (Fig. 1B).

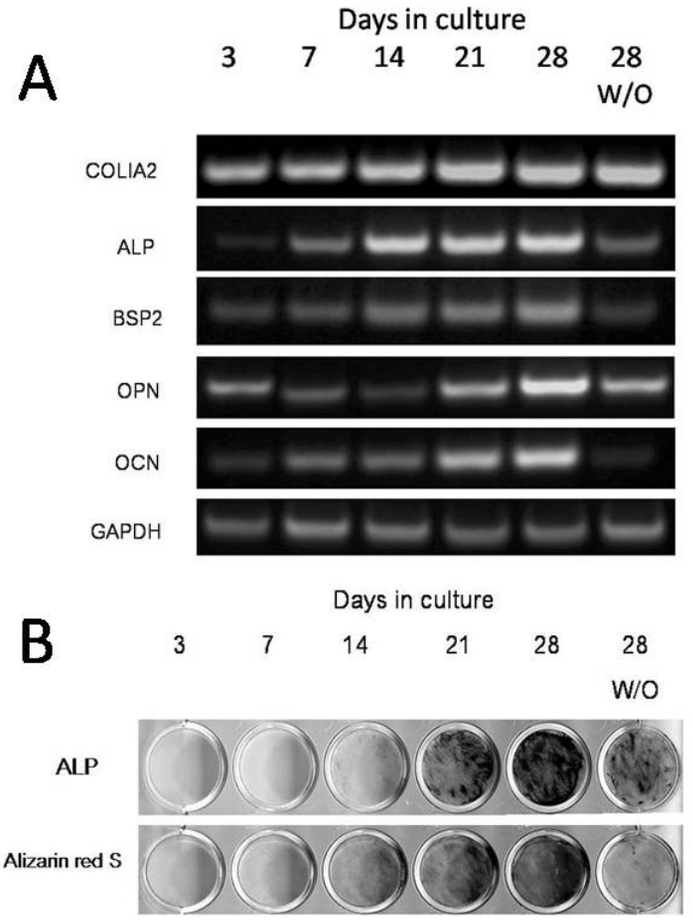

Fig. 1 Osteoblastic phenotype of the primary human osteoblasts. A) mRNA expression of COLIA2, ALP, BSP2, OPN, OCN and GAPDH. B) Alkaline phosphatase activity (ALP) and Alizarin red S staining. Cells were cultured in medium supplemented with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days. w/o = cells grown in medium without ascorbic acid and β-glycerophosphate.

Cytotoxicity test

From MTT assay, the percentages of cell viability related to the control group (cell growth in the regular culture medium) were calculated. The percentages of cell viability of the primary human osteoblasts treated with material extracts for 24 and 72 hours were shown in figure 2. IRM® extract was confirmed to be highly toxic, showing cell viability at 0.23 % and 14.44% at 24 and 72 hours, respectively (data not shown).

Relative percentages of cell viability at 24 hours in all material extracts were more than 90%. Relative percentages of cell viability at 72 hours tended to be more than those at 24 hours in all material extracts especially in day 3 and 7 extracts in which the relative percentages of cell viability were more than 100%. The only exception was for the day 1-PCB extract at both 24 and 72 hours, which showed the percentage of cell viability as 12.81% and 7.38%, respectively.

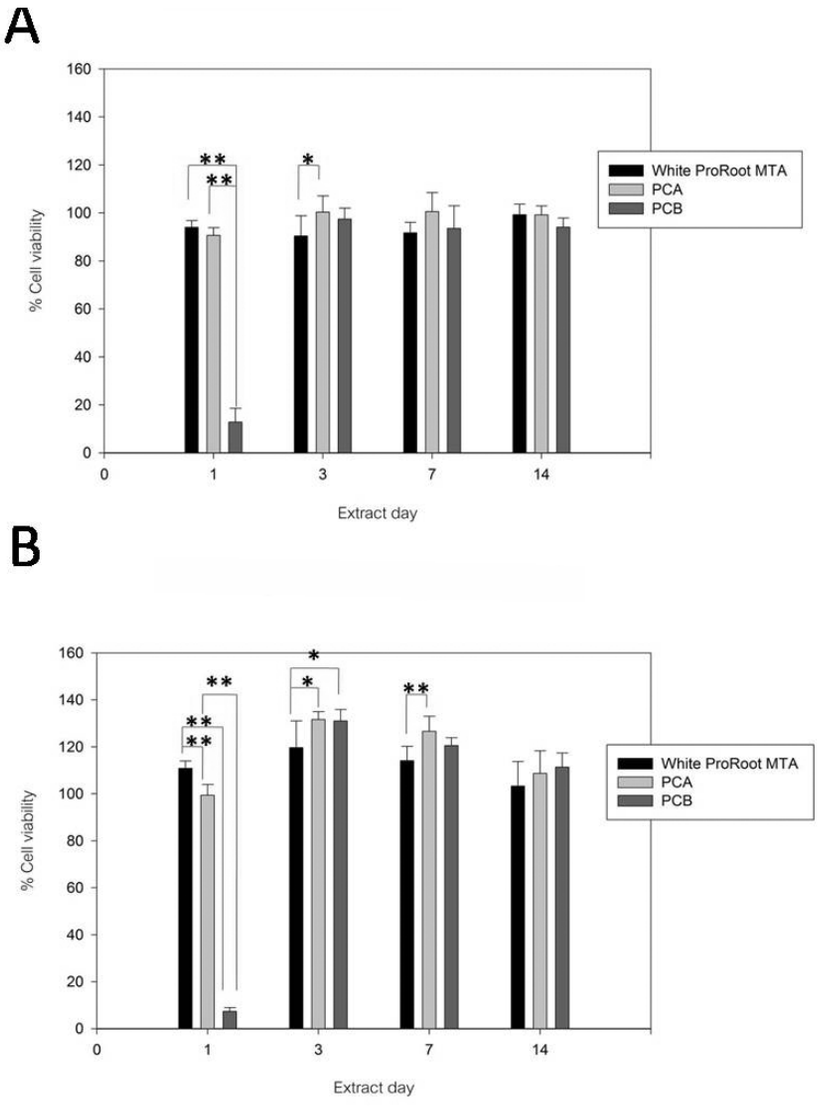

Fig. 2 The percentages of cell viability of primary human osteoblasts treated with material extracts for 24 hours (A) and 72 hours (B) relative to untreated control (medium) by MTT assay (* = statistically significant difference at $p < 0.05$, ** = statistically significant difference at $p < 0.001$).

The cytotoxicity of day 1-PCB extract was statistically significant more than that of day 1-white ProRoot® MTA extract ($p < 0.001$) at 24 and 72 hours. In contrast, the percentage of cell viability of day 1-PCA extract was not statistically different to day 1-white ProRoot® MTA extract when incubated for 24 hours. However, at 72 hours, the day 1-PCA extract showed a significantly lower percentage of cell viability than that of white ProRoot® MTA ($p < 0.001$). Nonetheless, the percentage of cell viability of the day 1-PCA extract was nearly 100% suggesting that the day 1-PCA extract was not cytotoxic. The day 3-PCA and PCB extracts showed higher percentages of cell viability than that of white ProRoot® MTA with a statistically significant difference for PCA extract at both 24 and 72 hours ($p < 0.05$) and for PCB extract at 72 hours ($p < 0.05$). There was no significant difference of percentages of cell viability among tested materials on days 7 and 14, except for the day 7-PCA extract which had a significantly higher percentage of cell viability at 72 hours than that of white ProRoot® MTA.

Cell morphology

Primary human osteoblasts grown on glass cover slips demonstrated that a large number of cells attached and spread onto the glass surface at 24 hours. At 72 hours, there were more cellular extensions and processes shown under scanning electron microscope (Fig. 3).

Within 24 hours, primary human osteoblasts, seeded on white ProRoot®MTA, attached and spread on the surface of the material discs. At 72 hours, there were more cells with their cellular extensions and processes interacting with the underlying MTA surface and with adjacent cells. Parts of lamellipodia inserted into the material surface (Fig. 3). Scanning electron micrographs showed that cell attachment and spreading on PCA and PCB were similar to those on the white ProRoot®MTA (Fig. 3). The morphology of human osteoblasts observed on IRM® was different from the others. The cells were round and did not attach to the IRM® surface at both 24 and 72 hours (Fig. 3).

Discussion

In this study, we compared cytotoxicity of two Thai white portland cements mixed with bismuth oxide (PCA and PCB) in primary human osteoblasts to that of white ProRoot® MTA. A previous study showed that white ProRoot® MTA and two Thai white portland cements mixed with bismuth oxide used in this study have comparable chemical constituents and physical properties. [24]

There are several *in vitro* studies about the biocompatibility of MTA.[5,30,31] Most studies used established cell lines because they are high proliferation, easily available and more reproducible than primary cells. However, established cell lines and primary cells have some differences in biological properties. For example, primary rat calvaria osteoblasts cultured in medium with β-glycerophosphate and dexamethasone could produce mineralized nodules, but MG-63 which is an osteosarcoma cell line could not.[32] In addition, ISO recommended the use of primary cell strains derived from living tissues for specific sensitivity testing to simulate the *in vivo* situation.[33] Osteoblast is one of the important cells for periradicular tissue healing after endodontic surgery. Therefore, primary human osteoblasts were used in this study to test the cytotoxicity effect of retrofilling materials. It was confirmed by RT-PCR of osteogenic marker genes, alkaline phosphatase activity test and Alizarin red S staining that the primary cell strain used in this study expressed osteoblastic markers and could produce mineralized nodules when cultured in medium with ascorbic acid and β-glycerophosphate.

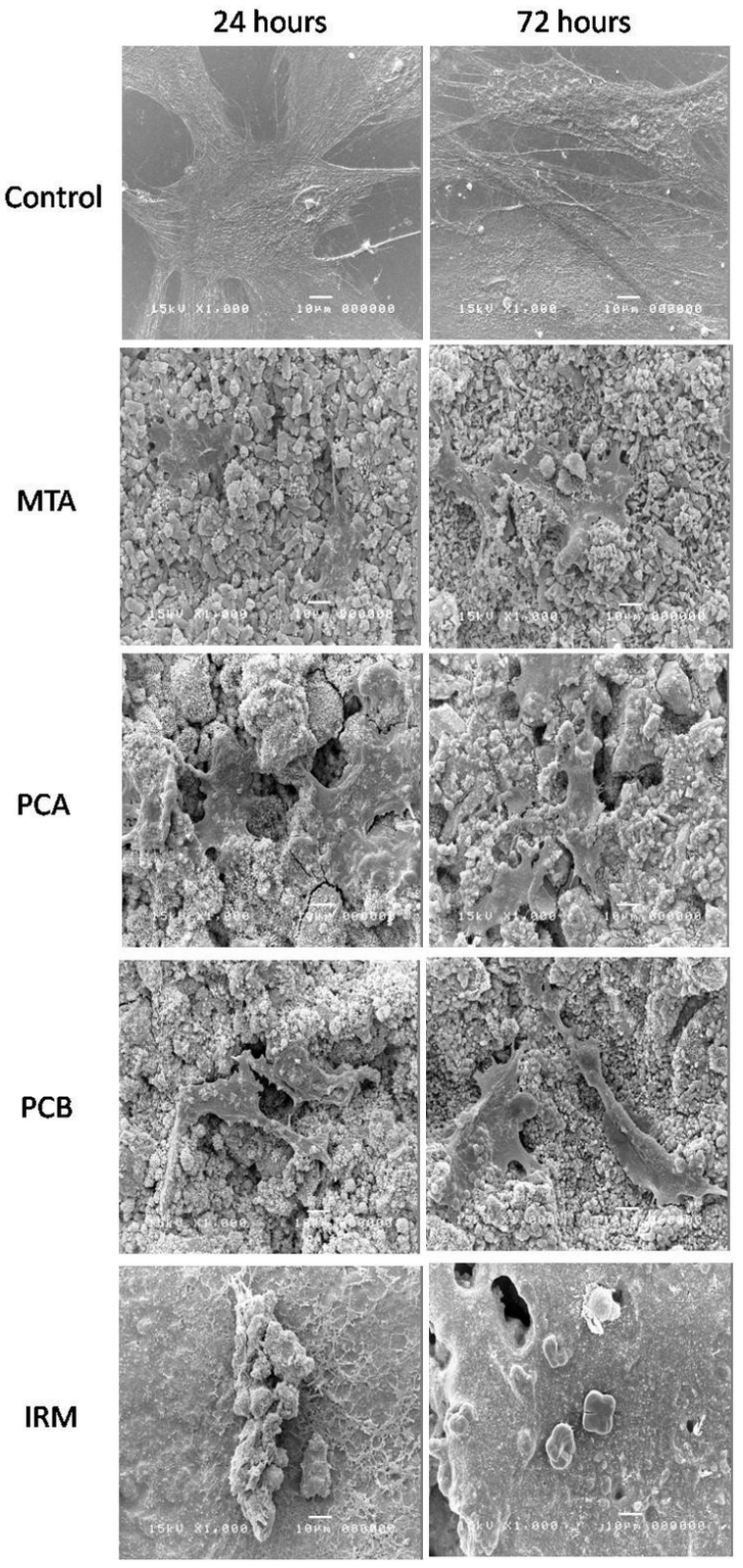

Fig. 3 Primary human osteoblasts were seeded onto glass coverslip (control), white ProRoot® MTA (MTA), PCA, PCB and IRM® discs and incubated for 24 and 72 hours. Scanning electron micrographs are shown at magnification 1000X.

Cell viability after exposure to extract medium was determined by MTT assay because tetrazolium salt reacts with mitochondrial dehydrogenase which is found only in living, metabolically active cells.[34] Moreover, this assay is highly sensitive and also inexpensive and suitable for screening purposes of a large number of samples in a short time.[35] The results from MTT assay showed that at 24 hours, all day 1-material extracts were toxic; however, white ProRoot® MTA and PCA extracts were less toxic than PCB extract. Although day 1-white ProRoot® MTA extract was slightly toxic at 24 hours, there were more viable cells than the control group after 72 hours. This result was in agreement with a previous study which reported that the survival rate of cells cultured in MTA extract was more than that in the control culture medium.[36] These results supported that white ProRoot® MTA is a biocompatible material.

Although lower number of viable cells was found when cultured in PCA extract than in white ProRoot® MTA extract on day 1, there were more viable cells in day 3- and 7-PCA extracts compared to white ProRoot® MTA extracts. Like PCA, day 1-PCB extract was highly toxic but day 3-PCB extract was not toxic and also enhanced cell proliferation at 72 hours. However, PCB extract was much more toxic than PCA extract on day 1. The reason is that there are differences in their pH and final setting time. From the previous study, the pH of PCB at the first 20 minutes after mixing was higher than that of PCA,[24] thus high pH from the day 1-PCB extract may affect cell viability. In addition, PCB had approximately 30 minutes longer final setting time than PCA,[24] therefore PCB may release toxic substances for a longer period.

The percentage of cell viability when cultured for 72 hours in day 3, 7 and 14 extracts of PCA, PCB and white ProRoot® MTA were more than 100. This result means that the extracts of these materials on days 3, 7 and 14 were not toxic. The results of the present study showed that all materials, especially PCB, tended to be toxic on the first day, but their toxicity later decreased and also enhanced cell proliferation. This result was similar to the study of Camilleri *et al.* which reported that after 24 hours incubated in white MTA and white portland cement extracts, cells increased activity compared to those in the control medium.[14]

Besides the extract test, our study investigated cell morphology of primary human osteoblasts in contact with materials by scanning electron microscope. Al-Rabeah *et al.* reported that primary human osteoblasts well attached and spread out over white MTA surface within 24 hours and their processes also interacted with adjacent cells.[37] Our study found that cells could attach onto white ProRoot® MTA surface within 24 hours and spread further in 72 hours like the study of Al-Rabeah *et al.*

Osteoblast morphology on two Thai white portland cements mixed with bismuth oxide was similar to that on white ProRoot® MTA. They were polygonal in shape as well as they also spread well over the materials. The result was in agreement with the study of Abdullah *et al.,* which showed that SaOS-2 could attach and spread on MTA and portland cement within 12, 24, 48 and 72 hours.[19] Furthermore, Gandolfi *et al.* showed that SaOS-2 were polygonal and could attach well on tetrasilicate cement which was white portland cement and bismuth oxide.[20] PCA, PCB and white ProRoot® MTA were less toxic than IRM® which showed marked rounding of the cells and depletion of cell number.

Conclusion

The results of this study demonstrated that two Thai white portland cements mixed with bismuth oxide were not toxic to primary human osteoblasts on days 3, 7 and 14. However, on day 1, PCB was more toxic than white ProRoot® MTA and PCA. The primary

human osteoblasts could attach to PCA and PCB in a similar fashion to white ProRoot® MTA. Therefore, this study suggests that PCA may be an alternative material of white ProRoot® MTA for retrofilling procedure.

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ความเป็นพิษของพอร์ตแลนด์ซีเมนต์สองชนิดที่ผลิตในประเทศไทยที่ผสมกับบิสมัทออกไซด์ต่อเซลล์สร้างกระดูกมนุษย์

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บทคัดย่อ

วัตถุประสงค์ เพื่อเปรียบเทียบความเป็นพิษของพอร์ตแลนด์ซีเมนต์สองชนิดที่ผลิตในประเทศไทยที่ผสมกับบิสมัทออกไซด์ (พีซีเอ และ พีซีบี) กับ ไวท์มิเนอรอลไตรออกไซด์แอกกริเกต (ไวท์โปรรูทเอ็มทีเอ) ต่อเซลล์สร้างกระดูกมนุษย์ และตรวจสอบรูปร่างของเซลล์บนวัสดุ

วัสดุและวิธีการ นำพีซีเอ พีซีบี และไวท์โปรรูทเอ็มทีเอผสมกับน้ำกลั่น และขึ้นรูปเป็นแผ่นวงกลม เซลล์สร้างกระดูกมนุษย์ถูกเลี้ยงในอาหารเลี้ยงเซลล์ที่ผ่านการแช่วัสดุดังกล่าวในช่วงเวลาที่แตกต่างกันคือ ณ วันที่ 1 3 7 และ 14 หลังการขึ้นรูป ความมีชีวิตของเซลล์ถูกวัดด้วยวิธีเมธิวเตตระโซเลียม วิเคราะห์ความแตกต่างของค่าเฉลี่ยร้อยละความมีชีวิตของเซลล์เปรียบเทียบระหว่างวัสดุด้วยสถิติความแปรปรวนแบบทางเดียว ที่ระดับนัยสำคัญ 0.05 ลักษณะรูปร่างของเซลล์ที่เลี้ยงบนวัสดุเป็นเวลา 24 และ 72 ชั่วโมง ถูกตรวจสอบด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด

ผลการศึกษา สารสกัดที่ 1 วันของพีซีบี มีความเป็นพิษต่อเซลล์มากกว่าสารสกัดจากวัสดุอื่น อย่างไรก็ดีเมื่อเซลล์ถูกเลี้ยงในสารสกัดจากวันที่ 3 เป็นเวลา 72 ชั่วโมง ร้อยละความมีชีวิตของเซลล์ที่ได้สารสกัดจากพีซีเอ และพีซีบี มีมากกว่าไวท์โปรรูทเอ็มทีเออย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) และสารสกัดที่ 7 วัน เมื่อเซลล์ถูกเลี้ยง 72 ชั่วโมง ร้อยละความมีชีวิตของเซลล์ที่ได้สารสกัดจากพีซีเอมีมากกว่าไวท์โปรรูทเอ็มทีเออย่างมีนัยสำคัญ ($p< 0.001$) สำหรับความเป็นพิษของสารสกัดที่ 14 วันของวัสดุทั้ง 3 ชนิด ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ที่เวลา 24 และ 72 ชั่วโมงเซลล์สามารถยึดเกาะและแผ่ตัวได้ดีบนวัสดุทั้ง 3 ชนิด

สรุป สารสกัดที่ 3 7 และ 14 วันของพีซีเอ และ พีซีบี ไม่เป็นพิษต่อเซลล์สร้างกระดูกมนุษย์ อย่างไรก็ดีสารสกัดที่ 1 วันของพีซีบี มีความเป็นพิษมากกว่าของไวท์โปรรูทเอ็มทีเอ และพีซีเอ เซลล์สร้างกระดูกมนุษย์สามารถเกาะบนพีซีเอ และพีซีบีได้เหมือนกับบนไวท์โปรรูทเอ็มทีเอ

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