

Research Article



White mineral trioxide aggregate mixed with calcium chloride dihydrate: chemical analysis and biological properties

Hany Mohamed Aly Ahmed ,^{1*} Norhayati Luddin ,¹
Thirumulu Ponnuraj Kannan ,^{1,2} Khairani Idah Mokhtar ,^{1,3} Azlina Ahmad ^{1,2}

¹Universiti Sains Malaysia School of Dental Sciences, Kubang Kerian, Malaysia

²Human Genome Centre, Universiti Sains Malaysia School of Medical Sciences, Kubang Kerian, Malaysia

³International Islamic University Malaysia, Kulliyah of Dentistry, Kuantan, Malaysia

OPEN ACCESS

Received: Nov 5, 2016

Accepted: Mar 7, 2017

Ahmed HMA, Luddin N, Kannan TP,
Mokhtar KI, Ahmad A

*Correspondence to

Hany Mohamed Aly Ahmed, BDS, HDD, PhD
Senior Lecturer, Department of Conservative
Dentistry, Universiti Sains Malaysia School
of Dental Sciences, Kubang Kerian, Kelantan
16150, Malaysia.
Tel: +60-1-2985-7937
Fax: +60-9-767-5505
E-mail: hany_endodontist@hotmail.com

Copyright © 2017. The Korean Academy of
Conservative Dentistry

This is an Open Access article distributed
under the terms of the Creative Commons
Attribution Non-Commercial License ([https://
creativecommons.org/licenses/by-nc/3.0/](https://creativecommons.org/licenses/by-nc/3.0/))
which permits unrestricted non-commercial
use, distribution, and reproduction in any
medium, provided the original work is properly
cited.

Funding

This study was supported by the Universiti
Sains Malaysia Research University Grant
number 1001/PPSG/813056.

Conflict of Interest

No potential conflict of interest relevant to this
article was reported.

ABSTRACT

Objectives: This study aimed to evaluate the chemical and biological properties of fast-set white mineral trioxide aggregate (FS WMTA), which was WMTA combined with calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), compared to that of WMTA.

Materials and Methods: Surface morphology, elemental, and phase analysis were examined using scanning electron microscope (SEM), energy dispersive X-ray microanalysis (EDX), and X-ray diffraction (XRD), respectively. The cytotoxicity and cell attachment properties were evaluated on human periodontal ligament fibroblasts (HPLFs) using methyl-thiazol-diphenyltetrazolium (MTT) assay and under SEM after 24 and 72 hours, respectively.

Results: Results showed that the addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA affected the surface morphology and chemical composition. Although FS WMTA exhibited a non-cytotoxic profile, the cell viability values of this combination were lesser than WMTA, and the difference was significant in 7 out of 10 concentrations at the 2 time intervals ($p < 0.05$). HPLFs adhered over the surface of WMTA and at the interface, after 24 hours of incubation. After 72 hours, there were increased numbers of HPLFs with prominent cytoplasmic processes. Similar findings were observed with FS WMTA, but the cells were not as confluent as with WMTA.

Conclusions: The addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA affected its chemical properties. The favorable biological profile of FS WMTA towards HPLFs may have a potential impact on its clinical application for repair of perforation defects.

Keywords: Calcium chloride; Cytotoxicity; Fibroblasts; Mineral trioxide aggregate; Periodontal ligament






INTRODUCTION

Advances in endodontic bio-materials are at the forefront of endodontic research. Gray mineral trioxide aggregate (GMTA) was introduced to the field of endodontics at Loma Linda University as a root-end filling material [1]. More recently, a white version (white mineral trioxide aggregate [WMTA]) was developed to address esthetic concerns. Many studies have demonstrated the favorable biological properties, good sealing ability, and hard tissue

Author Contributions

Conceptualization: Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Data curation: Ahmed HMA; Formal analysis: Ahmed HMA; Funding acquisition: Luddin N; Investigation: Ahmed HMA; Methodology: Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Project administration: Luddin N; Resources: Ahmed HMA, Luddin N; Software: Ahmed HMA; Supervision: Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Validation: Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Visualization: Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Writing - original draft: Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A; Writing - review & editing: Ahmed HMA.

ORCID iDs

Hany Mohamed Aly Ahmed 
<https://orcid.org/0000-0003-0776-9288>
Norhayati Luddin 
<https://orcid.org/0000-0002-7081-462X>
Thirumulu Ponnuraj Kannan 
<https://orcid.org/0000-0001-5015-9771>
Khairani Idah Mokhtar 
<https://orcid.org/0000-0002-7456-7865>
Azlina Ahmad 
<https://orcid.org/0000-0002-1393-5775>

formation and induction of WMTA [2-5]. However, prolonged setting time continues to be the main shortcoming [6,7].

The long setting time of mineral trioxide aggregate (MTA) can make handling of the material rather challenging [8]. To overcome this drawback, a number of setting accelerators applied at different concentrations have been examined [6,9-12]. CaCl₂ is the most common setting accelerator examined as a potential additive to MTA [6,9,13-17]. Investigations have proven the ability of the MTA/CaCl₂ combination to achieve good sealing ability and high pH values, especially at the concentration of 10% [18,19]. However, contradictions in the biological profiles of this combination have been reported. Bortoluzzi *et al.* [20] and Jafarnia *et al.* [21] reported favorable biological responses to the WMTA/CaCl₂ combination when applied in an animal model and to L929 mouse cells, respectively. A recent study has reported favorable cytotoxic properties of this combination on dental pulp stem cells [17]. Conversely, another report demonstrated undesirable cellular responses when this combination is applied to challenge MG-63 cells [22].

According to the discussion above, available reports on the biological properties of WMTA/CaCl₂ combination are mostly based on non-dental cell lines and animal models, which may respond differently compared to human dental cells [7]. Therefore, the present study aimed to examine the chemical composition of the WMTA/CaCl₂ combination (fast-set WMTA [FS WMTA]), and assess the biological properties of this FS formulation to the human periodontal ligament fibroblasts (HPLFs). The research hypotheses were that: 1) the addition of calcium chloride dihydrate (CaCl₂·2H₂O) to WMTA affects the surface morphology and chemical composition; 2) WMTA and FS WMTA show comparable cell viability values and cell attachment properties on HPLFs.

MATERIALS AND METHODS

Preparation of normal and FS formulations

1. Normal set formulation

One gram of WMTA (ProRoot MTA, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) was mixed with 250 µL of the liquid provided by the manufacturer. The mix was then introduced into acrylic molds (5 mm in diameter and 2 mm in height) [23].

2. FS formulation

Calcium chloride in the dihydrate form (CaCl₂·2H₂O, Merck, Darmstadt, Germany) was selected to be the setting accelerator [23,24]. One gram of WMTA was mixed with 10% CaCl₂·2H₂O and 200 µL of liquid provided from the manufacturer, and introduced into acrylic molds.

Scanning electron microscope/Energy dispersive X-ray microanalysis (SEM/EDX)

SEM/EDX microanalysis was performed after one day of curing [25]. The study samples were fitted onto aluminium stubs, coated with gold using a sputter coating machine (Leica EM SCD005, Leica Microsystems, Vienna, Austria), and then viewed under SEM (Quanta 450 FEG, FEI Netherlands, Eindhoven, The Netherlands) at 2 magnification steps (× 600 and × 20,000). For elemental analysis using EDX, 3 stubs were made for each group, and each stub was examined at 3 different areas [26]. The mean weight percentages for every element were then calculated.

X-ray diffraction (XRD)

The phase analysis of samples was examined using XRD (PANalytical X'Pert Pro MRD PW3040, PANalytical B. V., Almelo, The Netherlands). The generator of the machine was operated at 30 mA and 40 kV, and the detector was rotated between 10 and 80 ($^{\circ}2\theta$), at 0.05 ($^{\circ}2\theta$) per 2 seconds. Phase identification was accomplished by the use of search-match software utilizing International Centre for Diffraction Data (ICDD) database.

Cytotoxicity evaluation using extraction dilution method (methyl-thiazol-diphenyltetrazolium [MTT] assay)

1. Culture of HPLFs cell line

The complete growth medium — 500 mL of stromal cell growth medium (Lonza, Walkersville, MD, USA), fetal bovine serum (Lonza), human fibroblast growth factor-B (Lonza), human recombinant insulin (Lonza), and gentamycin sulphate (Lonza) — was prepared for the culture of HPLFs cell line (Lonza). HPLFs were then thawed and cultured.

2. Preparation of the materials

The study samples were prepared as described in the chemical analysis section, and then sterilized using ultraviolet light in a class II biological safety cabinet (Labconco, Kansas, MO, USA) for 30 minutes [27]. Subsequently, the samples were introduced into sterile 15 mL centrifugation tubes, and the prepared media were then added into each tube before incubation for 7 days at 37°C.

3. Cells preparation

Prepared media which is 5×10^3 cells in 100 μ L were added into each well of sterile 96-well plate (Nunc 96-well plate, Thermo Fisher Scientific, Roskilde, Denmark). Five replicates were prepared for each concentration (each group has 5 concentrations) then incubated at 37°C and 5% CO₂ for 24 and 72 hours. Each experiment was performed 3 times on 3 successive passages (P10–12).

4. Application of the extracts

On day 7, the extracts were passed through a sterile 0.2 μ m filter (Pall Corp., Ann Arbor, MI, USA). The extracts were then prepared at 5 serial dilutions (50, 25, 12.5, 6.25, 3.125 mg/mL). The maximum concentration (50 mg/mL) was selected based on a pilot study. The media in the seeded 96-well plate were then replaced by the materials extracts, and the last row served as the control. The plates were then incubated at 37°C and 5% CO₂ for 24 and 72 hours.

5. Application of MTT assay

MTT solution which is 30 μ L was added into each well at each interval of time. After 3–4 hours, the content of each well was replaced by 100 μ L of dimethyl sulfoxide (DMSO). The optical density (OD) was measured using an enzyme linked immunosorbent assay (ELISA) reader (Sunrise Remote/Touch Screen, Tecan, Grödig, Austria) at a test wavelength of 570 nm, and a reference wavelength of 600 nm. The cell viability values were then calculated:

$$\text{Cell viability (\%)} = \{(A - B)/(C - B)\} \times 100$$

where A is the OD of test group, B is the OD of blank wells, and C is the OD of control group.

The cytotoxicity profile was classified according to Zhang *et al.* [28] (> 90%, non-cytotoxic; 60%–90%, slight cytotoxicity; 30%–60%, moderate cytotoxicity; 0%–30%, severe cytotoxicity). Mann-Whitney *U* test was performed for statistical analysis ($p = 0.05$).

Cell attachment properties

The cell attachment properties were evaluated as described in previous studies [23,29]. Sterile acrylic molds filled with the study samples were prepared, and 250 μL of prepared medium having 10^5 cells was added onto the top of every sample and left for 30 minutes. Subsequently, 5 mL of prepared medium was added, and the plate was incubated at 37°C and 5% CO_2 for 24 and 72 hours. After each time interval, the samples were washed, 2.5% glutaraldehyde (Merck) was added and the samples were then dehydrated in ethanol. The samples were then fitted onto aluminium stubs, coated with gold, and then viewed under the SEM. Empty acrylic molds and acrylic molds filled with a dental amalgam filling material served as control groups.

RESULTS

Surface morphology

The surface morphology of WMTA was characterized by the presence of amorphous, globular, and crystalline particles in a general ground mass of finer amorphous material. At high magnification, WMTA exhibited areas of coarse amorphous crystalline structures. The addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ resulted in a more homogenous and less porous surface morphology compared to the normal set formulation (**Figures 1A, 1B, 2A, and 2B**).

Chemical analysis

EDX results showed that WMTA comprised Ca, O, C, Si, Bi, Al, and Mg. Cl was only identified in FS WMTA because of the addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The peaks and mean weight percentage for every element are listed in **Figures 1C and 2C**. XRD analysis showed that WMTA and FS WMTA composed of calcium silicate ($\text{Ca}_3\text{SiO}_5/\text{Ca}_3[\text{SiO}_4]\text{O}$), calcite (calcium carbonate [CaCO_3]), and bismuth oxide (Bi_2O_3). Small peaks of calcium aluminum silicate ($\text{CaAl}_2\text{Si}_2\text{O}_8/\text{Ca}_3\text{Al}_6\text{Si}_2\text{O}_{16}$) were also detected in both formulations. FS WMTA differed from WMTA by the presence of calcium silicate chloride ($\text{Ca}_2\text{SiO}_3\text{Cl}_2$) (**Figures 1D, 1E, 2D, and 2E**).

Cytotoxicity evaluation

Results showed that although FS WMTA exhibited favorable cytotoxicity profile, the addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA reduced the cell viability values, and the difference was significant in 7 out of 10 concentrations at 2 time intervals ($p < 0.05$; **Figure 3**). In terms of cytotoxicity classification, both groups were severely cytotoxic at 50 mg/mL after 24 and 72 hours. At concentrations ≤ 25 mg/mL, both WMTA and FS WMTA exhibited a non-cytotoxic profile at both time intervals except for one concentration of WMTA (3.125 mg/mL at 24 hours), which demonstrated a slight cytotoxic activity (**Figure 3**).

Cell attachment properties

After 24 hours of incubation, HPLFs adhered over the surface and at the interface of WMTA. The cells showed prominent cytoplasmic processes, especially at the interface (**Figure 4A and 4B**). After 72 hours of incubation, the HPLFs increased in number with prominent cytoplasmic processes interacting with the surface of the study samples and the neighbouring cells (**Figure 4C and 4D**). FS WMTA showed similar findings, but the cells were not as confluent as with WMTA (**Figure 4E-4H**). **Figure 4I-4N** show the cell attachment properties of the control groups (favorable and unfavorable profiles with acrylic mold and amalgam, respectively).

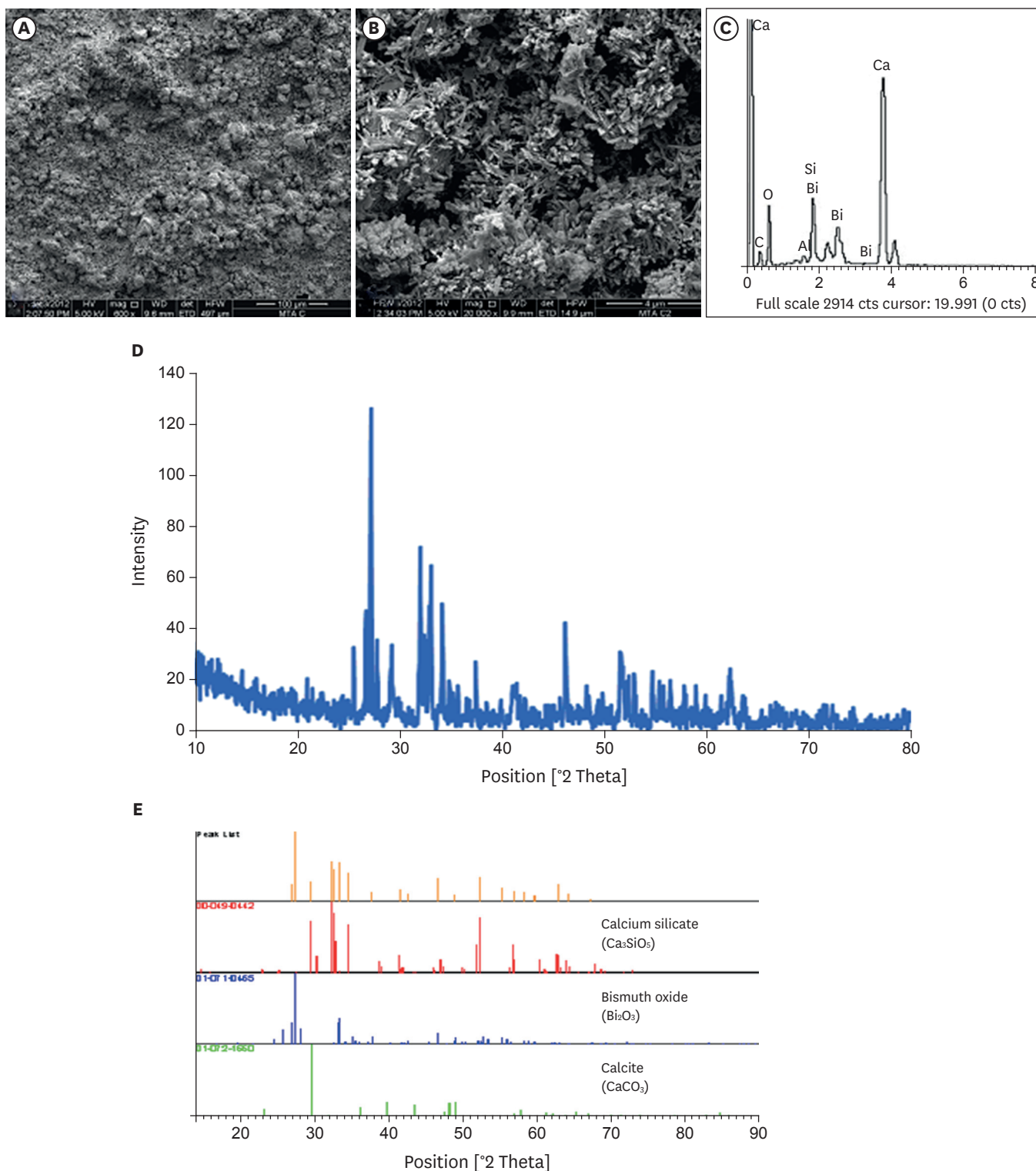


Figure 1. (A, B) Surface morphology of WMTA (A: $\times 600$, B: $\times 20,000$); (C) EDX microanalysis. Mean of weight percentages in WMTA: Ca = 32.26%, O = 40.76%, C = 7.82%, Si = 5.47%, Al = 0.32%, Mg = 0.06%, and Bi = 13.31%; (D, E) XRD analysis of WMTA. WMTA, white mineral trioxide aggregate; EDX, energy dispersive X-ray microanalysis; XRD, X-ray diffraction.

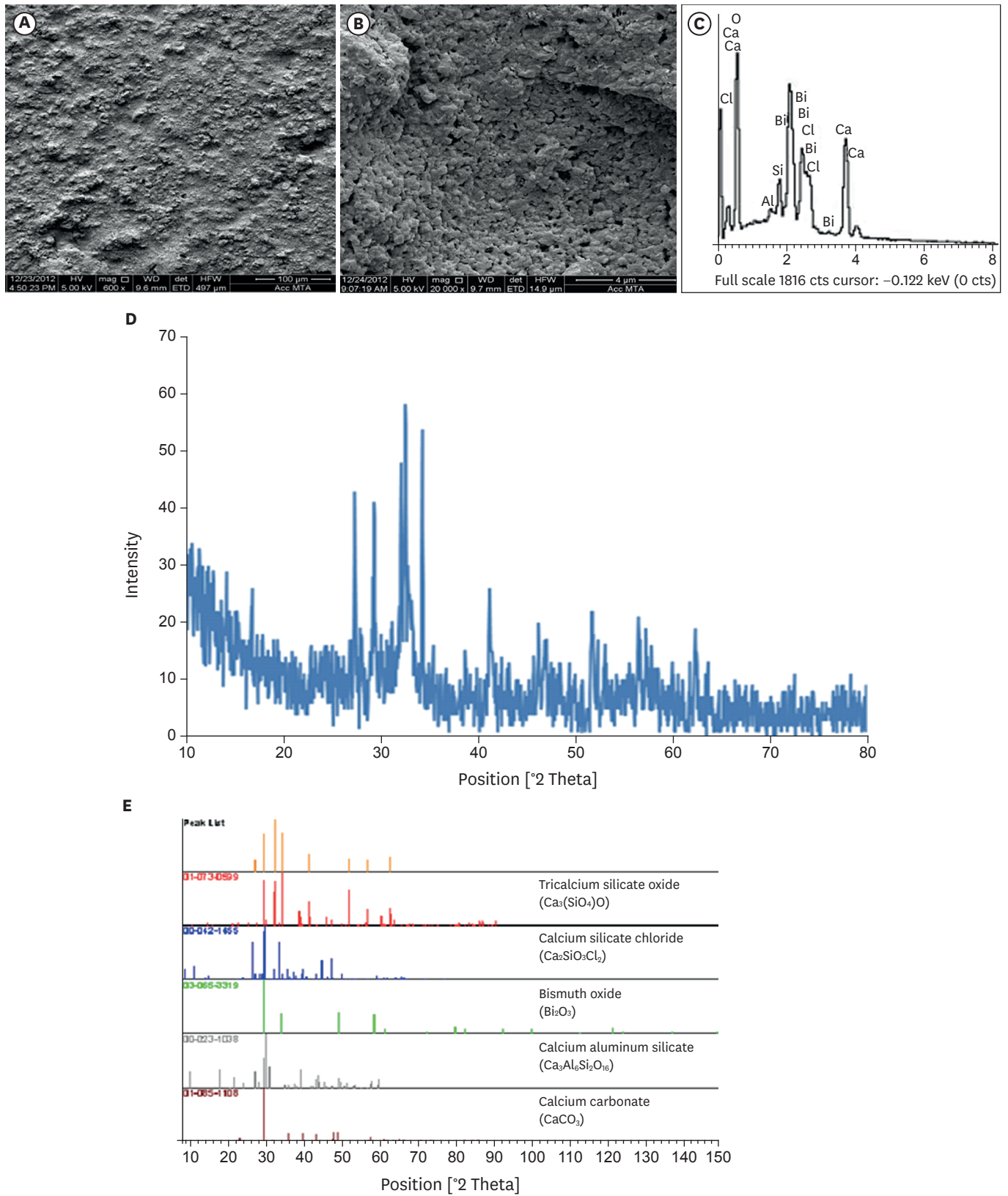


Figure 2. (A, B) Surface morphology of FS WMTA (A: $\times 600$, B: $\times 20,000$); (C) EDX microanalysis. Mean of weight percentages in FS WMTA: Ca = 30.85%, O = 34.19%, C = 3.28%, Si = 4.90%, Al = 0.47%, Mg = 0%, Bi = 19.30%, and Cl = 7.01%; (D, E) XRD analysis of FS WMTA. FS WMTA, fast-set white mineral trioxide aggregate; EDX, energy dispersive X-ray microanalysis; XRD, X-ray diffraction.

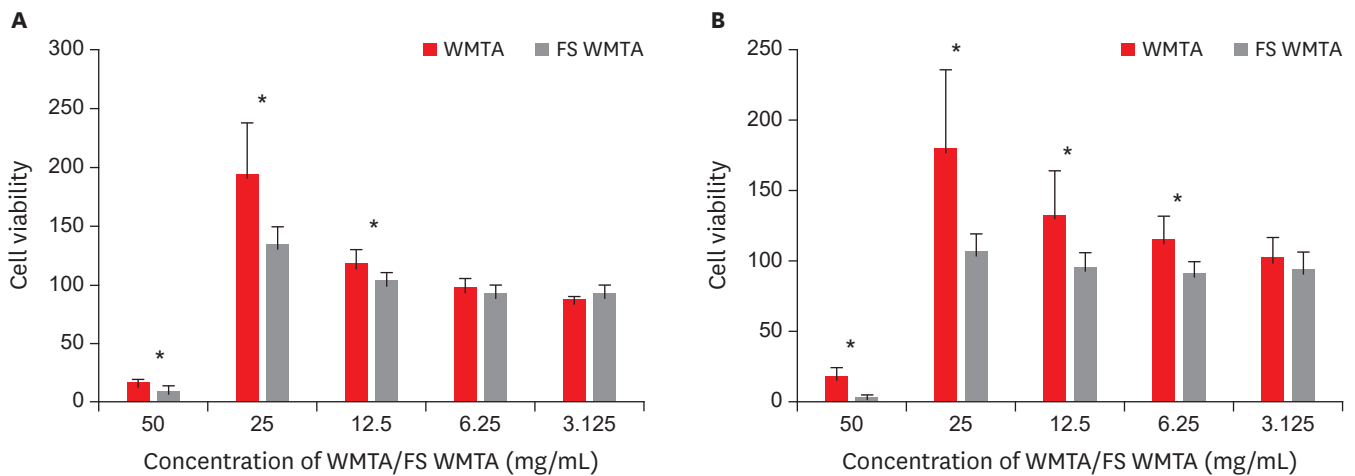


Figure 3. Intergroup comparisons between the cell viability values according to the concentrations of WMTA and FS WMTA extracts (A) after 24 hours; (B) after 72 hours. WMTA, white mineral trioxide aggregate; FS WMTA, fast-set white mineral trioxide aggregate.

*There is a statistically significant difference between WMTA and FS WMTA at the concentration.

DISCUSSION

The chemical composition, physical properties, and biological profile of WMTA have been examined in previous studies. However, information on the properties of WMTA when combined with CaCl_2 in the dihydrate formula is scarce. The present study showed that addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA results in a more homogenous and less porous surface morphology compared to WMTA. The porosity in the set calcium silicate-based formulations has been identified as a potential drawback for the material [7,18]. It can potentially allow the penetration of bacteria or their by-products; however, studies have shown that after the placement of MTA, a layer of hydroxyapatite forms over the material that fills the voids and surface defects [7]. The influence of CaCl_2 on the surface morphology of calcium silicate cements has been reported, and this effect appears to mainly depend on the concentration of CaCl_2 and hydration time [30,31].

EDX is a chemical microanalysis technique used to study the compositions of dental materials, including the cements [32]. Numerous studies have shown several detectable variations in the elemental composition of WMTA via EDX. Although Ca, O, Si, and Bi were identified in all related investigations [26,33-37], consistent with the current study, the presence of Al [26,33,34,36,37], S [33,34,36,37], C [26,33,36], and to a lesser extent, Mg and K [33] has also been reported. The present study demonstrated the presence of small/moderate peaks of C as well as small peaks of Al and Mg. S was not detected in any WMTA or FS WMTA samples.

The heterogeneity of elemental compositions of WMTA among different studies could be mainly attributed to 2 reasons. First, the formula of commercial WMTA products vary chemically, probably because of distinct manufacturing practices in different countries [38], and/or the continuous attempts of manufacturers to enhance the quality of the material. Similar observations have been reported with GMTA [35,38]. Second, the sample size used for EDX examination is critical for determining minor/trace elements. Two studies did not mention the sample size for EDX analysis [34,36]. Other studies prepared 2 stubs for each sample, and each sample was examined twice [35,39]. In the present study, 3 samples were analyzed 3 times, and each sample was analyzed at 3 different areas.

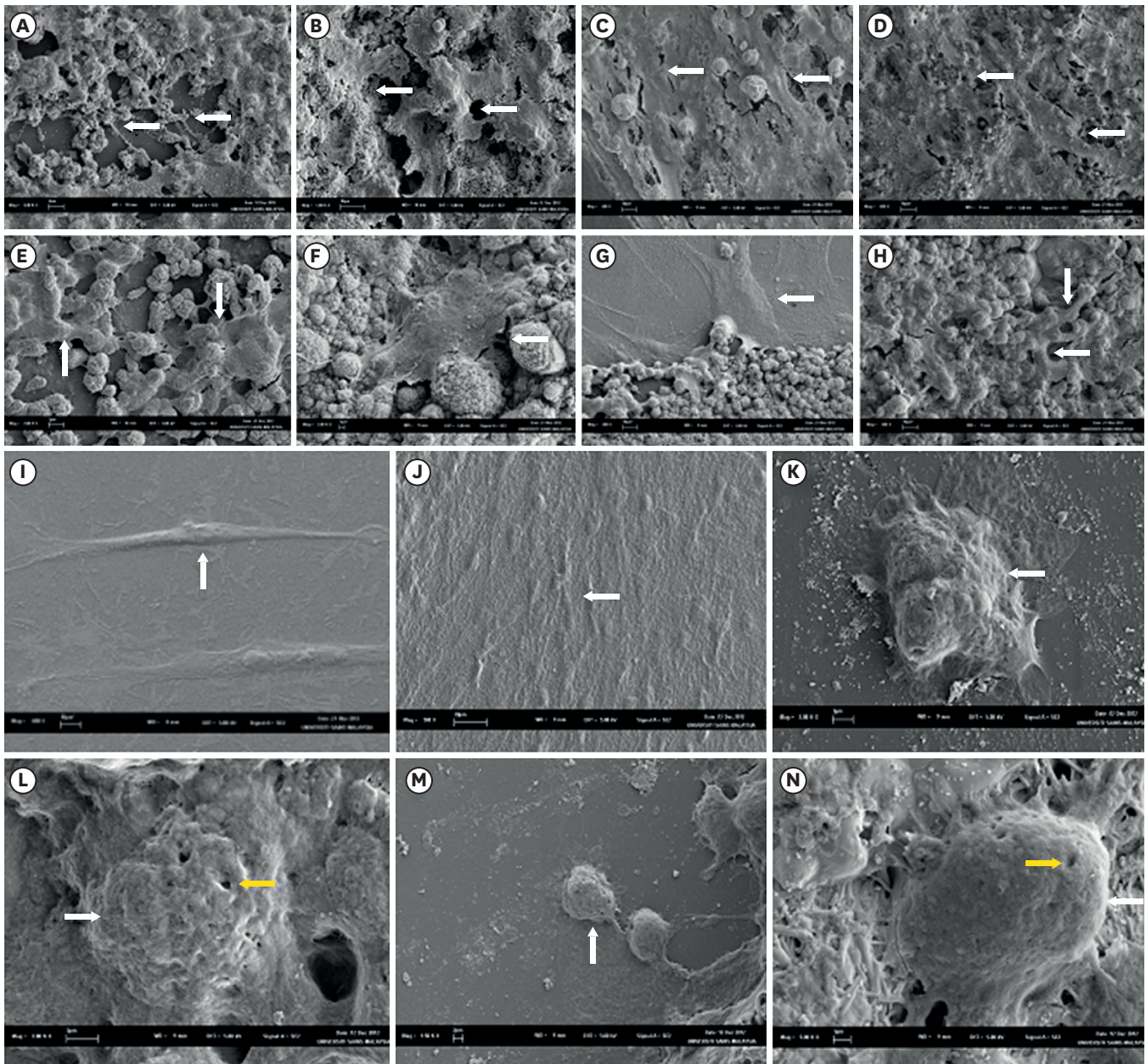


Figure 4. Cell attachment properties of WMTA and FS WMTA (white arrows). (A) WMTA at the interface (24 hours); (B) WMTA on the top (24 hours); (C) WMTA at the interface (72 hours); (D) WMTA on the top (72 hours); (E) FS WMTA at the interface (24 hours); (F) FS WMTA on the top (24 hours); (G) FS WMTA at the interface (72 hours); (H) FS WMTA on the top (72 hours); (I) Acrylic mold (control, 24 hours); (J) Acrylic mold (control, 72 hours); (K) Amalgam at the interface (control, 24 hours); (L) Amalgam on the top (control, 24 hours); (M) Amalgam at the interface (control, 72 hours); (N) Amalgam on the top (control, 72 hours). Yellow arrows indicate surface perforations. WMTA, white mineral trioxide aggregate; FS WMTA, fast-set white mineral trioxide aggregate.

XRD is an analytical technique used to study cements [40]; it enables identification of the major crystalline products in a cement sample. XRD analysis showed that WMTA primarily comprises tricalcium silicate, CaCO_3 , and Bi_2O_3 , similar to findings in previous studies [35,36,41]. Addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA resulted in formation of a number of components, including $\text{Ca}_2\text{SiO}_3\text{Cl}_2$. To the best of our knowledge, the available information on $\text{Ca}_2\text{SiO}_3\text{Cl}_2$ is limited [42], and the formation of a chloride complex during the hydration

reaction of WMTA requires further investigation. Based on the results and discussion above, the research hypotheses with regards to surface morphology and chemical composition can be accepted.

In vitro cytotoxicity tests are essential stages of the biocompatibility screening process [43]. The decision to use a given protocol for cytotoxicity evaluations must be based on its consonance with the chemical nature of the material being tested. Because MTA is a hydrophilic substance likely to release ionic components, it is apt to interfere with intracellular enzyme activities [44]. Therefore, MTT assay was selected for this study. The extraction dilution method was selected in this study to examine the cytotoxic effects of leachable elements from study samples on cells that are both distant to and in close contact with the cement [44]. This method can also simulate the clinical scenario where toxic elements of the materials leach into the surrounding fluids and bony crypt.

During extraction, the color of the samples notably changed from white to black after incubation in the culture medium of HPLFs. This finding has previously been reported in the literature [45-47], and may be attributed to the presence of Bi in WMTA. HPLFs showed higher cell viability values after exposure to WMTA than FS WMTA, although the cytotoxic profiles of both formulations appear favorable. This observation is consistent with 2 studies [17,21].

Cell adhesion must occur before cells can proliferate, differentiate, and produce an extracellular mineralized matrix on a substrate [48]. The quality and quantity of cell attachment onto retrograde filling materials is generally agreed to be a valid criterion for evaluations of the biocompatibility of dental bio-materials [49,50]. Despite differences in methodologies, the favorable attachment properties of WMTA to HPLFs observed in this work are consistent with others [43,51,52].

Few studies have examined the cell attachment properties of WMTA mixed with CaCl_2 . One investigation compared 3 combinations of FS WMTA including CaCl_2 [22]. SEM observations of MG-63 osteosarcoma cells demonstrated unfavorable attachment to the surfaces of WMTA mixed with CaCl_2 . Despite this unfavorable biological response, stating that this combination is not suitable for clinical application may be inappropriate because the cell types that would come into contact with this combination in the clinical setting may react differently [4], which has been confirmed in the current study. A recent study showed favorable cell attachment properties of this FS formulation on dental pulp stem cells isolated from teeth extracted from young adult patients [17].

Based on the results, the research hypotheses with regards to biological properties (cytotoxicity and cell attachment properties) can be partially accepted. The favorable sensitivity of HPLFs towards FS WMTA may have an impact on its clinical application for repair of perforation defects. Future *in vivo* studies are warranted to validate the findings demonstrated in this experimental investigation.

CONCLUSIONS

The addition of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to WMTA affected its chemical properties. The favorable biological profile of FS WMTA towards HPLFs may have a potential impact on its clinical application for repair of perforation defects.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Mrs. Ee Bee Choo, Mr. Hazhar Hassan, and Mr. Yushamdan Yusof (Nor lab, School of Physics), Ms. Jamilah Afandi and Mr. Johary Othman (Microscopy unit, School of Biological Sciences), Mr. Nik Fakuruddin (SEM unit, School of Health Sciences), and members of the craniofacial laboratory (School of Dental Sciences, Universiti Sains Malaysia) for their technical support.

REFERENCES

1. 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;19:591-595.
[PUBMED](#) | [CROSSREF](#)
2. Al-Rabeah E, Perinpanayagam H, MacFarland D. Human alveolar bone cells interact with ProRoot and tooth-colored MTA. *J Endod* 2006;32:872-875.
[PUBMED](#) | [CROSSREF](#)
3. Nair PN, Duncan HF, Pitt Ford TR, Luder HU. Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. *Int Endod J* 2008;41:128-150.
[PUBMED](#)
4. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations. *J Endod* 2010;36:190-202.
[PUBMED](#) | [CROSSREF](#)
5. Hakki SS, Bozkurt SB, Ozcopur B, Purali N, Belli S. Periodontal ligament fibroblast response to root perforations restored with different materials: a laboratory study. *Int Endod J* 2012;45:240-248.
[PUBMED](#) | [CROSSREF](#)
6. Wiltbank KB, Schwartz SA, Schindler WG. Effect of selected accelerants on the physical properties of mineral trioxide aggregate and Portland cement. *J Endod* 2007;33:1235-1238.
[PUBMED](#) | [CROSSREF](#)
7. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part III: clinical applications, drawbacks, and mechanism of action. *J Endod* 2010;36:400-413.
[PUBMED](#) | [CROSSREF](#)
8. Lee ES. A new mineral trioxide aggregate root-end filling technique. *J Endod* 2000;26:764-765.
[PUBMED](#) | [CROSSREF](#)
9. Kogan P, He J, Glickman GN, Watanabe I. The effects of various additives on setting properties of MTA. *J Endod* 2006;32:569-572.
[PUBMED](#) | [CROSSREF](#)
10. Huang TH, Shie MY, Kao CT, Ding SJ. The effect of setting accelerator on properties of mineral trioxide aggregate. *J Endod* 2008;34:590-593.
[PUBMED](#) | [CROSSREF](#)
11. Lee BN, Hwang YC, Jang JH, Chang HS, Hwang IN, Yang SY, Park YJ, Son HH, Oh WM. Improvement of the properties of mineral trioxide aggregate by mixing with hydration accelerators. *J Endod* 2011;37:1433-1436.
[PUBMED](#) | [CROSSREF](#)
12. Ahmed HMA, Saini R, Rahman IA, Saini D. Effect of bee products on the setting properties of mineral trioxide aggregate mixed with calcium chloride dihydrate. A preliminary study. *J ApiProduct ApiMedical Sci* 2011;3:123-127.
[CROSSREF](#)
13. Ber BS, Hatton JF, Stewart GP. Chemical modification of proroot mta to improve handling characteristics and decrease setting time. *J Endod* 2007;33:1231-1234.
[PUBMED](#) | [CROSSREF](#)
14. Lee BN, Kim HJ, Chang HS, Hwang IN, Oh WM, Kim JW, Koh JT, Min KS, Choi CH, Hwang YC. Effects of mineral trioxide aggregate mixed with hydration accelerators on osteoblastic differentiation. *J Endod* 2014;40:2019-2023.
[PUBMED](#) | [CROSSREF](#)
15. Zapf AM, Chedella SC, Berzins DW. Effect of additives on mineral trioxide aggregate setting reaction product formation. *J Endod* 2015;41:88-91.
[PUBMED](#) | [CROSSREF](#)

16. Prasad A, Pushpa S, Arunagiri D, Sawhny A, Misra A, Sujatha R. A comparative evaluation of the effect of various additives on selected physical properties of white mineral trioxide aggregate. *J Conserv Dent* 2015;18:237-241.
[PUBMED](#) | [CROSSREF](#)
17. Kulan P, Karabiyik O, Kose GT, Kargul B. Biocompatibility of accelerated mineral trioxide aggregate on stem cells derived from human dental pulp. *J Endod* 2016;42:276-279.
[PUBMED](#) | [CROSSREF](#)
18. Antunes Bortoluzzi E, Juárez Broon N, Antonio Hungaro Duarte M, de Oliveira Demarchi AC, Monteiro Bramante C. The use of a setting accelerator and its effect on pH and calcium ion release of mineral trioxide aggregate and white Portland cement. *J Endod* 2006;32:1194-1197.
[PUBMED](#) | [CROSSREF](#)
19. Bortoluzzi EA, Broon NJ, Bramante CM, Felipe WT, Tanomaru Filho M, Esberard RM. The influence of calcium chloride on the setting time, solubility, disintegration, and pH of mineral trioxide aggregate and white Portland cement with a radiopacifier. *J Endod* 2009;35:550-554.
[PUBMED](#) | [CROSSREF](#)
20. Bortoluzzi EA, Broon NJ, Bramante CM, Consolaro A, Garcia RB, de Moraes IG, Bernadineli N. Mineral trioxide aggregate with or without calcium chloride in pulpotomy. *J Endod* 2008;34:172-175.
[PUBMED](#) | [CROSSREF](#)
21. Jafarnia B, Jiang J, He J, Wang YH, Safavi KE, Zhu Q. Evaluation of cytotoxicity of MTA employing various additives. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:739-744.
[PUBMED](#) | [CROSSREF](#)
22. Kang JY, Lee BN, Son HJ, Koh JT, Kang SS, Son HH, Chang HS, Hwang IN, Hwang YC, Oh WM. Biocompatibility of mineral trioxide aggregate mixed with hydration accelerators. *J Endod* 2013;39:497-500.
[PUBMED](#) | [CROSSREF](#)
23. Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A. Chemical analysis and biological properties of two different formulations of white Portland cements. *Scanning* 2016;38:303-316.
[PUBMED](#) | [CROSSREF](#)
24. Ong RM, Luddin N, Ahmed HMA, Omar NS. Cytotoxicity of accelerated white MTA and Malaysian white Portland cement on stem cells from human exfoliated deciduous teeth (SHED): an *in vitro* study. *Singapore Dent J* 2012;33:19-23.
[PUBMED](#) | [CROSSREF](#)
25. Camilleri J. Characterization and chemical activity of Portland cement and two experimental cements with potential for use in dentistry. *Int Endod J* 2008;41:791-799.
[PUBMED](#) | [CROSSREF](#)
26. Hwang YC, Lee SH, Hwang IN, Kang IC, Kim MS, Kim SH, Son HH, Oh WM. Chemical composition, radiopacity, and biocompatibility of Portland cement with bismuth oxide. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:e96-e102.
[PUBMED](#) | [CROSSREF](#)
27. Ahmed HMA, Omar NS, Luddin N, Saini R, Saini D. Cytotoxicity evaluation of a new fast set highly viscous conventional glass ionomer cement with L929 fibroblast cell line. *J Conserv Dent* 2011;14:406-408.
[PUBMED](#) | [CROSSREF](#)
28. Zhang W, Li Z, Peng B. *Ex vivo* cytotoxicity of a new calcium silicate-based canal filling material. *Int Endod J* 2010;43:769-774.
[PUBMED](#) | [CROSSREF](#)
29. Ahmed HMA, Luddin N, Kannan TP, Mokhtar KI, Ahmad A. Cell attachment properties of Portland cement-based endodontic materials: biological and methodological considerations. *J Endod* 2014;40:1517-1523.
[PUBMED](#) | [CROSSREF](#)
30. Traetteberg A, Ramachandran VS, Grattan-Bellew PE. A study of the microstructure and hydration characteristics of tricalcium silicate in the presence of calcium chloride. *Cem Concr Res* 1974;4:203-221.
[CROSSREF](#)
31. Wang X, Sun H, Chang J. Characterization of Ca₃SiO₅/CaCl₂ composite cement for dental application. *Dent Mater* 2008;24:74-82.
[PUBMED](#) | [CROSSREF](#)
32. Oliveira MG, Xavier CB, Demarco FF, Pinheiro AL, Costa AT, Pozza DH. Comparative chemical study of MTA and Portland cements. *Braz Dent J* 2007;18:3-7.
[PUBMED](#) | [CROSSREF](#)
33. Asgary S, Parirokh M, Eghbal MJ, Brink F. A comparative study of white mineral trioxide aggregate and white Portland cements using X-ray microanalysis. *Aust Endod J* 2004;30:89-92.
[PUBMED](#) | [CROSSREF](#)

34. Dammaschke T, Gerth HU, Züchner H, Schäfer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. *Dent Mater* 2005;21:731-738.
[PUBMED](#) | [CROSSREF](#)
35. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. The constitution of mineral trioxide aggregate. *Dent Mater* 2005;21:297-303.
[PUBMED](#) | [CROSSREF](#)
36. Song JS, Mante FK, Romanow WJ, Kim S. Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102:809-815.
[PUBMED](#) | [CROSSREF](#)
37. Belío-Reyes IA, Bucio L, Cruz-Chavez E. Phase composition of ProRoot mineral trioxide aggregate by X-ray powder diffraction. *J Endod* 2009;35:875-878.
[PUBMED](#) | [CROSSREF](#)
38. Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Simon JH, Rotstein I. MTA preparations from different origins may vary in their antimicrobial activity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:e85-e88.
[PUBMED](#) | [CROSSREF](#)
39. Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *Int Endod J* 2005;38:834-842.
[PUBMED](#) | [CROSSREF](#)
40. Islam I, Chng HK, Yap AU. X-ray diffraction analysis of mineral trioxide aggregate and Portland cement. *Int Endod J* 2006;39:220-225.
[PUBMED](#) | [CROSSREF](#)
41. Park JW, Hong SH, Kim JH, Lee SJ, Shin SJ. X-Ray diffraction analysis of white ProRoot MTA and Diadent BioAggregate. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:155-158.
[PUBMED](#) | [CROSSREF](#)
42. Taylor HF. *Cement chemistry*. 2nd ed. London: Thomas Telford Ltd.; 1997. p321-322.
43. Al-Hiyasat AS, Al-Sa'Eed OR, Darmani H. Quality of cellular attachment to various root-end filling materials. *J Appl Oral Sci* 2012;20:82-88.
[PUBMED](#) | [CROSSREF](#)
44. Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. *J Endod* 2000;26:288-291.
[PUBMED](#) | [CROSSREF](#)
45. Watts JD, Holt DM, Beeson TJ, Kirkpatrick TC, Rutledge RE. Effects of pH and mixing agents on the temporal setting of tooth-colored and gray mineral trioxide aggregate. *J Endod* 2007;33:970-973.
[PUBMED](#) | [CROSSREF](#)
46. Boutsioukis C, Noula G, Lambrianidis T. *Ex vivo* study of the efficiency of two techniques for the removal of mineral trioxide aggregate used as a root canal filling material. *J Endod* 2008;34:1239-1242.
[PUBMED](#) | [CROSSREF](#)
47. Belobrov I, Parashos P. Treatment of tooth discoloration after the use of white mineral trioxide aggregate. *J Endod* 2011;37:1017-1020.
[PUBMED](#) | [CROSSREF](#)
48. Thomson TS, Berry JE, Somerman MJ, Kirkwood KL. Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. *J Endod* 2003;29:407-412.
[PUBMED](#) | [CROSSREF](#)
49. Zhu Q, Haglund R, Safavi KE, Spangberg LS. Adhesion of human osteoblasts on root-end filling materials. *J Endod* 2000;26:404-406.
[PUBMED](#) | [CROSSREF](#)
50. Ma J, Shen Y, Stojicic S, Haapasalo M. Biocompatibility of two novel root repair materials. *J Endod* 2011;37:793-798.
[PUBMED](#) | [CROSSREF](#)
51. Asgari S, Moosavi SH, Yadegari Z, Shahriari S. Cytotoxic effect of MTA and CEM cement in human gingival fibroblast cells. Scanning electronic microscope evaluation. *N Y State Dent J* 2012;78:51-54.
[PUBMED](#)
52. Trichaiyapon V, Torrungruang K, Panitvisai P. Cytotoxicity of flowable resin composite on cultured human periodontal ligament cells compared with mineral trioxide aggregate. *J Investig Clin Dent* 2012;3:215-220.
[PUBMED](#) | [CROSSREF](#)