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Original Article

Analysis of pH and Cytotoxic Activity of Locally Produced Radiopaque White Portland Cement

Soh Chin Fen[1], Loh Wei Lek[1], Thirumulu Ponnuraj Kannan[1,2], Siti Fadilah Bin Abdullah[1], Adam Husein[1], Mohamed Shady Nabhan[3], Hany Mohamed Aly Ahmed[4]

[1]School of Dental Sciences, Universiti Sains Malaysia, [2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia, [3]Department of Prosthetic Dentistry, Faculty of Dentistry, Ain Shams University, Cairo, Egypt, [4]Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract

Background: Portland cement (PC)-based formulations show continuous developments. **Purpose:** This study examined the pH and cytotoxic activity of a locally produced Malaysian white PC (MAWPC) mixed with different radiopacifying agents (barium sulfate [BS], niobium oxide [NO], and bismuth oxide [BO]) on human periodontal ligament fibroblasts (HPLFs). **Materials and Methods:** 0.8 g of MAWPC was mixed with 0.2 g of each radiopacifying agent and sterile distilled water. Five tablets of each group were prepared. After setting, the samples were immersed in 10-ml sterile distilled water and stored at 37°C, and the pH was measured at intervals of 0, 1, 3, 7, and 14 days using a calibrated digital pH meter. One-way ANOVA was used for data analysis ($P = 0.05$). For cytotoxic activity, the material extracts were prepared at three serial concentrations (25, 12.5, and 6.25 mg/ml), and 200 ml of each concentration was added into each well seeded with cultured HPLFs. The plates were then incubated for 48 h. The cell viability was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and the data were analyzed using Kruskal–Wallis test ($P = 0.05$). **Results:** The pH values of all groups were significantly higher compared to the control group ($P < 0.001$). With the exception of day 0, the pH values of all groups at all day intervals ranged from 9.9 to 10.9, and some significant differences were detected. Although the addition of radiopacifying agents decreased the cell viability values of MAWPC extracts ($P < 0.05$), all groups showed favorable cytotoxicity profile. MAWPC/BO combination showed higher cell viability values compared to MAWPC/NO and MAWPC/BS. **Conclusions:** The addition of radiopacifying agents to MAWPC maintained its high pH and favored the viability of HPLFs.

Keywords: Barium sulfate, bismuth oxide, cell viability, cytotoxicity, human periodontal ligament fibroblasts, niobium oxide, pH value, Portland cement

Introduction

An ideal endodontic filling material should maintain a hermetic seal in the pathway of communication between the root canal and its surrounding tissues.[1] It should be biocompatible, dimensionally stable, exhibit favorable host tissue response, insoluble in tissue fluids, nontoxic, noncarcinogenic, and radiopaque.[2]

Mineral trioxide aggregate (MTA) is an endodontic material first introduced as a root-end filling material in 1993.[3] It is composed of tricalcium silicate, tricalcium oxide, silicate oxide, and other mineral oxides. MTA exhibits many advantageous properties including optimum biocompatibility, good sealing ability, and favorable hard-tissue induction[4,5] which paved the way for its use in pulp capping, root-end filling, repairing furcal perforations, and resorption defects.[6,7] Despite favorable properties, white MTA (WMTA) has extended setting time, difficult handling properties, and discoloration potential and it is an expensive material.[8]

Bismuth oxide (BO) is the radiopacifying agent in WMTA, and studies showed that BO negatively affects the physiochemical and biological properties, can interact with collagen in hard tissue, causes coronal discoloration, and can react with sodium hypochlorite.[9,10]

WMTA is a Portland cement (PC)-based material, and therefore, white PC (WPC) was suggested as a viable substitute for

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Address for correspondence: Dr. Hany Mohamed Aly Ahmed, Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail: hany_endodontist@hotmail.com

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WMTA. It is inexpensive and exhibits many common chemical and physical properties. Both are composed of calcium phosphate, calcium, and silicon oxide. Because these materials exhibit compatibility among their compounds, the possibility of clinical use of PC has been considered as an alternative to MTA.

Pure WPC is not radiopaque enough to be distinguished from tooth and bone.[11] Accordingly, a number of radiopacifying agents, such as BO, zirconium oxide, and barium sulfate (BS), have been introduced as potential additives to WPC. One study showed that the addition of BO to WPC significantly reduces the cell viability during early evaluation time.[12] BS is commonly used as a radiopaque agent in medical biomaterials and endodontics. It is added to intracanal medicaments such as calcium hydroxide and showed no detrimental effects.[13] Niobium oxide (NO) is used to increase the radiopacity of methacrylate-based root canal sealers.[14] Its oxidized form exhibited good biocompatibility when it was used to cover dental implants.[15]

Malaysian WPC (MAWPC) is a locally produced WPC characterized by its whiteness, uniform composition, and performance.[16] It is much cheaper than MTA and is more available. Recently, MAWPC has been a subject to research studies and was found to be a potential substitute to WMTA.[17,18] However, the study of radiopacifying agents as potential additives to MAWPC is essential. The aim of this study was to examine the pH and cytotoxic effects of MAWPC mixed with BS, NO, and BO radiopacifying agents.

Materials and Methods

Material preparation

MAWPC (Aalborg, Malaysia) powder material was passed through a sieve of 45 μm (Retsch, Germany, ISO 3310-1) to ensure a homogenous fineness of the material. Table 1 shows the list of groups examined in this study and proportioning of WAWPC with the radiopacifying agents and sterile distilled water.

Examination of pH values

Five sample tablets (2 mm thickness × 10 mm diameter) were prepared for each group. Sterile distilled water was used as a control. All samples were incubated for 24 h at 37°C to set. After setting, each sample was immersed in 10-ml sterile distilled water in a 15-mm centrifugation tube and stored at 37°C throughout experiment. At each time interval (0, 1, 3, 7, and 14 days), the pH values were measured immediately using a calibrated digital pH meter (Hanna, Hanna Instruments, RI, USA). The solution from each tube was then replaced with 10-ml sterile distilled water for the next time interval. Data entry was performed in SPSS (the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 22). One-way ANOVA was used for statistical analysis, and P was set at 0.05.

Table 1: Proportioning of the test groups
(The amount of liquid for each group was optimized based on the best consistency for each group)

Test materials	Proportioning
Pure MAWPC	1 g of 45-μm sieved MAWPC (MAWPC, Aalborg, Malaysia) + 350-μl sterile distilled water
MAWPC + BS	0.8 g of 45-μm sieved MAWPC (MAWPC, Aalborg, Malaysia) + 0.2 g BS + 450-μl sterile distilled water
MAWPC + NO	0.8 g of 45-μm sieved MAWPC (MAWPC, Aalborg, Malaysia) + 0.2 g NO + 350-μl sterile distilled water
MAWPC + BO	0.8 g of 45-μm sieved MAWPC (MAWPC, Aalborg, Malaysia) + 0.2 g BO + 350-μl sterile distilled water

MAWPC – Malaysian white Portland cement, BO – Bismuth oxide, NO – Niobium oxide, BS – Barium sulfate

Cytotoxicity evaluation

Cell culture

All experimental procedures were carried out in a purifier Class II biological safety cabinet under aseptic condition. All surfaces will be disinfected with 70% prepared ethanol spray, before and after use. After preparation and setting of materials, the samples were retrieved and sterilized using ultraviolet (UV) radiation.

As recommended by the manufacturer, the complete growth medium for human periodontal ligament fibroblast (HPLF) cell line (Lonza, USA) was prepared by supplementing 500 ml of stromal cell growth medium (Lonza, USA), with fetal bovine serum (Lonza, USA), human fibroblast growth factor-B (Lonza, USA), human recombinant insulin (Lonza, USA), and gentamicin sulfate (Lonza, USA).

Preparation of the material extracts

After mixing, placement in acrylic molds, and setting, the study samples were retrieved, weighed, and sterilized using UV light. After that, the samples were introduced into sterile 15-ml centrifugation tubes. Prepared culture media were then added into each tube at the determined maximum concentrations. The immersed samples were incubated for 7 days at 37°C.

Application of material extracts

HPLFs were cultured, and 1 day before starting the experiment (day 6 of extracts incubation), HPLFs were harvested and counted. One set of sterile 96-well plate (Thermo Scientific Nunc, Denmark) was retrieved at 48 h time interval. About 100 μl of prepared media having 5×10^3 cells was added into each well. Six replicates were prepared for each concentration (each group has three serial concentrations, i.e., 25, 12.5, 6.25 mg/ml) and then incubated at 37°C and 5% CO_2 for 48 h. The experiment was performed two times.

On day 7, the extracts were transferred through a sterile, nonpyrogenic needle (Gauge 21, Terumo Corporation, Japan) fitted in a 10-ml nonpyrogenic syringe (B-D, Singapore) into another sterile centrifugation tube after passing through a sterile 0.2-μm filter (Pall, USA). The extracts were then prepared at three serial dilutions (25, 12.5, 6.25 mg/ml). The media in the seeded 96-well plate were then replaced by the material extracts. The last row served as the control. The plate was then incubated at 37ºC and 5% CO_2 for 48 h.

Cytotoxicity examination

30 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide solution at a concentration of 5 mg/ml was added into each well, and each plate was then incubated for 3–4 h. After that, all the contents of each well was replaced by 100 µl of dimethyl sulfoxide. The optical density (OD) of the solution was measured using an enzyme-linked immunosorbent assay reader (Sunrise, Tecan, Austria) at a test wavelength of 570 nm (reference wavelength was 600 nm). Control cells without material extracts served as cell viability of 100%, and the cell viability values were then calculated using the following formula: Cell viability (%) = [A − B]/[C − B] × 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 cytotoxic profile of the materials was classified according to Zhang *et al*.[19] (>90%: noncytotoxic, 60%–90%: slight, 30%–60%: moderate, and 0%–30%: severe cytotoxicity).

Kruskal–Wallis test followed by pairwise comparisons using Mann–Whitney tests (Bonferroni correction) was used to analyze the data obtained from the cytotoxicity test on HPLFs. The statistical level of significance was set at 0.05 ($P < 0.05$).

Results

Analysis of pH values

The pH values of all groups were significantly higher compared to the control group ($P < 0.001$). With the exception of day 0, the pH values of all groups at all day intervals ranged from 9.9 to 10.9, and some significant differences were detected. Table 2 shows the details of the demonstrated in this study.

Analysis of cytotoxicity values

Results showed that the cell viability values of MAPWC extracts on HPLFs were the highest among all groups, and the difference was statistically significant ($P < 0.05$), though the cytotoxicity profile for all groups is favorable. Figure 1 shows the cell viability values among MAWPC and MAWPC combined with various radiopacifying agents (NO, BS, and BO) on HPLFs. At 25 and 12.5 mg/ml concentration, all groups showed favorable cytotoxicity profile. At 6.25 mg/ml concentration, all groups showed slight cytotoxicity on HPLFs except for MAWPC which showed no cytotoxicity on HPLFs.

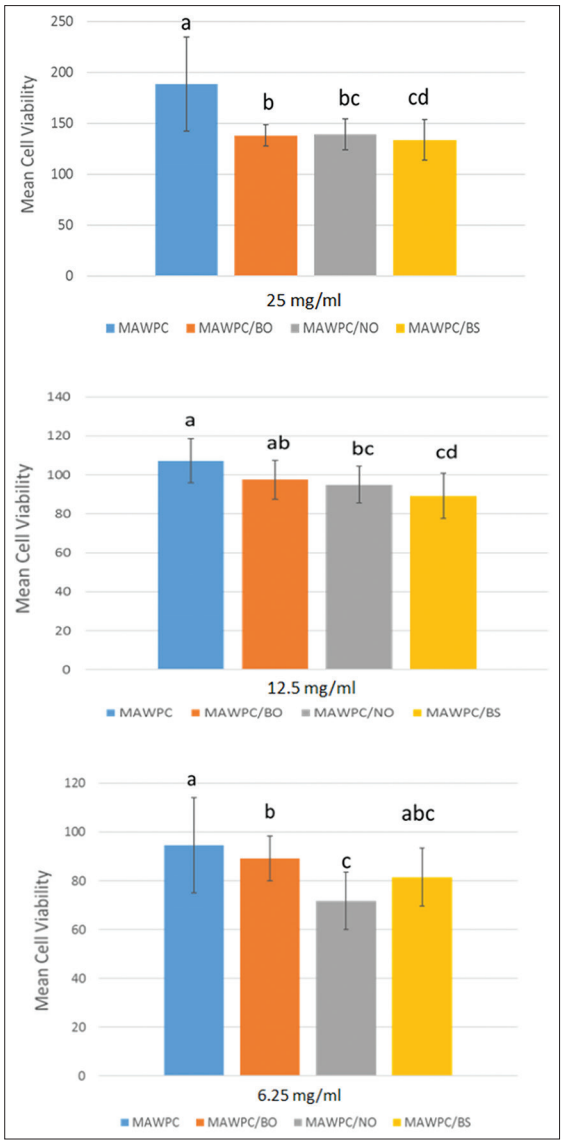

Figure 1: Analysis of cell viability values among MAWPC, MAWPC with NO, WPC with BS, and MAWPC with BO on HPLFs using Kruskal–Wallis test. Different letters: Statistically significant ($P \leq 0.05$). MAWPC: Malaysian white Portland Cement, NO: Niobium oxide, BS: Barium sulfate, BO: Bismuth oxide, HPLFs: Human periodontal ligament fibroblasts

Discussion

The ideal radiopacifying material should provide the necessary radiopacity to the cement while maintaining the favorable properties. It should be inert, free from any contaminants, nontoxic and be added in minimal amounts.[20] Together with the fact that PC of different origins has different chemical and biological properties,[17,18] this study investigated the pH and cytotoxic effects of a locally produced WPC (MAWPC) mixed with BS, NO, and BO radiopacifying agents.

The addition of radiopacifying agents to PC has been a subject of research investigations.[20-22] Studies have been performed to examine the effect of such radiopaque combinations on the physical and biological properties of PC. The high pH value of PC-based materials is an important property that plays a critical role in its biological profile, antimicrobial properties, and others.[20-22] Investigators[20] found that the addition of a number of radiopacifying agents (including BS) to PC originated from Denmark resulted in an alkaline pH at day 1 up to day 28. Another two studies found that the addition of BO, zirconium oxide, and other radiopacifying agents of different particle sizes did not affect the pH of PC originated from Brazil.[21,22] These findings are in accordance to the current study. Notably, the pH value at day 0 (immediate test) ranged from 6.1 to 7.2, which indicates that cement is able to obtain a high pH after some time. This finding is in accordance to one recent study performed on a novel root-end filling material.[23]

Table 2: Mean and standard deviation of the pH values of the test and control groups

	Day 0		Day 1		Day 3		Day 7		Day 14		*P*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
MAWPC	7.3^{A}	0.2	10.4^{A}	0.0	$10.8^{a,A,B}$	0.1	$10.9^{a,A}$	0.1	$10.8^{a,A}$	0.2	<0.001
MAWPC + NO	7.0	0.1	9.9	0.0	$10.6^{a,A}$	0.2	10.9^{A}	0.0	10.5^{a}	0.1	<0.001
MAWPC + BO	6.4	0.1	10.3^{A}	0.1	$10.8^{a,b,B}$	0.0	$10.9^{a,A}$	0.1	$10.7^{b,A}$	0.0	<0.001
MAWPC + BS	6.1	0.0	10.1	0.1	$10.8^{a,A,B}$	0.1	$10.9^{b,A}$	0.1	$10.8^{a,b,A}$	0.0	<0.001
Control	7.2^{A}	0.1	7.1^{a}	0.1	7.0^{a}	0.0	7.0^{a}	0.1	7.0^{a}	0.0	0.001
P	<0.001		<0.001		<0.001		<0.001		<0.001		

One-way ANOVA was used for statistical analysis. Similar small superscript letters indicate no significant difference between follow-up periods within same group. Similar capital superscript letters indicate no significant difference between groups within same follow-up period. SD – Standard deviation, MAWPC: Malaysian white Portland cement, NO: Niobium oxide, BS: Barium sulfate, BO: Bismuth oxide

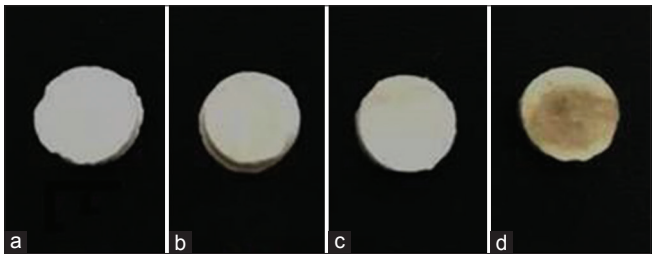

Figure 2: Left to right: (a) MAWPC, (b) MAWPC/BS, (c) MAWPC/NO, and (d) MAWPC/BO. MAWPC/BO showed grayish discoloration. MAWPC: Malaysian white Portland cement, NO: Niobium oxide, BS: Barium sulfate, BO: Bismuth oxide

but in contrast to another study.[24] which could be attributed to different materials used and methodological procedures for measuring the pH values.

Biological testing of endodontic biomaterials on dental cell lines is at the forefront of endodontic research. HPLFs were selected in this study because they are in close relation with cement in contact with the periapical tissue.[25] Different concentrations of the extracts were used to represent the clinical situation that biomaterials are diluted by tissue fluids and in turn examining the biological responses of cells near and far from the material.[25]

In this study, MAWPC/BO showed the highest cell viability values among all three radiopacifying agents. Favorable biological properties have been reported with this combination.[26] However, discoloration of MAWPC/BO samples was observed [Figure 2]. The discoloration of PC-based materials mixed with BO (such as MTA) has been documented in the literature.[27] As such, other radiopacifying agents have been introduced as an alternative to WPC/BO combinations, which can possibly be used when the esthetics is not of a concern.

BS is used routinely in gastroenterology as a contrast medium. The incorporation of BS in PC has been examined which usually has a limited effect on the hydration chemistry and mechanisms of the cement because the bond between barium and sulfate is strong.[28] In addition, BS is insoluble in acids and water, and thus, it is considered to be chemically inert.[29] One study showed favorable biological properties when PC-based material was mixed with barium chloride,[30] which is consistent to results of the current study.

NO has been indicated for the treatment of implant surfaces, due to its capacity to favor the osseointegration.[31] The use of NO as a radiopacifying agent of dental materials has also been studied.[22,32] One study found that PC mixed with NO micro- and nanoparticles can obtain acceptable radiopacity, maintain alkaline pH, and exert antimicrobial activity.[22] Another study compared the cytotoxic effects of PC/NO combination with that of MTA (Angelus) on osteoblast cell line.[32] Results showed favorable cytotoxic effects of PC/NO combinations, similar to findings reported in this study.

Despite favorable results of the current *in vitro* study, it only examines the pH and cytotoxic effects on one dental cell line. Future studies are necessary to evaluate other properties such as mechanical properties and biological profile including cell attachment properties and dentinogenic differentiation potential on different cell lines.

Conclusions

The addition of radiopacifying agents to MAWPC maintained its high pH and favored the viability of HPLFs. Future studies are warranted to substantiate results demonstrated in this study.

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Conflicts of interest

There are no conflicts of interest.

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