



Evaluation of the properties of Mineral Trioxide Aggregate mixed with Zinc Oxide exposed to different environmental conditions

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ABSTRACT

Addition of zinc oxide (ZnO) to Mineral Trioxide Aggregate (MTA) has been shown to rectify tooth discoloration caused by Angelus MTA. This study evaluated the microhardness, compressive strength, calcium ion release and crystalline structures of MTA mixed with ZnO in different environmental conditions. Molds with a diameter of 4 mm and a height of 6 mm were used for compressive strength, calcium ion release and X-ray diffraction (XRD) evaluations. Molds with 6 mm diameter and 4 mm height were used for surface microhardness evaluations. Cements evaluated include Angelus MTA (Angelus, Brail), Angelus MTA + ZnO, ProRoot MTA (Dentsply Tulsa Dental, OK), and ProRoot MTA + ZnO. Each group was divided into 3 subgroups according to exposure conditions: normal saline (NS), phosphate buffered saline (PBS) or blood. After 7 days incubation, surface microhardness, compressive strength and XRD analysis was performed. Calcium ion release was evaluated after 3, 24 and 168h incubation using atomic absorption spectrophotometry. Data were analyzed by One Way Anova followed by the Tukey HSD Post hoc tests and T-Test. The significance level was set at 0.05. Addition of ZnO to Angelus and ProRoot MTA significantly decreased the compressive strength of these cements regardless of the environmental conditions ($P < 0.001$); however, it had no significant effect on their microhardness or calcium ion release. In conclusion, adding ZnO to Angelus and ProRoot MTA can adversely affect the compressive strength of Angelus and ProRoot MTA.

1. Introduction

Tooth discoloration subsequent to regenerative endodontic treatments (RET) is considered as an unfavorable outcome [1–3]. Many materials used in the RET procedure may contribute to tooth discoloration including the use of triple antibiotic paste and calcium-silicate based cements such as Mineral Trioxide Aggregate (MTA) [1,4,5].

In the case of MTA, the contact of bismuth oxide which is the radiopacifying agent in this cement, with strong oxidizing agents, i.e., sodium hypochlorite or amino acids present in dentin collagen, attributes to destabilization of bismuth oxide and therefore, tooth discoloration [6,7]. This tooth discoloration is also seen when this cement is used for other treatment modalities such as vital pulp therapy [8], sealing root canal perforations and in root resorptions [9,10].

Several methods have been proposed to prevent tooth discoloration due to the application of calcium-silicate cements including the use of calcium-silicate cements containing alternative radiopacifying agent

(i.e. zirconium oxide or calcium tungstate) [11–13]. However, this leads to reduction of material radiopacity. As increasing the concentration of these radiopacifying agents may resolve this problem, it has detrimental effects on physical and chemical properties of these cements [14,15].

Marciano et al., [16] suggested adding ZnO to the composition of Angelus MTA for preventing tooth discoloration. They showed that addition of ZnO inhibited the destabilization of bismuth oxide upon contact with oxidizing agents and subsequently prevented tooth discoloration. They added different percentages of ZnO to Angelus MTA (5%, 15% and 45%) and evaluated the physical, chemical and biological properties of the mixture. According to the findings of their study, upon addition of 5% ZnO, the properties of Angelus MTA such as radiopacity, setting time, volume changes and biocompatibility were similar to the original cement and the calcium ion release was significantly higher.

The physical and chemical properties of materials used in

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endodontics have a major influence on clinical outcomes [17,18]. Complete hydration and maturation of MTA is necessary for this cement to acquire all its favorable properties. During the hydration of MTA, calcium hydroxide (CH) is produced in two forms of crystalline and soluble (i.e. Ca^{2+} and OH^-). While the formation of OH^- increases pH, activates alkaline phosphatase and thus favors mineralization [19], formation of Ca^{2+} induces the expression of osteopontin and bone morphogenetic protein [20]. Furthermore, when MTA is in contact with phosphate-containing physiological fluids, increase in the levels of Ca^{2+} results in the precipitation of apatite-like structures [21,22]. Apatite-like precipitate formation is considered as the basis for the favorable biological properties of MTA and contributes to sealing and integration between MTA and hard tissues [21,22] as well as occlusion of dentinal tubules and biomineralization [23,24].

The microhardness and compressive strength of calcium-silicate cements (CSCs) such as MTA are important properties and known as indicators of the quality and progression of hydration process [25–28]. Moreover, the hydration and maturation of MTA can also be evaluated through evaluation of crystalline phase formation with X-ray diffraction testing (XRD) [29,30].

Considering the mechanism of ZnO for inhibiting tooth discoloration and that the tooth discoloration caused by ProRoot MTA is also attributed to bismuth oxide [31], the aim of the current study was to evaluate the effect of mixing 5% ZnO with Angelus and ProRoot MTA on the hydration and maturation of these cements via evaluation of the microhardness, compressive strength, crystalline formation and calcium ion release of these two cements when in contact with stimulated tissue fluids (blood and phosphate buffered saline (PBS)) or normal saline (NS). The null hypothesis was that the addition of ZnO would not affect the aforementioned properties of Angelus and ProRoot MTA.

2. Materials and methods

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.DENTISTRY.REC.1397.042).

Cylindrical molds made of plexiglass with an internal diameter of 6 mm and height of 4 mm (according to ASTM E384 standard) were fabricated by CNC laser cutting (Laser Prol; GCC, New Taipei City, Taiwan) for microhardness testing.

Plexiglas split molds with an internal diameter of 4 mm and height of 6 mm were made by CNC laser cutting (Laser Prol; GCC, New Taipei City, Taiwan) for compressive strength, calcium ion release (according to ISO 9917_1 (2007) standard) and X-ray diffraction evaluations.

Cements evaluated were as follows:

1. White Angelus MTA (Angelus, Londrina, PR, Brazil)
2. White Angelus MTA mixed with 5% zinc oxide (ZnO) (Sigma Aldrich, Dorset, UK)
3. White ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK)
4. White ProRoot MTA mixed with 5% ZnO

For mixing ZnO with the cements, 0.05 g ZnO was added to 1 g of the powder of each cement and then was mixed in a Dry Powder Rotator (Glas-Col, IN, USA) to create a homogenous mixture.

Samples were then divided into 3 subgroups according to their exposure conditions:

1. Normal Saline (NS)
2. Phosphate Buffered Saline (PBS) (Merck, Darmstadt, Germany)
3. Whole fresh human blood (blood)

PBS was prepared by dissolving one PBS tablet in 500 ml distilled water. Whole fresh human blood was obtained by phlebotomy using a 23-gauge needle from a healthy volunteer member of the research group after giving an informed consent in accordance with the

declaration of Helsinki ethical principles [32].

Cement preparation was done by mixing 1g of each cement powder (with or without zinc oxide) with 0.33 ml of their liquid according to manufactures' instructions.

Prior to cement placement in molds, they were filled with either NS, PBS and blood according to the specific subgroup, and the excess liquid was aspirated after 20 s. Cement placement was done by using minimal pressure [33].

2.1. Surface microhardness test

After cement placement, specimens ($n = 10$ for each subgroup) were then wrapped in gauze soaked with one of NS, PBS or blood according to their subgroup then incubated at 37 °C in fully saturated conditions for 1 week. Then their surfaces were polished with 400–2000 grit silicon carbide sandpaper (3 M, St Paul, MN, USA). Specimens were then subjected to Vickers tester (Bareiss prüfgeratebau GmbH, Oberdischingen, Germany) with a pyramidal-shaped diamond indenter using a load of 300 g for 10s. Three separate indents were made on the polished surface of each sample. The distance between indentations with each other and from the edge of the sample was 2.5 times the diameter of the indents (in accordance with ASTM E384 standard for Vickers microhardness test).

The Vickers microhardness values were calculated by the testing machine on the following formula:

$$HV = \frac{2F \sin 136^\circ / 2}{d^2} \quad HV = 1.854 \frac{F}{d^2}$$

F is the load in kg^{-1}

d is the mean of the 2 diagonals in mm,

and HV stands for Vickers microhardness value.

2.2. Compressive strength

After cement placement, specimens ($n = 10$ for each subgroup) were then wrapped in gauze soaked with one of NS, PBS or blood according to their subgroup then incubated at 37 °C in fully saturated conditions for 1 week. Then samples were removed from the incubator and the molds were split. To evaluate the compressive strength, the samples were placed lengthwise between the platens of a Universal Testing Machine (Instron, Zwick roell group, Germany). The samples were compressed at the speed of 1 mm/min and the load of fracture was recorded in mega Pascal (MPa).

The Compressive strength value was calculated by following equation: [34]

$$RC = \frac{F \times 9.807}{A}$$

RC: Compressive Strength (MPa)

F: Force/unit area (kg)

A: Base Area

2.3. X-ray diffraction testing (XRD)

After cement placement, specimens were then wrapped in gauze soaked with one of NS, PBS or blood according to their subgroup then incubated at 37 °C in fully saturated conditions. After one week, the surface of the specimens were grind to a fine powder and XRD analysis was performed by X-ray diffractometer (XPert PRO MPD, Panalytical, Almelo, Netherlands).

2.4. Calcium ion release

After cement placement, the filled molds were then placed in 5 mL conical Eppendorf tubes (Fisher Scientific, Loughborough, UK) which

were half-filled (2.5 mL) with either D NS, PBS or blood according to their subgroup so that, only the lower surfaces of the molds were in contact with the content of the tubes. To maintain humidity, damp cotton pellets were placed on the top of the tubes and then the tubes were incubated at 37 °C in fully saturated conditions. Specimens were divided into three subgroups according to the incubation time; 3, 24 and 168h (n = 10 for each subgroup).

At the end of each time interval, the molds were removed from the tubes and 1 mL of the content of each tube was then diluted with 9 mL of deionized distilled water. A sample volume of 1 mL of the solution and 1 mL of 2500 ppm lanthanum chloride solution (Merck, Darmstadt, Germany) were introduced into another tube. Afterwards, 3 mL of hydrochloric acid (1% w/v) was also added [35].

Calcium ion release was measured using an atomic absorption spectrophotometer (Model GBC 904; CG Corp, Melbourne, Australia), equipped with a hollow calcium cathode lamp under the following operating conditions: lamp current 10 mA, flame type nitrous oxide/air acetylene, wavelength 422.7 nm and slit width 0.5 nm. A standard calibration curve was obtained using standard solutions containing calcium concentrations of 0, 1, 3, 5, 7 and 10 ppm. The released calcium ion from each specimen was measured according to the equation of the standard curve line.

2.5. Statistical analysis

To evaluate the effect of environment One Way Anova and Tukey HSD Post hoc tests were used and to evaluate the effect of the addition of ZnO and cement type, T-Test was used. P < 0.05 was considered statistically significant.

3. Result

The compressive strength values are summarized in Table 1. Adding ZnO to both Angelus MTA and ProRoot MTA significantly decreased the compressive strength values of these cements regardless of the environmental conditions (P < 0.001).

The surface microhardness values are summarized in Table 2. Mixing ZnO with ProRoot MTA and Angelus MTA had no significant effect on the surface microhardness of these cements regardless of their exposure conditions (P > 0.05).

For understanding the presumptive reason, we analyzed the samples with XRD measurement. XRD results are shown in Figs. 1 and 2. The crystalline form of CH was only detected in ProRoot MTA and Angelus MTA without ZnO exposed to PBS and saline. ZnO was detected in subgroups in which it was added to.

The calcium ion release values are shown in Tables 3 and 4. The addition of ZnO to both types of MTA decreased the calcium ion release from the cements irrespective of the time interval and exposure conditions however these differences were not statistically significant (P > 0.05). These values significantly increase over time in all subgroups (P < 0.001).

4. Discussion

Marciano et al. [16], proposed a method for preventing tooth

Table 1
Compressive strength values of different experimental groups. Groups identified by the same superscript letters show no significant different.

Cement type	ZnO	NS	PBS	Blood
Angelus MTA	+	5.8 ± 2.4 ^{A,B}	6.11 ± 2.2 ^A	3.57 ± 1.9 ^B
	-	14.4 ± 4.6 ^{CD}	16.76 ± 4.3 ^C	10.54 ± 3.3 ^D
ProRoot MTA	+	5.12 ± 1.1 ^F	5.25 ± 0.6 ^F	5.16 ± 0.6 ^F
	-	24.38 ± 3.4 ^E	28.6 ± 3.9 ^E	25.32 ± 3.6 ^E

Table 2
Microhardness values of different experimental groups. Groups identified by the same superscript letters show no significant different.

Cement type	ZnO	NS	PBS	Blood
Angelus MTA	+	19.6 ± 4.7 ^{A,B}	23.42 ± 6.02 ^A	17.3 ± 4.7 ^B
	-	23.6 ± 4.5 ^A	21.9 ± 4.6 ^A	19.9 ± 4.6 > ^{A,B}
ProRoot MTA	+	28.97 ± 2.5 ^F	25.22 ± 4.8 ^G	19.6 ± 2.1 ^H
	-	26.27 ± 4.3 ^F	22.54 ± 4.3 ^{F,G,H}	18.6 ± 4.5 ^H

discoloration caused by white Angelus MTA by adding ZnO to this cement. They concluded the addition of 5% ZnO to Angelus MTA was sufficient to prevent the tooth discoloration while not influencing the properties of the material; therefore, in the current study 5% ZnO was used. In addition, as the main reason for tooth discoloration caused by ProRoot MTA is also bismuth oxide, we decided to add ZnO to this cement as well.

MTA comes in contact with blood and phosphate containing physiologic solutions in its clinical applications. Exposure to different media has been shown to affect the hydration and maturation of MTA and thus the properties of this cement [26,28–30,36]. Thus evaluating properties of MTA with and without ZnO when exposed to physiologic solutions is of clinically significance.

The results of this study showed that the compressive strength of both cements significantly decreased with the addition of 5% ZnO in all exposure conditions. Gawlicki et al. [37], have shown that the addition of ZnO to Portland cement caused the formation of zinc hydroxide which is amorphous and cannot be detected by XRD. Zinc hydroxide creates an impermeable layer around tricalcium silicate and therefore impairs the hydration of the cement. As the composition and hydration of the Portland cement and MTA are similar, this negative effect may also occur in MTA causing decrease in compressive strength values. The detection of tricalcium silicate, absence of calcium hydroxide crystalline structures and lower amounts of calcium ion release seen in our results may also indicate the impairment of hydration in specimens containing ZnO. Contrarily, Marciano et al. [16] reported impairment in hydration when higher concentrations of ZnO were added. Note that they evaluated the hydration after 28 days of emersion in HBSS; therefore, differences seen in our results may be due to the effect of time and exposure conditions. Gawlicki et al. [37], also reported that transformation of amorphous zinc hydroxide into crystalline calcium hydrozincate destructs the impermeable layer, thereby promotes tricalcium silicate hydration. Evaluation of hydration and properties of the mixture of MTA with ZnO in different time intervals is suggested. Anshul et al. [38] evaluated the effect of adding ZnO nanoparticles to concrete on its compressive strength. They reported that adding up to 1% ZnO nanoparticles to concrete increased its compressive strength but amounts higher than that decreased this property.

According to the results of our study, the addition of ZnO did not affect the microhardness of these cements. As ZnO was detected on the surface of cements that it was added to, and considering the similarity of the microhardness of Zn with Ca, Si and P [39], these results can be justified.

The calcium ion release decreased in specimens mixed with ZnO although these differences were not statistically significant in each time interval and exposure condition. The lower values reported in specimens mixed with ZnO may be due to the impairment in hydration cause by adding ZnO as previously described. These results are inconsistent with the results of Marciano et al. [16] which reported higher calcium ion release in specimens mixed with 5%ZnO. This contradiction may be due to differences in exposure conditions as they exposed MTA to distilled water.

The calcium ion release significantly increase over time in all groups. In the case of specimens mixed with ZnO, the destruction of the impermeable layer surrounding tricalcium silicate is expected that over

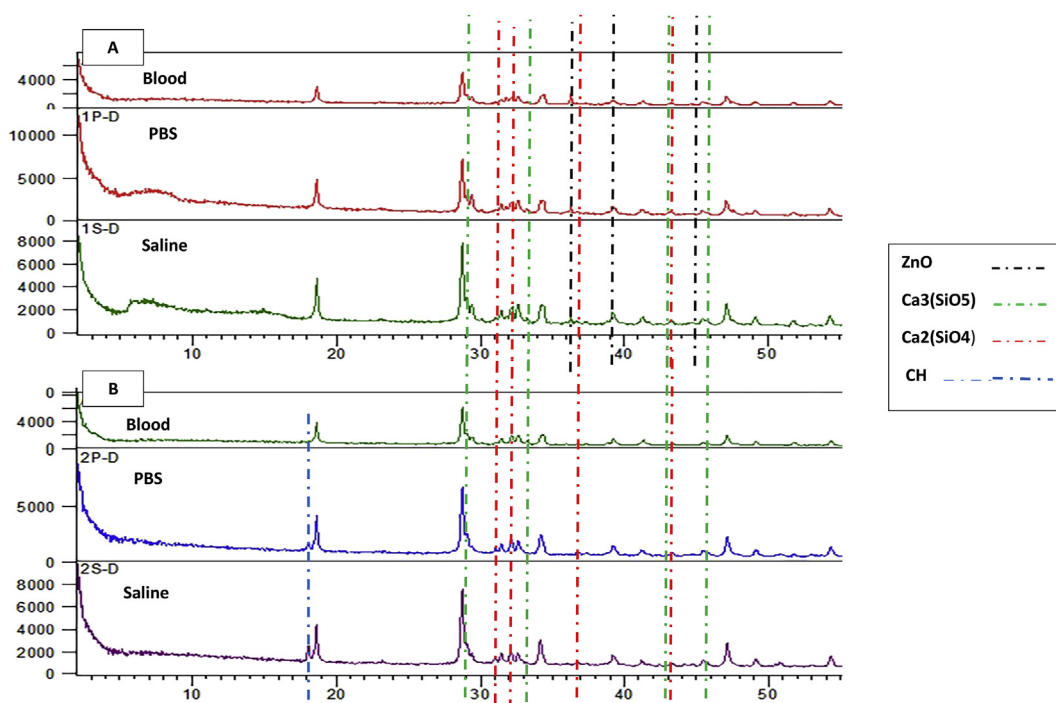


Fig. 1. X-ray diffraction testing (XRD) results of A. Angelus MTA + ZnO and B. Angelus MTA in different exposure conditions.

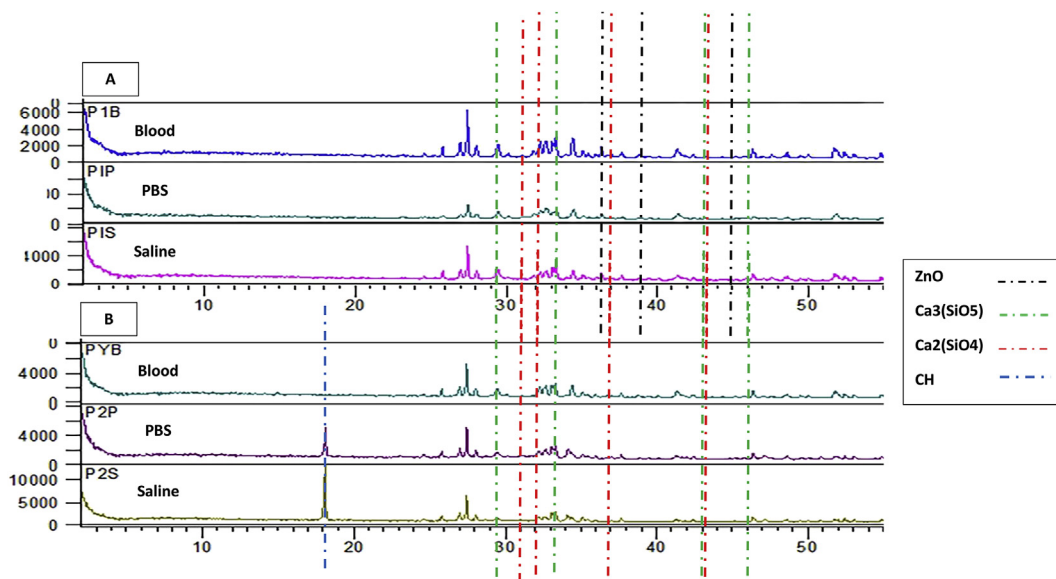


Fig. 2. X-ray diffraction testing (XRD) results of A. ProRoot MTA + ZnO and B. ProRoot MTA in different exposure conditions.

Table 3
Calcium ion release values of different subgroups of Angelus MTA. Groups identified by the same superscript letters show no significant different.

Cement type	ZnO	Exposure	Mean CIR ± SD (ppm*)		
			3 h	24 h	168 h
Angelus MTA	-	Normal Saline	102.11 ± 12.38 ^A	203.55 ± 14.53 ^D	235.28 ± 24.87 ^G
		PBS	109.71 ± 15.01 ^B	198.85 ± 14.67 ^E	247.22 ± 29.79 ^H
		Blood	109.71 ± 15.01 ^C	198.85 ± 14.67 ^F	247.22 ± 29.79 ^I
	+	Normal Saline	97.7 ± 10.99 ^A	162.64 ± 17.86 ^D	228.67 ± 18.84 ^G
		PBS	107.43 ± 11.58 ^B	194.01 ± 10.48 ^E	223.13 ± 19.78 ^H
		Blood	88.41 ± 13.31 ^C	136.93 ± 18.94 ^F	156.99 ± 1.69 ^I

Table 4

Calcium ion release values of different subgroups of ProRoot MTA. Groups identified by the same superscript letters show no significant different.

Cement type	ZnO	Exposure	Mean CIR ± SD (ppm*)		
			3 h	24 h	168 h
ProRoot MTA	-	Normal Saline	111.59 ± 38.31 ^a	201.71 ± 35.56 ^d	251.71 ± 28.04 ^s
		PBS	116.21 ± 12.96 ^b	204.63 ± 19.11 ^e	317.72 ± 35.62 ^h
		Blood	91.85 ± 8.51 ^c	152.56 ± 27.72 ^f	182.82 ± 22.98 ⁱ
	+	Normal Saline	102.13 ± 17.01 ^a	189.67 ± 19.5 ^d	221.19 ± 19.64 ^s
		PBS	113.52 ± 16.04 ^b	175.2 ± 15.36 ^e	246.39 ± 30.36 ^h
		Blood	82.83 ± 9.88 ^c	124.87 ± 12.37 ^f	164.46 ± 25.94 ⁱ

time [37]; therefore, promotion in the hydration of tricalcium silicate and subsequently increase of calcium ions is likely. Reyes-Carmona et al. [40] reported a decrease in calcium ion release over time. They placed MTA in root sections and suspended them in PBS. They described this decrease over time to be a consequence of “controlled biomineralization”, suggesting the involvement of non-collagen proteins of dentin, such as DMP1, in guiding calcium precipitation. As we used plexiglass molds instead of dentin slices, this phenomenon is not expected to have occurred in our study. Duarte et al. [41] also reported a decrease in calcium ion release from MTA over time. Furthermore, the values they reported were lower than that of our study. This difference in results may be due to different experimental condition and differences in specimen preparation in the two studies.

Conducting future research on the effect of lower doses of ZnO on cement properties and evaluating them over time is suggested.

5. Conclusion

Although the addition of 5% ZnO to MTA can prevent tooth discoloration, this modification had adverse effects on the hydration and compressive strength of the investigated cements; while no adverse effects were seen on the surface microhardness of Angelus and ProRoot MTA. Therefore, in cases in which calcium-silicate cements endure higher compressive forces, the use of other methods for discoloration prevention may be better.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioactmat.2020.04.001>.

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