Evaluation of pH, calcium ion release and antimicrobial activity of a new calcium aluminate cement

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Abstract: This study evaluated the pH, calcium ion release and antimicrobial activity of EndoBinder (EB), containing different radiopacifiers: bismuth oxide (Bi_2O_3), zinc oxide (ZnO) or zirconium oxide (ZrO_2), in comparison to MTA. For pH and calcium ion release tests, 5 specimens per group (n = 5) were immersed into 10 mL of distilled and deionized water at 37°C. After 2, 4, 12, 24, 48 h; 7, 14 and 28 days, the pH was measured and calcium ion release quantified in an atomic absorption spectrophotometer. For antimicrobial activity, the cements were tested against *S. aureus*, *E. coli*, *E. faecalis* and *C. albicans*, in triplicate. MTA presented higher values for pH and calcium ion release than the other groups, however, with no statistically significant difference after 28 days ($p > 0.05$); and the largest inhibition halos for all strains, with no significant difference (*E. coli* and *E. faecalis*) for pure EB and EB + Bi_2O_3 ($p > 0.05$). EB presented similar performance to that of MTA as regards pH and calcium ion release; however, when ZnO and ZrO_2 were used, EB did not present antimicrobial activity against some strains.

Descriptors: Endodontics; Root Canal Filling Materials; Disk Diffusion Antimicrobial Tests.

Introduction

Among the desirable characteristics of sealing cements, reparative capacity and antimicrobial activity stand out in particular. Mineral Trioxide Aggregate (MTA) was originally developed for retrograde filling and treatment of root and furcal perforations,[1,2] but its good clinical performance and biocompatibility[1,2] led to its use in other situations, including pulp capping and pulpotomy.[1-3]

Studies have reported the antimicrobial activity of MTA;[4,5] however, its most interesting property is its ability to stimulate mineralized tissue formation,[6] promoting less inflammatory reaction after direct application than calcium hydroxide cement.[7,8]

During MTA hydration, calcium disilicate and trisilicate react to form calcium hydroxide and hydrated calcium silicate gel, thus making the pH of the medium alkaline.[9] The calcium ions released during MTA setting are diffused through the dentinal tubules, increasing their concentration as the setting time increases, thus ensuring the good reparative capacity of the cement.[6-8]

Nonetheless, MTA has some disadvantages that warrant the devel-

opment of new materials with better properties, including long setting time,[10] low flow capacity[11] and high incidence of dental structure staining.[12,13]

With this in mind, a new calcium aluminate-based cement[14] called EndoBinder (Binderware, São Carlos, Brazil) was developed. It preserves the properties and clinical applications of MTA, thus offering an alternative to compensate for MTA disadvantages. According to several studies, EndoBinder presents good biological, mechanical and anti-microbiological properties,[15-17] and does not promote dental structure staining.[18]

Bismuth oxide, used as a radiopacifier in the MTA composition, increases MTA radiopacity, producing values higher than those equivalent to the Al scale.[14] However it is not inert, and interferes in the hydration mechanism of MTA, altering its physicochemical properties.[13]

Thus, the aim of this study was to evaluate the pH, calcium ion release and antimicrobial activity of EndoBinder, composed with different radiopacifiers, as compared with MTA. The null hypothesis tested was that there would be no difference among the cements tested, in regard to the properties evaluated.

Methodology

The cements used in this study are described in Table 1.

Table 1 - Cements used in the study.

Materials	Group	Manufacturer
EndoBinder pure	EBP	Binderware, São Carlos, Brazil
EndoBinder + 20% (weight) Bi_2O_3	EBBO	
EndoBinder + 20% (weight) ZnO	EBZnO	
EndoBinder + 20% (weight) ZrO_2	EBZrO	
MTA	MTA	Ângelus, Londrina, Brazil

Evaluation of pH and calcium ion release

In performing the calcium ion release and the pH tests, 1 mL of the tested cements (n = 5) were inserted into polyethylene tubes (10 mm long × 2 mm in diameter), using a plastic syringe. The cements were manipulated according to the manufacturers' recommendations, in the proportion of 1 g of powder to 0.21 mL of distilled water for EndoBinder; and 1 dose of powder to 1 drop of distilled water for MTA. After filling the tubes, their interiors were radiographed to evaluate whether they had been completely filled.

The specimens were immersed individually into Falcon tubes (BD Falcon, Franklin Lakes, USA), containing 10 mL of distilled and deionized water with a neutral pH (7.0), and stored at 37°C. The pH was assessed with a microprocessor controlled pH analyzer (pH 2000, Instrutherm, São Paulo, Brazil) at time intervals of 2, 4, 12, 24, 48 h and 7, 14 and 28 days of immersion. At each time interval, the polyethylene tube containing the cement was transferred to a new Falcon tube under the same conditions as the initial one, to assess pH and the ability of the cements to release calcium ions continuously. The resulting solution from each period of analysis was used to quantify the calcium ion release in the atomic absorption spectrophotometer (Perkin-Elmer Analyst 100, Perkin-Elmer Inc., Norwalk, USA).

Antimicrobial activity

The double-layer agar diffusion method was employed to evaluate the antimicrobial activity of the cements. Using a Teflon matrix (5 mm in internal diameter × 5 mm thick), 12 specimens of each cement (n = 3) were made in accordance with the manufacturers' instructions, as previously described.

S. aureus (ATCC 25923) and *E. coli* (ATCC 25922) were cultured aerobically on Muller Hinton Agar, *E. faecalis* (ATCC 29212), on Tryptic Soy Agar under microaerobic conditions in a candle jar with 3%–5% CO_2, and *C. albicans* (ATCC 10231), on Sabouraud Dextrose Agar under aerobic conditions.

Recent and pure bacterial cultures (37°C for 24 h) were placed on the appropriate culture media and transferred to test tubes with a 0.85% saline solution to obtain standard bacterial (~10^8 CFU/mL) and fungal (~10^6 CFU/mL) inocula, which were measured in a spectrophotometer (absorbance 0.08 to 0.1 and λ = 625 nm).

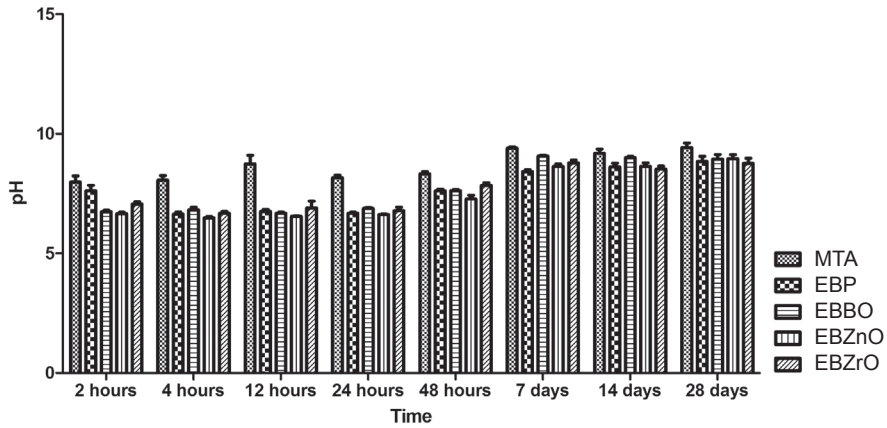
The culture media was poured into Petri plates to form the base layer, composed of 12 mL. After solidification, specimens of each cement type were

Table 2 - Mean and standard deviation of pH values at different time intervals.

	MTA	EBP	EBBO	EBZnO	EBZrO
2 h	8.0 (0.6) ^{a,A}	7.6 (0.5) ^{ac,A}	6.7 (0.1) ^{b,A}	6.7 (0.1) ^{b,AB}	7.1 (0.2) ^{bc,A}
4 h	8.1 (0.4) ^{a,A}	6.6 (0.2) ^{b,B}	6.8 (0.2) ^{b,A}	6.5 (0.1) ^{b,A}	6.7 (0.2) ^{b,A}
12 h	8.7 (0.8) ^{a,AB}	6.7 (0.2) ^{b,B}	6.7 (0.1) ^{b,A}	6.6 (0.0) ^{b,AB}	6.9 (0.6) ^{b,A}
24 h	8.2 (0.2) ^{a,A}	6.7 (0.1) ^{b,B}	6.9 (0.1) ^{b,AB}	6.6 (0.1) ^{b,AB}	6.8 (0.3) ^{b,A}
48 h	8.3 (0.2) ^{a,A}	7.6 (0.2) ^{ab,A}	7.6 (0.1) ^{ab,B}	7.3 (0.3) ^{b,B}	7.9 (0.3) ^{ab,BC}
7 days	9.4 (0.1) ^{a,B}	8.4 (0.2) ^{b,C}	9.1 (0.1) ^{ab,C}	8.6 (0.2) ^{b,C}	8.8 (0.2) ^{ab,D}
14 days	9.2 (0.4) ^{a,B}	8.6 (0.3) ^{a,C}	9.0 (0.1) ^{a,C}	8.6 (0.3) ^{a,C}	8.5 (0.3) ^{a,CD}
28 days	9.4 (0.4) ^{a,B}	8.9 (0.5) ^{a,C}	8.9 (0.4) ^{a,C}	9.0 (0.4) ^{a,C}	8.8 (0.5) ^{a,D}

*Different lower case letters in the line and capital letters in the column indicate a statistically significant difference (2-way ANOVA, Bonferroni test, $P < 0.05$).

Figure 1 - Graphic representation of the pH mean values at different time intervals.



applied to the base layer, and 8 mL of culture media, melted at 45°C with 1% standard microbial inoculum, was added to the base layer to form the seed layer. The plates were kept at room temperature for 2 h to allow pre-diffusion of the cements, and were then incubated at 37°C for 24 h.

The inhibition zones around the test specimens were measured by a millimeter ruler with a 0.5 mm accuracy. The tests were performed in triplicate, and the mean values of each test were submitted to statistical analysis.

Statistical analysis

The values obtained for the pH and the calcium ion release tests (2-way ANOVA, Bonferroni test, $p < 0.05$) and antimicrobial activity (1-way ANOVA, Tukey's test, $p < 0.05$) were statistically analyzed with Graphpad Prism 4.0 software (GraphPad Software, La Jolla, USA).

Results

pH and calcium ion release

The pH mean values are presented in Table 2.

MTA presented the highest pH values, ranging from 8.0 to 9.4 at 28 days. In the initial period, there was no significant difference for EBP ($p > 0.05$); however, MTA differed from the other groups ($p < 0.05$). Only EBP presented a statistically significant difference for EBBO and EBZnO ($p < 0.05$). At 14 and 28 days, all cements presented similar results, with no statistically significant difference among them ($p > 0.05$).

Comparing the same material during the different periods (Figure 1), the pH of MTA increased progressively. EBP presented an increase in pH up until 28 days, but with no statistically significant difference as of the 7th day ($p > 0.05$).

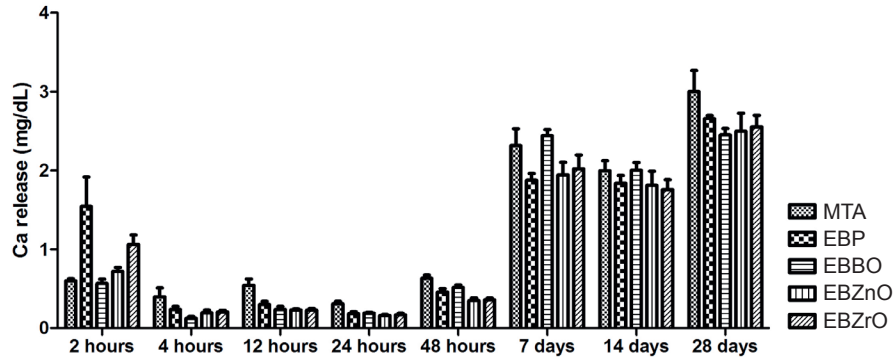
The calcium ion release mean values may be seen

Table 3 - Mean values and standard deviation for calcium ions (mg/dL) released at the different time intervals tested.

	MTA	EBP	EBBO	EBZnO	EBZrO
2 h	0.60 (0.01) ^{a,A}	1.54 (0.02) ^{b,A}	0.57 (0.01) ^{a,A}	0.68 (0.01) ^{a,A}	1.06 (0.02) ^{ab,A}
4 h	0.40 (0.01) ^{a,A}	0.24 (0.01) ^{a,B}	0.13 (0.01) ^{a,A}	0.19 (0.00) ^{a,A}	0.21 (0.01) ^{a,B}
12 h	0.54 (0.02) ^{a,A}	0.30 (0.01) ^{a,B}	0.24 (0.01) ^{a,A}	0.23 (0.00) ^{a,A}	0.23 (0.01) ^{a,B}
24 h	0.31(0.01) ^{a,A}	0.18 (0.00) ^{a,B}	0.19 (0.01) ^{a,A}	0.16 (0.00) ^{a,A}	0.17 (0.01) ^{a,B}
48 h	0.63(0.01) ^{a,A}	0.46 (0.01) ^{a,B}	0.52 (0.01) ^{a,A}	0.35 (0.01) ^{a,A}	0.36 (0.01) ^{a,B}
7 days	2.32 (0.04) ^{a,B}	1.88 (0.04) ^{a,A}	2.44 (0.05) ^{a,B}	1.94 (0.02) ^{a,BC}	2.02 (0.03) ^{a,CD}
14 days	2.00 (0.02) ^{a,B}	1.84 (0.05) ^{a,A}	2.00 (0.05) ^{a,B}	1.81 (0.03) ^{a,B}	1.76 (0.03) ^{a,C}
28 days	3.00 (0.07) ^{a,C}	2.66 (0.06) ^{a,C}	2.45 (0.07) ^{a,B}	2.50 (0.05) ^{a,C}	2.56 (0.06) ^{a,D}

*Different lower case letters in the line and capital letters in the column indicate a statistically significant difference (2-way ANOVA, Bonferroni test, $p < 0.05$).

Figure 2 - Graphic representation of the calcium ion (mg/dL) release mean values at different time intervals.



in Table 3.

When the different materials were compared according to the same period (Figure 2), only EBP (2 h) presented a statistically significant difference in relation to the other groups ($p < 0.05$), which showed no significant difference among one another ($p > 0.05$). In the other periods of analysis, there was no statistically significant difference among the groups ($p > 0.05$).

Antimicrobial activity

The inhibition halo mean values may be seen in Table 4.

MTA presented an inhibition halo similar to that of EBZnO ($p > 0.05$) for *S. aureus*, but differing statistically from those of the other groups ($p < 0.05$). EBZrO and EBZnO presented no antimicrobial activity for *E. coli* and *E. faecalis*. The other cements presented activity against these strains, but with no statistically significant difference among one another ($p > 0.05$). EBZrO and EBZnO also presented no antimicrobial activity for *C. albicans*. MTA presented a statistically significant difference for EBP and EBBO ($p < 0.05$), which did not differ statistically between each other ($p > 0.05$).

Table 4 - Mean values of inhibition halo diameters (mm).

	MTA	EBP	EBBO	EBZrO	EBZnO
S. aureus	11.0 ^{a}	7.0 ^{b}	7.3 ^{b}	7.0 ^{b}	8.3 ^{ab}
E. coli	10.0 ^{a}	7.0 ^{a}	8.0 ^{a}	-	-
E. faecalis	10.0 ^{a}	7.3 ^{a}	8.0 ^{a}	-	-
C. albicans	13.7 ^{a}	7.0 ^{b}	8.3 ^{b}	-	-

*Different letters in the lines indicate a statistically significant difference (1-way ANOVA, Tukey test, $p < 0.05$).

Discussion

In the present study, the pH, the calcium ion release and the antimicrobial activity of EndoBinder with different radiopacifiers were evaluated in comparison with MTA. Thus, the null hypothesis tested was partially accepted, insofar as the cements showed a similar behavior for pH and calcium ion

release. However, in terms of antimicrobial activity, the different radiopacifiers played a relevant role in the results for EB.

Unlike the results of this study, in which MTA presented a maximum pH value of 9.4, Cutajar *et al.*[19] found values higher than 12.0. This may be explained by the difference in the methodology used for pH measurement. Cutajar *et al.*[19] performed pH measurements directly in the cement after manipulation, and not in tubes containing distilled and deionized water, as in the present study. The method used in the present study has the advantage of allowing pH to be measured over longer periods of time, and reveals not only the pH of the material during setting, but also the medium alkalinization capacity of the material after setting.[19]

The initial pH value of MTA was higher than that of EndoBinder in its different forms, because of the calcium chloride in the MTA formula, added to accelerate its setting time.[20] This component significantly increases the pH after manipulation, thus keeping the medium alkaline, due to the additional calcium added to the formula.[20] However, some authors[20,21] observed a reduction in pH when calcium chloride was added to MTA, because of the repulsion between chlorine and hydroxyl ions within the material.

During its hydration process, MTA yields hydrated calcium silicate, a by-product of calcium hydroxide.[9] The reactions involving calcium silicate in the presence of water promote the hydrogenation of $Ca(OH)_2$ and CaO; therefore, MTA releases a high concentration of calcium ions into the medium[9] produced from the calcium hydroxide, and from the decomposition of calcium silicate hydrate, which is released more slowly than calcium hydroxide.[22] Thus, the dissociation of calcium hydroxide into calcium and hydroxyl ions results in a pH increase, and consequently, increased antimicrobial activity.[23]

On the other hand, the hydration process of EndoBinder yields calcium aluminate and aluminum hydroxide hydrates.[24] The release of calcium and hydroxyl ions may be attributed to the decomposition of calcium aluminate hydrate, which occurs more slowly in EndoBinder than in MTA, and could explain the lower pH of EndoBinder in the initial periods, and the slower increase of pH in EndoBinder, in comparison with MTA.[25]

In the particular case of EndoBinder, the water and cement (w/c) ratio, and also temperature, are determinants of the phases that will result from the hydration process.[25] Under the conditions in which the tests were performed (temperature of 37°C and w/c ratio = 0.21), the AH3 ($Al_2O_3.3H_2O$) and C3AH6 ($3CaO.Al_2O_3.6H_2O$) phases stood out particularly.[26] This considered, the results obtained for calcium ion release by EndoBinder are coherent with the above explanation. Because the dissolution and precipitation of the hydrated phases are quick for EBP, the concentration of calcium ions in solution is high in the first few hours.[26]

Hydration process phases are more stable after 2 h, after which the calcium ion release rate is reduced.[25] This rate only begins to increase with the hydration of CaO, which occurs in the anhydrous composition of calcium aluminate cement, as a result of how the cement is obtained, i.e., from the calcination of aluminum (Al_2O_3) and calcium carbonate ($CaCO_3$).[25] The results demonstrated an increase in calcium ion release as of 48 h in an aqueous medium. The tendency is the same when distinct radiopacifiers are added to EBP. The same increased calcium ion release as of 48 hours applies to the pH results, although, in the first hours, these results are slightly influenced by the calcium chloride, which tends to act as a buffer of the solution in which the EndoBinder phases are hydrated.[20]

Regarding antimicrobial activity, MTA inhibited all microorganisms effectively, as corroborated by the increase in the pH of the medium, due to calcium hydroxide formation.[23] Likewise, the antimicrobial activity of EndoBinder may be attributed to the decomposition of the calcium aluminate hydrate formed, which, in turn, releases calcium and hydroxyl ions.[24]

It is known that the radiopacifiers present in cement formulation are not inert and interfere in the mechanism of cement hydration, decreasing the calcium ion release rates and affecting several physicochemical properties.[13,22] This interference could be observed in the present study, insofar as the different radiopacifiers used influenced the antimicrobial activity of EB. Only EBZrO and EBZnO presented no antimicrobial activity against *E. coli*, *E. faeca-*

lis and *C. albicans*; however, they were effective against *S. aureus*. The other forms of EndoBinder, namely, EBP and EBBO, also showed antimicrobial activity against *E. faecalis*, the microorganism most present in recurrent endodontic infections.[27,28]

In another study, Jacobovitz *et al.*[15] evaluated the antimicrobial activity of MTA and EndoBinder + ZnO through apical microleakage tests, and found an absence of microbial growth after 30 days in both cements. The discrepant results could be explained by an important aspect of EndoBinder, namely, its good rheological properties (flowability and plasticity),[25] which offer an alternative to compensate for the negative features of MTA-based cements, such as sealing ability.

Conclusions

EndoBinder presented similar behavior to that of MTA for pH and calcium ion release, irrespective of the radiopacifier used. However, when ZnO and ZrO_2 were added, EndoBinder presented no antimicrobial activity against some microbial strains. In spite of the good performance of EndoBinder, further studies must be conducted before it can be validated as a sealing cement for root and furcal perforation treatment.

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