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Influence of 2% chlorhexidine on pH, calcium release and setting time of a resinous MTA-based root-end filling material

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Abstract: The addition of chlorhexidine (CHX) to a resinous experimental Mineral Trioxide Aggregate (E-MTA) based root-end filling material is an alternative to boost its antimicrobial activity. However, the influence of chlorhexidine on the properties of this material is unclear. The aim of this study was to evaluate the influence of 2% chlorhexidine on the pH, calcium ion release and setting time of a Bisphenol A Ethoxylate Dimethacrylate/Mineral Trioxide Aggregate (Bis-EMA/MTA) based dual-cure experimental root-end filling material (E-MTA), in comparison with E-MTA without the addition of CHX and with conventional white MTA (W-MTA). The materials were placed in polyethylene tubes, and immersed in deionized water to determine pH (digital pH meter) and calcium ion release (atomic absorption spectrometry technique). The setting time of each material was analyzed using Gilmore needles. The data were statistically analyzed at a significance level of 5%. E-MTA + CHX showed an alkaline pH in the 3 h period of evaluation, the alkalinity of which decreased but remained as such for 15 days. The pH of E-MTA + CHX was higher than the other two materials after 7 days, and lower after 30 days ($p < 0.05$). All of the materials were found to release calcium ions throughout the 30 days of the study. The addition of CHX increased the calcium ion release of E-MTA to levels statistically similar to W-MTA. E-MTA showed shorter initial and final setting time, compared with W-MTA ($p < 0.05$). The addition of 2% CHX to MTA prevented setting of the material. The addition of CHX to E-MTA increased its pH and calcium ion release. However, it also prevented setting of the material.

Keywords: Chlorhexidine; Hydrogen-Ion Concentration; Dental Materials.

Introduction

Periapical surgery usually consists of root-end resection and root-end filling to seal the communication between the apical tissues and the root canal system.[1] Root-end filling materials should prevent leakage, promote antimicrobial activity, and provide an effective environment for healing of the apical tissues.[2,3] Mineral Trioxide Aggregate (MTA) as a root-end filling material has proved to be the most effective material in preventing leakage[3] and stimulating tissue repair.[4]

Chlorhexidine (CHX), a cationic antimicrobial agent frequently used as a root canal irrigant[5] or as an inter-appointment root canal dressing,[6] has been added to conventional MTA as a sterile water substitute.[7] Studies have shown that MTA mixed with CHX produces a biocompatible material – insofar as it created weak inflammatory responses characterized by the presence of a fibrous connective tissue capsule in the subcutaneous tissues of rats[8] – with enhanced antimicrobial activity.[9]

Although MTA has many favorable properties that support its clinical use, there are also several drawbacks, *e.g.*, it is a material of difficult manipulation. Moreover, the extended setting time of MTA may cause the material to be washed out of the cavity during root-end surgery. The addition of light-curable resinous monomers to MTA has been proposed to improve its properties and reduce its setting time.[10] However, this addition to MTA cements inhibits MTA calcium ion release, impairing its ability to stimulate tissue repair.[11,12]

Chlorhexidine not only acts as an antimicrobial agent but also has been shown to increase the calcium release of calcium hydroxide medication.[13] The addition of chlorhexidine to a resinous MTA-based root-end material could enhance its antimicrobial activity and improve its ability to release calcium ions, thus increasing the success rate of root-end endodontic surgery. However, there is no data in the literature in respect to the effect of CHX addition on the properties of resinous MTA-based root-end material. Therefore, the objective of this study was to investigate the influence of 2% CHX on pH, calcium ion release and setting time of Bisphenol A Ethoxylate Dimethacrylate/Mineral Trioxide (Bis-EMA/MTA) based root-end filling material.

Methodology

The compositions of the experimental root-end filling material (E-MTA) (containing Bis-EMA/MTA), of the E-MTA + 2% CHX powder, and of the white-MTA (W-MTA) are shown in Table 1.

W-MTA (Angelus, Londrina, Brazil) was prepared following the manufacturer's instructions. E-MTA was prepared using equal parts of Paste 1 and Paste 2.

Calcium ion release and pH evaluation

The materials were manipulated and inserted in single, open-ended polyethylene tubes with 1.0 mm internal diameter and 10.0 mm length, using a lentulo spiral (Dentsply Maillefer, Toronto, Canada). E-MTA and E-MTA + CHX were light-cured for 40 seconds using a light-cure unit (Ultralux-Dabi Atlante, Ribeirão Preto, Brazil). After the tubes were filled, they were weighed to ensure standardization of the amount of cement in each tube. Five specimens of each material were prepared. Each specimen was immediately immersed in test tubes containing 10 mL of deionized water (Permution, Curitiba, Brazil). The tubes were then sealed with Parafilm (American National Can, Menasha, USA) and incubated at 37°C (Farmen, São Paulo, Brazil), and kept as such throughout the study. Previous to the immersion of the specimens, the pH and calcium ion concentration of the deionized water was verified (attesting pH 7.0). All laboratory equipment was previously treated with nitric acid to avoid interference in the results. Evaluations were performed at periods of 3 hours, 24 hours, 7 days, 15 days and 30 days. The specimens were carefully transferred to new tubes with fresh deionized water after each measurement. The measurement of pH was performed with a pH meter (Quimis Q400A, Diadema, Brazil). The release of calcium ions was measured using an atomic absorption spectrophotometer (AA6300, Shimadzu, Tokyo, Japan). The conditions for using the appliance were determined following the manufacturer's instructions: wavelength of 422.70 nm, gap of 0.5 nm, lamp current of 10 mA, and slightly reduced stoichiometry, and were maintained by an air-supported acetylene flow of 2.0 L per minute. A lanthanum chloride solution at 1 g/L was used to eliminate the interference of phosphates and sulfates, as well as the possible formation of refractory oxides. A standard stock solution of 100 mg/L was diluted in water to produce the following concentrations: 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 2.0 mg/L. The results were calculated according to a standard curve, established on the basis of solutions with predefined calcium concentrations.

Setting Time

The setting time was determined according to the American Society for Testing and Materials 2008, C266-08 standard test method for setting time of hydraulic-cement pastes using Gillmore needles.

Table 1. Composition of materials used in the study.

Materials	Composition	Type of cure	Proportion
E-MTA	Paste 1: MTA, Bis-EMA 10, Bis-EMA 30, camphorquinone, DHEPT, EDAB Paste 2: Ytterbium Fluoride, Bis-EMA 10, Bis-EMA 30, benzoyl peroxide	Dual-cure	1:1
E-MTA + CHX	Paste 1: MTA, Bis-EMA 10, Bis-EMA 30, camphorquinone, DHEPT, EDAB; chlorhexidine Paste 2: Ytterbium Fluoride, Bis-EMA 10, Bis-EMA 30, benzoyl peroxide	Dual-cure	1:1
W-MTA	Powder: Bl_2O_3, CaO, MgO, K_2O, Na_2O, Fe_2O_3, SO_3, SiO_2, Al_2O_3 Liquid: distilled water	Chemical	3:1

MTA: Mineral trioxide aggregate; DHEPT-N: N-dihidroxietil-p-toluidine; EDAB: Ethyl-4-dimethylamino benzoate, Bis-EMA: Ethoxylate bisphenol A glycol dimethacrylate; SiO_2: Silicon dioxide; Al_2O_3: Aluminum oxide; CHX: Chlorhexidine; Bl_2O_3: Bismuth dioxide; CaO: Calcium oxide.

The materials were mixed and placed in stainless steel rings with a 10 mm internal diameter and 2 mm height. Three stainless steel rings were filled with each material and stored in an incubator at 37°C and 95% relative humidity. Next, a 113.4 g Gillmore needle was used to determine the initial setting times, and a 453.6 g Gillmore needle, to determine the final setting times. This procedure was repeated every 60 s until the material set. In both analyses, the setting times were recorded at the moment in which the needle failed to leave a complete circular indentation on the surface of the specimen.

Statistical analysis

The assumptions of equality variances and normal distribution of errors were checked for all the response variables tested, and those that were not suitable were transformed. pH and calcium data were ranked, transformed and analyzed using two-way repeated measures of variance (ANOVA), followed by the Holm-Sidak method. Setting time was analyzed using one-way ANOVA followed by the Holm-Sidak method. Statistical analysis was carried out using the SigmaStat® software package (Version 3.5 for Windows®, Systat Software Corporation, San Jose, USA). Values of $p < .05$ were considered significant.

Results

W-MTA showed an alkaline pH during the 30 days of the experiment. E-MTA showed an alkaline pH in the first 24 h and then acid pH at the 7- and 15-day evaluations, ending with a neutral pH at the 30-day evaluation. E-MTA + CHX showed an alkaline pH in the 3 h evaluation period; although the level decreased, it remained alkaline for 15 days. Table 2 shows the mean and standard deviation pH values for each group. No statistical differences were observed between the 3 materials in the 3 h period. The pH of E-MTA + CHX was higher than that of the other two materials after 7 days, and lower after 30 days ($p < 0.05$).

All of the materials released calcium ions throughout the 30 days of the study. Table 3 presents the mean and standard deviation values of calcium ion release in different periods up to 30 days. Statistical differences were observed between E-MTA and W-MTA ($p < 0.05$), and between E-MTA and

Table 2. pH values (mean ± standard deviation, n = 5 for each material) of soaking water after immersion of the samples in different evaluation periods (3 h to 30 days).

Periods	W-MTA	E-MTA	E-MTA+CHX
3 h	9.92(±0.26)^a	9.32(±0.27)^a	9.63(±0.32)^a
24 h	10.06(±0.55)^a	7.86(±0.78)^b	7.83(±0.84)^b
7 d	7.02(±0.14)^b	6.25(±0.20)^c	7.35(±0.12)^a
15 d	8.05(±0.67)^a	6.63(±0.13)^b	7.35(±0.16)^a
30 d	7.78(±0.13)^a	7.04(±0.20)^b	6.75(±0.06)^c

Different lowercase letters indicate significant differences between groups. ($p \leq 05$)

Table 3. Calcium released (mean ± standard deviation, expressed as ppm, n = 5 for each material) in soaking water after immersion of the samples in different evaluation periods (3 h to 30 days).

Periods	W-MTA	E-MTA	E-MTA+CHX
3 h	9.97(±3.08)[a]	6.67(±1.99)[a]	6.50(±1.14)[a]
24 h	12.25(±2.03)[a]	5.87(±1.45)[b]	5.83(±0.82)[b]
7 d	5.43(±1.20)[a]	1.67(±0.47)[b]	5.58(±0.90)[a]
15 d	5.81(±0.80)[a]	3.18(±0.76)[b]	6.67(±0.74)[a]
30 d	6.54(±1.66)[a]	2.79(±0.82)[b]	7.65(±0.87)[a]

Different lowercase letters indicate significant differences between groups. ($p \leq 05$)

E-MTA + CHX ($p < 0.05$). In general, the addition of CHX increased the calcium ion release of E-MTA to levels statistically similar to W-MTA.

Table 4 shows the mean setting time for all the materials. E-MTA showed immediate setting, whereas W-MTA showed a mean final setting of 81.67 minutes. E-MTA + CHX remained unset even after a 7-day incubation period.

Discussion

The present in vitro study demonstrated the influence of 2% CHX on pH, calcium ion release and setting time of a resinous experimental MTA (E-MTA). The formulation of the experimental material includes Bis-EMA, a high-molecular-weight monomer that does not require diluents, which could result in a material with less cytotoxic effects. Linhares *et al*.[14] evaluated the same experimental material and found that the addition of $CaCl_2$ to E-MTA improved the release of calcium ions. In the present study, the addition of CHX increased the pH and calcium ion release of E-MTA, although it prevented the setting of this material.

The study model using cylindrical polyethylene tubes was chosen to standardize the amount of material used in each sample. Atomic absorption spectrophotometry has been used successfully in earlier studies for analyzing calcium ions,[15,16] whereas the setting time evaluation using Gillmore needles is a standard test method that has been used previously to evaluate the setting time of MTA-based materials.[15,17]

Table 4. Initial and final setting time (mean ± standard deviation, n = 3 for each material) determined by Gillmore needles at 37°C and 98% relative humidity. E-MTA was light-cured for 40 s.

Materials	Initial setting time (min)	Final setting time (min)
W-MTA	9.33±1.15[b]	81.67±7.63[b]
E-MTA	0.66±0[a]	0.66±0[a]
E+2 CHX	-[c]	-[c]

Different lowercase letters indicate significant differences between groups. ($p \leq 05$)

In the present study, W-MTA showed the highest pH values. It is well-established that MTA promotes an alkaline pH in a physiological solution.[11,15,18] The alkalization of the medium, promoted by MTA in a surgical environment, activates alkaline phosphatase – an important enzyme for the formation of mineralized tissue and consequent apical healing.[19] Overall, E-MTA presented the lowest pH values, whereas the addition of CHX to this material allowed the pH to remain alkaline for 15 days. A possible explanation is that E-MTA had already set as of the first immersion in water, whereas E-MTA + CHX had not set during the experiment, thus allowing hydroxyl ions to be released into the solution.

Structural characteristics of MTA allow a continuous hydration reaction, forming calcium hydroxide, which dissociates and releases calcium ions into the medium. Calcium ion release has been reported for both conventional MTA,[20] and light-curable MTA.[21] Although all materials tested in the present study released calcium ions, E-MTA showed a statistically lower release of these ions than W-MTA.

The basis for the biological properties of MTA has been attributed to the production of hydroxyapatite when the released calcium ions come into contact with tissue fluids.[22] The low calcium release shown by E-MTA could reduce the ability of the material to stimulate tissue mineralization. However, the addition of CHX to E-MTA increased the calcium ion release to levels statistically similar to those of W-MTA. When CHX is associated with $Ca(OH)_2$, it has a potential to induce the formation of *para*-chloroaniline, but may

also produce reactive oxygen species (ROS), which play a critical role in the cellular wall and membrane structure of microorganisms.[23] Moreover, it has been shown that the association between a mixture of MTA and 0.12% CHX has biocompatible effects when implanted subcutaneously in rats.[8] It is important to point out that, in the present study, CHX powder was added to the material instead of CHX solution or gel, in order to avoid the interference of vehicles normally associated with CHX, such as NATROSOL.

Nevertheless, the addition of CHX to E-MTA affected the setting of the material. An extended setting time poses a disadvantage for root-end fillings, insofar as it facilitates leakage and cement dislodgement during apical surgery.[24] The addition of antibacterial agents to dental materials has frequently resulted in changes in physical properties or in bond strength,[25] which may affect the clinical performance of these materials. It has been observed that the cationic properties of CHX interfere with the setting mechanisms of glass ionomer cements[26] and resins.[27] Moreover, Kogan *et al*.[28] showed that MTA mixed with CHX gel did not set until the end of a 4-hour observation period.

The CHX molecules added to E-MTA could have remained enclosed within linear polymer networks upon light curing. The addition of drugs to copolymers has been seen to interfere with the polymerizing process.[29] Consequences of clinical use of an unset resinous MTA could include dissolution of the material into the surgical cavity, and release of monomers that would exert a cytotoxic effect. As such, the results of this study showed that CHX should not be added to light-curable MTA.

Conclusion

In conclusion, the addition of CHX to E-MTA increased its pH and calcium ion release. However, it prevented the setting of the material. Therefore, the addition of CHX to light-curable MTA derails its clinical use as a root-end filling material.

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