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Atomic absorption spectrometry evaluation of calcium ion release from different calcium silicate-based endodontic materials used with new irrigants.

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Abstract

Objective: The aim of the present study was to measure, using Atomic Absorption Spectrometry (AAS), calcium ions released from three calcium silicate-based materials, white MTA (WMTA), Bioaggregate (BA), and Biodentine, after their immersion in 0.2% chitosan, 10% propolis, 1% acetic acid, and 17% EDTA and also in distilled water.

Methods: One hundred and fifty silicon tubes were prepared in this study. The tubes were randomly divided into three groups and filled as follows. Group I: MTA, Group II: BA Group III: Biodentine. After that, the set material was scraped from a silicon tube. Each group was then randomly divided into five subgroups of 10 samples each (n=10). For subgroup 1, a fresh 0.2% chitosan solution was prepared. For subgroup 2, 10% propolis solution was prepared. Subgroup 3 was treated with distilled water. For subgroup 4, 1% acetic acid was used. In subgroup 5, 17% EDTA was used. The samples were immersed in the test solutions for 10 minutes. AAS (GBC-GF; model Avanta PM) was used to determine the Ca ions released. The data were analysed using the SPSS 20 software. Kruskal-Wallis and Mann-Whitney U tests were applied (P<0.05).

Results: The lowest Ca release values were observed in distilled water, while the highest values were found in the samples incubated in the EDTA, and there are no statistically significant differences between propolis, chitosan, and distilled water.

Conclusions: Chitosan and propolis natural irrigation solutions may be preferred to EDTA, when used with MTA, BA, and Biodentine.

Keywords: Calcium ion release, Propolis, Chitosan.

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Introduction

Various irrigation solutions are used to disinfect the root canal during endodontic treatment [1]. The routinely used irrigants, such as sodium hypochlorite (NaOCl) and Ethylene Diamine Tetra-Acetic Acid (EDTA), have a detrimental effect on periapical tissues [2]. EDTA, one of the preferred irrigants for removing the smear layer, is a chelating agent and effectively removes inorganic material from the root canal [3]. Its reaction with the calcium ions in dentine results in calcium chelation and also causes deterioration of the root-end filling materials that have structure similar to dentine [4]. Smith et al. [4] have shown that EDTA and MTAD can initiate chemical reactions causing MTA degradation. Uyanık et al. [5] concluded that EDTA and BioPure MTAD decreased the sealing efficacy of MTA and Super-EBA.

At the acidic pH, the solubility of root repair materials may increase, which, in turn, adversely affects their sealing abilities [6,7]. Recently, new calcium silicate MTA-like cements, such as Bioaggregate and Biodentine, have been introduced. The irrigation solutions preferred by clinicians may not affect MTA-like repair materials used for apical resection, pulp capping, and perforation repair. Thus, new and different irrigation solutions, which have antibacterial efficiency, capable to remove the smear layer, and are tissue friendly, have been searched for. In this respect, solutions of some natural organic materials seem to be promising in endodontics [8,9]. Chitosan is a natural polysaccharide that has been studied for dental applications because of its antibacterial activity, biocompatibility, biodegradability, bioadhesivity, and non-toxicity [10]. Kishen et al. [11] showed that treatment of dentine with chitosan nanoparticles significantly reduced the number of Enterococcus faecalis cells. However, Rabea et al. [12] indicated that chitosan had lower antibacterial efficiency against many other microorganisms.

Propolis is a resinous material that honeybees (*Apis mellifera* L.) collect from various plant sources and mix with wax and other substances [13]. The use of propolis has increased due to its therapeutic and biological properties [14]. Current research involving propolis in oral surgery, pathology, periodontics, and endodontics highlights antimicrobial and antienflammatory activities of this material [15-18]. However, no studies to date have evaluated effects of natural irrigation solutions, such as chitosan or propolis, on root repair materials. The aim of the present study was to measure, using Atomic Absorption Spectrometry (AAS), the concentrations of calcium ions released from three calcium silicate-based materials, white MTA (WMTA), Bioaggregate (BA), and Biodentine, after their immersion in 0.2% chitosan, 10% propolis, 1% acetic acid, and 17% EDTA solutions and also in distilled water. The following null hypothes tested that there would be no differences between the root-end filling materials in the Ca ion emission levels.

Materials and Methods

One hundred and fifty open-ended silicon tubes (10 mm in length and 2 mm in internal diameter) were prepared in this study. The tubes were randomly divided into three groups of 50 tubes each and filled using a lentulo spiral (Dentsply Maillefer, Toronto, Canada) as follows. Group MTA: White MTA (WMTA) (Angelus, Londrina, Brazil) powder was mixed with distilled water according to the manufacturer's instructions, inserted into the silicon tubes and condensed by pluggers (Angelus, Londrina, Brazil). Group BA: The tubes were filled with BA (DiaDent Group International, Canada) which was prepared as per the protocol provided with the kit. Group Biodentine: Biodentine (Septodont, Saint Maur des Fosses, France) liquid from a single-dose container was emptied into a powder-containing capsule and mixed for 30 seconds at 4,000-4,200 rpm. Biodentine was placed into the silicon tubes and condensed using pluggers. After the tubes were filled, they were weighed to ensure standardization of the amount of cement in each tube. All samples were stored at 37°C and 100% humidity for 7 days. After that, the set material was scraped from a silicon tube with a scalpel. Each group was then randomly divided into five subgroups of 10 samples each (n=10), to be immersed in different irrigation solutions. For group 1, a fresh 0.2% chitosan solution was prepared by diluting 0.2 g of 90% deacetylated chitosan (from Ankara University, Department of Chemical Engineering) in 100 mL of 1% acetic acid and stirring the mixture for 2 h on a magnetic stirrer. For group 2, 10 g of propolis was dissolved in 100 mL of 70% ethanol by shaking for one hour, and the extract was filtered through a Whatman paper. Group 3 was treated with distilled water. For Group 4, 1% acetic acid was freshly prepared in the laboratory. In Group 5, 17% EDTA was used.

5 mL of each test solution was inserted into 15 mL Falcon tubes. The samples were immersed in the test solutions for 10 minutes. AAS (GBC-GF; model Avanta PM) with a calcium-specific hollow cathode lamp was used to determine the mean concentration, in parts per million (ppm), of the Ca ions released after 10 min. To verify the instrument calibration, a standard stock solution was diluted in water to produce the following concentrations: 100, 200, 400, 600, and 800 ppm. The results were calculated according to a standard curve, established on the basis of solutions with predefined calcium concentrations, for each sample and each immersion solutions. The data were analysed using the SPSS 20 software. Kruskal-Wallis and Mann-Whitney U test were applied, and $P<0.05$ was defined as statistically significant.

Results

Ca release values of the experimental groups are shown in Table 1. According to the statistical evaluation of the Ca release, there are statistically significant differences between Biodentine and MTA or BA in all irrigation solution groups. There are no statistically significant differences between MTA and BA groups.

Table 1. *Calcium released (mean ± standard deviation, expressed as ppm, n=10 for each material) in irrigation solutions after immersion of the samples.*

Solutions	Materials		
	MTA	BA	Biodentine
	Mean ± SD	Mean ± SD	Mean ± SD
Chitosan	131.58 ± 8.32^{Aa}	157.32 ± 12.59^{Aa}	308.02 ± 11.24^{Ba}
Propolis	110.06 ± 5.87^{Aa}	138.13 ± 9.74^{Aa}	201.34 ± 9.58^{Ba}
Distilled water	96.10 ± 3.02^{Aa}	97.25 ± 5.56^{Aa}	526.91 ± 15.21^{Ba}
Acetic acid	607.95 ± 15.14^{Ab}	338.94 ± 12.04^{Ab}	327.51 ± 12.63^{Bb}
EDTA	758.81 ± 11.23^{Ac}	495.42 ± 10.45^{Ac}	621.21 ± 14.03^{Bc}

Different letters represent statistically significant differences ($P<0.05$) in the same line (capital letters) or in the same column (lowercase letters). ppm = parts per million.

The lowest Ca release values were observed in the samples incubated in distilled water, while the highest values were found in the samples incubated in the EDTA solution. There are no statistically significant differences between acetic acid and EDTA in the Ca ion emission levels, and also there are no statistically significant differences between propolis, chitosan, and distilled water. The release of Ca ions from the materials was lower in the chitosan and propolis solutions compared to EDTA and acetic acid, and the differences were statistically significant.

Discussion

A number of root-end filling materials have been developed by various manufacturers [19], including MTA which has adequate biological and physical properties of a good root-end filling material [20]. Despite many advantages of MTA, researchers have searched for alternative materials because of its prolonged setting time and high price [21]. Two materials based on tricalcium silicate cement, Biodentine and BA, have been introduced as alternatives to MTA [19]. Biodentine is a new calcium silicate-based material that has recently been developed as a dentine replacement [22]. It sets, however, in 10 to 12 minutes, which is a much shorter time than that of MTA and BA. Biodentine is sold as a powder packaged in capsules

(0.7 g) to be mixed with a liquid phase (0.18 mL) containing calcium chlorid [23]. BA contains 41% tricalcium silicate cement and no aluminium, and so is similar to white MTA in its chemical composition. The major difference is that tantalum oxide is used as a radiopacifier in BA instead of bismuth oxide in MTA [24].

The present study indicated that there were statistically significant differences between Biodentine and MTA or BA, but no statistically significant difference between MTA and BA. Thus, the hypothesis was rejected. Due to its short setting time and viscosity, Biodentine may be more affected by the solutions. Also, the liquid of Biodentine has calcium chloride, while the liquid of MTA and BA is distilled water, which may as well contribute to the results. Clinicians have to use different irrigation solutions, like NaOCl, EDTA, and different acids, for disinfection and also smear removal, as well as different calcium silicate-based materials for different treatment procedures such as apical resection, perforations, apexifications, and pulp cappings. So, irrigation solutions may directly contact to calcium silicate-based materials. However, different solutions can affect, negatively or positively, the performance of repair materials, and indeed some studies have shown that acidic pH and chelating agents adversely affect MTA or MTA-like materials [22,25].

Watts et al. [25] concluded that low pH causes significant decrease in compressive strength of MTA. Camilleri et al. [22] also reported that acid-etch procedures affect the compressive strength and surface micro-hardness of MTA, a calcium silicate-based cement similar to Biodentine. Biodentine demonstrated both structural and chemical changes when etched with 37% phosphoric acid [22]. Also, the calcium to silicon ratio of the etched Biodentine was lower than that of the unetched Biodentine surface. 37% phosphoric acid caused both decrease in microhardness and release of Ca from Biodentine surface in the same study [22]. Chitosan is a polysaccharide with chelating capacity [26], and also propolis possesses antibacterial, antifungal, antiviral, antiinflammatory, antioxidant, and immunomodulatory properties [27]. Therefore, we have chosen these natural materials to compare with acetic acid and EDTA for their effects on calcium silicate-based materials.

According to Silva et al. [28], 15% EDTA and 0.2% chitosan had the greatest effect on root dentine demineralisation, followed by 10% citric acid and 1% acetic acid. Pimenta et al. [9] shave concluded that the similar chelating effect of 0.2% chitosan compared to 15% EDTA and 10% citric acid, combined with its already advantageous properties and low concentration, may make chitosan a preferred solution for dentine demineralisation. In the present study, 1% acetic acid solution was chosen as a control of the effectiveness of chitosan; however, the effect of 1% acetic acid on the materials was higher than that of chitosan. An interesting finding obtained in this study was that, although chitosan had similar chelation activity to that of EDTA, the chitosan was less effective on tricalcium silicate-based materials than EDTA and acetic acid. Thus, the chitosan solution may be safely used together with root repair materials.

In previous studies demonstrated that Ca ion release from Biodentine was higher than MTA in distilled water after 28 days [23,29]. In the present study, we tried to simulate clinical conditions, where irrigation solutions are in contact with root repair materials only for a short time in clinic treatment process. Thus, a 10 min immersion time was chosen, and the results obtained that Ca ion release from Biodentine was higher than that from MTA and BA in the distilled water group. This is consistent with the results of Han's [29] and Gandolfi's [23] studies. The high Ca release can be correlated with the presence of calcium chloride in Biodentine liquid [23]. In addition, the fast hydration reaction of Biodentine may be correlated with the high calcium release.

Limitations of the present study should be noted. First, ph-measurement of the irrigation solutions was not measured, a chemical reaction between irrigation solutions and the test materials will be possible. This possible reaction is irrefutable, of course. Therefore, further research is needed to understand the chemical reactions between irrigation solutions of Biodentine, BA, and MTA. Second, surface structure evaluation of test materials with SEM was not done. SEM evaluation can be more informative fort his study.

Conclusion

Propolis and chitosan used for experimental purposes showed similar effects as distilled water on MTA, BA, and Biodentine. Thus, chitosan and propolis natural irrigation solutions may be preferred to EDTA, when used with MTA, BA, and Biodentine. Further research is needed to complement the results of the present study.

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References

1. Yan P, Peng B, Fan B, Fan M, Bian Z. The effects of sodium hypochlorite (5.25%), Chlorhexidine (2%), and Glyde File Prep on the bond strength of MTA-dentin. J Endod 2006; 32: 58-60.
2. Zhu WC, Gyamfi J, Niu LN. Anatomy of sodium hypochlorite accidents involving facial ecchymosis - a review. J Dent 2013; 41: 935-948.
3. Spano JC, Silva RG, Guedes DF. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. J Endod 2009; 35: 727-730.
4. Smith JB, Loushine RJ, Weller RN. Metrologic evaluation of the surface of white MTA after the use of two endodontic irrigants. J Endod 2007; 33: 463-467.

5. Uyanik MO, Nagas E, Sahin C, Dagli F, Cehreli ZC. Effects of different irrigation regimens on the sealing properties of repaired furcal perforations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107: e91-95.
6. Arnold JW, Rueggeberg FA, Anderson RW. The disintegration of superEBA cement in solutions with adjusted pH and osmolarity. J Endod 1997; 23: 663-668.
7. Roy CO, Jeansonne BG, Gerrets TF. Effect of an acid environment on leakage of root-end filling materials. J Endod 2001; 27: 7-8.
8. Awawdeh L, Al-Beitawi M, Hammad M. Effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against Enterococcus faecalis: a laboratory study. Aust Endod J 2009; 35: 52-58.
9. Pimenta JA, Zaparolli D, Pecora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J 2012; 23: 212-217.
10. Akncbay H, Senel S, Ay ZY. Application of chitosan gel in the treatment of chronic periodontitis. J Biomed Mater Res B Appl Biomater 2007; 80: 290-296.
11. Kishen A, Shi Z, Shrestha A, Neoh KG. An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod 2008; 34: 1515-1520.
12. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 2003; 4: 1457-1465.
13. Hu F, Hepburn HR, Li Y. Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. J Ethnopharmacol 2005; 100: 276-283.
14. de Rezende GP, da Costa LR, Pimenta FC, Baroni DA. In vitro antimicrobial activity of endodontic pastes with propolis extracts and calcium hydroxide: a preliminary study. Braz Dent J 2008; 19: 301-305.
15. Park YK, Koo MH, Abreu JA. Antimicrobial activity of propolis on oral microorganisms. Curr Microbiol 1998; 36: 24-28.
16. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. Caries Res 1991; 25: 347-351.
17. Koo H, Rosalen PL, Cury JA. Effect of Apis mellifera propolis from two Brazilian regions on caries development in desalivated rats. Caries Res 1999; 33: 393-400.
18. Bretz WA, Chiego DJ, Marcucci MC. Preliminary report on the effects of propolis on wound healing in the dental pulp. Z Naturforsch C 1998; 53: 1045-1048.
19. Grech L, Mallia B, Camilleri J. Characterization of set Intermediate Restorative Material, Biodentine, Bioaggregate and a prototype calcium silicate cement for use as root-end filling materials. Int Endod J 2013; 46: 632-641.
20. Lamb EL, Loushine RJ, Weller RN, Kimbrough WF, Pashley DH. Effect of root resection on the apical sealing ability of mineral trioxide aggregate. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 732-735.
21. Saghiri MA, Lotfi M, Saghiri AM. Effect of pH on sealing ability of white mineral trioxide aggregate as a root-end filling material. J Endod 2008; 34: 1226-1229.
22. Camilleri J. Investigation of Biodentine as dentine replacement material. J Dent 2013; 41: 600-610.
23. Gandolfi MG, Siboni F, Botero T. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. J Appl Biomater Funct Mater 2015;131:43-60.
24. 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.
25. 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.
26. Kurita K. Chitin and chitosan: functional biopolymers from marine crustaceans. Mar Biotechnol (NY) 2006; 8: 203-226.
27. Kayaoglu G, Omurlu H, Akca G. Antibacterial activity of Propolis versus conventional endodontic disinfectants against Enterococcus faecalis in infected dentinal tubules. J Endod 2011; 37: 376-381.
28. Silva PV, Guedes DF, Nakadi FV, Pecora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J 2013; 46: 332-338.
29. Han L, Kodama S, Okiji T. Evaluation of calcium-releasing and apatite-forming abilities of fast-setting calcium silicate-based endodontic materials. Int Endod J 2015; 48:124-130.

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