Effects of various additives on antimicrobial, physical and chemical properties of mineral trioxide aggregate (MTA)

Flaviana Bombarda de **ANDRADE**[1]
Murilo Priori **ALCALDE**[2]
Bruno Martini **GUIMARÃES**[3]
Pedro **BELEZE NETO**[4]
Marcela Paola Castro **ARIAS**[5]
Clovis Monteiro **BRAMANTE**[1]
Ivaldo Gomes de **MORAES**[1]
Marco Antonio Hungaro **DUARTE**[1]

doi: http://dx.doi.org/10.14436/2178-3713.5.1.019-029.oar

ABSTRACT

Objective: To assess the antimicrobial activity, solubility, setting time, flowability, pH, calcium release and surface characteristic of mineral trioxide aggregate (MTA) associated with different substances: 1% sodium hypochlorite gel, 2% chlorhexidine gel, K-Y gel, distilled water associated with 10% propylene glycol (CCPG), saline solution, and distilled water alone. **Methods:** The antimicrobial test included *Streptococcus mutans, Lactobacillus casei, Enterococcus faecalis and Candida albicans*, and the direct contact method was used. To assess setting time and flowability, ASTM 266/08 and ADA 57/2007 specifications were used. To assess pH and calcium release, the different types of material were inserted into retrograde cavities of acrylic resin teeth and immersed in ultrapure water for reading at different periods. The latter was performed with a pHmeter and an atomic absorption spectrophotometer. To assess the surface characteristics, acrylic teeth were analyzed under scanning electron micrograph (SEM). **Results:** The results yielded by the antimicrobial activity test revealed that additives associated with MTA were more inhibitory than bactericidal, with chlorhexidine achieving the best results. The K-Y vehicle made the additive more soluble than others. There were statistical differences amongst groups ($p > 0.05$) for flowability. The greatest setting time was observed in the CCPG group. The K-Y group presented the lowest pH and calcium release values in the 3-hour period. In the other periods, there was greater uniformity amongst groups. As regards the surface characteristics analysis, the CCPG group presented the greatest porosity ($p < 0.05$). **Conclusions:** Only the chlorhexidine gel brought some improvement to the antimicrobial effect, K-Y gel interfered in the physical-chemical properties of MTA and the addition of CCPG provided the greatest porosity.

Keywords: Antimicrobial sensitivity test. Calcium release. Solubility. pH level. Physical-chemical properties. MTA.

How to cite this article: Andrade FB, Alcalde MP, Guimarães BM, Beleze Neto P, Arias MPC, Bramante CM, Moraes IG, Duarte MAH. Effects of various additives on antimicrobial, physical and chemical properties of mineral trioxide aggregate (MTA). Dental Press Endod. 2015 Jan-Apr;5(1):19-29.
DOI: http://dx.doi.org/10.14436/2178-3713.5.1.019-029.oar

[1]Professor, Universidade de São Paulo, School of Dentistry, Department of Cosmetic Dentistry, Endodontics and Dental Material, Bauru, São Paulo, Brazil.

[2]Masters student in Endodontics, Universidade de São Paulo, School of Dentistry, Bauru, São Paulo, Brazil.

[3]PhD resident in Endodontics, Universidade de São Paulo, School of Dentistry, Bauru, São Paulo, Brazil.

[4]Undergraduate in Dentistry, Universidade de São Paulo, School of Dentistry, Bauru, São Paulo, Brazil.

[5]MSc in Endodontics, Universidade de São Paulo, School of Dentistry, Bauru, São Paulo, Brazil.

» The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

Submitted: December 9, 2014. Revised and accepted: January 5, 2015.

Contact address: Bruno Martini Guimarães
Faculdade de Odontologia de Bauru – USP
Al. Octávio Pinheiro Brisolla, 9-75 – Bauru/SP – CEP: 17012-901 - Brazil
E-mail: brunomgui@usp.br

Introduction

Mineral trioxide aggregate (MTA) has been recognized as a bioactive material with excellent chemical and physical properties, such as low toxicity, non-carcinogenicity, low solubility in tissue fluids, and dimensional stability.[1] These advantages are responsible for its successful use in many clinical applications, including direct pulp capping,[2] apexification, external root resorption repair and root-end filling material.[3,4]

The main advantages of this cement include biocompatibility and the ability to stimulate hard tissue repair.[5,6] The hydrophilic nature of its powder particles allows its use in humid environments, such as in perforation seal and apical surgeries.[7,8]

With regards to setting time, the characteristics of MTA depend on the size of particles, the powder-liquid ratio, temperature and presence of humidity.[9] In the case of ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland), the setting occurs within 2 hours and 47 minutes;[8] the setting time is related with the presence of calcium sulphate. The manufacturer of MTA Angelus (Angelus, Londrina/PR, Brazil) removed calcium sulfate from the composition, which provides a setting time within 10 and 15 minutes.[6]

However, when MTA is manipulated with distilled water, it produces a grainy and sandy mixture, which hardens easily and hinders insertion, filling and condensation, particular in regions of difficult access.[10] Some alternatives to distilled water have been studied so as to improve MTA properties, for instance, propylene glycol.[11]

Kogan et al[10] identified the types and quantities of additives necessary to obtain better properties of ProRooT MTA used in clinical procedures. They found that MTA mixed with sodium hypochlorite (NaOCl) presented with good physico-chemical properties and working times. Other substances, such as K-Y gel and 2% chlorhexidine (CHX) gel favoured better flowability, but did not interfere in the chemical properties of the material.

MTA antimicrobial activity is a controversial issue due to different results found in the literature.[12] Its antimicrobial activity is caused by its high pH level and setting time as a result of MTA hydration which, in turn, results in CaOH.[2 8,13] pH diffusion in antimicrobial activity is poor in solid culture media. Agar diffusion requires that substances have similar diffusion gradients fin order to have effectiveness compared.[14]

Thus, the objective of the present study was to assess the antimicrobial activity, solubility, setting time, flowability, pH, calcium release and surface characteristic of MTA associated with different substances: 1% sodium hypochlorite gel, 2% chlorhexidine gel, K-Y gel, distilled water associated with 10% propylene glycol (CCPG), saline solution, and distilled water alone. The null hypothesis is that the substance associated with MTA does not interfere in its antimicrobial activity and physical-chemical properties.

Material and Methods

The MTA cement (Angelus, Londrina, Paraná, Brazil) used in this study was mixed with different vehicles, according to the following groups: Group 1, 1% NaOCl gel; Group 2, 2% CHX gel; Group 3, water - propylene glycol mix (90% water and 10% propylene glycol); Group 4, K-Y gel (Johnson & Johnson, São José dos Campos, São Paulo, Brazil); Group 5, saline solution; Group 6, distilled water (control group). Samples were weighed on a precision scale and the ratio of manipulation was 1.0 g of MTA powder for 0.35 mL of the tested substance (according to the group).

Antimicrobial analysis

Samples of each substance were inserted into sterilized glass tubes, 3 mm in height and 4 mm in internal diameter, obtained from glass tubes cut by an Isomet cutting machine (Buehler, Lake Bluff, Illinois, USA) with a diamond disc (Extec Corp., Enfield, Ct, USA). Cement was tested in two experimental periods: immediately and 24 hours after mixing. Subsequently, samples were kept in a sterilized box at 37 °C under controlled humidity.

Streptococcus mutans (ATCC 25175), *Lactobacillus casei* (ATCC 7469), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (NTCC 3736) were used for antimicrobial activity assessment. Brain Heart Infusion (BHI, Difco, BD, Sparks, MD, USA) was the culture media used for bacterial growth, and Sabouraud (dextrose) media (Difco, BD, Sparks, MD, USA) was used for fungal growth. 15% percent agar was added to the solid medium and spread on 15 x 150 mm Petri dishes.

The reactivated cultures were grown in tubes containing 3 mL of BHI, changed once a day for three

days, to achieve the exponential growth of microorganisms. Each specimen was frozen and stored.

Cell suspension of each tube was spectrophotometrically assessed (Bel Photonics do Brasil Ltda, Osasco, São Paulo, Brazil) at 540 nm, in order to match absorbance equivalent to the 0.5 tube (1.5×10^8 CFU/mL) in the McFarland scale.

For the direct contact method, the test was carried out during the exponential phase for each microorganism and determined during the pilot experiment. The inoculum and the samples were immersed in tubes and tested in triplicate. For positive controls, only microorganisms were used; whereas for negative controls, only cement samples without microorganisms were used in the culture media. Samples had been previously mixed and stored in sterilized boxes.

Subsequently, the 24-hour and immediate samples were placed into glass tubes and the antimicrobial activity was registered every 2 hours, for up to 10 hours, using a spectrophotometer. A total of 50 µL of cell suspension from each tube were seeded onto BHI agar plates which were then incubated from 24 to 48 hours at 37 °C, so as to monitor microbial growth by means of CFU (colony forming units). Samples were categorized as follows: 0 = no colonies; 1 = up to 100 CFUs; 2 = more than 100 CFUs; 3 = more than 100 CFUs, with confluent growth. Purity of cultures was confirmed by Gram staining and colony morphology.

Solubility test

Solubility was assessed by means of the method described by Estrela et al,[15] following #57 ADA specification. Cements were mixed and compacted into ring molds, producing round specimens, 20 mm in diameter and 1.5 mm thick. A nylon string was inserted into the cement in order to keep specimens suspended. Subsequently, they were immersed into distilled water during the experimental period. The specimens were covered with a glass plate with a sheet of plastic in between. Samples were stored in an oven at 37 °C, for three times MTA setting time.

The specimens were then removed from the molds, particles removed from the surface and weighed for an accuracy of 0.001 g. All samples were then placed in a shallow dish and 50 mL of distilled water were added for 7 days, without touching the dish walls. After this period, the specimens were removed, washed, dried and heated in a dehumidifier for 24 hours so as to be weighed again. Solubility was calculated by recording the difference in mass as a percentage of the original mass during the immersion period. This difference was multiplied by 100 and divided by total initial mass.

Setting time

Samples were made according to #57 ADA specifications. The determination for the setting time of the tested cements was the #C266-08 ASTM (American Society for Testing and Materials). The setting time was analyzed with specimens kept in a humidity chamber at 37 °C with relative humidity of 100%.

The cements were proportioned and inserted into metallic rings, 10 mm in internal diameter and 2 mm in height. Afterwards, surfaces were penetrated with a 113.5-g Gillmore needle at the initial setting time and a 453.6-g Gillmore needle at the final setting time. This procedure was repeated at two-minute intervals. In both records, the setting time was recorded at the moment when the needle failed to leave a complete circular indentation. Three specimens were used for each type of material.

Flowability test

The flowability of each group was determined following methods similar to that described by #57 ADA specifications.

A total of 0.5 mL of the tested cement was placed on a glass plate (40 x 40 x 5 mm) by means of a graduated disposable 3-mL syringe. After three minutes of mixing, another glass plate, with a mass of 20 ± 2 g and a load of 100 g, totaling 120 g, was applied centrally on top of the material and maintained at a temperature of 230 ± 20 °C and a humidity of 50 ± 5%. Ten minutes after the initial mixing, the load was removed, and the average of the major and minor diameters of the compressed discs was measured by means of a digital caliper with resolution of 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan). If both measurements were consistent within 1.0 mm, the results were recorded. If the major and minor diameter discs were not uniformly circular or did not match within 1.0 mm, the test was repeated. The mean of the three measurements for each cement was considered the flowability

of the material. According to #57 ADA specification, for the flowability test, a disc of at least 25 mm in diameter must be obtained.

pH level and calcium release

Sixty five artificial teeth made of resin that included a root-end preparation were filled with the different tested cements and immersed individually in 10 mL of deionized water. An artificial tooth with no filling material was immersed and used as the control. After 3, 24, 72 and 168 hours, the teeth were placed in new flasks and the water in which they had been kept in had its pH level determined and its calcium release was measured (mg/dL) as well.

pH level measurements were obtained by means of a pHmeter of which precision was calibrated using buffer solutions with pH levels of 4, 7 and 14.

Calcium ion release was measured by means of an atomic absorption spectrophotometer (Thermo Scientific, Solaar M Series AA Spectrophotometer, Cambridge, England), equipped with a calcium specific hollow cathode lamp.

In order to avoid potential interference of alkaline metals, 0.20 mol/L lanthanum chloride solution was used. Standard calcium solutions containing 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, and 80 mg/L were used. Ultrapure water was used as control. Ca++ ion release readings were carried out at the same time when pH level was determined.

Surface characteristics analysis

For this analysis, 30 acrylic teeth were filled with the different types of material tested, thereby totalling five specimens per group. After filling, the teeth were stored at 37 °C and 100% humidity for 24 hours for cement setting. At the end of the 24-hour period, the specimens were prepared and analyzed under a scanning electron microscope (Aspex Express, FEI Company, Eindhoven, Netherlands),observing the surface characteristics of the different types of material tested as well as their porosity (Fig 1).

Statistical analysis

Data obtained by means of direct contact, solubility, pH level, calcium ion release, setting time and flowability tests were normally distributed. Analysis of variance (ANOVA) was used to perform multiple comparison tests, whereas Tukey-Kramer test was used for individual comparisons. For comparison of surface characteristics, Kruskal-Wallis test for global comparison was used while Dunn test was performed for individual comparisons. Significance level was set at 5% for all tests.

Results

Direct contact test

None of the cements tested were able to completely eliminate microorganisms, thereby showing an

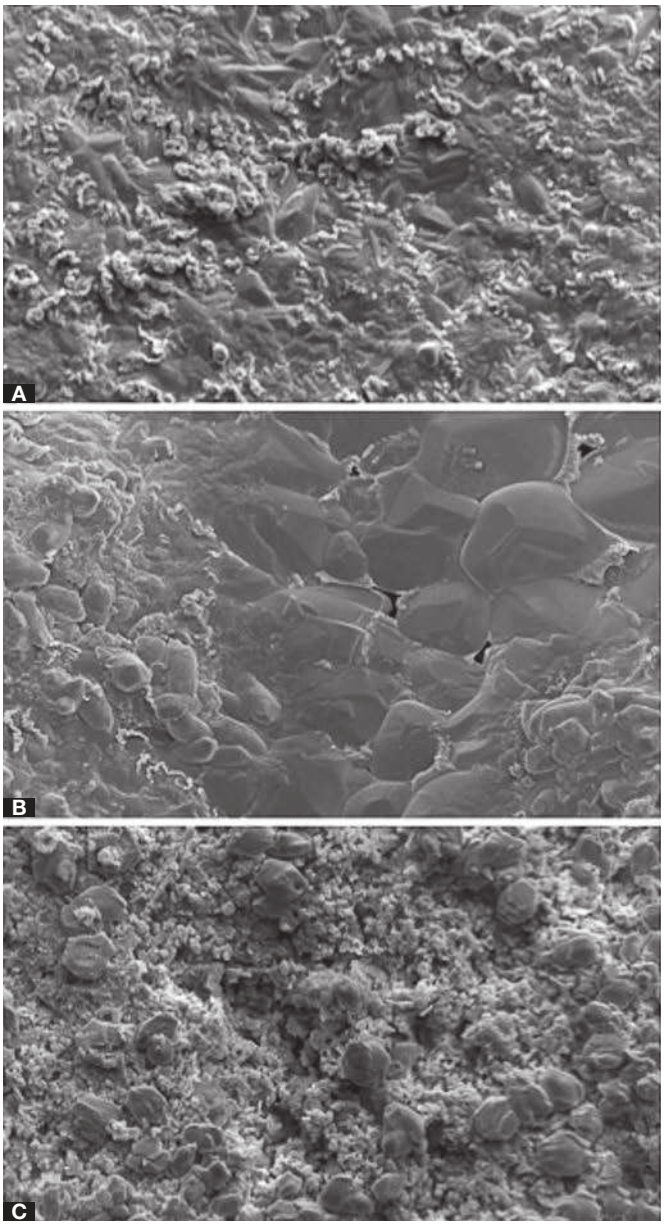

Figure 1. Representative scanning electron microscope (SEM) images of porosity scores for the surface characteristics analysis of the different types of material tested. The first image represents low porosity (**A**) followed by medium (**B**) and high (**C**) porosity.

inhibitory effect, only. *Enterococcus faecalis* showed lower proliferation between the 2nd and 6th hour when in contact with the CHX group, in comparison to the 1% NaOCl gel, K-Y gel, CCPG and saline solution groups (Fig 2). There were no statistically significant differences among groups after 8 and 10 hours, but it is possible to establish a decreasing order of inhibition: CHX > K-Y > NaOCl > CCPG > saline solution. The 24-hour MTA samples associated with CHX and K-Y vehicles promoted higher inhibition when compared to CCPG.

The effective inhibition count of CFUs was significant for 2% CHX gel after 2 hours. After 4 hours, no statistically significant differences were found ($p > 0.05$); and after 6 and 10 hours, K-Y promoted the largest growth when compared to all of the other tested vehicles (Fig 2). The different groups tested were similar after 24 hours, and showed a confluent score 3 growth.

Propylene glycol and 2% CHX gel were significantly different ($P < 0.05$) in terms of inhibition of *Lactobacillus casei*. After 4 hours, 2% CHX gel yielded better results when compared to the other groups, and K-Y gel was the most effective, followed by CCPG (Fig 3). Although CHX was the most effective, no statistically significant differences ($p < 0.05$) were found among the vehicles after 6, 8, 10 and 24 hours. Moreover, CFU counts showed no statistically significant differences ($p > 0.05$) amongst all vehicles for all time periods.

There were statistically significant differences ($p < 0.05$) between K-Y gel and CCPG after 2 hours of direct contact with *Streptococcus mutans* (Fig 4), but no differences after 4, 6, 8, 10 and 24 hours. However, on the Petri dishes, significant differences ($p < 0.05$) were found after 2, 4 and 6 hours for CHX, in comparison to the other vehicles (Fig 4) which showed few CFUs.

The results for *Candida albicans* showed statistically significant difference ($P < 0.05$) between CCPG and CHX after 2 hours. Similar results were found for CHX and distilled water (Fig 5). CFUs count had confluent growth on all dishes, without statistical significance. After 4 and 10 hours, K-Y was the weakest and promoted less inhibition. Amongst the 24-hour samples, CHX was the most effective (Fig 5).

Calcium release

For the 3-hour calcium release period, there were statistically significant differences ($P < 0.05$) among the following groups: K-Y, CCPG, distilled water and saline solution. Additionally, there were statistically significant differences ($P < 0.05$) when 1% NaOCl and 2% CHX gel were compared to distilled water. For all other comparisons, no statistically significant differences were found ($P > 0.05$).

For the 24-hour period, there were statistically significant differences ($P < 0.05$) when 2% CHX gel was compared to saline solution, 1% NaOCl gel, CCPG and K-Y gel. Moreover, there was statistically significant difference ($P < 0.05$) when distilled water was compared to K-Y and 1% NaOCl gel. For the 72-hour analysis, there were statistically significant differences ($p < 0.05$) among saline

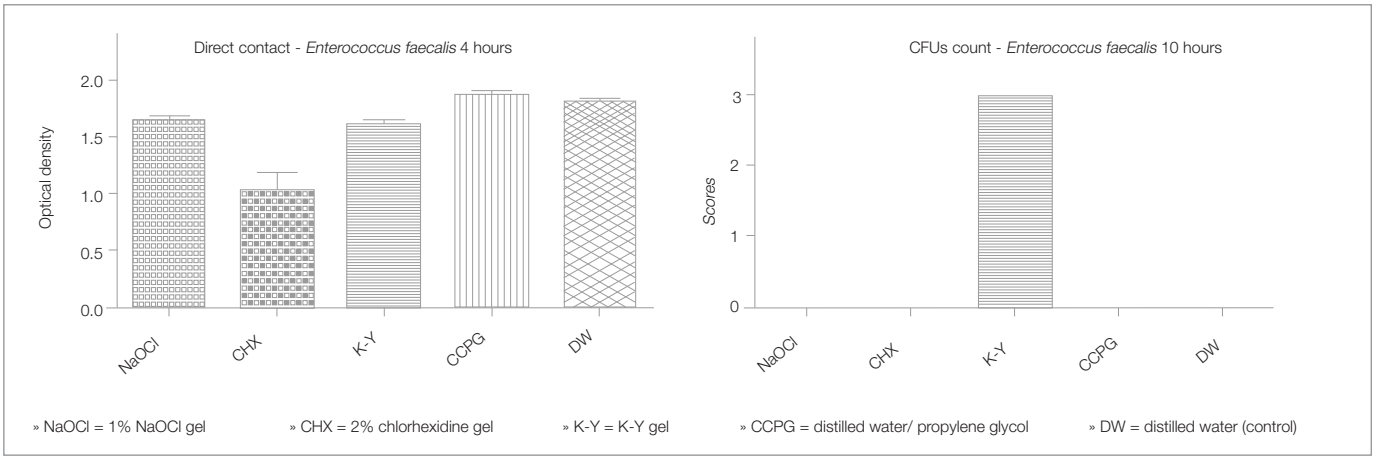

Figure 2. Graphic representation of absorbance and colony forming unit (CFU) counts on the culture media with *Enterococcus faecalis*, after 4 and 10 hours of cement manipulation with five different vehicles.

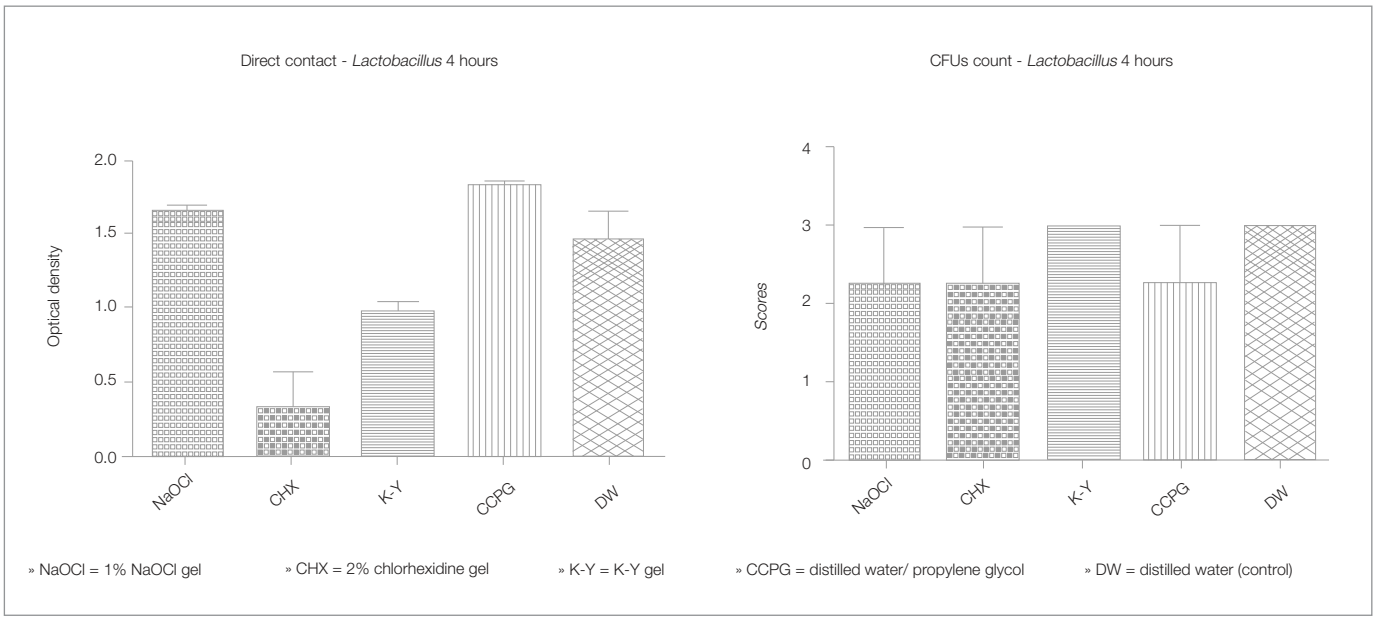

Figure 3. Graphic representation of absorbance and colony forming unit (CFU) counts on the culture media with *Lactobacillus Casei,* after 4 hours of cement manipulation with five different vehicles.

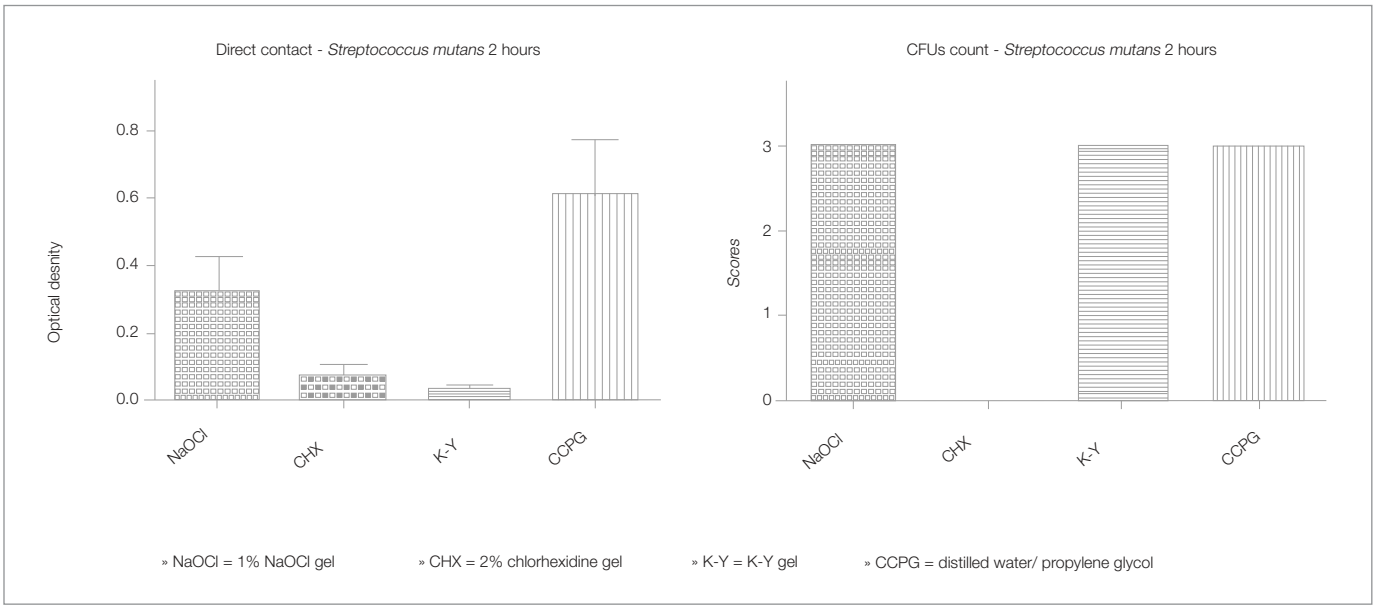

Figure 4. Graphic representation of absorbance and colony forming unit (CFU) counts on the culture media with *Streptococcus mutans,* after 2 hours of cement manipulation with five different vehicles.

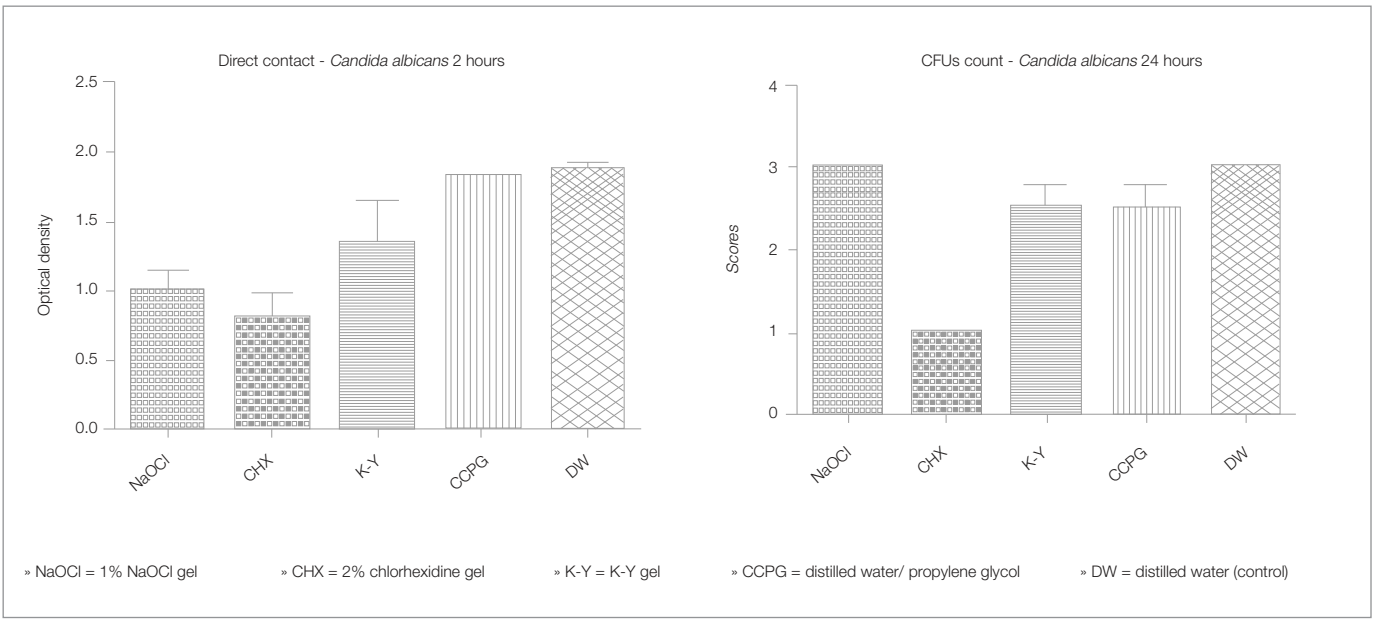

Figure 5. Graphic representation of absorbance and colony forming unit (CFU) counts on the culture media with *Candida albicans*, after 2 and 24 hours respectively of cement manipulation with five different vehicles.

solution, distilled water and 2% CHX gel groups. For the 168-hour period, there were statistically significant differences ($P < 0.05$) among distilled water, saline solution and CCPG groups (Table 1).

pH level

As regards the pH level, for the 3-hour period, there were statistically significant differences ($p < 0.05$) among CCPG, saline solution and 1% NaOCl gel groups when compared to K-Y and distilled water groups. In addition, statistically significant differences ($p < 0.05$) also occurred when 2% CHX gel, K-Y and distilled water were compared. For the 24-hour period, there were statistically significant differences ($p < 0.05$) among CCPG, saline solution and 2% CHX gel groups. Statistically significant differences ($p < 0.05$) were also found when 2% CHX gel, K-Y gel, 1% NaOCl gel and distilled water groups were compared.

For the 72-hour analysis, statistically significant differences ($p < 0.05$) occurred among 1% NaOCl gel and CCPG, saline solution, K-Y and distilled water groups. In addition, there was statistically significant difference ($p < 0.05$) when 2% CHX gel and saline solution were compared. For the 168-hour period, there were statistically significant differences ($p < 0.05$) between distilled water and CCPG, 2% CHX gel, saline solution and K-Y groups (Table 1).

Solubility test

Results reveal increased solubility of MTA manipulated with the tested vehicles, with K-Y being the most soluble and showing statistically significant differences($p < 0.05$) when compared to the other groups (Table 2).

Flowability and setting time

In terms of flowability, no statistically significant difference ($p < 0.05$) was found amongst the tested groups. For initial and final setting times, the lowest values were found for the saline solution group, whereas the highest values were found for the 2% CHX gel group. There were no statistically significant differences ($p > 0.05$) among 1% NaOCl gel, CCPG and distilled water, K-Y and saline solution, and distilled water and CCPG. The other comparisons revealed statistically significant differences ($p < 0.05$) (Table 2).

Surface characteristics

Table 2 contains the median, minimum and maximum values of scores attributed during analysis of surface characteristics of each group studied. Statistically significant difference ($p < 0.05$) were found when CCPG and CHX groups were compared.

Table 1. Mean and standard deviation of pH level and calcium ion (Ca) (mg/L) for the different types of material tested in different periods.

		3 hours	24 hours	72 hours	168 hours
NaOCl gel	pH	28.05 ± 15.77	11.15 ± 5.34	9.65 ± 3.86	14.15 ± 4.85
	Ca	11.17 ± 0.76	7.92 ± 0.77	6.75 ± 0.35	7.48 ± 0.20
CHX gel	pH	43.35 ± 16.75	37.10 ± 12.23	14.85 ± 4.23	10.10 ± 5.56
	Ca	11.43 ± 0.36	9.00 ± 1.25	7.10 ± 0.42	7.44 ± 0.49
K-Y gel	pH	17.15 ± 10.07	10.20 ± 4.79	9.90 ± 4.33	14.65 ± 7.52
	Ca	7.270 ± 1.73	7.77 ± 0.43	7.23 ± 0.26	7.06 ± 0.43
CCPG	pH	63.10 ± 26.70	12.95 ± 4.49	9.55 ± 3.42	9.35 ± 2.36
	Ca	11.97 ± 0.37	7.12 ± 0.36	7.25 ± 0.17	7.27 ± 0.44
Saline solution	pH	71.45 ± 26.43	13.35 ± 4.44	8.50 ± 2.90	8.75 ± 3.29
	Ca	11.87 ± 0.89	7.98 ± 0.89	7.68 ± 0.30	7.21 ± 0.17
Distilled water	pH	114.5 ± 51.61	24.50 ± 10.83	18.50 ± 6.21	26.00 ± 11.83
	Ca	8.10 ± 0.67	8.00 ± 0.03	7.45 ± 0.15	7.90 ± 0.23

Table 2. Mean and standard deviation of flowability (mm), initial and final setting times (minutes), solubility (percentage) and median, minimum and maximum values of scores of the surface characteristics analysis.

	Flowability	Initial setting time	Final setting time	Solubility	Surface characteristics
NaOCl gel	9.14 ± 0.84	16.78 ± 0.63	58.60 ± 2.07	0	1.0 (1.0 – 2.0)
CHX gel	9.47 ± 0.14	25.11 ± 0.07	72.62 ± 0.86	0.10 ± 0.06	0.0 (0.0 – 1.0)
K-Y gel	10.56 ± 0.82	13.11 ± 0.07	47.63 ± 0.52	0.75 ± 0.18	0.0 (0.0 – 2.0)
CCPG	8.37 ± 0.45	16.50 ± 0.70	60.62 ± 0.86	0.28 ± 0.33	2.0 (2.0 – 2.0)
Saline solution	9.22 ± 0.28	12.63 ± 0.52	45.58 ± 0.60	0	1.0 (0.0 – 1.0)
Distilled water	8.64 ± 0.87	15.25 ± 0.35	60.62 ± 0.86	0	1.0 (0.0 – 1.0)

Discussion

The interest in studying a Brazilian MTA (Angelus, Londrina, Paraná, Brazil) associated with viscous vehicles arouses from the tendency towards using these vehicles so as to improve MTA consistency, handling and clinical insertion,[6,10] particularly in sites of difficult access. The null hypothesis tested was rejected, since all vehicles negativelly affected the material antimicrobial activity and physicochemical properties.

The antimicrobial effect of MTA cement was studied by direct contact tests only, which is important for evaluation of restorative material.[16] The association between MTA and gel-like vehicles revealed limited antimicrobial activity with inhibitory effects, only. The conflicting results yielded by antibacterial and antifungal investigations on MTA might be attributed to the different specimen of microorganisms tested, the source of the preparation material[12] and the variability of methods used to study these properties.[17]

The direct contact test was used to assess the antimicrobial effect because it is considered reliable to assess the antiseptic action of restorative material. Moreover, it is indicated for low-solubility material, such as MTA.[18]

The microorganisms tested herein, *Enterococcus faecalis*[19] and *Candida albicans,*[20] were chosen due to being resistent in root canal infections. Others, however, are more prevalent in the etiology of dental caries: *Streptococcus mutans* and *Lactobacillus casei*.[21]

In previous studies, MTA associated with distilled water revealed inhibitory action over many microorganisms.[13] Against *Candida albicans*, *E. faecalis*, *Micrococcus luteus*, *S. aureus*, *S. mitis*, *Pseudomonas aeruginosa* and *E. coli*,[22] MTA was not significantly inhibitory when compared to endodontic sealers (Fill canal and Sealapex). Our results reveal differences in antimicrobial activity with positive inhibition in the culture tubes, but variability on the Petri dishes, displaying only inhibitory and non bactericidal effects.

CHX was the vehicle that stood out in the antimicrobial activity promoted by MTA, similar to previous studies. Ellepola, Samaranayake[23] and Shurrab[24] showed that modulation of CHX for *Candida ssp*, in *in vivo* colonization, promoted reduction in adherence due to changes in oral mucosa cells.[25]

The growth of *E. faecalis* and *Candida albicans* on Petri dishes proved interesting when K-Y gel was associated with MTA, mainly after 6 to 10 hours (Figs 2, 5), with high growth (CFUs) which had not been previously visible in the culture tubes.

The association between K-Y gel and MTA resulted in the highest solubility ($p < 0.05$), whereas saline solution associated with MTA resulted in the lowest solubility. Solubility is an important point to be assessed, since it is directly related to antimicrobial effects and might be influenced by the powder-water ratio and the kind of vehicle used.[26] Nevertheless, in the case of K-Y gel, high solubility did not favor a great antimicrobial activity, thereby suggesting material dissolution, however, with low release of hydroxyl and calcium ions.

Low solubility could be an option to fill the apical zone of root canals,[27] but more soluble vehicles, such as K-Y gel, might be advantageous due to hydroxide and calcium release. Large amounts of calcium hydroxide and calcium oxide are important to enhance the mineralization process that occurs during apical repair.[6]

The pH level and calcium release are important chemical properties which are directly related to mineralization process[28,29,30] and antimicrobial activity.[28,31]

The pH level analysis revealed that, within the first 3 hours, results were similar among CCPG, saline solution, 1% NaOCl gel and 2% CHX gel groups, and that there were statistically significant differences between the K-Y gel and th distilled water (control) group. The CCPG group showed a high pH level, which might be due to the presence of calcium chloride in this mixture, corroborating previous other studies.[32,33] For the 24-hour period, the 2% CHX gel group presented the highest pH level, followed by distilled water (control), saline solution and 1% NaOCl gel groups. CHX (2% gel) probably favoured prolonged hydration of MTA Angelus, thereby producing a larger amount of calcium hydroxide.

As regards calcium ion release, the distilled water group presented higher values, particularly at the 3, 72 and 168-hour periods, presenting similar results when this substance was associated with calcium hydroxide,[34] showing lower calcium release values in comparison to 2% CHX gel in the 24-hour period. Although another study[33] showed that the addition of calcium chloride increased calcium release; in the present study, calcium release values were lower than in the distilled water group. The probable reason was

that calcium chloride solution was associated with propylene glycol while the other study associated calcium chloride solution with distilled water.

For the 2 and 24-hour periods, the highest calcium release values were obtained in the distilled water and saline solution groups, whereas the lowest values were found in the K-Y gel group although this association presented the highest solubility. The possible reason was a reduction in the hydration of MTA in the initial periods and the occurrence of lower calcium hydroxide formation. For the 72 and 168-hour periods, the results yielded by the K-Y gel group were similar to the other groups tested.

Surface analysis revealed that the CCPG group presented the greatest porosity, possibly due to interference cased by hydration, once the setting time of this group was slower. The delayed setting time could have favored the great solubility and surface degradation of this type of material.

Regarding flowability, no statistically significant differences were found among vehicles, and none of them had flowability values above the minimum recommended by ADA #57 standards, which is 20 mm. All vehicles presented values lower than 10 mm, except for the K-Y gel group, which presented mean values of 10.56 mm. The present study found that K-Y gel, 1% NaOCl gel and 2% CHX gel groups enhanced the handling of MTA, in agreement with another study.[35]

With regards to setting time, all vehicles favoured MTA setting, especially when it was associated with K-Y gel and 1% NaOCl gel, both of which sped setting time up when compared to distilled water (control), also agreeing with other studies.[10,35]

The use of 2% CHX gel caused a delay in setting time, which is disagreement with a previous study[10] associating MTA with 2% CHX gel. Possibly, the vehicle used in the preparation of the CHX gel was different in terms of composition. The present study used the Biodinâmica CHX gel (Ibiporã, Paraná, Brazil), which includes distilled water in its composition, while the other study[10] used Ultradent CHX gel (Consepsis V) which probably did not have distilled water in its composition, hence the non-occurrence of setting.

In the saline solution group, setting time was sped up, in contrast with another study[10] which observed a delay. Quite possibly, the reason for this difference involves the type of MTA used. In this study, the MTA Angelus used does not have calcium sulfate in its composition;[6] while the other study[10] used Pro Root MTA (Dentsply) which had calcium sulfate in its composition, which acts as a setting time retarder.

Results reveal a reduction in setting time when MTA was associated with distilled water. This is in contrast with other studies[10,35] using ProRoot MTA, but similar to Bortoluzzi et al[32] and Vivan et al[6] who also used MTA Angelus. This leads us to believe that calcium sulfate present in the Pro Root MTA delayed setting time.

Associating calcium chloride solution, a setting time accelerator,[32] with distilled water, causes a reduction in MTA setting time. However, in the present study, CCPG did not have such reduction, probably due to propylene glycol association in this mixture. Propylene glycol associated with distilled water retarded MTA Angelus setting time, which corroborates the results of another study.[36]

Conclusion

Powder-water ratio, in addition to the individual characteristics of the vehicles and the handling time could change MTA properties, including its antimicrobial activity, in agreement to Parirokh and Torabinejad.[12] Changes in solubility and introduction of new vehicles with potential antimicrobial inhibition effects can bring benefits to the clinical use of MTA, mainly with regards to improvements in consistency. However, further studies are warranted to assess all physical and biological properties of these formulations, so as to allow their clinical indication. In the present study, 2% CHX gel was the only vehicle to bring some improvements to the antimicrobial activity of MTA.

References

1. Ozdemir HO, Ozcelik B, Karabucak B, Cehreli ZC. Calcium ion diffusion from mineral trioxide aggregate through simulated root resorption defects. Dent Traumatol. 2008;24(1):70-3.
2. Hayashi M, Shimizu A, Ebisu S. MTA for obturation of mandibular central incisors with open apices: case report. J Endod. 2004;30(2):120-2.
3. Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod. 1995;21(6):295-9.
4. Vizgirda PJ, Liewehr FR, Patton WR, McPherson JC, Buxton TB. A comparison of laterally condensed gutta-percha, thermoplasticized gutta-percha, and mineral trioxide aggregate as root canal filling materials. J Endod. 2004;30(2):103-6.
5. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E. Reaction of dogs' teeth to root canal filling with mineral trioxide aggregate or a glass ionomer sealer. J Endod. 1999;25(11):728-30.
6. Vivan RR, Zapata RO, Zeferino MA, Bramante CM, Bernardineli N, Garcia RB, et al. Evaluation of the physical and chemical properties of two commercial and three experimental root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110(2):250-6.
7. Abedi HR, Ingle JI. Mineral trioxide aggregate: a review of a new cement. J Calif Dent Assoc. 1995;23(12):36-9.
8. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod. 1995;21(7):349-53.
9. Torabinejad M, Watson TF, Ford TRP. Sealing ability of a Mineral Trioxide Aggregate when used as a root end filling material. J Endod. 1993;19(12):591-5.
10. Kogan P, He JN, Glickman GN, Watanabe I. The effects of various additives on setting properties of MTA. J Endod. 2006;32(6):569-72.
11. Holland R, Ferreira LB, Souza V, Otoboni JA, Murata SS, Dezan E. Reaction of the lateral periodontium of dogs' teeth to contaminated and noncontaminated perforations filled with mineral trioxide aggregate. J Endod. 2007;33(10):1192-7.
12. Parirokh M, Torabinejad M. Mineral Trioxide Aggregate: a comprehensive literature review-part I: Chemical, Physical, and Antibacterial Properties. J Endod. 2010;36(1):16-27.
13. Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci. 2007;49(1):41-5.
14. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, et al. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(5):709-16.
15. Estrela C, Estrela CR, Guimaraes LF, Silva RS, Pecora JD. Surface tension of calcium hydroxide associated with different substances. J Appl Oral Sci. 2005;13(2):152-6.
16. Asgary S, Parirokh M, Eghbal MJ, Brink F. Chemical differences between white and gray mineral trioxide aggregate. J Endod. 2005;31(2):101-3.
17. Ribeiro CS, Scelza MF, Hirata Júnior R, Buarque de Oliveira LM. The antimicrobial activity of gray-colored mineral trioxide aggregate (GMTA) and white-colored MTA (WMTA) under aerobic and anaerobic conditions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(6):e109-12.
18. Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of mineral trioxide aggregate. J Endod. 2003;29(12):826-7.
19. Gomes BP, Pedroso JA, Jacinto RC, et al. In vitro evaluation of the antimicrobial activity of five root canal sealers. Braz Dent J. 2004;15(1):30-5.
20. Ferreira FB, Ferreira AL, Gomes BP, Souza-Filho FJ. Resolution of persistent periapical infection by endodontic surgery. Int Endod J. 2004;37(1):61-9.
21. Botelho MP, Maciel SM, Cerci Neto A, Dezan CC, Fernandes KB, Andrade FB. Cariogenic microorganisms and oral conditions in asthmatic children. Caries Res. 2011;45(4):386-92.
22. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. Int Endod J. 2005;38(8):539-43.
23. Ellepola ANB, Samaranayake LP. Adjunctive use of chlorhexidine in oval candidoses: a review. Oral Dis. 2001;7(1):11-7.
24. Shurrab MY. Antimicrobial efficiency of some antiseptic products on endodontic microflora isolated from gangrenous pulp tissue. J Contemp Dent Pract. 2006;7(4):53-62.
25. Pizzo G, Giuliana G, Milici ME, D'Angelo M. Effect of antimicrobial mouthrinses on the in vitro adhesion of Candida albicans to human buccal epithelial cells. Clin Oral Investig. 2001;5(3):172-6.
26. Fridland M, Rosado R. MTA solubility: a long term study. J Endod. 2005;31(5):376-9.
27. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TRP. The constitution of mineral trioxide aggregate. Dent Mater. 2005;21(4):297-303.
28. Estrela C, Sydney GB, Bammann LL, Felippe Junior O. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 1995;6(2):85-90.
29. Estrela C, Pesce HF. Chemical analysis of the formation of calcium carbonate and its influence on calcium hydroxide pastes in connective tissue of the dog--Part II. Braz Dent J. 1997;8(1):49-53.
30. Narita H, Itoh S, Imazato S, Yoshitake F, Ebisu S. An explanation of the mineralization mechanism in osteoblasts induced by calcium hydroxide. Acta Biomater. 2010;6(2):586-90.
31. Kontakiotis EG, Wu MK, Wesselink PR. Effect of calcium hydroxide dressing on seal of permanent root filling. Endod Dent Traumatol. 1997;13(6):281-4.
32. Bortoluzzi EA, Broon NJ, Bramante CM, Felippe WT, Tanomaru Filho M, Esberard RM. The influence of calcium chloride on the setting time, solubility, disintegration, and pH of mineral trioxide aggregate and white Portland cement with a radiopacifier. J Endod. 2009;35(4):550-4.
33. Bortoluzzi EA, Broon NJ, Duarte MAH, Demarchi CCD, Bramante CM. The use of a setting accelerator and its effect on pH and calcium ion release of mineral trioxide aggregate and white Portland cement. J Endod. 2006;32(12):1194-7.
34. Simon ST, Bhat KS, Francis R. Effect of four vehicles on the pH of calcium hydroxide and the release of calcium ion. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;80(4):459-64.
35. AlAnezi AZ, Zhu Q, Wang YH, Safavi KE, Jiang J. Effect of selected accelerants on setting time and biocompatibility of mineral trioxide aggregate (MTA). Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(1):122-7.
36. Duarte MA, Alves de Aguiar K, Zeferino MA, Vivan RR, Ordinola-Zapata R, Tanomaru-Filho M, et al. Evaluation of the propylene glycol association on some physical and chemical properties of mineral trioxide aggregate. Int Endod J. 2012;45(6):565-70.