

Evaluation of the pH, calcium release and antibacterial activity of MTA Fillapex

Avaliação do pH, liberação de cálcio e atividade antibacteriana do MTA Fillapex

Milton Carlos KUGA^a, Gisele FARIA^a, Paulo Henrique WECKWERTH^b, Marco Antonio Hungaro DUARTE^c, Edson Alves de CAMPOS^a, Marcus Vinicius Reis SÓ^d, Kennia Scapin VIOLA^a

^aFaculdade de Odontologia, UNESP – Univ Estadual Paulista, Araraquara, SP, Brasil

^bUSC – Universidade Sagrado Coração, Bauru, SP, Brasil

^cFaculdade de Odontologia, USP – Universidade de São Paulo, Bauru, SP, Brasil

^dFaculdade de Odontologia, UFRGS – Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil

Resumo

Objetivo: O estudo avaliou, em diversos períodos de análise, o pH e liberação de cálcio e a atividade antibacteriana proporcionada pelo MTA Fillapex, em relação ao Sealapex e AH Plus. **Material e método:** Tubos de polietilenos foram preenchidos com um dos cimentos e imersos em água destilada. Após de 24 horas, 14 e 28 dias, o valor do pH e o cálcio liberado pelos cimentos foram avaliados diretamente na água destilada em que os espécimes permaneceram imersos. A atividade antibacteriana dos cimentos foi avaliada em culturas de *Enterococcus faecalis* ou *Staphylococcus aureus*, por meio do teste de difusão em ágar. Os dados obtidos foram submetidos aos testes de ANOVA e Tukey ($\alpha=0.05$). **Resultado:** Em todos os períodos analisados, o Sealapex proporcionou os maiores valores de pH ($p<0,05$) em relação aos demais cimentos e o MTA Fillapex proporcionou maiores valores que o dos AH Plus ($p<0,05$). Em 14 dias, o MTA Fillapex promoveu maior liberação de cálcio que o proporcionado pelo Sealapex ($p<0,05$). Em 28 dias, o Sealapex proporcionou maior liberação de cálcio que o MTA Fillapex ($p<0,05$). Em todos os períodos, o AH Plus apresentou a menor liberação de cálcio em relação aos outros cimentos ($p<0,05$). Em relação ao *E. faecalis*, não foram observadas diferenças ($p>0,05$) entre as zonas de inibição de crescimento bacteriano produzidas pelos diversos cimentos. Em relação ao *S. aureus*, o Sealapex apresentou maior atividade antibacteriana que o MTA Fillapex e o AH Plus ($p<0,05$), que por sua vez foram semelhantes entre si ($p>0,05$). **Conclusão:** Após o período final de avaliação, o pH e a liberação de cálcio proporcionado pelo MTA Fillapex foram menores que os do Sealapex e maiores que os do AH Plus. A atividade antimicrobiana do MTA Fillapex não diferiu dos demais cimentos endodônticos.

Descritores: Cálcio; endodontia; *Enterococcus faecalis*; *Staphylococcus aureus*.

Abstract

Objective: This study evaluated, in several analysis periods, pH and calcium release and antibacterial activity provided by MTA Fillapex sealer compared to Sealapex and AH Plus sealers. **Material and method:** Polyethylene tubes were filled with a sealer and immersed in distilled water. After 24 hours, 14 and 28 days, pH and calcium release by endodontic sealers were evaluated directly in water which the tubes were stored. Sealers antibacterial activity was evaluated against *Enterococcus faecalis* and *Staphylococcus aureus* by means of agar diffusion test. All data were submitted to ANOVA and Tukey tests ($\alpha=0.05$). **Result:** In all periods evaluated, Sealapex had the highest pH value ($p<0.05$) in comparison to other sealers and MTA Fillapex provided higher pH values than AH Plus ($p<0.05$). In 14-days period, MTA Fillapex had greater calcium release value than Sealapex ($p<0.05$). In 28-days period, Sealapex provided higher calcium release than MTA Fillapex ($p<0.05$). In all periods, AH Plus provided lower calcium release than other sealers ($p<0.05$). In relation to *E. faecalis*, there were no differences among the sealers, in relation to antibacterial activity ($p>0.05$). In relation to *S. aureus*, Sealapex presented better antibacterial effectiveness than the MTA Fillapex and AH Plus ($p<0.05$), which were similar each other ($p>0.05$). **Conclusion:** In final evaluation period, pH values and calcium release provided by MTA Fillapex were lower than provided by Sealapex and higher than provided by AH Plus. The MTA Fillapex antimicrobial action was similar to other endodontic sealers.

Descriptors: Calcium; endodontics; *Enterococcus faecalis*; *Staphylococcus aureus*.

INTRODUCTION

Mineral trioxide aggregate (MTA) is indicated to be used in root perforations, root-end cavities, for obturation of the apical portion of immature teeth, pulp capping, and pulpotomy¹. Despite the favorable biological properties, MTA does not exhibit the physical properties needed to be used as an endodontic sealer². Newer developments of MTA include its use as root canal sealer. Currently, three MTA sealer formulations are available: Endo-CPM-Sealer (EGEO, Buenos Aires, Argentina), ProRoot Endo Sealer (Dentsply Maillefer, Ballaigues, Switzerland), and experimental cement (MTAS - mixture of 80% white Portland cement and 20% bismuth oxide with addition of water soluble polymer)³.

MTA-based sealers are biocompatible, stimulate mineralization⁴, and exhibit higher push-out strengths than traditional oxide zinc and eugenol cement⁵. Recently a new formulation of MTA-based cement (MTA Fillapex, Angelus Soluções Odontológicas, Londrina, PR, Brazil) was launched to be used as root canal sealer. Its satisfactory biological properties, antibiofilm activity properties and easy handling enable the use in root canal^{2,6,7}.

MTA contain calcium oxide and presents a similar mechanism of action to calcium hydroxide⁸. Calcium hydroxide or calcium oxide-containing cements have been suggested as obturating materials because of their ability to dissociate into calcium and hydroxyl ions, resulting in a higher pH in the adjacent medium and inducing mineralized tissue formation^{4,9}. Similar condition occurs to MTA-based sealers¹⁰.

Besides inducing mineralized tissue formation, another desirable property of root canal sealers is the antibacterial activity. Root canal sealers should be able to eliminate residual pathogens, and prevent canal reinfection in order to create a favorable environment for the healing process¹¹. Several endodontic bacterial strains are inhibited by Sealapex and AH Plus sealers¹¹. Therefore, these endodontic sealers can be used in comparison test with new materials.

Thus, the aim of this study was to evaluate pH and calcium release and antibacterial activity of MTA Fillapex sealer compared to AH Plus and Sealapex (new formulation) sealers.

MATERIAL AND METHOD

1. Sample Preparation

Root canal sealers used, their formulations and manufacturers are listed in Table 1.

All sealers were mixed according to manufacturer's instructions. Thirty polyethylene tubes measuring 10 mm in length and 1.5 mm in internal diameter were filled with the fresh mixtures of evaluated sealers, using a Lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland). For pH and calcium release evaluation, 10 samples from each studied sealer were prepared. The tubes filled with the fresh mixtures were weighed to check the standardization of sealer amount. They were placed in polypropylene flasks (Injeplast, São Paulo, SP, Brazil) containing 10 mL of neutral pH distilled water and kept in an oven at 37 °C (Farmen, São Paulo, SP, Brazil). Previous to the immersion of specimens, the pH and calcium concentration of distilled water were verified, attesting pH 6.8 and total absence of calcium. Evaluations of pH and calcium release were carried out after 24 hours, 14 and 28 days. After each period of immersion, the tubes were removed and placed into another flask with the same volume of new distilled water.

2. Analysis of pH and Calcium Release

Measurement of pH was performed with a pH meter (model DM22, Digimed, São Paulo, SP, Brazil) previously calibrated with solutions of known pH in constant temperature (25 °C)¹². After removal of the specimens, the flasks were placed in a shaker (251, Farmen) for 5 seconds, before pH measurement. The control procedure included measuring the pH of the distilled water in which no samples were immersed.

The calcium release was measured using an atomic absorption spectrophotometer (AA6800, Shimadzu, Tokyo, Japan), equipped with a calcium specific hollow cathode lamp as described by Vasconcelos et al.¹² (2009). Briefly, the conditions for use of the appliance were determined following the manufacturer's instructions, using a wavelength of 422.70 nm, gap of 0.2 nm, current of 10 mA in the lamp, and slightly reducing stoichiometry, kept by an acetylene flow of 2.0 L per minute, supported by the air. A lanthanum chloride solution at concentration 10 g/L was

Table 1. Constituents and manufacturers of the root canals sealers

Sealer	Composition	Manufacturer
MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, pigments	Angelus Ind Prod Odontológicos S/A, Londrina, PR, Brazil
AH Plus	Paste A: epoxy resin; calcium tungstate; zirconium oxide; aerosil and iron oxide Paste B: aminoadamantane; N,N'-dibenzyl-5-oxa-nonandiamine-1,9; TCD-diamine; calcium tungstate; zirconium oxide; aerosil, and silicone oil	Dentsply De Trey, Konstanz, Germany
Sealapex (new formulation)	Base: calcium oxide, zinc oxide, sulfonamides, and silica Catalyst: bismuth trioxide, polymethyl methacrylate, methyl salicylate, titanium dioxide, silica, pigments, isobutyl salicylate	Kerr/Sybron, Romulus, MI, USA

used to eliminate the interference of phosphates and sulfates and the possibility of formation of refractory oxides. A standard stock solution of 10 mg/dL was diluted in water to achieve the following concentrations: 0.025 mg/dL, 0.05 mg/dL, 0.1 mg/dL, 0.25 mg/dL, 0.5 mg/dL, and 1.0 mg/dL. The results were calculated according to a standard curve, established on the basis of solutions with pre-defined calcium concentrations.

3. Antibacterial Activity Test

To evaluate the antibacterial activity of tested materials, the radial agar diffusion test was used on Mueller-Hinton agar plates. *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923) were reactivated from lyophilized frozen stock for observation of cell and colony morphology, which confirmed the culture purity. The microorganisms were reactivated in Brucella agar added with 5% sheep blood followed by incubation at 37 °C for 24 h. For the inoculums, 5 colonies were transferred to 5 mL BHI tubes and incubated for a period of 15 to 18 h and were used to standardize the final concentration of 1.5×10^8 CFU/mL equivalent to the 0.5 standard of the McFarland scale using a 630-nm-wavelength spectrophotometer (Pharmacia Biotech, São Paulo, SP, Brazil). Ten Petri plates (100 × 10 mm) with Mueller-Hinton agar (Merck, Rio de Janeiro, RJ, Brazil) were inoculated with microbial suspensions, 5 to each bacterial specimen, using sterile swabs obtaining growth in junction. Three wells (3 mm in depth × 5 mm in diameter) were made in each plate at equidistant points, using a cooper coil and were immediately filled with 0.2 mL of freshly prepared sealers. In order to have the same sealer volume, was introduced into wells. The plates were maintained for 2 h at room temperature for pre-diffusion of materials, and then incubated at 37 °C during 24 h. The diameter of the zones of bacterial growth inhibition formed around the well containing the sealers was measured with a digital caliper with a resolution of 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan) under reflect light. Positive and negative controls

were done with and without inoculum for the same periods and under identical incubation conditions. All assays were carried out under aseptic conditions¹³.

Data of pH, calcium release and antibacterial activity were analyzed statistically by ANOVA and Tukey's test at a significance level of 5%.

RESULT

pH values for the materials evaluated in the different experimental periods are described in Table 2. pH values were statistically different among the materials ($p < 0.05$). Sealapex had the highest pH followed by MTA Fillapex and AH Plus in all periods evaluated ($p < 0.05$).

Table 3 shows the calcium release values for the sealers in the different experimental periods. MTA Fillapex had greater calcium release than Sealapex in 14-day period, but in 28 days the inverse was observed ($p < 0.05$). AH Plus showed lower calcium release than the other sealers in all periods ($p < 0.05$).

Figure 1 show the values expressed as means of 6 repetitions and standard deviations of the zones of bacterial growth inhibition obtained for the tested materials. All sealers showed zones of bacterial growth inhibition against the bacterial strains. There were no statistically significant differences among the inhibition zones produced by the tested materials for *E. faecalis* ($p > 0.05$). Sealapex presented better antibacterial effectiveness against *S. aureus* than MTA Fillapex and AH Plus ($p < 0.05$).

DISCUSSION

The ideal endodontic filling material should be biocompatible and able to induce mineralized tissue formation. These properties are dependent on the cement ability to release hydroxyl and calcium ions^{4,9}. The methodology for evaluating the material

Table 2. Comparison of pH values obtained for the sealers in the different experimental periods

	MTA Fillapex		AH Plus		Sealapex	
	Mean	SD	Mean	SD	Mean	SD
24h	9.39 ^a	0.30	8.10 ^b	0.29	9.82 ^c	0.18
14d	8.89 ^a	0.54	6.80 ^b	0.52	9.77 ^c	0.35
28d	9.15 ^a	0.48	6.11 ^b	0.58	9.83 ^c	0.10

^{a,b,c}Different letters in each period indicate significant difference ($p < 0.05$) SD, standard deviation.

Table 3. Comparison of calcium release values (mg/L) obtained for the sealers in the different experimental periods

	MTA Fillapex		AH Plus		Sealapex	
	Mean	SD	Mean	SD	Mean	SD
24h	9.15 ^a	4.03	1.56 ^b	1.20	7.45 ^a	1.41
14d	9.30 ^a	3.00	2.37 ^b	0.06	6.92 ^c	0.63
28d	7.64 ^a	2.12	3.01 ^b	0.07	10.68 ^c	1.86

^{a,b,c}Different letters in each period indicate significant difference ($p < 0.05$) SD, standard deviation.

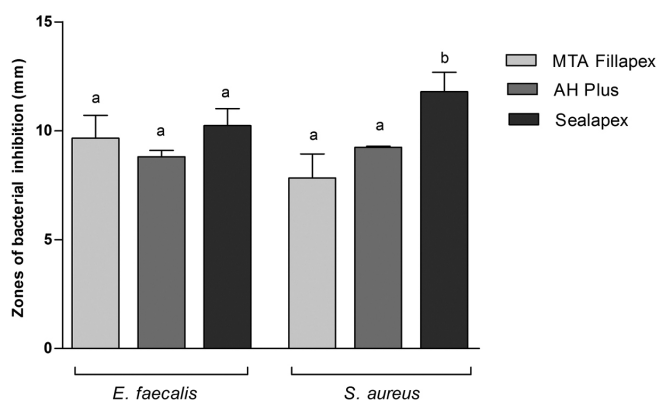


Figure 1. Comparison of diameter (in mm) of the zones of bacterial growth inhibition against the bacterial strains. The values are expressed as means of 6 repetitions and standard deviations (SD). ^{a,b}Different letters in each bacteria indicate significant difference between sealers (ANOVA and Tukey's test/ $p < 0.05$).

pH and calcium release is well established in the literature^{9,14,15}. It consists in filling standardized tubes with the materials to be tested and immersing them in distilled water. After specific periods pH is determined with pH meter and calcium release is measured with atomic absorption spectrophotometer¹⁶.

MTA Fillapex pH was lower than Sealapex pH in all studied periods. In MTA-based materials, the chemical reaction that takes place during setting results is the formation of calcium hydroxide, which subsequently dissociates into calcium and hydroxyl ions which increase the pH of the area⁸.

The reason for the difference between MTA Fillapex pH and Sealapex pH may be related to differences in the percentage of extractable calcium hydroxide in the content of sealers or to the intrinsic properties of these materials which may lead to different chemical reactions interfering in hydroxyl and calcium ions release and in their solubility¹⁷. It has been shown that set Sealapex has a poorly formed matrix and this porous material permits ingress of water over time, promoting continued reaction between calcium powder and binder¹⁸, which could explain its greater release of hydroxyl ions. It is known that there is similarity in the chemical composition of Sealapex and MTA Fillapex¹⁹. Both materials contain salicylate resin, bismuth trioxide and silica, as noted in Table 1. Other MTA-based sealer, the Endo CPM, has a pH value similar to Sealapex^{9,12}. The results of Sealapex pH (new formulation) are consistent with previous studies that evaluated the Sealapex (old formulation)^{14,15}.

MTA Fillapex and Sealapex showed greater pH and calcium release compared with AH Plus. This is consistent with previous study which showed that calcium oxide and calcium hydroxide-containing sealers favor an alkaline pH and calcium release¹⁵. Recent study also showed that MTA Fillapex has alkaline pH²⁰.

High levels of Ca^{2+} ion release were observed in MTA Fillapex and Sealapex, but not in AH Plus, in accordance with our study^{19,20}. The low calcium release of AH Plus is in agreement with Duarte et al.¹⁶ (2004) who observed low values in calcium release of pure AH Plus, similar to zinc oxide plus eugenol sealer.

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials^{11,13,21,22}. The advantage

of this method is that it allows direct comparisons of root canal sealers against the tested microorganisms, indicating which sealer has the potential to eliminate bacteria in the local microenvironment of the root canal system²¹. A disadvantage of the agar diffusion test is that the result of this method does not depend only on the toxicity of the material for the particular microorganism, but it is also highly influenced by diffusion of the material across the medium^{13,21}. A material that diffuses more easily will probably provide larger zones of microbial growth inhibition¹³. In the present study, great care was taken to keep the plates for 2 h at room temperature (pre-incubation) to allow the diffusion of sealers through the agar. This is an important factor to evidence the antimicrobial activity of the calcium hydroxide-based materials¹¹.

Studies evaluating antibacterial activity of endodontic sealers have used *E. faecalis* and *S. aureus* strains which are facultative anaerobes, Gram-positive coccus^{11,21,22}. All sealers produced zones of bacterial growth inhibition against *E. faecalis*, with no statistically significant difference. These findings agree with previous studies which showed no differences in antibacterial activity of AH Plus and Sealapex using agar-diffusion test²³. Antibacterial activity of Sealapex, as well as other calcium hydroxide-based materials, depends on ionization that releases hydroxyl ions causing a pH increase¹¹. However, *E. faecalis* is considered as one of the most resistant microorganisms to calcium hydroxide-based intracanal medications²⁴. Morgental et al.²⁵ (2011) showed that MTA Fillapex had an antibacterial effect against *E. faecalis* only before setting. On the other hand, Sealapex was the most effective in producing zones of bacterial growth inhibition against *S. aureus*. This result is consistent with previous studies which showed that Sealapex has antibacterial activity against this microorganism and promotes greater antibacterial zone than AH Plus^{7,22}. The lower antibacterial activity against *S. aureus* of MTA Fillapex in relation to Sealapex can be explained by the same reasons cited above to explain its lower pH. The antibacterial effect of AH Plus observed in the present study would be related to bisphenol diglycidyl ether present in its composition and the formaldehyde release²² by this sealer during polymerization process^{23,26,27}.

This study showed that MTA Fillapex provided high pH value and adequate calcium release during all periods evaluated and its antibacterial activity was similar to AH Plus, which is the endodontic sealer used as gold standard in endodontic material tests. Through results obtained, supported with the adequate biological results described in previous studies, it is possible indicate MTA Fillapex as an endodontic sealer to solving problems in root canal system^{1,6,10}.

CONCLUSION

In final evaluation period, pH values and calcium release provided by MTA Fillapex were lower than provided by Sealapex and higher than provided by AH Plus. The MTA Fillapex antibacterial action was similar to other endodontic sealers. Therefore, MTA Fillapex is an adequate endodontic sealer to be used in problem solving in root canal system.

REFERENCES

1. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review-part III: clinical applications, drawbacks, and mechanism of action. *J Endod.* 2010; 36(3): 400-13. PMID:20171353. <http://dx.doi.org/10.1016/j.joen.2009.09.009>
2. Silva EJ, Rosa TP, Herrera DR, Jacinto RC, Gomes BPEA, Zaia AA. Evaluation of cytotoxicity and physicochemical properties of calcium silicate-based endodontic sealer MTA Fillapex. *J Endod.* 2013; 39(2): 274-7. PMID:23321245. <http://dx.doi.org/10.1016/j.joen.2012.06.030>
3. Camilleri J, Gandolfi MG, Siboni F, Prati C. Dynamic sealing ability of MTA root canal sealer. *Int Endod J.* 2011; 44(1): 9-20. PMID:20646079. <http://dx.doi.org/10.1111/j.1365-2591.2010.01774.x>
4. Gomes-Filho JE, Watanabe S, Bernabé PF, de Moraes Costa MT. A mineral trioxide aggregate sealer stimulated mineralization. *J Endod.* 2009; 35(2): 256-60. PMID:19166785. <http://dx.doi.org/10.1016/j.joen.2008.11.006>
5. Huffman BP, Mai S, Pinna L, Weller RN, Primus CM, Gutmann JL, et al. Dislocation resistance of ProRoot Endo Sealer, a calcium silicate-based root canal sealer, from radicular dentine. *Int Endod J.* 2009; 42(1): 34-46. PMID:19125978. <http://dx.doi.org/10.1111/j.1365-2591.2008.01490.x>
6. Bin CV, Valera MC, Camargo SE, Rabelo SB, Silva GO, Balducci I, et al. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. *J Endod.* 2012; 38(4): 495-500. PMID:22414836. <http://dx.doi.org/10.1016/j.joen.2011.11.003>
7. Faria-Júnior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. *Int Endod J.* 2013; 46(8): 755-62. PMID:23441819. <http://dx.doi.org/10.1111/iej.12055>
8. Holland R, de Souza V, Nery MJ, Faraco Júnior IM, Bernabé PF, Otoboni Filho JA, et al. Reaction of rat connective tissue to implanted dentin tube filed with mineral trioxide aggregate, Portland cement and calcium hydroxide. *Braz Dent J.* 2001; 12(1): 3-8. PMID:11210247.
9. Tanomaru-Filho M, Chaves Faleiros FB, Saçaki JN, Hungaro Duarte MA, Guerreiro-Tanomaru JM. Evaluation of pH and calcium ion release of root-end filling materials containing calcium hydroxide or mineral trioxide aggregate. *J Endod.* 2009; 35(10): 1418-21. PMID:19801243. <http://dx.doi.org/10.1016/j.joen.2009.07.009>
10. Gomes-Filho JE, Watanabe S, Lodi CS, Cintra LT, Nery MJ, Filho JA, et al. Rat tissue reaction to MTA Fillapex. *Dent Traumatol.* 2012; 28(6): 452-6. PMID:22151741. <http://dx.doi.org/10.1111/j.1600-9657.2011.01096.x>
11. Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifácio KC, Ito IY. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. *J Endod.* 2000; 26(7): 391-4. PMID:11199762. <http://dx.doi.org/10.1097/00004770-200007000-00003>
12. Vasconcelos BC, Bernardes RA, Cruz SM, Duarte MA, Padilha Pde M, Bernardineli N, et al. Evaluation of pH and calcium ion release of new root-end filling materials. *Oral Surg Oral Med Oral Path Oral Radiol Endod* 2009;108(1): 135-9. PMID:19451009. <http://dx.doi.org/10.1016/j.tripleo.2009.02.026>
13. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. *J Endod.* 2000; 26(5): 274-7. PMID:11199734. <http://dx.doi.org/10.1097/00004770-200005000-00005>
14. Silva LA, Leonardo MR, da Silva RS, Assed S, Guimaraes LF. Calcium hydroxide root canal sealers: evaluation of pH, calcium ion concentration and conductivity. *Int Endod J.* 1997; 30(3): 205-9. PMID:9477806. <http://dx.doi.org/10.1111/j.1365-2591.1997.tb00698.x>
15. Duarte MAH, Demarchi ACCO, Giaxa MH, Kuga MC, Fraga SC, Souza LCD. Evaluation of pH and calcium ion release of three root canal sealers. *J Endod.* 2000; 26(7): 389-90. PMID:11199761. <http://dx.doi.org/10.1097/00004770-200007000-00002>
16. Duarte MA, de Demarchi AC, de Moraes IG. Determination of pH and calcium ion release provided by pure and calcium hydroxide-containing AHPlus. *Int Endod J.* 2004; 37(1): 42-5. PMID:14718056. <http://dx.doi.org/10.1111/j.1365-2591.2004.00756.x>
17. Schäfer E, Zandbiglari T. Solubility of root-canal sealers in water and artificial saliva. *Int Endod J.* 2003; 36(10): 660-9. <http://dx.doi.org/10.1046/j.1365-2591.2003.00705.x>
18. Caicedo R, Von Fraunhofer JA. The properties of endodontic sealer cements. *J Endod.* 1988; 14(11): 527-34. [http://dx.doi.org/10.1016/S0099-2399\(88\)80084-0](http://dx.doi.org/10.1016/S0099-2399(88)80084-0)
19. Borges RP, Sousa-Neto MD, Versiani MA, Rached-Júnior FA, De-Deus G, Miranda CE, et al. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. *Int Endod J.* 2012; 45(5): 419-28. PMID:22150403. <http://dx.doi.org/10.1111/j.1365-2591.2011.01992.x>
20. Keine KC, Guiotti FA, Leonardo RT, Kuga MC, Duarte MAH, Campos EA, et al. Influence of the addition of calcium hydroxide powder on some physical and chemical properties of the sealer MTA Fillapex. *World J Dent.* 2012; 3(2): 190-3.
21. Gomes BP, Pedrosa JA, Jacinto RC, Vianna ME, Ferraz CC, Zaia AA, et al. *In vitro* evaluation of the antimicrobial activity of five root canal sealers. *Braz Dent J.* 2004; 15(1): 30-5. PMID:15322642. <http://dx.doi.org/10.1590/S0103-64402004000100006>
22. Queiroz AM, Nelson-Filho P, Silva LAB, Assed S, Silva RAB, Ito IY. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex and EndoREZ. *Braz Dent J.* 2009; 20(4): 290-6. PMID:20069251. <http://dx.doi.org/10.1590/S0103-64402009000400005>
23. Çobankara FK, Altinöz HC, Erganis O, Kav K, Belli S. In vitro antibacterial activities of root-canal sealers by using two different methods. *J Endod.* 2004; 30(1): 57-60. PMID:14760911. <http://dx.doi.org/10.1097/00004770-200401000-00013>
24. Siqueira JF Jr. Aetiology of root canal treatment failure: why well treated teeth can fail. *Int Endod J.* 2001; 34(1): 1-10. <http://dx.doi.org/10.1046/j.1365-2591.2001.00396.x>
25. Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM. Antibacterial activity of two MTA-based root canal sealers. *Int Endod J.* 2011; 44(12): 1128-33 PMID:21895702. <http://dx.doi.org/10.1111/j.1365-2591.2011.01931.x>

26. Pumarola J, Berastegui E, Brau E, Canalda C, Jiménez de Anta MT. Antimicrobial activity of seven root canal sealers. Results of agar diffusion and agar dilution tests. *Oral Surg Oral Med Oral Pathol.* 1992; 74(2): 216-20. [http://dx.doi.org/10.1016/0030-4220\(92\)90386-5](http://dx.doi.org/10.1016/0030-4220(92)90386-5)
27. Leonardo MR, Bezerra da Silva LA, Tanomaru Filho M, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1999; 88(2): 221-5. [http://dx.doi.org/10.1016/S1079-2104\(99\)70119-8](http://dx.doi.org/10.1016/S1079-2104(99)70119-8)

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

CORRESPONDING AUTHOR

Milton Carlos Kuga

Departamento de Odontologia Restauradora, Faculdade de Odontologia, UNESP – Univ Estadual Paulista, Rua Humaitá, 1680, 14801-903 Araraquara - SP, Brasil

e-mail: kuga@foar.unesp.br

Received: May 16, 2013

Accepted: July 25, 2013