Penetration degree of sealer in artificial lateral canal after passive ultrasonic irrigation with EDTA for different times

Daylana P. da Silva[1], Ingrid Magna R. Silva[2], Lucas F. Falcão[3], Daniel F. Falcão[4], Maria Ângela A.L. Ferraz [2], Carlos Alberto M. Falcão[2]

[1] Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba, Departamento de Odontologia Restauradora, Piracicaba, São Paulo, Brasil.

[2] Universidade Estadual do Piauí, Faculdade de Odontologia e Enfermagem, Departamento de Odontologia Restauradora, Parnaíba, Piauí, Brasil.

[3] São Leopoldo Mandic, Faculdade de Odontologia, Departamento de Odontologia Restauradora, Campinas, São Paulo, Brasil.

[4] Universidade Federal do Piauí, Faculdade de Odontologia, Departamento de Odontologia Restauradora, Teresina, Piauí, Brasil.

ABSTRACT

The aim of this study was to evaluate the degree of penetration of obturation cement in artificial lateral canals after Passive Ultrasonic Irrigation (PUI) with ethylenediaminetetraacetic acid (EDTA) for different times. Fifty upper molar palatine roots were used, in which two artificial lateral canals were made at distances of 7 and 3 millimeters from the root apex. After instrumentation and drying the canal, the final toilet stage was performed on five groups (n = 10), as follows: G1 - EDTA 17% + PUI for 10 seconds; G2 - EDTA 17% + PUI for 20 seconds; G3 - EDTA 17% + PUI for 30 seconds; G4 - EDTA 17% + PUI for 60 seconds; G5 - EDTA 17% + activation by instrument R50 for 5 minutes (Control). The canals were sealed by the single cone technique, and after 72 hours, sectioned in two planes transverse to the artificial canal, to see the degree of penetration of the sealing cement. In the radiographic analysis, there was no statistical difference (p> 0.05) between groups in the two artificial lateral canals. However, PUI of EDTA for 60 seconds produced a significant difference in the degree of penetration of the sealing cement (p <0.05) at 7 mm from the apex. Therefore, PUI with EDTA for 60 seconds promoted a higher degree of penetration of the obturator cement in the artificial lateral canal.

Received: March 2019; Accepted: June 2019

Keywords: *dentin; EDTA; root canal.*

Grau de penetração do cimento obturador em canais laterais simulados após ativação ultrassônica passiva do EDTA em diferentes tempos

RESUMO

O objetivo deste trabalho foi avaliar o grau de penetração do cimento obturador em canais laterais artificiais, após Irrigação Ultrassônica Passiva (IUP) do ácido etilenodiaminotetracético (EDTA), em diferentes tempos. Foram utilizadas 50 raízes palatinas de molares superiores, e em seguida confeccionados dois canais laterais artificiais a 7 e 3 milímetros do ápice radicular. Após a instrumentação e secagem dos canais, foi iniciada a etapa de toillet final, de acordo com os seguintes grupos (n=10): G1- EDTA 17%+IUP durante 10 segundos; G2 - EDTA 17%+IUP durante 20 segundos; G3 - EDTA 17%+IUP durante 30 segundos; G4- EDTA 17%+IUP durante 60 segundos; G5- EDTA 17%+ativação pelo instrumento R50 durante 5 minutos (Controle). Os canais foram obturados pela técnica do cone único, e após 72 horas, seccionados em dois planos transversais dos canais artificiais, para se visualizar o grau de penetração do cimento obturador. Na análise radiográfica, não houve diferença estatística (p>0,05) entre os grupos, nos dois canais laterais artificiais. Entretanto, a IUP do EDTA por 60 segundos conseguiu um obter resultado significativo, sobre o grau de penetração do cimento obturador (p<0,05) a 7 milímetros do ápice. Portanto, a IUP do EDTA no tempo de 60 segundos promoveu maior grau de penetração do cimento obturador nos canais laterais artifciais.

Palavras-chave: *dentina; EDTA; canal radicular.*

INTRODUCTION

The aim of root canal endodontic treatment is to eliminate microbes and promote periapical tissue health. It involves maintaining aseptic conditions throughout canal biomechanical preparation and filling[1].

During root canal instrumentation, a smear layer forms, which consists of bacteria, debris and

necrotic tissue, with negative impact on dentin tubules and on penetration of drugs and intracanal sealers[2]. Cleaning the root canal enables better adaptation of sealer materials[3] and adhesion of resin-based endodontic cements to the dentin[4], and prevents apical and coronal microbial leakage [5].

Complex root canal anatomy should also be considered, because it prevents complete disinfection of the lateral walls and therefore requires the use of auxiliary substances and methods with physicochemical action [6,-8]. Irrigation solutions should provide antimicrobial action, organic tissue dissolution, root canal debridement, and compatibility with periapical tissues[9]. Among the most frequently used substances are chlorhexidine, sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) [10,11].

In order to improve the smear layer removal technique, the irrigation solution may be associated with ultrasonic devices. Versiani et al. (2015)[6] observed that the conventional mechanical irrigation method (CMIM) was unable to act on all the root walls and the apical area. Passive Ultrasonic Irrigation (PUI) has therefore been proposed as a technique to activate the irrigating solution, since its active tips produce mechanical-vibrating effects on the dentin walls, providing more effective cleaning and better sealing of the dentinal tubules[12,13]. Previous studies have shown that PUI promoted greater debris removal from the lateral canal[14,15] in straight and curved roots[16,17]. However, complete irrigation penetration may not be achieved at all stages of root canal treatment[18].

In view of the controversial data in the literature on the use of EDTA 17% and ultrasound to seal dentinal tubules, the aim of this study was to evaluate the degree of penetration of the obturation cement in artificial lateral canals after passive ultrasonic activation of EDTA for different times. The hypothesis tested was that the degree of penetration of the obturation cement is not influenced by the PUI with EDTA.

MATERIALS AND METHODS

Preparation of teeth

After approval by the Research Ethics Committee of FACIME/UESPI (1,376,991), 50 human teeth with intact, straight roots and fully formed apices (palatine root of the 1st and 2nd upper molars) were obtained from the University's Tooth Bank, Brazilian State of Piauí.

The dental crowns were sectioned with a double-faced diamond disk (KG Sorensen®, Cotia, São Paulo, Brazil), at slow rotation, in order to facilitate canal instrumentation. Canals were measured with a K-file file (Dentsply® Maillefer, Petrópolis, Rio de Janeiro, Brazil) #15 by reaching the apical foramen and withdrawing 1 mm in order to obtain the working length. Subsequently, files #20 and #25 #30 were used with the Reciproc System R 50 instrument (VDW, Germany). Root canal preparation steps included irrigation with 2 mL of 1% sodium hypochlorite (Milton's solution; Biodynamics®, Ibiporã, Paraná, Brazil) each time instruments were changed, with metal vacuum suction (Endo Points®, Rio de Janeiro, Brazil), and absorbent paper points (Denstply®, Petrópolis, Brazil). A total 12 mL of solution were used.

Two lateral canals were prepared perpendicular to the long axis of the root, in the middle third (apex of 7 mm) and in the apical third (apex of 3 mm), using a rotating instrument with a Kerr file (Dentsply®, Petrópolis, Brazil) size 10 (100 micrometers diameter). This instrument was modified by beveling the tip to form a chamfer. In order to adapt it for low rotation, the other end was removed and the instrument was fixed with cyanoacrylate to a mandrel[19].

After instrumentation and drying the canal, the final toilet stage was begun according to the following groups (n=10): G1-EDTA 17% + PUI for 10 seconds; G2-EDTA 17% + PUI for 20 seconds; G3-EDTA 17% + PUI for 30 seconds; G4-EDTA 17% + PUI for 60 seconds; G5- EDTA 17% + activation by instrument R50 for 5 minutes (Control).

PUI was performed using the E-1 insert (Irrisonic, Helse São Paulo, Brazil) coupled to an ultrasound device (CVDentus, São Paulo, Brazil) at a power of 20%, according to the study group. Then the canals were filled using the single cone technique, in which Sealer 26 cement (Dentsply®, Petrópolis, Rio de Janeiro, Brazil) was placed in the canal using a Lentulo spiral No. 40 (Dentsply®, Petrópolis, Rio de Janeiro, Brazil). The canal opening was sealed with light-cured composite resin (Dentsply®, Petrópolis, Rio de Janeiro, Brazil) and the roots were radiographed.

After 72 hours, the teeth were sectioned with diamond discs (KG Sorensen®, Cotia, São Paulo, Brazil) at two transverse planes 1 mm below the

artificial canals, in order to not remove the sealant cement. Then a medium-grain diamond bur (KG Sorensen®, Cotia, São Paulo, Brazil) was used to wear away 1 mm of the cross-sectional area, revealing the degree of sealant cement penetration.

A single examiner trained for this purpose (Kappa = 0.76) used a 10x stereomicroscope to evaluate the degree of cement penetration in the lateral canal. The microscopic and radiographic results were classified into four groups: Grade 0 (without cement penetration); Grade 1 (cement penetration in the proximal third); Grade 2 (cement penetration to the middle third); Grade 3 (cement penetration to the final third).

Statistical analysis

The data were analyzed using the statistical program SPSS (Statistical Package for Social Sciences) version 20.0 specific for Windows. The Shapiro-Wilk and Wilcoxon tests were used to analyze the normality and values of the means between groups. The level of significance was set at 5%.

RESULTS

Table 1 shows the descriptive results of the degree of penetration of the endodontic cement in the lateral canal, based on radiographic and microscopic analyses.

Table 2 shows that for the radiographic analysis, there was no significant difference ($p > 0.05$) between experimental groups. However, under microscopic analysis, a difference was observed between groups ($p < 0.05$), where the highest degree of penetration of the filler cement in the artificial lateral canals was seen in group 4 (Table 3).

DISCUSSION

Several factors may influence the degree of penetration of the sealant cement, such as the root canal anatomy, the efficacy of smear layer removal, the final irrigation technique and obturation, and the physicochemical properties of the sealing materials. Under microscopic analysis, this study found the greatest degree of sealing cement penetration in lateral canals at 7 mm from the root apex for canals treated with EDTA PUI for 60 seconds. Thus, the null hypothesis was rejected.

Table 1: Descriptive analysis of the degree of penetration of endodontic cement in the lateral canals, according to treatment group and distance from the apex.

Type of Analysis	Groups	Distance from the apex	Score 0	Score 1	Score 2	Score 3
Radiographic	G1	3 mm	10	-	-	-
		7 mm	10	-	-	-
	G2	3 mm	9	-	-	1
		7 mm	8	1	-	1
	G3	3 mm	7	1	-	2
		7 mm	7	2	-	1
	G4	3 mm	8	-	1	1
		7 mm	5	1	-	4
	G5	3 mm	7	-	1	2
		7 mm	8	-	1	1
Microscopic	G1	3 mm	8	-	1	1
		7 mm	10	-	-	-
	G2	3 mm	10	-	-	-
		7 mm	7	1	1	1
	G3	3 mm	6	1	1	2
		7 mm	6	2	-	2
	G4	3 mm	5	1	1	3
		7 mm	4	1	0	5
	G5	3 mm	7	-	1	2
		7 mm	6	-	1	3

G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

Table 2: Radiographic analysis of the degree of penetration of the endodontic cement in the lateral canals (apical third).

Distance from the apex	Groups	Mean	p
Apex	G1	21.00	
	G2	23.65	
	G3	28.40	0.36
	G4	25.90	
	G5	28.55	
Middle	G1	19.50	
	G2	24.35	
	G3	26.45	0.11
	G4	32.60	
	G5	24.60	

p <0.05 is statistically significant. G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

Table 3: Microscopic analysis of the degree of penetration of the endodontic cement in the lateral canals (apical and middle third).

Distance from the apex	Groups	Mean	p
Apex	G1	22.10	
	G2	23.90	
	G3	26.80	0.69
	G4	29.70	
	G5	25.00	
Middle	G1	17.50	
	G2	22.15	
	G3	26.80	0.04
	G4	33.15	
	G5	27.90	

p <0.05 is statistically significant. G1, EDTA 17% + PUI for 10 seconds; G2, EDTA 17% + PUI for 20 seconds; G3, EDTA 17% + PUI for 30 seconds; G4, EDTA 17% + PUI for 60 seconds; G5, EDTA 17% + activation by instrument R50 for 5 minutes (Control).

EDTA has chelating and disinfecting properties[20]. It removes calcium from the crystalline phosphate network, causing superficial demineralization and exposure of collagen fibers in the dentin tubules. Galler et al [21] suggest that irrigation with EDTA may potentiate cell differentiation by exposing growth factors trapped in the dentin matrix.

There was greater penetration in the middle third than in the apical third, as observed in previous studies[22,23]. Other authors further state that the EDTA solution is able to remove the smear layer only in the coronal and middle thirds due to the presence of a greater amount of dentin tubules and their diameter, which enable greater contact between the solution and the dentin tissue[24-26]. However, some studies report that the association of EDTA with sodium hypochlorite may potentiate its cleaning action and enable it to reach the apical third[10,27].

Another strategy used is activation of the EDTA using the memory instrument or ultrasonic tip. This procedure induces an increase in temperature and rupture of the surface tension of the solution[13], causing a high activity energy release. Thus, this mechanism acts on the smear layer, promoting its removal and greater diffusion of drugs and sealing of the dentinal tubules[28]. Thus, several root canal irrigation techniques and systems have been developed to improve final canal irrigation[29].

Some studies, such as Jiang et al,[28], Stamos et al[30] and Sabins et al[31] concluded that the ultrasonic system is more effective than the sonic system for cleaning root canals; however, it can damage the dentinal tissue. On the other hand, Van Der Sluis et al[32] report that there is no consensus in the literature to support the idea that one form of energy is better than the other.

When comparing PUI and CMIM, Rodig et al[33] and Paragiola et al[34] observed that PUI showed greater efficacy in root canal cleansing. Other authors compared the degree of penetration of the filling cement through these two techniques, and showed that ultrasonic activation of EDTA 17% presented a greater number of sealed dentinal tubules[35,36].

Contradicting the microscopic analysis, the radiographic analyses revealed that even using PUI, there was no difference between degree of penetration in the apical and middle thirds, as observed in previous studies[37,38]. In addition, Costa et al[39] and Ciucchi et al[40] emphasize that this can occur due to the greater amplitude of displacement that occurs at the tip of the insertion, where the apical zone would probably be. These contrasts may be related to differences in the methodology, such

as quantity of irrigating solution, activation time, selection of activator tip, and canal conicity after instrumentation.

Considering the foregoing discussion, further studies should be conducted combining chelating and disinfecting chemicals applied for different times and using different techniques, with the aim of increasing the cleaning and degree of penetration of the obturator materials in both the middle and apical thirds.

In view of the results, it may be concluded that the PUI with EDTA for 60 seconds promoted a higher degree of penetration of the obturation cement in the artificial lateral canals.

ACKNOWLEDGMENTS

The authors thank Prof. MsC. Patrick Veras Quelemes and the Laboratory of Biodiversity and Biotechnology (UFPI) for enabling the execution and analysis proposed in this study.

FUNDING

None

CORRESPONDENCE

Dr. Daylana Pacheco da Silva
Faculdade de Odontologia de Piracicaba,
Universidade de Campinas
Av. Limeira, nº. 901- Areão - 13414-903,
Piracicaba - SP - Brasil.
daylanapachecos@gmail.com

REFERENCES

1. Zöllner NA, Ferreira MCA, Carvalho PL, Rodrigues JD, Clemente RGP, Medeiros JMF. Análise da remoção do smear layer pelo uso de três soluções irrigantes. RGO 2007; 55:349-356. URL:http://www.revistargo.com.br/viewarticle.php?id=697
2. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old Enterococcus faecalis biofilms in dentin canals. J Endod 2012; 38:1376-1379.
3. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J 2003; 36:810-830.
4. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of diferent irrigating solutions on bond strength of two root canal-filling systems. J Endod 2009; 35:537-540.
5. Economides N, Kokorikos I, Kolokouris I, Panagiotis B, Gogos C. Comparative study of apical sealing ability of a new resin-based root canal sealer. J Endod 2004; 30:403-405.
6. Versiani MA; De-Deus G; Vera J; Souza E, Steier L, Pécora JD, Sousa-Neto MD. 3D mapping of the irrigated areas of the root canal space using micro-computed tomography. Clin Oral Investig 2015;19:859-866.
7. Haapasalo M, Qian W, Portenier I, Waltimo T. Effects of dentin on the antimicrobial properties of endodontic medicaments. J Endod 2007; 33:917-925.
8. De-Deus G, Murad C, Paciornik S, Reis CM, Coutinho-Filho T. The effect of the canal-filled area on the bacterial leakage of oval-shaped canals. Int Endod J 2008; 41:183-190.
9. Cheung GS, Stock CJ. In vitro cleaning ability of root canal irrigants with and without endosonics. Int Endod J 1993; 26:334-343.
10. Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: a scanning electron microscopic study. J Endod 2009; 35:1573-1576.
11. Prado M, Gusman H, Gomes BP, Simão RA. Scanning electron microscopic investigation of the effectiveness of phosphoric acid in smear layer removal when compared with EDTA and citric acid. J Endod 2011; 37: 255-258.
12. Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ, Pecora JD. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. Int Endod J 2002; 35:418-421.
13. Kuah HG, Lui JN, Tseng PS, Chen NN. The Effect of EDTA with and without Ultrasonics on Removal of the Smear Layer. J Endod 2009; 35:393-396.
14. Van der Sluis LW, Vogels MP, Verhaagen B, Macedo R, Wesselink PR. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod 2010; 36: 737-740.
15. Castelo-Baz P, Varela-Patiño P, Cantatore G, Domínguez-Perez A, Ruíz-Piñón M, Miguéns-Vila R, Martín-Biedma B. In vitro comparison of passive and continuous ultrasonic irrigation in curved root canals. J Clin Exp Dent 2016; 8:437-441.
16. Al-Jadaa A, Paque F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod 2009; 35: 1408-1411.
17. Malki M, Verhaagen B, Jiang LM, Nehme W et al. Irrigant flow beyond the insertion depth of an ultrasonically oscillating file in straight and curved root canals: visualization and cleaning efficacy. J Endod 2012; 38:657-661.
18. Vera J, Arias A, Romero M. Effect of maintaining apical patency on irrigant penetration into the apical third of root canals when using passive ultrasonic irrigation: an in vivo study. J Endod 2011; 37:1276-1278.
19. Barbosa THM, Silva MS, Silva DP, Moura ACM, Ferraz MAAL, Falcão CAM. Influence of the ultrasonic activation of irrigating solutions on sealer penetration into lateral root canals. RGO 2018; 66: 117-121. URL: http://www.scielo.br/pdf/rgo/v66n2/1981-8637-rgo-66-02-00117.pdf
20. Marending M, Paqué F, Fischer J, Zehnder M. Impact of irrigant sequence on mechanical properties of human root dentin. J Endod 2007; 33:132-135.
21. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G. Influence of root canal

disinfectants on growth factor release from dentin. J Endod 2015; 41:363-368.
22. Moon YM, Shon WJ, Baek SH, Bae KS, Kum KY, Lee W. Effect of final irrigation regimen on sealer penetration in curved root canals. J Endod 2010; 36:732-736.
23. De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coutinho-Filho T. The influence of filling technique on depth of tubule penetration by root canal sealer: a study using light microscopy and digital image processing. Aust Endod J 2004; 30:23-28.
24. Generali L, Prati C, Pirani C, Cavani F, Gatto MR, Gandolfi MG. Double dye technique and fluid filtration test to evaluate early sealing ability of na endodontic sealer. Clin Oral Investig 2017; 21:1267-1276.
25. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. Lasers Surg Med 2016; 48:70-76.
26. Kara Tuncer A, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. J Endod 2012; 38: 860-863.
27. Mancini M, Armellin E, Casaglia A, Cerroni L, Cianconi L. A comparative study of smear layer removal and erosion in apical intraradicular dentine with three irrigating solutions: a scanning electron microscopy evaluation. J Endod 2009; 35:900-903.
28. Jiang, LM; Verhaagen, B; Versluis, M; van der Sluis, LW. Influence of the oscillation direction of an ultrasonic file on the cleaning efficacy of passive ultrasonic irrigation. J Endod 2010; 36:1372-1376.
29. Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a computational fluid dynamics study. Int Endod J 2009; 42:144-155.
30. Stamos DE, Sadeghi EM, Haasch GC, Gerstein H. An in vitro comparison study to quantitate the debridement ability of hand, sonic, and ultrasonic instrumentation. J Endod 1987; 13:434-440.
31. Sabins RA, Johnson JD, Hellstein JW. Comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. J Endod 2003; 29:674-678.
32. Van der Sluis L, Wu MK, Wesselink P. Comparison of 2 flushing methods used during passive ultrasonic irrigation of the root canal. Quintessence Int 2009; 40(10): 875-879.
33. Rodig T, Dollmann S, Konietschke F, Drebenstedt S, Hülsmann M. Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod 2010; 36:1983-1987.
34. Paragliola R, Franco V, Fabiani C, Mazzoni A, Nato F, Tay FR, Breschi L, Grandini S. Final rinse optimization: influence of different agitation protocols. J Endod 2010; 36:282-285.
35. Kanter V, Weldon E, Nair U, Varella C, Kanter K, Anusavice K, Pileggi R. A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112:809-813.
36. Hedge V, Arora S. Effect of advanced irrigation protocols on self-expanding Smart-Seal obturation system: A scanning electron microscopic push-out bond strength study. Contemp Clin Dent 2015; 6:26-30.
37. Uroz-Torres D, Gonzalez-Rodriguez MP, Ferrer-Luque CM. Effectiveness of the EndoActivator system in removing the smear layer after root canal instrumentation. J Endod 2010; 36:308-311.
38. Klyn SL, Kirkpatrick TC, Rutledge RE. In vitro comparisons of debris removal of the EndoActivator system, the F file, ultrasonic irrigation, and NaOCl irrigation alone after hand-rotary instrumentation in human mandibular molars. J Endod 2010; 36:1367-1371.
39. Costa WF, Antoniazzi JH, Campos MNM, Pécora JD, Robazza, CRC. Avaliação comparativa. Sob microscopia ótica, da capacidade de limpeza da irrigação manual convencional versus ultra-sônica dos canais radiculares. Rev. Paul. Odont. 1986; 8:50-60. URL:http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=108650&indexSearch=ID
40. Ciucchi B, Khettabi M, Holz J. The effectiveness of different endodontic irrigation procedures on the removal of the smear layer: a scanning electron microscopic study. Int Endod J 1989; 22:21-28.