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Original Article

Comparative in-vitro evaluation of the efficiency of two different single file systems in reducing bacterial load from contaminated root canals followed by, evaluation of the efficiency of 2% Chlorhexidine gel in preventing bacterial regrowth when placed in the instrumented canals for 7 days

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ABSTRACT:

Background & Objectives: Shaping and cleaning of the root canals have a major role in reducing microorganisms. In this study, two different single file systems were evaluated for their efficiency in reducing E.faecalis from mesial root canals of extracted lower first molars quantitatively using bacterial culture method. The efficiency of 2% Chlorhexidine gel intracanal medicament was also evaluated, by placing them in the instrumented canals for 7 days. **Method**: 65 intact severely curved mesial root canals were selected using CBCT and divided into 5 groups (n=13). Samples in Groups I, II, III, and IV, were instrumented using One Shape, WaveOne, ProTaper, and Hand K files respectively as per manufacturer instructions and standardized irrigation protocol. Specimens were contaminated with E.faecalis for 21 days. Sample 1 confirmed the presence of organisms, Sample 2 showed a reduction in bacterialload after instrumentation. For Sample 3 specimens were divided into 2 subgroups. In subgroup 1, 2% Chlorhexidine gel was placed and in Subgroup 2, Tryptic soy agar nutrient was placed for 7 days. **Results:** Group I showed reduction in bacterial count by 70.13%, group II by 78.75%, group III by 74.7%, and group IV by 57.85%. In subgroup 1, statistically significant reduction in bacterial count was observed in group I only ($p = 0.02$). **Conclusion:** From the results obtained, WaveOne file in reciprocating motion showed the highest reduction in the bacterial count by 78.75%. 2% Chlorhexidine gel used as intracanal medicament for 7 days was not effective in reducing bacterial regrowth.

Key words: OneShape, Wave One primary, 2% Chlorhexidine gel.

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INTRODUCTION:

The ultimate goal in root canal treatment is to create a microbe-free environment preserving the original canal anatomy corono apically.[1,2] Achieving this goal is quite tedious because of the intricacies in the root canal systems and the plethora of microorganisms.[3]

'Less is more' is the dictum which is recently used in endodontics which refers to the use of less number of files for instrumentation of canals.[4,5,6] Single files which worked in continuous rotation motion include One Shape (Micro-Mega, France), KOMET F 360 (Komet, USA) and NeoNiTi files (Neolix SAS, France). Single reciprocating file systems include WaveOne (Dentsply Maileffer, Switzerland), Reciproc (VDW, Munich, Germany) and Unicone files (Medin, Czech Republic).[5]

E.faecalis organisms are the most common pathogen found in persistent endodontic infections and retreatment cases.[7,8] They are capable of invading deeper into dentinal tubules by means of bacterial adhesins and are difficult to be removed from canals by instrumentation.[7,8,9]

Intracanal medicaments help in preventing the multiplication of organisms, preventing reinfections until the completion of root canal treatment.[10] Chlorhexidine is a cationic biguanide which has positive charges and acts by absorbing to negatively charged surfaces.[11]

Chlorhexidine gel contains 1% natrosol as gel base and chlorhexidine gluconate in a pH of 5.5 to 7.[11] They are slowly released from these sites and thus prolongs their antimicrobial activity.[12] Substantivity depends on the number of chlorhexidine molecules available to interact with dentine.[14]

This study was done, 1) to compare the efficiency of single file rotary & reciprocating systems in reducing E.faecalis colony count after instrumenting severely curved mesial root canals of extracted lower first molars and 2) to assess the efficiency of 2% Chlorhexidine gel as an intracanal medicament in preventing regrowth of organisms when placed in the canals for a duration of 7 days.

METHODOLOGY:

100 freshly extracted mandibular first molar teeth were collected, disinfected in 5.25% sodium hypochlorite (Prime Dental products, India) for 30 minutes and stored in saline (Fresenius Kabi, India) for 7 days. 65 teeth having two intact mesial canals with fully formed apex, and a canal curvature ranging between 20˚-35˚ were selected using Schneider's method with the CBCT (Sirona Orthophosphos XG, France) image(Galileos) analysis. Mesial root was separated from the furcation region using a diamond disc (Moon Dental, China). Patency was established in mesiobuccal (MB) and mesiolingual (ML) canals using No.10 K file (Mani, Japan) and the working length (WL) was kept 1mm short of the apex. The apex of the samples was sealed using composite resin (Filtek, 3M ESPE, USA), root surfaces were covered in silicone impression material (Soft Putty, 3M ESPE, Korea) and embedded in autopolymerizing acrylic resin mould (DPI, Apexion Dental Products, India).MB and ML canals were enlarged circumferentially using No.15 size K files (Mani, Japan) and irrigated using 2ml of 17% EDTA (AnabondStaedman, Pharma research, India) for 1 min followed by 5ml of distilled water (Super Amp, Claris Otsuka Pvt. Ltd, India) using 30G side vented needle (ProRinse, DentsplyMaileffer, Australia).

65 specimens were divided into 5 groups(n=13) based on the instrumentation technique used. Irrigation protocol was to use 2ml of 3% sodium hypochlorite (Venson's, India) and 5ml of distilled water in 30G side vented needle in all the groupswith the change of each file, except group 5.

In Group 1 One Shape files (Micro-Mega,France) with 6% taper (tip diameter of 0.25mm) was used for instrumentation ofmesial canals in rotating motion in endomotors (X Smart Plus, Dentsply, Germany) at 400rpm and 2.5Ncm torque. Instrumentation of canals was completed in three consecutive steps using in and out pecking motion up to WL.

In Group 2 WaveOne primary files (DentsplyMaileffer, Switzerland) with 8% taper (tip diameter of 0.25mm) was used for instrumentation of mesial canals in reciprocating motion in endomotor (X Smart Plus, Dentsply, Germany) at 300rpm and 5Ncm torque. Instrumentation was completed in three consecutive steps.

In Group 3 ProTaper universal files (Dentsply Maileffer, Switzerland) SX, S1, S2, F1, F2 (tip diameter 0.25mm) were used in sequences for the instrumentation of mesial canals in endomotor (X Smart Plus, Dentsply, Germany) set at a speed of 250rpm and torque 5Ncm.

In Group 4 canals were prepared using SS hand K files (DentsplyMaileffer, United States) in crown-down pressureless technique.[2] GG drills (Mani, Japan) of sizes no.1, 2 and 3 was used to enlarge the coronal third of canal. Instrumentation started with SS hand K-file size 45, followed by the sizes 40, 35, 30, 25 to the WL with mild apically directed force.

In Group 5 canals were uninstrumented. (Negative control group)

Contamination of samples with E.faecalis

All the 65 specimens were autoclaved (Confident Dental Equipments, India) at 121˚C, 15psi for 30 minutes. Reference strain E.faecalis ATCC 29212 was isolated from m-Enterococcus agar plates (Difco m-Enterococcus agar, BD, France) and incubated for 24hr at 37˚C. E.faecalis suspension was prepared in Tryptic Soy Broth (TSB)(Hi-Media, India) and standardized to 4 McFarland scale. 52 specimens were filled with broth containing the bacterial suspension and 13 specimens were filled with plain TSB using Insulin syringe (Dispo Van, U-40 1ml, India). Coronal portion of roots was sealed with cavit(3M ESPE, Germany) and specimens were incubated (Rotek, Cyrix healthcare, India) at 37˚C for 21 days.

Sample 1: Collected before instrumentation

It was collected 21 days after the inoculation of the specimens to confirm the presence of bacterial colonies. The coronal seal was removed and the canals were filled with 5ml of distilled water. 3 sterile 15 size paper points (Dentsply Maileffer, Switzerland) were introduced one after the other into the canal for 1min each and stored in tubes (Labtech,25 x 75mm, India) containing 500µL of peptone water (Hi-Media, India) and incubated. From the master solution, 4 serial dilutions were made and plated on m-Enterococcus agar medium. It wasthen incubated at 37˚C for 48 hrs. The grown bacterial colonies were recorded as CFU/mL.

Sample 2: Collected immediately after instrumentation of samples

After instrumentation of 4 groups, 5ml of distilled water was added to the instrumented canals. A circumferential filing was done using 25 size H file (DentsplyMaileffer, United States) to the respective WL. The file was sectioned below the handle and dropped into a sterile test tube containing 500µL of Peptone water. Bacterial culture was done using the same method used for sample 1. Colony formed were recorded as CFU/mL. Mean, the standard deviation of bacterial colony count was tabulated. For comparing the values between the samples Kruskal Wallis test was used.

Sample 3: Collected to assess regrowth evaluation of E.faecalis after 7 days

52 instrumented samples were subdivided into 2 groups. Subgroup 1 contained 24 samples and was filled with 2% CHX (Concepsis V, Ultradent, South Jordan) gel. Subgroup 2 contained 28 samples and was filled with plain TSB. Samples were then incubated at 37°C for 7 days. 5mL distilled water was added to the specimens after 7 days to remove the2 % CHX gel and TSB from the canals. 3 sterile paper points of size 25 were introduced into canals one after the other for 1 minute and dropped in test tubes containing 500µL of Peptone water. Bacterial culture was done using the same method used for sample 1. The grown bacterial colonies were recorded as CFU/mL.

RESULTS:

The presence of microorganisms was confirmed in the Groups I, II, III and IV from the Sample 1 in all the four dilutions (Table 1). Group V showed an absence of microorganisms. In Sample 2, there was a statistically significant reduction ($p<0.05$) in the bacterial count in the groups I, II, III, IV by 70.13%, 78.75%,74.7% and 57.85% respectively (Table 2). Between group I and II, there was a statistically significant difference in the reduction of microbial load after instrumentation ($p=0.00$) with group II, WaveOne showing higher efficiency in reducing the microbial load by 78.75%. Between group I and III,a statistically significant difference was seen in reducing the microbial load ($p=0.00$) and, group III showed higher efficiency by 74.7%. Statistically, significant difference was seen in between group I and group IV post instrumentation ($p=0.00$) with the group I showing higher efficiency by 70.13%. Sample 3 showed the presence of organisms in subgroup 2 in the groups I, II, III and IV (Table 2) (Graph 1). In Sample 3 there was no statistically significant difference between the subgroups 1 and 2 ($p>0.05$) in the groups II, III and IV. In Subgroup 1, statistically a significant reduction in bacterial growth was seen only in group I ($p=0.002$). There was no significant difference among the subgroups in group II ($p>0.05$).

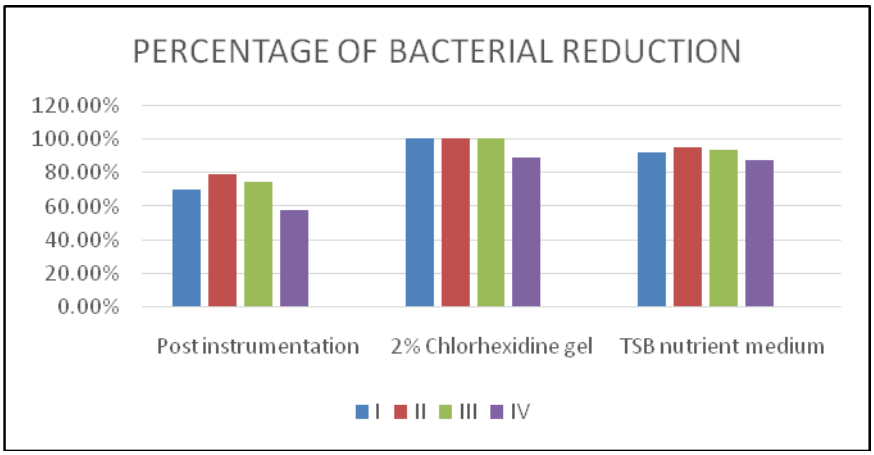
Table 1: Mean and standard deviation of bacterial colony count of different groups in sample 1, sample 2, and sample 3

Groups	Dilutions	Mean SD		P Value	Mean SD		P Value
		S1	S2		S3.1	S3.2	
I	10^{-1}	2.32596 .26956	1.86881 .39663	.000	.00000 .0000	.06816 18033	.000
	10^{-2}	2.03299 .50236	1.44866 .72706	.000	.00000 .0000	.00000 .0000	.000
	10^{-3}	1.09106 .91630	.96807 .77550	.002	.00000 .0000	.00000 .0000	.002
	10^{-4}	.20645 50401	.35614 .5548	.141	.00000 .0000	.00000 .0000	.041
II	10^{-1}	2.33071 .20494	1.98700 .20313	.001	.00000 .0000	.111164 .196537	.445
	10^{-2}	1.66912 .97232	1.70310 .57934	.000	.00000 .0000	.00000 .0000	1
	10^{-3}	1.20855 .09773	.84684 .82291	.001	.00000 .0000	.00000 .0000	1
	10^{-4}	.502650 .77889	.40793 .56069	.180	.00000 .0000	.00000 .0000	1
III	10^{-1}	2.19730 .56114	1.77829 .34798	.000	.00000 .0000	.079520 19478	.628
	10^{-2}	1.50504 .05690	1.05212 .80183	.000	.00000 .0000	.00000 .0000	1
	10^{-3}	1.13498 .91886	.48146 .70121	.001	.00000 .0000	.00000 .0000	1
	10^{-4}	.7719 .4243	.24782 .55249	.052	.00000 .0000	.00000 .0000	.052
IV	10^{-1}	2.25328 .30277	1.83989 .28574	.000	.05017 .1228	.111164 19653	.731
	10^{-2}	1.43023 .02147	1.68659 .31352	.000	.00000 .0000	.04300 .1137	.731
	10^{-3}	1.16248 .89813	.63942 .64191	.001	.00000 .0000	.00000 .0000	1
	10^{-4}	.18702 .45681	.36794 .51594	.052	.00000 .0000	.00000 .0000	1
V	10^{-1}	.00000 .00000	.00000 .00000	1	.00000 .00000	.00000 .00000	1
	10^{-2}	.00000 .00000	.00000 .00000	1	.00000 .00000	.00000 .00000	1
	10^{-3}	.00000 .00000	.00000 .00000	1	.00000 .00000	.00000 .00000	1
	10^{-4}	.00000 .00000	.00000 .00000	1	.00000 .00000	.00000 .00000	1

Table 2: Percentage of bacterial reduction of different groups using the mean average values from sample 2, and sample 3 (Sub group 1 & 2)

Groups	SAMPLE 2	SAMPLE 3	
	Reduction percentage Post Instrumentation	SUB GROUP 1 Reduction Percentage 2% Chlorhexidine gel	SUBGROUP 2 Reduction percentage TSB nutrient medium
I	70.13%	100%	91.72%
II	78.75%	100%	95.01%
III	74.7%	100%	93.5%
IV	57.85%	89.03%	87.19%
V	0	0	0

Graph 1: Bar chart showing percentage of bacterial reduction in sample 2 and sample 3 (sub groups 1 and 2)



DISCUSSION:

Shaping and cleaning of the root canals have a major role in reducing the microbial load from contaminated root canals.[1,3] Single file NiTi systems which work in continuous rotation and continuous reciprocation have the advantages of increased fatigue resistance, reduced cross contaminations and reduced chairside time.[5,15] Severely curved patent mesial root canals were selected with the aid of 3D-CBCT imaging systems. OneShape rotary file and WaveOne reciprocating files were compared in this study. Protaper universal multifile system was used as positive control for this study.

E.faecalis overcomes challenges of survival in canals by 1) exhibiting genetic polymorphism, 2) presence of serine proteases, gelatinases and Ace collagen binding protein, 3) its smaller size helps to invade and live within dentinal tubules, 4) survive a longer period of starvation until nutrition (serum) is available.[16] For the same E.faecalis ATCC 29212 strain was selected as the pathogen of choice in this study. Sample 1 confirmed the presence of bacteria in the groups I, II, III and IV. Sample 2 showed a reduction in bacterial load after instrumentation.

One Shape single file with 6% taper made up of conventional Austenite 55 NiTi which works in continuous rotation, was used for instrumentation in group 1. It has a variable pitch, non-cutting safety tip. At the apical third, it has three sharp triangular cutting edges, a modified triangular cross section at the middle third and an 'S' shaped cross-section with two cutting edges near the shaft.[5,17] There was a reduction in the bacterial count by 70.13 % using this file system. Similar results were seen in some of the ex-vivo studies conducted.[18,20]

Group II instrumented using the WaveOne file, works in reciprocation with 160˚ clockwise and 41˚ counter-clockwise motion. This file has left angled working blades made using the M wire alloy by the thermal treatment processing to the NiTi wire blanks.[4,5] M wire is known to improve the cyclic fatigue, torsional resistance and therefore is suitable for instrumentation of severely curved canals.[20] Reduction in microbial load was high by 78.75 %. This result was in accordance with some of the previous studies wherein increased reduction in microbial load with WaveOne file was attributed to the increased core diameter, increased taper and increase in the stiffness at apical 5mm of this file.[4,21]

ProTaper universal files used in group III works in continuous rotation. It has progressively tapering design with a convex triangular cross-section for the shaping files and U shaped cross- section for the finishing files.[15] Reduction in microbial load was by 74.7 %. Similar results obtained were seen in some of the studies. The progressively tapered design along the cutting surface lead to an excessive removal of dentin which helps in the better flow of irrigant to apex leading to the better removal of organisms.[21,22]

Group IV, instrumented using hand files in a crown down pressureless technique showed a reduction in the bacterial count by 57.85%.

Sample 3 was collected after placement of intracanal medicament for 7 days. Intracanal medicaments are used to prevent the growth of organisms between

appointments.[23,24] The results from previous studies showed the ability of 2% chlorhexidine in reducing the regrowth of organisms. [25,26,27] In this study, a statistically significant reduction in regrowth with 2% Chlorhexidine gel was seen only in group I (p=.000). The lesser time of contact of the medicament with the organisms was suggested as the reason for the regrowth of organisms in the presence of the medicament. [28,29] In the presence of a nutrient medium presence of microorganism was noticed in all the groups after 7 days. The efficiency of the groups I, II, III, IV in reducing the regrowth of microorganisms following placement of nutrient medium was 91.72%, 95.01%, 93.5%, 87.19 % respectively. Statistically, no significant difference in bacterial regrowth was seen between the groups II, III and IV with and without placement of intracanal medicament. These findings suggest that the placement of 2% Chlorhexidine gel as an intracanal medicament for short duration (7 days) does not have much potential in preventing the regrowth of microorganisms.

SUMMARY & CONCLUSION:

Within the limits of this in vitro study, WaveOne group showed the highest reduction in the bacterial count by 78.75% after instrumentation. 2% Chlorhexidine gel, when used as an intracanal medicament for a duration of 7 days, was ineffective in preventing the regrowth of microorganisms.

REFERENCES:

1. Haapasalo M, Endhal U, Zandi H, Coil J.M. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic Topics.2005;10:77-102.
2. Ingle JI, Bakland LK, Baumgartner JC. Ingle's Endodontics.6th ed. 2008. BC Deecker Inc.Pg:573-587, 800-810,813-866, 909-915, 919-953, 992-1011.
3. Peters OA. Current challenges and concepts in the preparation of root canal systems: A Review. J Endod. 2004;30(8):559-567.
4. Anil Dhingra A, Kochar R, Banerjee S, Srivastava P. Comparative evaluation of the canal curvature modifications after instrumentation with One Shape rotary and Wave One reciprocating files. J Conserv Dent. 2014;17(2):138-14.
5. Kumar SR, Gade V. Single file niti rotary systems. Int J Med and Dent Sci. 2015;4(1):701-707.
6. Thompson S.A. An overview of nickel-titanium alloys used in dentistry. IntEndod J. 2000;33:297-310.
7. Portenier I, Waltimo T-M. T, Haapasalo M. Enteroccusfaecalis – the root canal survivor or "star" in post-treatment disease. Endod Top. 2003;6:135-59. 42.
8. Ran S, Wang J, W.Jiang, Zhu C, Liang J. Assessment of dentinal tubule invasion capacity of enterococcus faecalis under stress conditions ex vivo. Int Endod J. 2015;48:362-372.
9. Chivatxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by Enterococcus faecalis. IntEndod J. 2008;41:873-882.
10. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod. 2002;28(10):689-693.
11. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. IntEndod J. 2009; 42:288-302.
12. Mohammadi Z, Khademi AA, Davari AR. Evaluation of the antimicrobial substantivity of three concentrations of chlorhexidine in bovine root dentine. Iran Endod J. 2008;2:113-25.
13. Gomes B.P.F.A, Souza S.F.C, Ferraz C.C.R, TeixeiraF.B,Zaia A.A, Valdrighi L, Filho FJ. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against enterococcus faecalis in bovine root dentine in vitro. IntEndod J. 2003;36:267-275.
14. Barbosa C, Goncalves RB, Siqueira JF, DeUzeda M. Evaluation of the antibacterial activities of calcium hydroxide, chlorhexidine, and camphorated paramonochlorophenol as intracanal medicament. A clinical and laboratory study. J Endod. 1997;23(5):297-299.
15. Capar I.D, Arslan H. A review of instrumentation kinematics of engine driven nickel-titanium instruments. IntEndod J. 2016;49:119-135.
16. Kowalski WJ, Kasper EL, Hatton JF, Murray BE, Nallapareddy SR, Gillespie MJ. Enterococcus faecalis Adhesion, Ace, mediates attachment to particulate dentin. J Endod.2006;32(70):634-637.
17. Micro Mega, mmb@micro-mega.com: One Shape: Complete canal shaping with only one single file in continuous rotation. Date of accessing:15.12.2014.
18. Maryam Vossoghi, MitraVossoghi, Shahriar Shahriari, Farhad Farmarzi, Rasoul Yousefi Mashouf, Maryam Farhadian. Efficacy of a novel rotary system in reduction of intracanal bacteria: An invitro study. Iran Endod J.2016;11(3):219-222 .
19. Ferrer-Luque CM, Bejarano I, Ruiz-Linares M, Baca P. Reduction in enterococcus faecalis counts-a comparison between rotary and reciprocating systems. Int Endod J. 2014; 47:380-386.
20. Santa Rosa J, de Sousa-Neto MD, Versiani MA, Nevares G, Xavier F, Romeiro K, Cassimiro M, Leoni GB, de Menezes RF, Albquerque D. Shaping ability of single file systems with different movements. A microcomputed tomographic study. Iran Endod J. 2016;11:228-33.
21. Baek JY, Yoo HM, Park DS, Seok TS , Kum KY, Shin SY, Chang SW. Comparison of the shaping abilities of three nickel-titanium instrumentation systems using micro-computed tomography. Journal of Dental Sciences. 2014;9: 111-117.
22. Yang GB, Zhou XD, Zheng YL, Zhang H, Shu Y, Wu HK. Shaping ability of progressive versus constant taper instruments in curved root canals of extracted teeth. IntEndod J. 2007;40:707-14.
23. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod. 2002;28(10):689-693.

24. Manzur A, Gonzalez AM, Poroz A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: A randomized clinical trial. J Endod. 2007;33(2):114–118.
25. Wang CS, Arnold RR, Trope M, Teixera FB. Clinical efficiency of 2% chlorhexidine gel in reducing intra canal bacteria. J Endod. 2007;33(11):1283-1289.
26. Sharifian MR, Shokouhinejad N, Aligholi M, Emaneini M, Katebi A, Assadian H. In vitro comparison of the effectiveness of chlorhexidine and two calcium hydroxide formulations on enterococcus faecalis. Iran Endod J. 2008;3(3):50-56.
27. Vasudeva A, Sinha DJ, Tyagi SP, Singh NN,Garg P, Upadhyay D. Disinfection of dentinal tubules with 2% Chlorhexidine gel, CalciumHydroxide and herbal Intra canal medicaments against Enterococcus faecalis:An in-vitro study. Singapore Dent J.2017;38:39-44
28. Paquette L, LegnerM, Fillery E, Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. J Endod. 2007;33(7):788-795.
29. Schafer E, Bossmann K. Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against enterococcus faecalis. J Endod. 2004;31(1):53-56.

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