Final Rinse Optimization: Influence of Different Agitation Protocols

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Abstract

Introduction: This study examined the effect of different root canal irrigant agitation protocols in the penetration of an endodontic irrigant into dentinal tubules. **Methods:** Fifty-six human single-rooted teeth were shaped with nickel-titanium instruments, and a final rinse of 5% sodium hypochlorite labeled with 0.2% alizarin red was performed. Specimens were assigned to 7 groups (N = 8) and submitted to the following rinse activation protocols: no agitation (control group), K-File or gutta-percha agitation, or different sonic (EndoActivator [Advanced Endodontics, Santa Barbara, CA] and Plastic Endo, Lincolnshire, IL) and ultrasonic (Satelec [Acteongroup, Merignac, France] and EMS, Nyon, Switzerland) agitations. Specimens were sectioned at 1, 3, and 5 mm from the apex in 1-mm-thick slabs, ground, and prepared for fluorescence microscopy at 100× with a wavelength of 450 milliseconds. Irrigant penetration into dentinal tubules was analyzed by using Kruskal-Wallis analysis of variance followed by post-hoc comparisons. **Results:** Groups were ranked in the following order: control = K-file = gutta-percha < EndoActivator = Plastic Endo < Satelec = EMS. At 1 mm from the apex, the highest score was found for the EMS group compared with the control, K-file, gutta-percha, EndoActivator, and Plastic Endo groups, whereas no difference was found with the Satelec group. **Conclusion:** The results support the use of an ultrasonic agitation to increase the effectiveness of the final rinse procedure in the apical third of the canal walls. *(J Endod 2010;36:282–285)*

Key Words

Cleaning, endodontic treatment, irrigation, sodium hypochlorite, ultrasound

Microorganisms and their end products are considered the main causes of pulp and periapical diseases (1), and their elimination by biomechanical procedures is crucial (2). Organic residues and bacteria located within the dentin tubules cannot be properly cleaned because of the anatomic complexities of many root canals, even after meticulous mechanical instrumentation and is a major concern for the clinical outcome (3).

Among currently used solutions, sodium hypochlorite (NaOCl) appears to satisfy most of the requirements for a root canal irrigant (4). It has the unique capacity to dissolve necrotic tissue (5) and the organic components of the smear layer (6). It also kills sessile endodontic pathogens organized in biofilms and in dentinal tubules as efficiently as chlorhexidine or iodine at a comparable concentration (7). It inactivates endotoxins (8) and also disintegrates endodontic biofilms (9, 10).

The application time of NaOCl solution is a factor that has gained little attention in endodontic studies. Even fast-acting biocides such as NaOCl require an adequate working time to reach their full potential (11). Because rotary root canal preparation techniques have expedited the shaping process, the optimal time that NaOCl at a given concentration needs to remain in the canal system is an issue yet to be resolved.

Apart from contact time, the mode of application is a matter of concern for clinicians. Moorer and Wesselink (12) opined that mechanical agitation or fluid flow was more important in the ability of NaOCl to dissolve tissue than the initial percentage of available active chlorine. The use of an irrigant in conjunction with ultrasonic vibration is directly associated with the cleaning effectiveness of the canal space (13, 14). This could reduce the time needed for the antimicrobial efficacy of the irrigating solution.

Different techniques have been proposed for the final rinsing step to reduce the time needed for an irrigant to be effective. Huang et al (15) showed that agitation of a canal irrigant using hand files or irrigation needles could significantly remove more test album medium or allow better apical irrigant replacement. In addition, manual dynamic irrigation (push-pull agitation) with a well-fitting gutta-percha point can improve the penetration and exchange of irrigant at the apical level (16). The use of a plastic file in conjunction with sonic and ultrasonic devices has also been tested. However, a recent Cochrane review (17) revealed insufficient evidence on ultrasonic instrumentation effectiveness either when it is used alone or in conjunction with hand instrumentation (18–20).

Alizarin red is a fluorescent organic compound used in biomorphologic assays for quantifying the presence of calcific depositions (21). The purpose of this study was to assess the penetration of 5% NaOCl labeled with 0.2% alizarin red into dentinal tubules

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0099-2399/$0 - see front matter

doi:10.1016/j.joen.2009.10.004

when used in root canals with different agitation protocols. The null hypothesis tested was that there is no difference in irrigant penetration using different agitation protocols.

Materials and Methods

Fifty-six recently extracted human single-rooted teeth with a straight single canal were selected for the study under a protocol approved by the University of Siena (Italy). Exclusion criteria were as follows: teeth shorter than 20 mm; apex larger than #25 before instrumentation; and presence of caries, root fissures, or fractures. All teeth were stored in saline at 4°C and used within 1 month after extraction.

To standardize canal instrumentation, crowns were removed by cutting the teeth 2 mm above the cementoenamel junction using a slow-speed Isomet saw (Buehler, Lake Bluff, IL) under copious water cooling. A size 10 K-type file was inserted into each canal until it was seen through the apical foramen. The working length was established by reducing this length by 0.5 mm.

The canals were shaped with nickel-titanium rotary instruments (FlexMaster, VDW, Munich, Germany). A size 40, 0.06 taper was the last file used at the working length. Irrigation with 5% NaOCl was performed during instrumentation using a syringe with a 30-G needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland). Smear layer removal was achieved after irrigation with 3 mL of 17% EDTA for 2 minutes followed by 3 mL of sterile saline. The teeth were randomly divided into seven groups (N = 8 for each group). The exterior part of the apical third of each root was covered with wax to prevent irrigant from dripping through the apical foramen. This was done after placing a calibrated FineMedium gutta-percha cone at the working length in order to avoid wax intrusion into the apex. The cone was removed after the wax had set.

A final rinse of each canal was performed by using 5 mL of 5% NaOCl labeled with 0.2% alizarin red using the 30-G endodontic needle at 5 mm from the working length. To standardize the procedures for all teeth, a flux of 1 mL/30 seconds was used for 90 seconds. The following groups had a different agitation procedure during the final rinse: (1) control group: no agitation (NaOCl with Alizarin red without activation), (2) K-file group: agitation with a size 10 K-file (20 up and down movements to the working length at a frequency of 3 per second), gutta-percha group: agitation with a fine medium gutta-percha cone (20 up and down movements to the working length at a frequency of 3 per second), (3) EndoActivator group: agitation with a sonic device (EndoActivator; Advanced Endodontics, Santa Barbara, CA) 10,000 cpm for 20 seconds, (4) Plastic Endo group: agitation with F-file (Plastic Endo LLC, Lincolnshire, IL) for 30 seconds at 500 rpm to 1 mm from the working length, (5) Satelec group: agitation with Passive Ultrasonic IrriSafe Satelec (Acteongroup, Merignac, France) with power setting at 5 for 20 seconds, and (6) EMS group: agitation with Passive Ultrasonic ESI File (EMS, Nyon, Switzerland) with power setting at 5 for 20 seconds. For gutta-percha and Plastic Endo groups, the respective device was inserted to and activated at 1 mm from the working length.

After drying the canal with paper points, each specimen was cut into three 1-mm thick slabs at 1, 3, and 5 mm from the apex. Slabs were then bonded onto glass slides and ground with wet silicon carbide papers to approximately 40-μm thick. The slides were examined with a fluorescence light microscope (Nikon Eclipse; Nikon, Tokyo, Japan) at 100× with a wavelength of 540 to 570 nm. If the whole canal could not fit completely in one image, two or more partial images were taken to produce a montage using Adobe Photoshop CS3 (Adobe Systems Italia S.r.l, Milan, Italy). Images from all specimens were evaluated by two blinded operators. In the case of disagreement between the operators, the lower score was assigned. The following set of scores was used to assess the penetration of the irrigant solution into the dentinal tubules (Fig. 1*A*): "0" = no visible alizarin red, "1" = minor traces of alizarin red, "2" = traces of alizarin red along the whole intraradicular surface of the canal, "3" = penetration of alizarin red in <50% of the dentinal tubules, and "4" = penetration of alizarin red in >50% of the tubules.

Additional specimens were prepared as controls as follows: (1) negative control: without adding 0.2% alizarin red to the final rinse solution and (2) positive control: 1-mm thick slabs were immersed in 0.2%

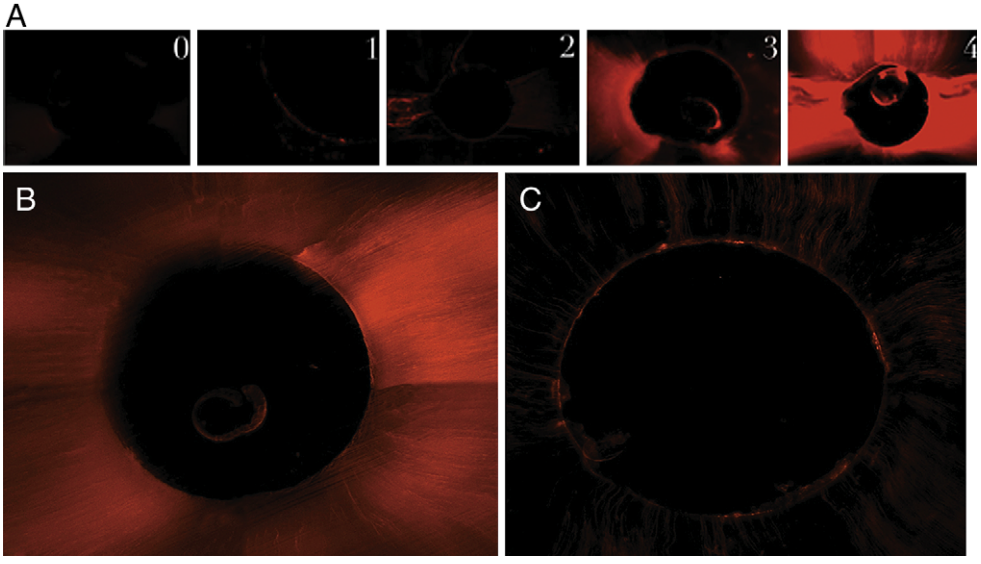

Figure 1. (*A*) Dye penetration scores based on the extent of fluorescence observed from dentinal tubules. "0": no visible alizarin red, "1": minor (incomplete) traces of alizarin red along the surface of the canal wall, "2": traces of alizarin red along the entire circumference of the canal wall, '3": penetration of alizarin red in less than 50% of the dentinal tubules, and "4": penetration of alizarin red in more than 50% of the dentinal tubules. (*B*) A representative example of fluorescence exhibited by group 7 at 1 mm from the apex. Evidence of alizarin red could be identified from the entire canal wall circumference and with penetration of the fluorescent dye into the patent dentinal tubules. (*C*) A representative example of fluorescence exhibited by group 4 at 1 mm from the apex. Traces of alizarin red could be partially identified along the canal wall circumference, with partial penetration into the dentinal tubules.

TABLE 1. Summary of Median Scores when the Dye Penetration Scores Derived from the Coronal, Middle, and Apical Thirds of the Canal Walls Were Pooled Together

Group*	N	Median	25%	75%	*p* value
1 (control)[c]	24	1.0	0	1.0	<0.001
2 (K-file)[b,c]	24	1.0	1.0	1.0	
3 (gutta-percha)[b,c]	24	1.0	1.0	1.0	
4 (Plastic Endo)[b,c]	24	1.0	1.0	1.0	
5 (EndoActivator)[b]	24	1.5	1.0	2.0	
6 (Satelec)[a]	24	3.0	3.0	4.0	
7 (EMS)[a]	24	4.0	3.0	4.0	

*Groups with the same superscripts are not statistically significant ($p > 0.05$).

alizarin red for 10 minutes to investigate dye uptake pattern. Statistical analysis was performed by using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among the groups at $\alpha = 0.05$. Data were investigated either pooled together or separately with respect to the distance from the apex.

Results

Statistically significant differences were found among groups in relation to the agitation mode. For the entire canal, groups were ranked in the following order: control = K-file = gutta-percha < EndoActivator = Plastic Endo < Satelec = EMS Group (Table 1, $p < 0.05$). For different sections of the canal space, the distance from the apex (1, 3, and 5 mm) did not influence alizarin red penetration within each group ($p > 0.05$).

Analysis of the irrigant agitation modes at different locations revealed that at 1 mm from the root apex, the EMS group exhibited the highest score (Fig. 1*B*) and was significantly different ($p < 0.001$) from the control, K-file, gutta-percha, EndoActivator, and Plastic Endo groups (Fig. 1*C*). There was no difference between the Satelec groups and the EMS group (Table 2). At 3 and 5 mm from the root apex, the Plastic Endo, Satelec, and EMS groups yielded similar scores that were significantly higher than the other groups ($p < 0.001$). No fluorescence was found in negative controls, whereas the intense presence of dye tracing within dentinal tubules was recorded in positive controls.

TABLE 2. Median Scores at 1, 3, and 5 mm from the Root Apex

Group	N	Median	25%	75%	*p* value
		1 mm			
Control[c]	8	0.0	0.0	1.0	<0.001
K-file[c]	8	1.0	0.5	1.0	
Gutta-percha[b,c]	8	1.0	1.0	1.0	
Plastic Endo[b,c]	8	1.0	1.0	1.0	
EndoActivator[b,c]	8	1.0	1.0	1.0	
Satelec[a,b]	8	3.0	3.0	3.5	
EMS 7[a]	8	4.0	3.0	4.0	
		3 mm			
Control[b]	8	1.0	0.5	1.0	<0.001
K-file 2[b]	8	1.0	1.0	1.0	
Gutta-percha 3[b]	8	1.0	1.0	1.0	
Plastic Endo[b]	8	1.0	1.0	1.0	
EndoActivator[a,b]	8	2.0	1.0	2.0	
Satelec[a]	8	3.0	3.0	4.0	
EMS[a]	8	4.0	3.0	4.0	
		5 mm			
Control[b]	8	1.0	0.5	1.0	<0.001
K-File[b]	8	1.0	1.0	2.0	
Gutta-percha[b]	8	1.0	1.0	2.0	
Plastic Endo[b]	8	1.0	1.0	1.5	
EndoActivator[a,b]	8	2.0	1.5	2.0	
Satelec[a]	8	3.5	3.0	4.0	
EMS[a]	8	4.0	3.5	4.0	

Group 1, control; group 2, K-file; group 3, gutta-percha; group 4, Plastic Endo; Group 5, EndoActivator; group 6, Satelec; and group 7, EMS.

[a,b,c]Groups with the same superscripts are not statistically significant ($p > 0.05$).

Discussion

Although mechanical instrumentation reduces bacteria from human root canals by approximately 50%, disinfecting irrigants are needed to eliminate the microbiota in locations where instruments cannot access (22–24). Although NaOCl is an effective disinfectant when it comes into direct contact with bacteria biofilms, it produced clean and debris-free dentin surfaces only in the coronal and middle thirds but not in the apical third of the canal wall when used in conjunction with nickel-titanium instruments (25). Consequently, different irrigant agitation techniques have been proposed to increase the efficacy of the irrigant solutions. Some of these techniques include manual agitation with hand files, manual agitation with gutta-percha cones, mechanical agitation with plastic instruments, and sonic and ultrasonic agitation (26).

In this study, alizarin red was used to label NaOCl. Because the validity of this methodology was confirmed with the control group, the protocol was used for investigating the penetration of the dye-labeled NaOCl within the root canal space after different final rinsing procedures. Tracing of NaOCl penetration into dentinal tubules with fluorescence microscopy enabled us to evaluate the effect of irrigant agitation techniques on irrigant penetration within the apical 1- to 5-mm region of the canal space. Additional studies should investigate the optimal concentration of the NaOCl to kill bacteria and deproteinize organic tissues without extracting collagen from the mineralized radicular dentin (27).

The null hypothesis tested was rejected because differences were found in irrigant penetration using different agitation protocols. Passive ultrasonic irrigation (28) improves the efficacy of irrigating solutions in removing organic and inorganic debris from root canal walls (29, 30). The term passive does not adequately describe the process because it is in fact active; however, when it was first introduced the term passive related to the "noncutting" action of the ultrasonically activated file. The technique relies on the transmission of acoustic energy from an oscillating file or smooth wire to an irrigant in the canal space. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming of the irrigant (31–33). A possible explanation for the improved irrigant penetration into those nonsclerotic tubules within the apical third of the canal wall is the better current flow and increased irrigant volume (34) associated with ultrasonic agitation.

Because a vapor lock exists in the apical third of the canal (35, 36) when the apical foramen is sealed with wax, it is prudent to elaborate on why better dye penetration was observed in the ultrasonic groups. Using a control and an experimental balanced design to compare the effect of vapor lock on the efficacy of canal debridement from the apical 0 to 2 mm of the canal walls, we recently observed that the use of NaOCl and EDTA was able to remove smear layers from that region irrespective of the presence or absence of a vapor lock (ie, same "smear score"). However, canals that simulated the presence of a vapor lock exhibited a significantly higher "debris score" compared with those simulating the absence of a vapor lock (37). Because this study examined only dye penetration into dentinal tubules (ie, smear layer removal), it is understandable that the ultrasonic agitation techniques produce better results. Further studies should be conducted to examine the effect of different agitation techniques on the "debris score." A novel way in accomplishing this objective is to stain soft-tissue debris with phosphotungstic acid so that both hard- and soft-tissue debris can be simultaneously evaluated by high contrast three-dimensional imaging

using microcomputed tomography. Investigations with this technique are in order.

Acknowledgments

The authors would like to thank Dr Luciano Giardino and Dr Emanule Ambu for the idea of using alizarine for this test.

References

1. Grossman LI, Meinmamn BW. Solution of pulp tissue by chemical agents. J Am Dent Assoc 1941;8:223–5.
2. Harrison J. Irrigation of the root canal system. Dent Clin North Am 1984;28:797–8.
3. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod 2004;30:559–67.
4. Zehnder M. Root canal irrigants review. J Endod 2006;32:389–98.
5. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004;30:785–7.
6. Haikel Y, Gorce F, Allemann C, et al. In vitro efficiency of endodontic irrigation solutions on protein desorption. Int Endod J 1994;27:16–20.
7. Spratt DA, Pratten J, Wilson M, et al. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001;34:300–7.
8. Sarbinoff JA, O'Leary TJ, Miller CH. The comparative effectiveness of various agents in detoxifying diseased root surfaces. J Periodontol 1983;54:77–80.
9. Clegg MS, Vertucci FJ, Walker C, et al. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod 2006;32:434–7.
10. Dunavant TR, Regan JD, Glickman GN, et al. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. J Endod 2006;32:527–31.
11. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12:147–79.
12. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J 1982;5:187–96.
13. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 1992;8:605–12.
14. Gu LS, Kim JR, Ling J, et al. Review of contemporary irrigant agitation techniques and devices. J Endod 2009;35:791–804.
15. Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. Int Endod J 2008;41:60–71.
16. McGill S, Gulabivala K, Mordan N, et al. The efficacy of dynamic irrigation using a commercially available system (RinsEndo) determined by removal of a collagen 'bio-molecular film' from an ex vivo model. Int Endod J 2008;41:602–8.
17. Pedrazzi V, Oliveira-Neto JM, Sepueira P, et al. Hand and ultrasonic instrumentation for orthograde root canal treatment of permanent teeth. Cochrane Database Syst Rev 2008;8:CD006384.
18. Chopra S, Murray PE, Namerow KN. A scanning electron microscopic evaluation of the effectiveness of the F-file versus ultrasonic activation of a K-file to remove smear layer. J Endod 2008;34:1243–5.
19. Jensen SA, Walker TL, Huttler JW, et al. Comparison of the cleaning efficacy of passive sonic activation and passive ultrasonic activation after hand instrumentation in molar root canals. J Endod 1999;25:735–8.
20. van der Sluis LW, Versluis M, Wu MK, et al. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40:415–26.
21. Shoji K. Alizarin red S staining of calcium compound crystals in synovial fluid. Nippon Seikeigeka Gakkai Zasshi 1993;67:201–10.
22. Dalton DC, Ørstavik D, Phillips C, et al. Bacterial reduction with nickel titanium instrumentation. Int Endod J 1998;24:763–7.
23. 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;22:21–8.
24. Zehnder M, Schmidlin P, Sener B, et al. Chelation in root canal therapy reconsidered. J Endod 2005;31:817–20.
25. Foschi F, Nucci C, Montebugnoli L, et al. SEM evaluation of canal wall dentine following use of MTwo and ProTaper NiTi rotary instruments. Int Endod J 2004; 37:832–9.
26. Gu LS, Kim JR, Ling J, et al. Review of contemporary irrigant agitation techniques and devices. J Endod 2009;35:791–804.
27. Zhang Z, Kim YK, Cadenaro M, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod 2010; accepted for publication.
28. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod 1980;6: 740–3.
29. Lee SJ, Wu MK, Wesselink PR. The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. Int Endod J 2004;37:672–8.
30. Townsend C, Maki J. An in vitro comparison of new irrigation and agitation techniques to ultrasonic agitation in removing bacteria from a simulated root canal. J Endod 2009;35:1040–3.
31. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod 1987;14:490–9.
32. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: an insight into the mechanism involved. J Endod 1987;13:93–100.
33. Ahmad M, Pitt Ford TR, Crum LA, et al. Ultrasonic debridement of root canals: acoustic cavitation and its relevance. J Endod 1988;14:486–93.
34. de Gregorio C, Estevez R, Cisneros R, et al. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. J Endod 2009;35:891–5.
35. Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. Oral Surg Oral Med Oral Pathol 1971;31: 96–103.
36. Pesse AV, Warrier GR, Dhir VK. An experimental study of the gas entrapment process in closed-end microchannels. Int J Heat Mass Transfer 2005;48: 5150–65.
37. Tay FR, Gu L-S, Schoeffel JG, et al. The effect of vapor lock on root canal debridement using a side-vented needle for positive pressure irrigant delivery. J Endod 2010; accepted for publication.