Effectiveness of Different Irrigation Agitating Devices on Debris and Smear Layer Removal: An In Vitro Comparative Study

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Abstract

Background: Sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid 17% (EDTA) are the most commonly used irrigants in endodontic treatment. However, delivering these irrigants into the entire length of the canal remains a point of interest as conventional needle irrigation is unable to reach and clean the entire length of the canal. Numerous devices have been proposed to increase the efficacy of irrigant delivery including sonic devices, ultrasonic devices, negative apical pressure irrigation, mechanical, as well as laser activation devices.

Objective: This study compared different irrigation agitation techniques to manual dynamic agitation using conventional side-vent needle irrigation in removal of smear layer and canal cleanliness when used with NaOCl and EDTA irrigation.

Methods: Forty extracted teeth were selected and prepared using ProTaper next files and irrigated with NaOCl & EDTA. Specimens were divided into 4 groups (n=40); Group 1 was irrigated with conventional side-vent needle without activation, Group 2 was irrigated and activated with sonic energy (EndoActivator), Group 3 was irrigated and mechanically activated using XP-Endo Finisher, Group 4 was irrigated and activated with ultrasonic energy (Irrisafe).

Results: Specimens showed similar score in coronal and middle segments for Groups 2, 3, 4, while XP-Endo finisher group was more effective in smear layer removal from the apical segment. Conventional needle irrigation group had the highest scores of remaining debris and smear layer in all segments.

Conclusion: EndoActivator, Irrisafe, and XP-Endo finisher were more effective in smear layer removal that conventional needle irrigation, XP-Endo finisher was more effective in smear layer removal at the apical segment.

Keywords: Root Canal Irrigants; Scanning Electron Microscope; Smear Layer; Sodium Hypochlorite; Ultrasonics.
Introduction

Endodontic treatment aims to eliminate or minimize the microorganisms present within the root canal system as well as in preventing recontamination. Sodium hypochlorite (NaOCl) whether in full concentration or diluted and Ethylenediaminetetraacetic acid 17% (EDTA) are the most commonly used irrigants in endodontic treatment. Their combinations have gained a wide acceptance and proved effective at removing pulpal remnants from within the canal system as well as smear layer from canal walls. However, delivering these irrigants into the full length of the canal remains a point of interest as conventional needle irrigation is unable to reach and clean the full length of the canal and even if that was possible; risk of extrusion into the periapical area is always present when using regular hypodermic needles. Using a side-vent needle allowed delivery of irrigants within 1mm of the working length. Numerous devices have been recommended to increase the efficacy of irrigant delivery and improve canal cleanliness including sonic devices, ultrasonic devices, negative apical pressure irrigation, mechanical, as well as laser activation devices. Passive ultrasonic irrigation when compared to conventional needle irrigation technique; demonstrated better results in the removal of the smear layer from the canal walls. Similar results were achieved by various authors when using sonic irrigation activation technique. Passive ultrasonic irrigation (PUI) has been shown to be effective in generating acoustic streaming effects that increase wall shear stress and enhance rupturing of intra-radicular biofilm through continuous cavitation and implosions. Irrisafe (Satelec Acteon Group, Merignac, France) is an ultrasonic device that operates in the range of 25–30 kHz and activates the irrigant solution by acoustic streaming and micro-cavitation, and in many cases able to reach the full working length (WL) of the root canal. However root contact with the oscillating instrument restricts its movement. Thus, ultrasonic instruments are less likely to oscillate freely in curved root canals. It has been shown that even in straight root canals; ultrasonic instruments often contact the canal wall during at least 20% of the working time. Furthermore, ultrasonic irrigation instruments usually possess a non-cutting design. nevertheless, they are made of a metal alloy that is harder than root dentin which adds to the risks of changing root canal morphology. The EndoActivator (DENTSPLY Tulsa Dental, Tulsa, OK) is a sonic device that uses a polyamide non-cutting tip to activate irrigant solutions and operates at frequencies ranging from 2–3 kHz. The manufacturer claims that this device produces a hydrodynamic activation of the irrigants that is able to safely clean the root canal system and morphologic irregularities Several studies showed no difference in cleaning efficacy between PUI and passive sonic activation at low frequency. XP-Endo Finisher (FKG Dentaire, Switzerland) has been recently introduced and used in disturbing the bacterial biofilm. The manufacturer claims it to provide an optimal cleaning of the root canal system while still preserving dentin. The file design is based on the shape-memory principle of NiTi alloy, with a small core size 25 it comprises of a C-shape arc in the apical half of the file. The limited literature availability about the effectiveness of XP in removing the smear layer demands further research that can justify their usage.

Aim of the study

The aim of the present study was to evaluate the effectiveness of three different methods used for the activation of root canal irrigants in debris and smear layer removal; namely the ultrasonic, sonic, and mechanical agitation devices compared to the manual dynamic agitation technique when using a standard irrigation protocol through Scanning electron microscope (SEM) assessment of canal cleanliness and score of open dentinal tubules.

Materials and methods

Single rooted extracted teeth were collected from the dental department at Bahrain Defence Force hospital for the study; extraction was done for orthodontic reasons or based on clinical need. The specimens were examined under magnification for cracks, resorption, or any defects. they were also examined radiographically to ensure they conceded with Vertucci Type I canal classification, the configuration of the single canal was confirmed.
through high magnification and bucco-lingual and mesio-distal radiographs. Finally, the specimens had their external surfaces cleaned using Ultrasonic scaler to ensure removal of any debris or remnants attached to the external tooth surface. Exclusion criteria included roots with cracks, decay, defects, or had more than one canal. Forty single rooted teeth with mature apices were selected for this study. This study was approved by the research ethics committee – Bahrain Defence Force hospital.

All Specimens were endodontically accessed, working length was determined by inserting a size #15 K-type file (DENTSPLY Maillefer, Ballaigues, Switzerland) inside the root canal until visible at the apex and then 1mm was subtracted. The crowns of all teeth were then adjusted to a standardized working length of 18 mm. The root canals were then cleaned and shaped using ProTaper Next system (DENTSPLY Maillefer, Ballaigues, Switzerland); the master Rotary file was set at size # X4.

Specimens (n=40) were randomly divided into four equal groups each having ten specimens (n=10) as follows:

**Group 1:** Only irrigation without activation

**Groups 2:** Irrigation + sonic activation using Endo-Activator tip # small at speed 10,000 cpm

**Group 3:** Irrigation + mechanical activation using Xp-Endo finisher at speed 800 rpm \ 1 ncm

**Group 4:** Irrigation + ultrasonic activation using Irrisafe tip # 20, power set at Level 2 peizosonic energy (Piezo smart, mectron, Italy)

Standard irrigation regime was applied for all groups; delivery was done using side venting irrigation needle gauge 30 (Endotec luer Mirajrct, Germany) inserted 1 mm short of the working length.

Two rounds of fresh 1 ml NaOCl 5.25% (Clorox, Kingdom of Saudi Arabia) concentration was used to irrigate each canal and flood the access cavity for 30 seconds each round. Then the canals were dried using ProTaper next paper points size # X4 (DENTSPLY Maillefer, Ballaigues, Switzerland), followed by 2 ml EDTA (tg cleanser 17%, Technical & General Ltd. UK) for 60 seconds then the canals were dried again using paper points, followed by two more rounds of fresh mix of 1 ml NaOCl 5.25% for 30 seconds each round. Finally, the canals were flushed using 5 ml distilled water then dried using paper points.

For each group, activation was done according to the manufacturer’s recommendations as described in table (1). Variation in activation protocol between groups was due to different instruction set by manufacturers so as to reach maximum efficiency according to the manufacturer recommendation.

After irrigation & activation the specimens were collected, longitudinally sectioned where the roots were split into 2 halves vertically and gold coated to allow for SEM imaging and then submitted for SEM evaluation at the coronal, middle and apical section of each one of the longitudinal segments.

**Table 1: Summary of irrigation protocol.**

| Group | Group 1 | Group 2 | Group 3 | Group 4 |
|-------|---------|---------|---------|---------|
| **Irrigation mode** | **Needle irrigation** | **EndoActivator** | **XP-Endo** | **Irrisafe** |
| Irrigation round 1 | 1ml NaOCl | 30 sec without activation | 30 sec without activation | 30 sec with activation |
| Irrigation round 2 | 1ml NaOCl | 30 sec without activation | 30 sec without activation | 30 sec with activation |
| Irrigation round 3 | 2ml EDTA | 60 sec without activation | 60 sec with activation | 60 sec with activation |
| Irrigation round 4 | 1ml NaOCl | 30 sec without activation | 30 sec with activation | 30 sec with activation |
| Irrigation round 5 | 1ml NaOCl | 30 sec without activation | 30 sec without activation | 30 sec with activation |
| Irrigation round 6 | 5 ml Distilled water | 30 sec without activation | 30 sec without activation | 30 sec without activation |
The results were compared according to the following scoring system advocated by Parente et al. as described in Table (2).

**Table 2: The scoring system.**

| Score | Description |
|-------|-------------|
| 0     | Smear layer is completely absent. Most tubules are patent and debris-free |
|       | Smear layer covering <25% of the canal wall. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris. |
| 1     | Smear layer is evident in 25–50% of the canal surface. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris. |
| 2     | Smear layer is evident in 50–75% of the canal surface. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris. |
| 3     | Smear layer covering 75–100% of the canal surface. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris. |

Separate blind evaluations were undertaken by two trained observers in the interpretation of SEM morphology. When discrepancies existed during the course of evaluation, the two examiners had to reach an agreement in between them regarding the score to be used for each specimen. Scores were then tabulated, and statistical analysis was carried out.

Statistical analysis was performed using IBM SPSS Statistics Version 2.0 for Windows. Data were presented as mean, standard deviation (SD) and percentages. The significance level was set at P ≤ 0.05. Kruskal-Wallis test followed by Mann-Whitney test were used to compare mean scores between irrigation protocols and different regions.

### Results

This study evaluated 40 specimens for different irrigation agitation techniques and made the following observations. Results (Table: 3) showed that there was a statistically significant difference in mean scores between irrigation protocols (P=0.012 in coronal section, P=0.012 in middle section, and P=0.015 in apical section). Within coronal section, Group 1 showed the significantly highest mean scores, followed by Group 2, then Group 3 and 4 which were statistically similar. Within middle section, mean scores of Group 1 was significantly higher than Group 4; while Group 2 and 3 did not significantly differ from Group 1 and 4. Within apical section, Group 1 yielded significantly higher mean scores compared to Groups 2, 3 and 4 which showed no significant difference.

Within Group 1, 2 and 3, there was no statistically significant difference in mean scores between different regions. Within Group 4, the highest mean scores were observed in the apical section and the least scores in the coronal section, while the scores of the middle section did not significantly differ from those of the coronal and apical sections.

**Table 3: Mean±SD of smear layer removal grading scores of different irrigation protocols at each region.**

| Irrigation protocol       | Coronal | Middle | Apical | p-value |
|---------------------------|---------|--------|--------|---------|
| Irrigation + No activation| 4±0a    | 4±0a   | 4±0a   | 1.000   |
| Irrigation + Sonic activation| 3±0b   | 3±0ab  | 3±0b   | 1.000   |
| Irrigation + Mechanical activation| 0.3±0.5c | 3±0ab | 3±0b   | 0.052   |
| Irrigation + Ultrasonic activation| 1±0A   | 2±0AB | 3±0AB  | 0.018*  |

*p-value 0.012* 0.012* 0.015*

*Significant at P≤0.05

*Means with different lowercase superscript letters within each column and uppercase superscript letters within each row are statistically significantly different at P≤0.05.
Discussion

The removal of smear layer produced after root canal instrumentation has been recommended due to its content of bacteria, bacterial by-products, and necrotic residue. The removal procedure compromises of disinfection process as well as decreasing dentin permeability and limits the antimicrobial and dissolving action of irrigants. Smear layer also prevents entry of endodontic sealers into the dentinal tubules forming a barrier between root canal filling material and canal wall which adversely affects sealing ability and increases chances of reinfection. However, there is no consensus regarding the irrigants type, role, sequence, and volume required to allow complete disinfection. Such debate also involves the ideal strength, optimal temperature, and the application time required for any given irrigant to fulfil its desired task.

NaOCl has the ability to destroy spores, viruses, and bacteria, and has the ability to digest vital and necrotic pulp tissue from all aspects of the root canal system. It contains hypochlorite ion (OCl) and hypochlorous acid (HOCl), which together constitute the active chlorine content that provides the protein-dissolving ability and antibacterial properties of this irrigant. Meanwhile, the recommended concentration remains debatable, different values were evaluated; 6%, 3%, and 1%. Many authors found high concentration of NaOCl yielded better results on bacterial biofilm disruption and antimicrobial activity when compared to lower concentrations. Nevertheless, higher concentrations presented a higher risk of toxicity to surrounding periodontal and periapical tissue. It is widely accepted that NaOCl when used as intra-canal irrigant; concentration has an inverse relation to application volume and time. In this study 5.25% concentration was used in order to evaluate its effect when activated by different methods without any interference or discrepancy that may result from using a low concentration NaOCl.

The use of EDTA to remove the inorganic constituents of the smear layer by a process of chelation was advocated as early as 1957. EDTA 17% solution when used for one minute after canal preparation has been shown to remove the smear layer. Many studies showed that alternating between EDTA & NaOCl during canal preparation reduced the amount of remaining debris and resulted in cleaner canals. Moreover, Clarkson et al. showed that the active chlorine content of NaOCl was greatly reduced when mixed with EDTA, they recommended that NaOCl and EDTA should not mix in the root canal at the same time but rather the canals should be flushed out before alternating to the next irrigant.

PUI has been shown to be more effective than conventional syringe and needle irrigation at eliminating debris. PUI is an irrigation protocol applied with ultrasonically activated files using ultrasonic energy that is transmitted from the ultrasonic file to the irrigant operating at a range.
between 25–30 kHz, producing acoustic streaming and cavitation of the irrigation solution, but such could only occurs when the file is allowed to vibrate freely in the canal.\textsuperscript{5,6} Mozo et al. found Ultrasonic activation of irrigants with Irrisafe tips to be an effective tool for eliminating the debris, removal of smear layer and opening up dentinal tubules.\textsuperscript{7}

There are many studies that evaluated the use of PUI to remove the smear layer. However, there is no consensus on which solution should be ultrasonically activated. Some authors activated EDTA alone, NaOCl alone, or both simultaneously.\textsuperscript{17,8,18} Furthermore, literature comparing the effect of PUI in each solution separately or together is limited. Similarly, there is no consensus on activation times of PUI, which could be anywhere from 20 seconds to 5 minutes.\textsuperscript{5} In this study, activation time for Irrisafe group was 3 interrupted minutes following manufacturer recommendation and in similarity to other studies.\textsuperscript{5,17-19}

Sonic irrigation operates at a lower frequency (1–6 kHz), in comparison to ultrasonic irrigation and produces smaller shear stresses, the sonic energy also generates higher amplitude which is defined as the back-and-forth tip movement.\textsuperscript{4} Sonic activation has been shown to be an effective method for disinfecting root canals.\textsuperscript{8,9} Some authors mentioned that Ultrasonic systems removed more dentin debris from the root canal compared to the sonic irrigation systems.\textsuperscript{5,7} The positive relationship between acoustic streaming velocity and frequency might explain the superior efficiency of the ultrasonic systems over the sonic systems. In contrast other authors found no significant difference in debris removal ability between these two techniques, the authors allowed more time for sonic irrigation which may explain the contrast in their findings.\textsuperscript{8,9} Thus, it is reasonable to assume that when sonic irrigation is applied for a longer period, there will probably be no significant difference in the remaining debris between these two agitation techniques. Similarly, in this study Irrisafe & EndoActivator groups showed similar values in coronal and middle segments but the Irrisafe group had lower score and was more effective in debris and smear layer removal at the apical region (figure:1), which could be explained that the ultrasonic tip was able to reach the apical segment and oscillate freely as the canals were enlarged to an equivalent of 0.4 mm apically, meanwhile those studies that found sonic activation to be more superior used more irrigation volume in contrast to this study where similar irrigation protocol in terms of time and volume were used for all groups.\textsuperscript{8,9}

XP-Endo finisher could expand up to 6 mm or 100 times more than its tip size when activated. The file is temperature sensitive being straight (Austenitic phase) at room temperature and C-shape arc like (Martensitic phase) at body temperature. It is intended for use in rotation mode and claimed by its manufacturer to allow better cleaning and smear layer removal. The file could return to its original shape manually once it cools by means of external source. The irregular design of the XP finisher allows it to contact more dentin than other ordinary NiTi instruments which could explain the significant difference and lower score recorded in this study (group: 4) and its effectiveness in debris and smear layer removal especially a the apical segment (figure:2).\textsuperscript{10} It is safe to conclude that due to the metal design and tip design of the XP finisher; it could mechanically remove the smear layer from the dentin wall with high efficiency and without inducing more cutting in comparison to the ultrasonic and Sonic techniques that have the disadvantages of diminished effect when the energizing file touches the canal walls, and they are totally dependent on the dissolving ability of the irrigation solution.

Most of the studies on the removal of smear layer including this study were performed by using conventional SEM, which means that it requires high vacuum and metal coating of the specimen surfaces to allow visualization of the area to be evaluated. This evaluation method has been repeatedly criticized as it only allows evaluation of post-treatment images that are acquired after the final irrigation; root canal areas that were not touched during instrumentation could be wrongfully scored as areas free of smear layer when such layer never existed at this particular area due to lack of instrumentation, also it could lead researchers to misguided conclusions by assigning higher values to areas that were previously free of smear layer.\textsuperscript{20} These concerns may contribute to
different values found in this study especially in the coronal & middle segments when compared to apical segments in the XP-Endo & Irrisafe groups. Similarly, the qualitative analysis of open dentinal tubules score is the most used method in research which was applied in the current study. However, the scoring of open dentinal tubules and qualitative analysis of smear layer removal only after final irrigation has been reported to experience technical failures. The methodology applied in most studies of smear layer removal has been questioned.20 In order to avoid observer bias and allow for quantitative analysis, automated evaluation by using software has been recommended.21 It allows identification of the number and corresponding areas of opened dentinal tubules.

In this study, the results showed relatively higher values (i.e., lower scores) of open dentinal tubule areas on cervical and middle thirds compared to apical thirds in groups 2, 3, and 4, whereas group 1 (control group without activation) showed the highest scores of remaining debris and the least number of open dentinal tubules regardless of the segment evaluated. The difference in the Irrisafe & XP-Endo finisher groups’ apical segments could also be attributed to the lower number and surface area of dentinal tubules found in the apical region.22 Another reason that may explain this result is that we used NaOCl after EDTA which may have contributed to dentin erosion resulting in areas that had smear layer removed but still appeared to be void of open dentinal tubules.23 Based on the findings, it can be concluded that none of the used devices were able to completely remove debris and smear layers. The limitations included difficulty in setting a common irrigation activation protocol as each device had different manufacturer recommendation to achieve maximum efficiency. The majority of previous studies when comparing different irrigation techniques utilized common irrigation protocol which often set doubts on the accuracy of the conclusion remarks as the efficiency of some of the devices used may have been undermined by the deliberate deviation from the manufacturer recommendation in an attempt to unify the irrigation protocol variable. Future studies should be directed towards reaching a consensus on irrigation protocols that could be used as a standardized method to in research as well as clinically.

**Conclusion**

Within the limitation of this study, it can be concluded that none of the activation techniques evaluated were able to completely remove the smear layer. XP-Endo finisher was able to remove more debris and was more effective in smear layer removal at the apical segment. Future similar studies are needed to evaluate the effect of XP-Endo finisher in curved canals and whether changes in irrigation type enhanced the XP-Endo finisher’s effectiveness in smear layer removal.

**Ethical Statement**

This study was approved by the research ethics committee – Bahrain Defence Force hospital.

**Declaration of Competing Interest**

The author has no conflict of interest to declare, this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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**References**

1. Mamootil K and Messer H. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. *Int Endod J*. 2007; 40:873-881.

2. Mohammadi Z, Sodium hypochlorite in endodontics: an update review. *International Dental Journal*. 2008; 58: 329-341

3. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. *Eur J Dent*. 2013; 7:135-42

4. Gu L, Kim JR, Ling J, Choi K, Pashley DH, Tay FR. Review of Contemporary Irrigant Agitation Techniques and Devices. *J Endod*. 2009; 35:791-804.

5. Van Der Sluis LWM, Shemesh H, Wu MK, Wesselink PR. An evolution of the influence of
passive ultrasonic irrigation on the seal of root canal fillings. *Int Endod J.* 2007; 40:356-361.

6. Plotino G, Pameijer CH, Maria Grande N, Somma F. Ultrasonics in Endodontics: A Review of the Literature. *J Endod.* 2007; 33: 81-95.

7. Mozo S, Llena C, Chieffi N, Forner L, Ferrari M. Effectiveness of passive ultrasonic irrigation in improving elimination of smear layer and opening dentinal tubules. *J Clin Exp Dent.* 2014; 6:47-52.

8. Mancini M, Cerroni L, Iorio L, Armellin E, Conte G, Cianconi L. Smear layer removal and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and passive ultrasonic irrigation): Field emission scanning electron microscopic evaluation in an in vitro study. *J Endod.* 2013; 39:1456-1460.

9. Ruddle C. Endodontic disinfection: Tsunami irrigation. *Saudi Endod J.* 2015; 5:1-12.

10. Saryilmaz E, and Keskin C. Apical Extrusion of Debris and Irrigant Using XP-Endo Finisher, EndoActivator, Passive Ultrasonic Irrigation or Syringe. *Med Dent J* 2018; 19:127-31.

11. Parente JM, Loushine RJ, Susin L. Root canal debridement using manual dynamic agitation or the EndoVac for final irrigation in a closed system and an open system. *Int Endod J.* 2010; 43:1001-1012.

12. Violich DR and Chandler NP. The smear layer in endodontics - A review. *Int Endod J.* 2010; 43:2-15.

13. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of Smear Layer on Sealing Ability of Canal Obturation: A Systematic Review and Meta-analysis. *J Endod.* 2007; 33:96-105.

14. Chang YC, Huang FM, Tai KW, Chou MY. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001;92 :446-450.

15. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. *J Endod.* 2011;37 :538-543.

16. Saito K, Webb TD, Imamura GM, Goodell GG. Effect of Shortened Irrigation Times with 17% Ethylene Diamine Tetra-acetic Acid on Smear Layer Removal after Rotary Canal Instrumentation. *J Endod.* 2008;34 :1011-1014.

17. Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without Surfactants or Ultrasonics on Removal of Smear Layer. *J Endod.* 2007;33: 472-475.

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 :1243-1245.

19. Blank-Gonalves LM, Nabeshima CK, Martins GHR, MacHado MEDL. Qualitative analysis of the removal of the smear layer in the apical third of curved roots: Conventional irrigation versus activation systems. *J Endod.* 2011; 37:1268-1271.

20. De-Deus G, Reis C, Paciornik S. Critical appraisal of published smear layer-removal studies: Methodological issues. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontontology.* 2011; 112:531-543.

21. George R, Rutley EB, Walsh LJ. Evaluation of Smear Layer: A Comparison of Automated Image Analysis versus Expert Observers. *J Endod.* 2008;34 :999-1002.

22. Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *J Endod.* 1984; 10:359-363.

23. Qian W, Shen Y, Haapasalo M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. *J Endod.* 2011; 37 :1437-1441.