Comparative evaluation of 15% ethylenediamine tetra-acetic acid plus cetavlon and 5% chlorine dioxide in removal of smear layer: A scanning electron microscope study

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Abstract

Aims: The purpose of this study was to compare the efficacy of smear layer removal by 5% chlorine dioxide and 15% Ethylenediamine Tetra-Acetic Acid plus Cetavlon (EDTAC) from the human root canal dentin. Materials and Methods: Fifty single rooted human mandibular anterior teeth were divided into two groups of 20 teeth each and control group of 10 teeth. The root canals were prepared till F3 protaper and initially irrigated with 2% Sodium hypochlorite followed by 1 min irrigation with 15% EDTAC or 5% Chlorine dioxide respectively. The control group was irrigated with saline. The teeth were longitudinally split and observed under Scanning electron microscope SEM (×2000). Results: At the coronal thirds, no statistically significant difference was found between 15% EDTAC and 5% Chlorine dioxide in removing smear layer. In the middle and apical third region 15% EDTAC showed better smear layer removal ability than 5% Chlorine dioxide. Conclusion: Final irrigation with 15% EDTAC is superior to 5% chlorine dioxide in removing smear layer in the middle and apical third of radicular dentin.

Keywords: Chlorine dioxide, ethylenediamine tetra-acetic acid plus cetavlon, smear layer removal

Introduction

Root canal treatment can be summarized as a sequence of procedures for cleaning, shaping, and filling the root canal system. One of the most important procedures during treatment is the chemo-mechanical preparation of the root canal, based on the appropriate use of instruments and irrigating solutions.

Mechanical preparation of the root canal inadvertently leads to formation of smear layer in root canal which is an amorphous layer composed of organic and inorganic material. Though, the influence of smear layer on treatment outcome is yet to be ascertained but its presence leads to increased leakage and acts as a provision of substrate for bacterial growth and ingress. The alternating use of NaOCl and Ethylenediamine tetra-acetic acid (EDTA) has been investigated for its ability to achieve removal of organic and inorganic components of smear layer. Various smear layer removal agents such as citric acid, phosphoric acid with pH being as low as 1.5 have been investigated, the extremely low pH of these agents might have adverse effects on the periapical tissues. Chlorine dioxide has recently come under consideration as a possible root canal irrigant and is reported to be tuberculocidal, bactericidal, virucidal, and fungicidal, it also exhibits antimicrobial activity over a wide range of pH ranging from 3 to 9 and is less cytotoxic with a better disinfectant action as compared to Sodium hypochlorite. Previous studies have used a concentration of 13.8% to 28% for chlorine dioxide but experimental results indicate that chlorine dioxide is unstable above the concentration of 9.5% ([ClO₂]/[air]) and is pungent at higher concentrations. To date, there are no studies which have reported on the smear layer removal capacity of Chlorine dioxide. Hence, the purpose of this study is to compare and contrast the efficacy of 5% Chlorine dioxide and 15% EDTAC on removing the smear layer from the prepared root canal wall.

Materials and Methods

Fifty single-rooted mandibular anterior teeth with healthy pulp and Type 1 spatial configuration (Weine, 1982) without significant canal curvature, extracted for periodontal reasons from 40 to 60 year old patients were selected. The teeth were devoid of caries, cracks, endodontic treatments, or restorations. Only teeth with intact and mature root apices
were selected. The teeth were stored in saline at 4°C until the root canal treatment, which was performed within 7 days.

**Sample preparation**

After access preparation, the working length was measured by subtracting 0.5 mm from length recorded when the tip of #10 K-file (Dentsply-Tulsa Dental, Tulsa, OK) was visible at the apical foramina. Two layers of utility wax were applied over the root tips to prevent the irrigating solutions from passing through the apical foramina. The instrumentation was initiated with hand files (Dentsply-Tulsa Dental, Tulsa, OK) up to #20 followed by ProTaper rotary files (Dentsply-Tulsa Dental, Tulsa, OK) from S1 to F3. Amidst the sequential use of instruments, the root canals were flushed with 2 mL of 2% NaOCl (Dentpro Ltd, Chandigarh, India) by a 30-gauge needle (NaviTip; Ultradent, South Jordan, UT) positioned in apical third of canal without binding.

At the end of preparation, Group I (20 teeth) was irrigated with 5 mL of 15% EDTAC (Largal Ultra, Septodont, Paris, France) for 1 min followed by 5 mL of 2% NaOCl for 1 min, Group II (20 teeth) was irrigated with 5 mL of 5% chlorine dioxide (Blo-Out, India) for 1 min followed by 5 mL of 2% NaOCl for 1 min and the Group III (10 teeth, control) was irrigated with 5 mL of saline for 1 min followed by 5 mL of 2% NaOCl. Then, the root canals were finally irrigated with 5 mL of saline to remove any precipitate that might have been formed. The canals were dried with sterile paper points (Dentsply-Maillefer, Ballaigues, China). The pH of 5% Chlorine dioxide was 3.9, as measured by a pH meter (Model LI-120, Elico Pvt. Ltd., Hyderabad, India).

To assess the degree of smear layer removal, utility wax was removed and two longitudinal grooves were prepared on the lingual and buccal surfaces of each root with a diamond bur used with a high-speed water-cooled handpiece to facilitate vertical splitting with a chisel after canal instrumentation. The roots were then split into two halves using a chisel and stored in deionized water at 37°C until SEM analysis. The split root samples were critical point dried, sputter-coated with platinum, and examined with an SEM (Leo 430, Leo. Electron Microscopy Ltd., Cambridge, England) at 15 kV and at a final magnification of ×2000. Several photomicrographs were taken to observe the surface morphology of the canal walls at the coronal (10-12 mm from apex), middle (6-7 mm from apex), and apical (1-2 mm from apex) thirds of each specimen. Two investigators calibrated and scored the images regarding the dentinal canal walls surface cleanliness, at the coronal, middle, and apical areas of each specimen according to the following criteria:

- No smear layer: No smear layer on the surface of the root canal; all the tubules were clean and open
- Moderate smear layer: No or little smear layer on the surface and debris inside the tubules
- Heavy smear layer: Smear layer covering the root canal surface and the tubules

**Results**

Interaction analyses using GLMM, indicated that there was no significant difference in the amount of debris with site between the two examiners ($P = 0.7$). Figure 1 shows representative images of the scores. The estimated means of the smear layer scores for each group are listed in Table 1. There was also minimal inter-observer variability (kappa = 0.96, $P < 0.001$). On comparison, there was significant difference between the smear layer removing ability of the three irrigants ($P < 0.001$). On pair-wise comparison, both 15% EDTAC and 5% Chlorine dioxide reduced the debris significantly in comparison with saline ($P < 0.001$ for both). Between EDTAC and chlorine dioxide, the debris was significantly less with the use of EDTAC in the middle and apical third ($P < 0.001$) and no statistical difference was observed in the coronal third ($P > 0.001$). Coronal third had lowest debris level followed by middle and apical third [Table 1]. The control group was incapable of removing any smear layer. Random variables used for this analysis were assessment by examiner, section of the tooth and the number of teeth.

![Figure 1: The scoring system used to analyze the SEM results. (magnification × 2000). (a) No smear layer: No smear layer on the surface of the root canal; all the tubules were clean and open. (b) Moderate smear layer: No or little smear layer on the surface and debris inside the tubules. (c) Heavy smear layer: Smear layer covering the root canal surface and the tubules](image-url)
Discussion

In this study, we compared the efficacy of 15% EDTAC and 5% chlorine dioxide as a final irrigant in the removal of the smear layer from the coronal, middle, and apical thirds of the human root canal system. Although, the findings revealed that a final flush of 15% EDTAC is more efficacious than 5% Chlorine dioxide in smear layer removal in the apical third of root canal, however the former failed to render a smear free apical third. The latter finding is in accordance with previous reports which have shown the inability of EDTAC to completely eliminate the smear layer from the apical third of root canal system. Presence of sclerosed dentin in the apical third is known to contain depleted amounts of non-collagenous proteins which may be one of the possible reasons for the reduced efficacy of 15% EDTAC. There are numerous guidelines for the optimum exposure time of EDTA, a majority of them agree with irrigation time of 1 min as it prevents intertubular and peritubular erosion.

The capacity of smear layer removal of chlorine dioxide could be because of its low pH, i.e., 3.97 as past studies confirm that pH of an irrigant is indirectly proportional to the amount of demineralization of root canal dentin. EDTAC was used in this study as it has been found that surfactant addition enhances the demineralizing capacity of EDTA. The irrigation regime followed is in agreement with current protocol, including the use of 5 mL EDTA final rinse as proposed by Mello et al. They showed that a final rinse with 5 mL of EDTA was as effective as 10 mL or 15 mL of EDTA for the removal of the smear layer. The findings also revealed that 5% chlorine dioxide, in comparison to 15% EDTAC, is an equally effective smear removal agent in coronal third of root canal but inferior in the middle and apical third of the root canal. Other potential variables which determine smear layer removal are irrigant concentration and duration of final flush. We used an experimental concentration of 5% for chlorine dioxide with duration set at 1 min, as till date, there are no studies which could determine the ideal concentration of chlorine dioxide to be used in the root canals.

In the control group, there was a heavy smear layer present on the coronal, middle, and apical thirds. This showed the inability of saline to remove endodontic smear layer. The result was in agreement with the findings of Ciucchi et al.

In this study, the entire canal length was utilized to simulate the clinical situation and to test the efficacy of the solutions in all segments of the root canal system. SEM was used in this study to assess the smear layer because it is easily available; a superior alternative could be digital image analysis which prevents evaluator bias, requires less time and enables to measure average tubule diameter and density.

The results of this study show the limited ability of 5% chlorine dioxide as a smear layer removal agent in the root canal. Further studies should be directed towards manipulating possible variables such as volume and concentration of chlorine dioxide, effects of various exposure times and evaluating its influence on various dental materials in order to enable its routine use in endodontics.

References

1. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975;1:238-42.
2. Karagöz-Kucukay I, Bayirli G. An apical leakage study in the presence and absence of the smear layer. Int Endod J 1994;27:87-93.
3. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. Int Endod J 1990;23:20-7.
4. Goldman M, Goldman LB, Cavalieri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions: A scanning electronic microscopic study. Part 2. J Endod 1982;8:487-92.
5. Berg MS, Jacobsen EL, DeGole EA, Remelikis NA. A comparison of five irrigating solutions: A scanning electron microscopic study. J Endod 1986;12;192-7.
6. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod 1987;13:147-57.
7. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. Int Endod J 1999;32:32-9.
8. Wei MK, Wu QP, Huang Q, Wu JL, Zhang JM. Plasma membrane damage to Candida albicans caused by chlorine dioxide (ClO2). Lett Appl Microbiol 2008:47:67-73.
9. Junli H, Nenqi R, Guanle Y. Disinfection effect of chlorine dioxide on viruses, algae and animal planktons in water. Water Res 1997;31:455-60.
10. Barnhart BD, Chuang A, Lucca JJ, Roberts S, Liewehr F, Joyce AP. An in vitro evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. J Endod 2005;31:613-5.
11. Deininger R, Ancheta A, Ziegler A. Chlorine dioxide. Paper
presented at the PAN American Health Organization (PAHO) Symposium: Water Quality: Effective Disinfection. Available from: http://www.bvsde.paho.org/bvsacg/fulltext/symposium/ponen11.pdf. [Last accessed on 2012 May 17].  
12. Nishikiori R, Nomura Y, Sawajiri M, Masuki K, Hirata I, Okazaki M. Influence of chlorine dioxide on cell death and cell cycle of human gingival fibroblasts. J Dent 2008;36:993-8.  
13. Cobankara FK, Ozkan HB, Terlemez A. Comparison of organic tissue dissolution capacities of sodium hypochlorite and chlorine dioxide. J Endod 2010;36:272-4.  
14. 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.  
15. US Environmental Protection Agency. Alternative Disinfectants and Oxidants Guidance Manual. [Last accessed on 2012 May 24].  
16. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K. A new solution for the removal of the smear layer. J Endod 2003;29:170-5.  
17. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. Int Endod J 1999;32:32-9.  
18. 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.  
19. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentin. J Dent 1999;27:43-6.  
20. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J 2002;35:934-9.  
21. Cruz-Filho AM, Sousa-Neto MD, Savioli RN, Silva RG, Vansan LP, Pécora JD. Effect of chelating solutions on the microhardness of root canal lumen dentin. J Endod 2011;37:358-62.  
22. Akçay I, Sen BH. The effect of surfactant addition to EDTA on microhardness of root dentin. J Endod 2012;38:704-7.  
23. Mello I, Robazza CR, Antoniauzzi JH, Coi J. Influence of different volumes of EDTA for final rinse on smear layer removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e40-3.

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