# Comparing Post-Obturation of Apical Microleakage Following the Use of Different Intracanal Chelators

**Yazdan Shantiaee[1] Omid Dianat[2] Mohammad Ali Mozayeni[2] Mohammad Tajedin[3] Soheila Darmiani*[4] Golbarg Kolahi Ahari[5]**

[1]*Dept. of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2]*Iranian Center for Endodontic Research, Dept. of Endodontic, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[3]*Dept. of Pediatric Dentistry, Tehran Azad University, Tehran, Iran*.
[4]*Dental Research Center, Dept of Endodontic, Dental School, Birjand University of Medical Sciences, Birjand, Iran.*
[5]*PHD Candidate of Biochemistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

**Abstract**

***Objectives*:** Different chelators may be used during root canal treatment, offering various advantages including lubricant effect inside the canal and smear layer removal. However, chelator residues in narrow root canals can lead to apical microleakage. The aim of this in-vitro study was to compare apical microleakage following the use of three root canal chelators via fluid filtration method in root canals instrumented with ProTaper rotary system.

***Methods*:** Sixty-eight distobuccal canals of maxillary first molars were randomly divided into six groups of four experimental (n=15) and a positive and a negative control group (n=4). In groups one to three, RC-Prep, 17% EDTA and Glyde File Prep were used as chelators, respectively and sodium hypochlorite (NaOCl) was used as irrigant in all groups except for group four. In group four, root canals were instrumented without chelators and only saline was used for irrigation. Root canals in all groups were prepared using ProTaper rotary system up to $F_2$ file and filled using cold lateral condensation technique with gutta-percha and AH26 sealer. Apical microleakage was assessed by fluid filtration method. The data were subjected to Kruskal-Wallis test.

***Results*** No significant differences were noted among the experimental groups regarding apical microleakage ($P$>0.05). However, preparations with RC Prep+ NaOCl and Glyde File Prep+ NaOCl yielded the highest and the lowest values of apical microleakage, respectively.

***Conclusion*:** Use of different chelators did not cause statistically significant difference in apical microleakage of root canals.

***Key Words*:** Chelating Agents, Dental Leakage, Root Canal Preparation

***How to cite:***

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*Corresponding Author:
Darmiani S.
E-mail:
soheiladarmiani@yahoo.com

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## Introduction

The aim of endodontic therapy is to clean and shape the root canal system to provide a fluid-tight seal gained by a three-dimensional obturation that does not allow apical microleakage (1, 2). Apical microleakage is a common reason of clinical endodontic failure, and is influenced by many variables such as the root canal filling technique, physical and chemical properties of sealers and presence or absence of smear layer (1, 3). Smear layer plays an important role in providing a fluid-tight seal in the root canal system. Lack of a tight apical seal results in leakage of fluid through the apex into the root canal and can result in endodontic failure (1). Application of lubricants along with irrigation with NaOCl has been widely recommended during the process of root canal preparation by rotary systems. Remnants of these materials in the apical portion of root canals can compromise the quality of the seal and lead to apical microleakage (2).

The efficacy of root canal filling techniques in providing optimal apical seal has been evaluated by various methods such as dye

penetration (4,5), radioisotope labeling (6), bacterial leakage (7) and electrochemical methods (8). Dye penetration is the most commonly used method but has some major limitations; samples are destroyed in this technique, thus assessment of changes in apical seal over time in individual samples cannot be done. Also, this method is not quantitative and assesses leakage in only one plane (2, 5, 9). Fluid filtration method is another technique of measuring microleakage, which does not have the limitations of previous methods. It does not require destruction of samples and enables measuring microleakage over extended periods of time. Furthermore, this method quantifies the leakage of the entire sample. Use of this method is recommended to increase the reliability and repeatability of assessments (10-13). Moradi et al. compared the accuracy of fluid filtration and bacterial leakage techniques for assessment of apical sealing ability and showed that both techniques yielded similar results (14). Wu et al. assessed the sensitivity, advantages and disadvantages of dye penetration and fluid filtration techniques. The results showed that fluid filtration technique had higher sensitivity for detection of voids in the root canal filling material compared with the dye penetration technique (15). Grandini et al. assessed the efficacy of Glyde File Prep in combination with NaOCl for root canal irrigation. The results showed that this combination failed to efficiently remove the smear layer and debris (16).

Use of chelating agents has been suggested to improve chemomechanical debridement during root canal treatment (16, 17). They were introduced to enhance the preparation of narrow root canals (18). Also, applying chelators as lubricants during rotary root canal preparation reduces the risk of file fracture (17). The chelating properties of these materials result in more effective smear layer removal and better penetration of sealers for an ultimately higher apical seal (18). On the other hand, the remnants of these chelators in the apical region hinder the penetration of sealers into dentine and thus compromise apical seal (17, 19). Biesterfield et al. compared the quality of periapical seal in root canals irrigated with RC-Prep, 2.5% NaOCl and 5% Salvizol using radioisotope method. The results revealed that RC-Prep provided the worst quality of seal. However, there was no significant difference in the efficacy of the other two agents (20). Farhad et al. evaluated the impact of smear layer on apical seal using dye penetration technique. They found that smear layer removal significantly enhanced apical seal and yielded satisfying endodontic outcome (21). Zarei et al. evaluated the effect of application of RC-Prep on the amount of apical microleakage using dye penetration technique and indicated that application of RC-Prep, as a chelating agent, had no effect on the amount of apical leakage and is useful for canal preparation by instruments. (22).

The purpose of this in-vitro study was to comparatively evaluate apical microleakage of obturated canals following the application of three chelating agents in root canals instrumented with ProTaper rotary system by fluid filtration method.

## Methods

This in vitro experimental study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Sample size was calculated using NCSS (PASS 11.0.8) software (Kaysville, UT, USA) and based on a previous study by Vasconcelos et al

(10). By considering the effect size of 0.44 in the four groups, α=0.05 and β=0.20, sample size was calculated to be 15 in each experimental group.

Sixty-eight human maxillary first molar teeth with closed apices and root curvature of less than 30˚ extracted due to periodontal disease were randomly selected for this study. Teeth with cracks, fractures or calcifications were excluded. The teeth were cleaned using 5% NaOCl and stored in normal saline until the experiment. Caries and previous restorations were removed with a diamond bur (Dentsply Maillefer, Tulsa, OK, USA) and a high-speed handpiece under air and water spray. Access cavity was prepared. A#15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was placed into the distobuccal root canal until its tip was visible at the apex. The working length was determined by subtracting 1mm from this length. In order to standardize the apical diameter, the apex of each distobuccal canal was enlarged with #10 and #15 K-files. The samples were then randomly divided into six groups. The positive and negative control groups each contained four samples, and the remaining 60 teeth were equally assigned to four experimental groups as follows:

*Group 1*: RC-Prep (Premier Dental Products, Philadelphia, PA, USA) was used as the chelator.

*Group 2:* 17% EDTA (Merck Co., Darmstadt, Germany) was used as the chelator.

*Group 3*: Glyde File Prep (Dentsply Maillefer, Ballaigues, Switzerland) was used as the chelator.

*Group 4*: Canals were instrumented without using chelators, and only normal saline was used as the irrigating solution.

*Group 5***:** (negative control group): The canals were prepared as in group 1. All teeth were covered with two layers of nail varnish, including the apical foramina.

*Group 6***:** (positive control group): After instrumentation, canals were left unfilled.

Canals were instrumented by ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) with Endo IT control motor (AsepticoWoodinville, WA, USA). According to the manufacturer's instructions, $S_1$ and $S_2$ files were used for the filing and $F_1$ and $F_2$ for the finishing process.

Next, the canals in all groups except for group four were irrigated with 5 mL of 2.5% NaOCl (Golrang, Tabriz, Iran) for five minutes using a disposable syringe and 27-guage needle. The root canals were completely dried with paper points (Aria Dent, Tehran, Iran) before obturation.

All distobuccal canals were obturated with gutta-percha (Gapadent Co., Daegu, Korea) and AH26 sealer (DeTrey, Dentsply, Konstanz, Germany) using lateral condensation technique. All instrumentation and obturation procedures were carried out by one operator to reduce inter-operator variability. After filling, all teeth were kept in 100% humidity at 37˚C for 72 hours to allow setting of the sealer.

The external surfaces of the samples were covered with two layers of nail varnish and one layer of cyanoacrylate glue (Interlock Co., LTD, Tokyo, Japan) from the coronal edge to 2.0 mm short of the apex. Seven days after obturation of root canals, apical microleakage was measured using the fluid filtration method employing a pressure equivalent to 1.2 atmospheres, as described in previous studies (10, 11). The amount of microleakage was expressed in $\mu L/cmH_2O$/minute (Figures 1 and 2).

The data were statistically analyzed using SPSS software and Kolmogorov-Smirnov and

Kruskal-Wallis tests. The level of significance was set at 5% for all tests.

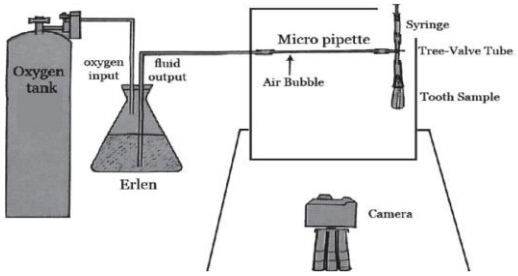


**Figure 1- A schematic view of fluid filtration apparatus**

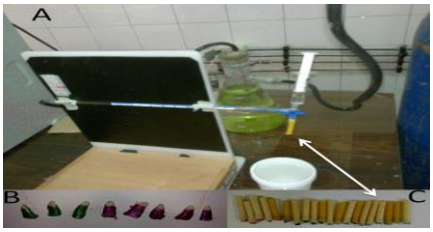


**Figure 2- (A) Fluid filtration apparatus (B) External surfaces of samples were covered with two layers of nail varnish and one layer of cyanoacrylate glue from the coronal edge to 2.0 mm short of the apex. (C) Teeth are ready for placement in the apparatus**.

## Result

The mean (±standard deviation) microleakage values in µL/cm$H_2O$/minute are shown in Table 1. Overall, RC-Prep+ NaOCl group demonstrated the greatest amount of microleakage and the saline group, EDTA+ NaOCl group and Glyde File Prep+NaOCl group ranked second, third and fourth in terms of microleakage, respectively. Overall, there was no statistically significant difference ($P$>0.05) in apical microleakage between groups treated with various chelators (RC-Prep, EDTA and Glyde File Prep) in combination with NaOCl and saline.

The Kolmogorov-Smirnov test failed to analyze the existing data and since the data did not have a normal distribution, the non-parametric Kruskal-Wallis test was applied to compare different irrigation protocols in terms of apical microleakage. The results showed that there was no significant difference among the four groups concerning the amount of microleakage ($P$ = 0.595).

**Table 1- The mean microleakage and standard deviation (SD) values in the four groups**

| Groups | Mean Microleakage µL/cmH2O/ minute | SD |
|---|---|---|
| Group 1: RC-Prep+NaOCl (n=15) | 147.38 | 120.8 |
| Group 2: EDTA+NaOCl (n=15) | 116.3 | 109.92 |
| Group 3: Glyde File Prep+ NaOCl (n=15) | 100.0 | 91.89 |
| Group 4: Normal saline (n=15) | 116.48 | 108.32 |

## Discussion

Smear layer formation is inevitable after root canal preparation. Elimination or preservation of smear layer in root canal treatment has long been a matter of controversy (23). The positive effects of smear layer include minimizing dentine permeability and blocking dentinal tubules against invasion of bacteria and endotoxins (24). However, smear layer may compromise the adaptation of the filling material to root canal walls and may enhance the accumulation of microorganisms (25). The idea of smear layer removal basically originated from the necessity of sealing open dentinal tubules (26). Sodium hypochlorite has the ability to remove organic components of the smear layer while RC-Prep, EDTA and Glyde file Prep as lubricants or chelators have the potential to remove the mineral content (27,28). Thus, using both agents together can lead to apical microleakage. Furthermore, these compounds have been recommended for use in narrow root canals instrumented with rotary

systems. However, the chelator residues in the apical region may interfere with the adhesion of sealer to canal walls and consequently increase apical microleakage.

In the current in-vitro study, we compared apical microleakage following the use of three different chelators by fluid filtration method. The results of this study indicated that Glyde File Prep+ NaOCl and RC-Prep+ NaOCl yielded the lowest and the highest degree of microleakage, respectively.

The main reason behind selection of distobuccal canals in the current study was that distobuccal canals do not often have any connection with other canals.

Fluid filtration system has various advantages. It provides quantitative data and it uses positive pressure, which helps eliminate the problems caused by air or fluid entrapment in other methods. It is nondestructive and therefore allows repeated measurements on the same samples. The sensitivity of the system can be adjusted by altering the pressure used and the diameter of the measuring micropipette (14, 22).

Fraser (29) showed that when the chelating agents were applied, they caused softening of the root canal dentine in the cervical and middle thirds of the root, but not in the apical third. In the current study, no statistically significant increase in apical microleakage was observed; it may be due to the fact that chelators cannot penetrate deep into the narrow apical parts of root canals. Biesterfeld *et al*. (20) compared the quality of apical seal following the use of RC-Prep, 2.5% NaOCl solution and 0.5% Salvizol. They reported that RC-Prep group showed the least sealing ability after 2 weeks; however, there was no statistically significant difference among the groups. Similar results were obtained in the current study; demonstrating that RC-Prep+ NaOCl group had the greatest amount of microleakage.

Grandini *et al*. (16) showed that applying Glyde File Prep with 2.5% NaOCl significantly decreased the smear layer and microleakage. The results of the current study also showed that Glyde File Prep+ NaOCl yielded the least amount of microleakage. This may be attributed to the superior efficacy of this chelator in removing the smear layer and providing better seal of dentinal tubules.

Some studies (18, 19) recommended EDTA in combination with NaOCl due to their antimicrobial properties and to decrease apical microleakage. The results of the current study also showed that the use of EDTA with NaOCl resulted in less microleakage in comparison with RC-Prep+ NaOCl or normal saline. Similar results were obtained by Yamashita *et al*. (30). They found that the apical third as well as the middle and coronal thirds of the root canals had not been cleaned in experimental groups (chlorhexidine, saline, NaOCl and NaOCl plus EDTA as irrigating solutions). They reported that cleaning with EDTA plus NaOCl was superior to cleaning with chlorhexidine and saline.

Attal *et al*. (31) and Dogan Buzoglu *et al*. (32) demonstrated that use of EDTA alone or in combination with NaOCl decreased the wettability of dentinal walls of root canals and therefore allowed better penetration of hydrophobic sealers into dentinal tubules and their superior adhesion to cleaned canal walls. These findings are consistent with the observations of the current study showing that use of EDTA along with NaOCl can effectively eliminate apical microleakage.

Prado *et al*. (33) showed no significant difference between chlorhexidine and sodium hypochlorite during chemo-mechanical

preparation followed by EDTA or phosphoric acid for smear layer removal. These findings are consistent with the current results since no statistically significant difference in apical microleakage was observed among the groups.

## Conclusion

Applied chelating agents showed no statistically significant difference in terms of apical microleakage in narrow canals, and they all can be used with NaOCl in root canal preparation. However, use of Glyde File Prep with NaOCl and RC-Prep with NaOCl yielded the lowest and the highest amount of microleakage, respectively.

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**Conflict of Interest: "**None Declared**"**

## References:

1. Pommel L, Jacquot B, Camps J. Lack of correlation among three methods for evaluation of apical leakage. J Endod. 2001 May;27(5):347-50.
2. Lucena-Martín C, Ferrer-Luque CM, González-Rodríguez MP, Robles-Gijón V, Navajas-Rodríguez de Mondelo JM. A comparative study of apical leakage of Endomethasone,Top Seal, and Roeko Seal sealer cements. J Endod. 2002 Jun;28(6):423-6.
3. Ersahan S, Aydin C. Solubility and apical sealing characteristics of a new calcium silicate-based root canal sealer in comparison to calcium hydroxide-, methacrylate resin- and epoxy resin-based sealers. Acta Odontol Scand 2012 Jul;71(3-4):857-62.
4. Ghoddusi J, Dibaji F, Marandi S. Correlation between sealer penetration and microleakage following the use of MTAD as a final irrigant. Aust Endod J. 2010 Dec;36(3):109-13.
5. Ballal NV, Kundabala M, Bhat KS. A comparative evaluation of postobturation apical seal following intracanal irrigation with maleic acid and EDTA: a dye leakage under vaccum study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Mar;109(3):e126-30.
6. Haïkel Y, Freymann M, Fanti V, Claisse A, Poumier F, Watson M. Apical microleakage of radiolabeled lysozyme over time in three techniques of root canal obturation. J Endod. 2000 Mar;26(3):148-52.
7. Yildirim T, Er K, Tasdemir T, Tahan E, Buruk K, Serper A. Effect of smear layer and root-end cavity thickness on apical sealing ability of MTA as a root-end filling material: a bacterial leakage study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Jan;109(1):e67-72.

8. Kustarci A, Arslan D, Kaya B. Effects of three different irrigating solutions and KTP laser irradiation on apical leakage: an electrochemical study. Acta Odontol Scand. 2012 Sep;70(5):377-83
9. Conte G, Mancini M, Cerroni L, Costantini M, Cianconi L. Fluid filtration and dye leakage testing of Resilon/Epiphany and guttapercha/Pulp Canal Sealer root canal fillings. OJST 2013 Nov; 3(9): 14-21.
10. Vasconcelos BC, Bernardes RA, Duarte MA, Bramante CM, Moraes IG. Apical sealing of root canal fillings performed with five different endodontic sealers: analysis by fluid filtration. J Appl Oral Sci. 2011 Aug;19(4):324-8.
11. Orucoglu H, Sengun A, Yilmaz N. Apical leakage of resin based root canal sealers with a new computerized fluid filtration meter. 2005 Dec;31(12):886-90.
12. Moradi S, Disfani R, Baziar H, Daneshvar F, Jafarzadeh H. Use of fluid filtration method to evaluate the effect of master cone size on the apical seal of severely curved root canals. 2013;55(2):93-8.
13. Kaya S, Yiğit Özer S, Adigüzel Ö, Oruçoğlu H, Deger Y, Tumen EC, et al. Comparison of apical microleakage of dual-curing resin cements with fluid-filtration and dye extraction techniques. Med Sci Monit. 2015 Mar 31;21:937-44.
14. Moradi S, Lomee M, Gharechahi M. Comparison of fluid filtration and bacterial leakage techniques for evaluation of microleakage in endodontics. Dent Res J (Isfahan). 2015 Mar-Apr;12(2):109-14.
15. Wu M-K, De Gee AJ, Wesselink PR. Fluid transport and dye penetration along root canal fillings. Int Endod J. 1994 Sep;27(5):233-8.
16. Grandini S, Balleri P, Ferrari M. Evaluation of Glyde File Prep in combination with sodium hypochlorite as a root canal irrigant. 2002 Apr;28(4):300-3.
17. Hülsmann M, Heckendorff M, Lennon Á. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003 Dec;36(12):810-30.
18. Ji Y, He M, Chang S, Zhang X, Yang H. Influence of EDTA on demineralization rate of dentine: calcification treatment in root canal therapy. Journal of Materials Science & Technology. 2014;30(7):692-8.
19. Nischith KG, Srikumar GP, Razvi S, Chandra RV. Effect of smear layer on the apical seal of endodontically treated teeth: An ex vivo study. J Contemp Dent Pract. 2012 Jan 1;13(1):23-6.

20. Biesterfeld RC, Taintor JF. A comparison of periapical seals of root canals with RC-Prep or Salvizol. Oral Surg Oral Med Oral Pathol. 1980 Jun;49(6):532-7.
21. Farhad A, Elahi T. The effect of smear layer on apical seal on endodontically treated teath. J Res Med Sci 2004. 9(3):130-133.
22. Zarei M, Barzanoni M, Esmaieli H. An in vitro evaluation of apical leakage after root canal preparation by RC Prep. J Mash Dent Sch; 2006 Mar; 30(3,4): 217-24.
23. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod. 2002 Dec;94(6):658-66.
24. 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 Feb;33(2):96-105.
25. Violich DR, Chandler NP. The smear layer in endodontics–a review. Int Endod J. 2010 Jan;43(1):2-15.
26. Kennedy WA, Walker WA, Gqugh RW. Smear layer removal effects on apical leakage. J Endod . 1986 Jan;12(1):21-7.
27. Behrend GD, Cutler C, Gutmann J. An in vitro study of smear layer removal and microbial leakage along root canal fillings. Int Endod J. 1996 Mar;29(2):99-107.
28. Calt S, Serper A. Smear layer removal by EGTA. J Endod. 2000 Aug;26(8):459-61.
29. Fraser JG. Chelating agents: Their softening effect on root canal dentin. Oral Surg Oral Med Oral Pathol. 1974 May;37(5):803-11.
30. Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LAB. Scanning electron microscopic study of the cleaning ability of chlorhexidine as a root canal irrigant. Int Endod J. 2003 Jun;36(6):391-4.
31. Attal JP, Asmussen E, Degrange M. Effect of surface treatment on the free surface energy of dentin. Dent Mater. 1994 Jul;10(4):259-64.
32. Dogan Buzoglu H, Calt S, Gümüsderelioglu M. Evaluation of the surface free energy on root canal dentine walls treated with chelating agents and NaOCl. Int Endod J. 2007 Jan;40(1):18-24.
33. Prado M, Simao RA, Gomes BP. A microleakage study of gutta-percha/AH Plus and Resilon/Real self-etch systems after different irrigation protocols. J Appl Oral Sci. 2014 Jun;22(3):174-9.