"Evaluation of the effect of EDTA, EDTAC, RC-Prep and BioPure MTAD on the microhardness of root canal dentine- An in vitro study"

SANDEEP SINGH * #
SHASHI RASHMI ACHARYA ** #
VASUDEV BALLAL *** #
RIJESH M **** ##

ABSTRACT

Aim: To determine the effect of Ethylenediaminetetraacetic acid (EDTA), Ethylenediaminetetraacetic acid plus Cetavlon (EDTAC) solutions, RC-Prep and a Mixture of tetracycline isomer/ an acid / a detergent (Biopure MTAD) on the Coronal, Middle and Apical root canal dentine.

Materials and Methods: Twenty intact freshly extracted single rooted maxillary and mandibular anterior human teeth were taken and stored in physiological saline solution until used.. The teeth were sectioned longitudinally by diamond abrasive disc, embedded in acrylic resin and polished with different grades of sand paper and finally with alumina suspension on felt cloth**.** 40 samples were divided in to four groups(n = 10). Group I samples were treated with 17% EDTA for 1 minute , Group II samples were treated with EDTAC for 1 minute (n = 10),Group III samples were treated with RC-Prep for 1 minute, Group IV samples were treated with BioPure MTAD for 2 minutes and 5 minutes respectively.

Result: There was no statistically significant difference in the microhardness reduction in the coronal, middle and apical third of the root canal dentin when treated with 17% EDTA, EDTAC, RC-Prep and BioPure MTAD.

Conclusion: In all the four groups, microhardness of the root canal dentin was reduced. BioPure MTAD was least effective in reducing the microhardness of root canal dentine and 17% EDTA had the maximum effect.

Keywords: EDTA, EDTAC, RC-Prep BioPure MTAD, Vicker's Microhardness Testing Machine

INTRODUCTION

Endodontic instrumentation using either manual or mechanized techniques, produces a smear layer and smear plugs which contains organic and inorganic particles of calcified tissue and organic elements such as pulp tissue debris, odontoblastic processes, microorganisms, and blood cells in dentinal tubules. [1]

The use of chelating agents and acids have been suggested to remove the smear layer from the root canal, because the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them highly soluble in acids. [2,3,4]

Chelation is a physico-chemical process which involves the uptake of multivalent positive ions by specific chemical substances. In the specific case of root dentine, the agent reacts with the calcium

* Post Graduate Student, ** Professor and Head, *** Reader, **** Research Scholar, # Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Manipal. ## Department of Metallurgical and Materials Engineering,NITKS, Surathkal.

ions in the hydroxyapatite crystals. This process can cause changes in the microstructure of the dentine and changes in the Ca : P ratio.

Various irrigating solutions have been tried, among which 17% EDTA has been popular as the most effective chelating agent for the removal of smear layer.

Initially, the use of EDTA solution in Endodontics was proposed by Østby (1957) who recommended the use of 15% EDTA to assist with the instrumentation of calcified, narrow or blocked canals, because of its ability to foster the chelation of the calcium ions at a pH close to neutral (Hill 1959).

Hill and Goldberg and Abramovich reported that addition of a quaternary ammonium bromide (Cetavlon) to 15% EDTA increased the action by reducing its surface tension, because EDTA solutions act only through direct contact with the substrate. Guerisoli et al[5] stated that the association of EDTA with a wetting agent enhances its bactericidal effectiveness.

RC-Prep introduced by Stewart et al. in 1969, contains 15% EDTA, 10% urea peroxide (UP), and glycol. Oxygen is set free by the reaction of RC-Prep with NaOCl irrigant so that pulpal remnants and blood coagulates can be easily removed from the root canal wall.

BioPure MTAD a new irrigant, based on a mixture of antibiotic [Doxycylcline Hyclate:- 150mg/5ml (3%), citric acid (4.25%), and a detergent (0.5 % Polysorbate 80 detergent or Tween 80)]. It has a pH of 2.15 that is capable of removing inorganic substances. The recommended final irrigation to be done by BioPure MTAD is 5 minutes. It has also been confirmed that the smear layer removing capability of BioPure MTAD is not compromised when used for 2 minutes as final irrigant. [6]

It has been reported that some chemicals used for endodontic irrigation are capable of causing alterations in the chemical composition of dentin. [7,8,9]

Any change in the Ca/P ratio may alter the original proportion of organic and inorganic components, which in turn change the microhardness, permeability, and solubility characteristics of dentin. [8,9,18,19]

Panighi and G'Sell[10] reported a positive correlation between hardness and the mineral content of the tooth. It has been indicated that microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues.[11] So as microhardness of root canal dentin is sensitive to its composition and surface changes, the present study aims to demonstrate that microhardness tests, being a simple and effective method to evaluate and compare the demineralization power of different chelating agents , given that the tests are carefully calibrated.

Thus the purpose of this study is to determine the effect of EDTA, EDTAC, RC-Prep and BioPure MTAD solutions on the microhardness of human root canal dentine.

MATERIALS AND METHODS

Twenty intact freshly extracted single rooted maxillary and mandibular anterior human teeth were taken and stored in physiological saline solution containing 0.1% Sodium azide until use. They were sectioned transversely at CEJ by

diamond disc and crowns were discarded**.**

The teeth were sectioned longitudinally and embedded in acrylic resin followed by polishing with different grades of sand paper and finally with alumina suspension on felt cloth**.** The samples were randomly divided in to 4 groups (n = 10) based on the test solution used.

Group I: 17% EDTA solution was freshly prepared by using EDTA solution (pH = 7.3) with the following composition:

- Disodium salt of EDTA (17.00g)
- Aqua dest. (100.00ml)
- 5M Sodium hydroxide (9.25mL)

Group II: EDTAC was freshly prepared by using 15% EDTA at (pH = 7.3).

0.75g of the detergent Cetyl-tri-methyl ammonium bromide is added to 100 ml of the solution .[12]

Group III: RC-Prep (Premier Dental Philadelphia, PA, USA), paste type chelator.

Group IV: BioPure MTAD Tulsa Dental, USA

Samples in Group I,II, III were treated with the chelating agent for 1 minute and in Group IV samples were treated for 2 and 5 minutes respectively.

The subjected samples were treated with 1ml of specific solutions and were irrigated with 0.9% saline after their prescribed time limit. Since RC-Prep is a paste type chelator, it was coated on to the dentin surface by a F1 Protaper File.

A MicroVicker's Hardness Tester (Fuel Instruments and Engineers Pvt. Ltd.) was used. The diamond-shaped indentations were carefully observed in an optical microscope with a digital camera and image analysis software, allowing the accurate digital measurement of their diagonals. The average length of the two diagonals was used to calculate the microhardness value (MHV). All experiments were completed under the same conditions: 50 g load and 15 s dwell time[13], following the suggestions by Cruz-Filho et al. (2001)[14]. In each sample, three indentations were made each in the coronal, middle and apical third of the root canal dentin sample.

At the beginning of the experiment, reference microhardness values (MHVs) were obtained for samples prior to application of the solutions (Before Application), so that the same samples can act as their own controls. In Group IV, after obtaining reference MHVs(Before Application), samples were subjected to the test solution for 2 minutes [After Application (2 min)] followed by additional 3 minutes i.e. a total time exposure of 5 minutes [After Application (5 min)] a second and a third set of measurements, adjacent to the previous ones, were obtained respectively.

STATISTICAL ANALYSIS

Statistical analysis for comparing microhardness values in the coronal, middle and apical area of the root canal dentin for the four different test groups were carried out using 2-way ANOVA with repeated measures ($P < 0.05$).

RESULTS

The mean and SD values of the coronal,middle and apical third root dentin microhardness data for various groups are listed in Table 1. In all the four groups, microhardness was

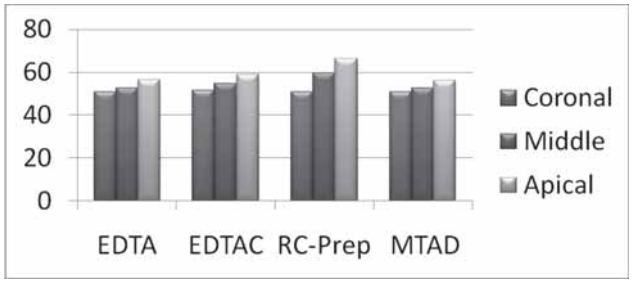
reduced in the coronal, middle and apical third of the root canal dentin. BioPure MTAD was least effective in reducing the microhardness of root canal dentine and 17% EDTA had the maximum effect.

There was no statistically significant difference in the microhardness reduction in the coronal, middle and apical third of the root canal dentin when treated with 17% EDTA, EDTAC, RC-Prep and BioPure MTAD.

Intergroup Comparison
Repeated Measures Anova Test (Table 1)

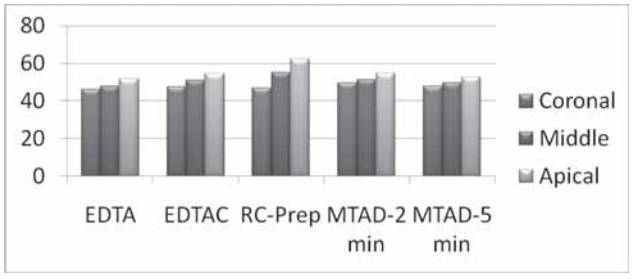
EDTA	Microhardness Mean	Std. Deviation	N
Before Application mean of Coronal 1/3rd	50.9600	8.03375	10
Before Application mean of Middle 1/3rd	52.6067	8.09037	10
Before Application mean of Apical 1/3rd	56.4200	13.07606	10
After Application (1 min)mean of Coronal 1/3rd	46.1933	7.79013	10
After Application (1 min) mean of Middle 1/3rd	48.0333	7.86653	10
After Application (1 min) mean of Apical 1/3rd	51.6533	13.17985	10
EDTAC	MicrohardnessMean	Std. Deviation	N
Before Application mean of Coronal 1/3rd	51.5867	3.50211	10
Before Application mean of Middle 1/3rd	54.7633	2.12327	10
Before Application mean of Apical 1/3rd	58.8900	1.63655	10
After Application (1 min)mean of Coronal 1/3rd	47.7233	3.73264	10
After Application (1 min) mean of Middle 1/3rd	51.0800	2.19760	10
After Application (1 min) mean of Apical 1/3rd	54.6833	1.79631	10
RC-Prep	Microhardness Mean	Std. Deviation	N
Before Application mean of Coronal 1/3rd	50.9000	6.51445	10
Before Application mean of Middle 1/3rd	59.6733	5.08636	10
Before Application mean of Apical 1/3rd	66.2400	3.91174	10
After Application (1 min)mean of Coronal 1/3rd	46.9533	6.56081	10
After Application (1 min) mean of Middle 1/3rd	55.1133	5.15182	10
After Application (1 min) mean of Apical 1/3rd	62.3200	3.70176	10
Bio-Pure - MTAD	Microhardness Mean	Std. Deviation	N
Before Application mean of Coronal 1/3rd	50.9500	8.00301	10
Before Application mean of Middle 1/3rd	52.5567	8.07983	10
Before Application mean of Apical 1/3rd	56.3267	12.96247	10
After Application (2 min)mean of Coronal 1/3rd	49.8567	8.13277	10
After Application (2 min) mean of Middle 1/3rd	51.5067	8.28553	10
After Application (2 min) mean of Apical 1/3rd	54.9367	12.97142	10
After Application (5 min)mean of Coronal 1/3rd	47.7967	8.12412	10
After Application (5 min) mean of Middle 1/3rd	49.5800	8.34076	10
After Application (5 min) mean of Apical 1/3rd	52.8300	12.98556	10

Befor Application Microhardness Comparison



In the 40 samples tested, comparison of the microhardness of the root canal dentin in coronal1/3rd, middle1/3rd and apical 1/3rd respectively was **statistically insignificant**.

Afteer Application Microhardness Comparison



In the 40 samples tested, comparison of the microhardness reduction of the root canal dentin by different chemicals in coronal1/3rd, middle1/3rd and apical 1/3rd respectively was **statistically insignificant**.

DISCUSSION

Smear layer is a negative factor when sealing the root canal because of its weak adherence to the root canal walls hindering sealer adhesion. Thus, the removal before obturation to allow intimate contact of the sealer with the dentin surface is mandatory. [15]

Serper et al [16], concluded in their study that 17% EDTA has the potential for causing excessive peritubular and intertubular dentinal erosion if the application time exceeds 1 min. Thus in the present study, all EDTA based chelating agents had application time which was limited to 1 minute.

The irrigation regimen for BioPure MTAD is initial rinse with 1.3% Sodium hypochlorite during instrumentation and 5 minute final rinse with BioPure MTAD for effective removal of smear layer and desired antibacterial effect. A recent study showed that the use of a 2 minute final irrigation time did not compromise the smear layer removal capability of BioPure MTAD. [6] Thus, in the present study, we used both the time periods i.e. 2 and 5 minutes for the application of BioPure MTAD and consequently checked the microhardness.

It is clear that the comparison between the MHV values would be biased by the underlying differences in dentine morphology. Thus, in the present work, the actual measurements were obtained from three indentations each in the coronal, middle and apical third of root canal dentin. This methodological approach differs from the clinical situation in which the chelator substances affect the dentine walls more strongly. However, this approach allows a much better control of experimental variables, leading to readily comparable results that are fundamental for the present study. [4]

It was also noted in all the samples that their was a variable increase in the microhardness from coronal to apical third of root canal dentin irrespective of treatment with any test agent. This may be attributed to the histology of the root canal dentin. Carrigan et al. [20] showed that tubule density decreased from cervical to apical dentine and Pashley et al. [21] reported an inverse correlation between dentine microhardness and tubular density.

In the present study, maximum decrease in microhardness was achieved by EDTA followed by RC-Prep, EDTAC and MTAD. But, the difference

in microhardness was statistically insignificant.

Zehnder et al. [22] (2005) reported that the association of an endodontic chelator solution with a wetting agent that reduces surface tension did not improve the effectiveness of Calcium ion removal. This conclusion is confirmed by the present work, as EDTAC was not more effective than 17% EDTA in reducing the microhardness.

In our study, BioPure MTAD treatment of root canal dentin resulted in least microhardness reduction of root canal dentin. This finding is in agreement with past study which reported that BioPure MTAD is effective in removing smear layer and at the same time is milder on the dentin structure. [17]

However, Gustavo et al [23] reported that there is full saturation of the demineralizing ability of BioPure MTAD after 30 seconds. In the present study, there was reduction in microhardness of root canal dentin after 2 minutes of treatment with BioPure MTAD and there was further decrease in microhardness after a total of 5 minutes of application. This implies that BioPure MTAD has demineralizing ability beyond 2 minutes and up to 5 minutes.

On the basis of the results obtained and experimental conditions of the present study we can conclude that..

1. There was no statistically significant difference in the microhardness reduction of the root canal dentin by 17% EDTA, EDTAC, RC-Prep and BioPure MTAD.

2. Overall, BioPure MTAD was least effective in reducing the microhardness of the root canal dentine and 17% EDTA had the maximum effect.

REFERENCES

1. Sen BH, Wesselink PR, Turkun M. The smear layer: a phenomenon in root canal therapy. International Endodontic Journal 1995;28:141– 8.

2. Pashley DH. Smear layer: overview of structure and function. Proc Finn Dent Soc 1992;88(Suppl I):215-24.

3. 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 Endod 2002;94:658-66.

4. De-Deus G, Paciornik S, Mauricio MH. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. International Endodontics Journal 2006;39:401-7.

5. Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ, Pecora JD. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. International Endodontic Journal. 2002 May;35(5):418-21.

6. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of BioPure MTAD to remove the smear layer. Journal of Endodontics 2003;29:233–9.

7. Dogan H, Calt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. Journal of Endodontics 2001;27:578-80.

8. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. Journal of Endodontics 1996;22:23-5.

9. Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. Journal of Endodontics 2005;31:187-9.

10. Panighi M, G Sell C. Effect of the tooth microstructure on the shear bond strength of a dental composite. J Biomed Mater Res 1993;27:975-81.

11. Rotstein I (1994) Effect of hydrogen peroxide and sodium perborate on the microhardness of human enamel and dentine. Journal of Endodontics 20, 61–3.

12. Pawlicka H (1982) Verwendung der Chelatverbindungen zur Erweiterung der Wurzelkanale: Mikroharteuntersuchung. Stomatologie der DDR 3,355-61.

13. Antonio M, Manoel D. Evaluation effect of EDTA, CDTA and EGTA on Radicular Dentin Microhardness. Journal of Endodontics 2001; 27;3; 183-184.

14. Cruz-Filho AM, Sousa-Neto MD, Saquy PC, Pecora JD (2001) Evaluation of the effect of EDTAC, CDTA and EGTA

on radicular dentine microhardness. Journal of Endodontics 27,183–4.

15. Sousa Neto MD, Marchesan MA, Pécora JD, Brugnera Junior A, Sousa YTCS, Saquy PC. Effect of Er:YAG laser on adhesion of root canal sealer. Journal of Endodontics 2002;28:185-187.

16. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. Journal of Endodontics 2002;28:17–9.

17. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. Journal of Endodontics 2003;29:170-5.

18. Hennequin M, Douillard Y. Effects of different pH values of citric acid solutions on the calcium and phosphorus contents of human dentin. Journal of Endodontics 1994;20:551– 4.

19. Dogan H, Calt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. Journal of Endodontics 2001;27:578–80.

20. Carrigan PL, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. Journal of Endodontics 1984;10:359–63.

21. Pashley D, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. Endod Dent Traumatol 1985;1:176 –9.

22. Zehnder M, Schicht O, Sener B, Schmidlin P (2005) Reducing surface tension in endodontic chelator solutions has no effect on their ability to remove calcium from instrumented root canals. Journal of Endodontics 31, 590–2.

23. Gustavo De-Deus, MD*, Claudia Reis, MS, Sandra Fidel, PhD, Rivail Fidel, PhD,†and Sidnei Paciornik, PhD Dentin Demineralization When Subjected to BioPure MTAD: A Longitudinal and Quantitative Assessment. Journal of Endodontics: 2007 ;33;11