Comparative evaluation of demineralization of radicular dentin with 17% ethylenediaminetetraacetic acid, 10% citric acid, and MTAD at different time intervals: An in vitro study

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Abstract

Background: The smear layer has the capability to protect the bacteria within the dentinal tubules from intracanal medicament. After removal of the smear layer from infected root canals, it allows disinfection of the entire root canal. The smear layer compromising the seal between the root canal sealer and root canal wall also decreases the penetration of irrigants into dentinal tubules. Aims: This study compares the amount of phosphorous liberated and demineralization of the radicular dentin with 17% ethylenediaminetetraacetic acid, 10% citric acid and mixture of doxycycline, citric acid, and a detergent at different time intervals. Materials and Methods: Extracted maxillary single-rooted teeth were prepared by using a combination of passive step-back and rotary 0.04 taper nickel-titanium files. Sodium hypochlorite 5.25% and sterile distilled water were used as an intracanal irrigant. The canals were then treated with 5 mL of one of the following solutions such as final rinse sterile distilled water, 5.25% sodium hypochlorite, 17% ethylenediaminetetraacetic acid (EDTA) or mixture of doxycycline, citric acid, and a detergent. The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portions of each canal were examined under a scanning electron microscope. Statistical Analysis: Data were analyzed by one-way analysis of variance (ANOVA) to determine whether there were significant differences between the groups. Results: The results show that mixture of doxycycline, citric acid, and a detergent is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules. Conclusions: In this study, 10% citric acid shows the maximum amount of dimeneralization of radicular dentine followed by mixture of doxycycline, citric acid, and a detergent, and 17% ethylenediaminetetraacetic acid. When all the subgroups were compared, it was seen that the maximum amount of phosphorus liberation was performed by 10% citric acid >mixture of doxycycline, citric acid, and a detergent >17% EDTA at a different time interval.

Key words: Citric acid, detergent, citric acid, smear layer, ethylenediaminetetraacetic acid, mixture of doxycycline, MTAD, smear layer
INTRODUCTION

The goal of endodontic therapy is to remove microorganisms from the canal and prevent their reentry.\[1\] The successful root canal therapy depends on open cleaning, shaping, and three-dimensional obturation of the canal. Access cavity preparation, root canal instrumentation with proper irrigation, intracanal medicaments, obturation, and finally coronal restoration under proper isolation are all parts of an aseptic chain.\[2\] Recently used methods of biomechanical preparation, especially rotary instrumentation techniques produce a smear layer that covers the dentinal tubules in the canal wall.\[3\] The compromised sealing ability of obturation material and colonization of bacteria in the dentinal tubules by smear layer are still controversial.\[4-6\] The smear layer itself can be contaminated and the ability to protect bacteria from intracanal medicament or irrigation. Removal of the smear layer from dentinal tubules with infected root canals and disinfection of the entire root canal are to be allowed.\[7\] Also, the smear layer decreases the penetration effect of irrigants and root canal sealers into dentinal tubules and has the potential of decrease the seal between the root canal sealer and the root canal wall.\[8\]

The property of the ideal root canal irrigant is able to disinfect the canal and remove the smear layer from dentinal tubules.\[9\] Single root canal irrigant solution is not able to allow both effects alone so the acids or chelating agents use with tissue solvents has been advocated. Sodium hypochlorite (NaOCl) at the concentrations of 0.5–5.25% is a commonly used root canal irrigating solution in endodontics. Used of chelating agent is 17% ethylenediaminetetraacetic acid (EDTA) that provides an excellent irrigation for calcified and narrow canals with little effect on periapical tissues. Various studies reported the efficacy of EDTA and sodium hypochlorite in removing the smear layer.\[10\] Organic acids such as citric acid may also be used for smear layer removal. Concentrations ranging 1–50% have been investigated.\[11\] Tidmarsh et al.\[12\] also reported that 50% citric acid irrigation was effective in removal of the smear layer. Wayman et al.\[13\] showed that the use of 2.5% NaOCl and 10% citric acid was a very effective approach for removal of the smear layer. Recently, Torabinejad et al.\[14\] introduced root canal irrigation material MTAD (mixture of a tetracycline isomer, an acid, and a detergent) for removal of the smear layer and disinfection of the canal. The MTAD is a more effective irrigant than the others as a final irrigant to remove the smear layer and clinically, the result shows that it also batters antibacterial property.\[15,16\] MTAD is commercially available as Biopure MTAD (Dentsply Tulsa Dental, Tulsa, OK).\[17\]

Aims and objectives

The aim of this study was to evaluate and compare the demineralization of radicular dentin and evaluate the amount of phosphorous liberated dentin with 17% EDTA [Dental Avenue India Pvt. Ltd. Mumbai India], 10% citric acid (Biochemistry Lab, Udaipur, Rajasthan, India), and MTAD (Dentsply Tulsa Dental, Tulsa, OK) at different time intervals.

MATERIALS AND METHODS

This study was conducted in the Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Loyara, Udaipur, Rajasthan, India. Sixty-five freshly extracted human permanent maxillary incisor teeth were free of visible caries, cracks, and restorations were used in this study.

Access cavity was prepared by using round no. 2 bur and removal of pulp tissue from canal was done by using a barbed broach. The working length was taken by inserting a no. 10 size k file into each root canal until it was just visible from the apical foramen; the file was windrow 1 mm and kept 1 mm short to achieve desired working length. Apically, one-third of the canals were prepared up to a size 40 by K-file (SybronEndo Mexico). After chemomechanical preparation, the teeth were decorated at the cementoenamel junction using a high speed diamond disc (Dfs M.I. Germany) with a cooling system to standardize the root length of 13 mm. The roots were cut longitudinally into two segments and the length and width of the roots were normalized to attain equal areas to be exposed to the irrigating solutions. The outer surface of the root was isolated with nail polish.

The specimens were then randomly divided into four experimental groups. Each experimental group was further divided into four subgroups on the basis of time interval consisting of 40 specimens each and the fourth experimental subgroup (control group) consisting of 10 specimens than 1 mL of final irrigating solution was taken into a test tube. Then the selected specimen placed into 17% EDTA, 10% citric acid, and MTAD solution for different time periods as we required for the test groups. The specimen was immersed in the solution for different time periods of 1 min, 5 min, 10 min, and 15 min.
Automated random access biochemistry analyzer” ERBA CHEM-5 Plus Germany was used to measure the amount of phosphorus liberated from the solution.

The final irrigation regimens for the experimental groups are as follows [Table 1]:
- Group I—17% EDTA—20 teeth
- Group II—10% citric acid—20 teeth
- Group III—MTAD—20 teeth
- Group IV—saline (control group)—5 teeth

Each group was further divided into four subgroups on the basis of time intervals [Table 1]:
- Subgroup I—1 min
- Subgroup II—5 min
- Subgroup III—10 min
- Subgroup IV—15 min.

RESULT

Group-wise assessment of the amount of phosphorus liberation was done using the automated random access biochemistry analyzer. Graph 1 show the amount of phosphorus liberation (mg/dL) for different time intervals. All the groups demonstrated phosphorus liberation. The control specimens demonstrated no phosphorus liberation.

After 1 min, 5 min, and 10 min immersion in irrigating solution, 10% citric acid had extracted the greatest amount of phosphorus followed by MTAD and 17% EDTA. The differences among the solutions were significant \( P < 0.05 \). After a 15-min immersion, MTAD showed the maximum amount of phosphorus liberation.

Table 1: Intergroup comparison by analysis of variance (anova) to determine statistical significance of difference of mean of each group

| (I) Groups of material | (J) Groups of material | Mean difference (I-J) | Standard error | Significant P value | 95% confidence interval |
|------------------------|------------------------|----------------------|----------------|-------------------|------------------------|
|                        |                        |                      |                |                   | Lower bound | Upper bound    |
| For 1 min              |                        |                      |                |                   | Lower bound | Upper bound    |
| 17% EDTA†              | Citric acid            | -12.73000            | 0.23672        | <0.001            | -13.4296   | -12.0304       |
|                        | MTAD                   | -8.78000             | 0.23672        | <0.001            | -9.4796    | -8.0804        |
| 10% citric acid        | EDTA                   | 12.73000             | 0.23672        | <0.001            | 12.0304    | 13.4296        |
|                        | MTAD                   | 3.95000              | 0.23672        | <0.001            | 3.2504     | 4.6496         |
| MTAD                   | EDTA                   | 8.78000              | 0.23672        | <0.001            | 8.0804    | 9.4796         |
|                        | Citric acid            | -3.95000             | 0.23672        | <0.001            | -4.6496   | -3.2504        |

†EDTA: Ethylenediaminetetraacetic acid

For 5 min

| (I) Groups of material | (J) Groups of material | Mean difference (I-J) | Standard error | Significant P value | 95% confidence interval |
|------------------------|------------------------|----------------------|----------------|-------------------|------------------------|
|                        |                        |                      |                |                   | Lower bound | Upper bound    |
| 17% EDTA†              | Citric acid            | -10.37000            | 0.36998        | <0.001            | -11.4634   | -9.2766        |
|                        | MTAD                   | -7.85000             | 0.36998        | <0.001            | -8.9434    | -6.7566        |
| 10% citric acid        | EDTA                   | 10.37000             | 0.36998        | <0.001            | 9.2766    | 11.4634        |
|                        | MTAD                   | 2.52000              | 0.36998        | <0.001            | 1.4266     | 3.6134         |
| MTAD                   | EDTA                   | 7.85000              | 0.36998        | <0.001            | 6.7566    | 8.9434         |
|                        | Citric acid            | -2.52000             | 0.36998        | <0.001            | -3.6134   | -1.4266        |

†EDTA: Ethylenediaminetetraacetic acid

For 10 min

| (I) Groups of material | (J) Groups of material | Mean difference (I-J) | Standard error | Significant P value | 95% confidence interval |
|------------------------|------------------------|----------------------|----------------|-------------------|------------------------|
|                        |                        |                      |                |                   | Lower bound | Upper bound    |
| 17% EDTA†              | Citric acid            | -32.42000            | 0.44586        | <0.001            | -33.7377   | -31.1023       |
|                        | MTAD                   | -11.41000            | 0.44586        | <0.001            | -12.7277   | -10.0923       |
| 10% citric acid        | EDTA                   | 32.42000             | 0.44586        | <0.001            | 31.1023    | 33.7377        |
|                        | MTAD                   | 21.01000             | 0.44586        | <0.001            | 19.6923    | 22.3277        |
| MTAD                   | EDTA                   | 11.41000             | 0.44586        | <0.001            | 10.0923    | 12.7277        |
|                        | Citric acid            | -21.01000            | 0.44586        | <0.001            | -22.3277   | -19.6923       |

†EDTA: Ethylenediaminetetraacetic acid

Contd...
DISCUSSION

The microleakage might be a result of the presence a fluid-filled space in between the sealers and the wall of the canal. This space could be a result of the lack of sealing between filling material to the root canal walls’ presence of smear layer on the root canal walls. This smear layer acts as a physical barrier between dentinal tubules and sealers and interference with adhesion and penetration of sealers into the dentinal tubules, thereby preventing the bonding and adherence of the root canal filling material to the dentinal wall.[18] Therefore, removal of the smear layer may enhance the seal of the root canal filling.[19] Removal of the smear layer can be achieved by chelating agents such as citric acid, EDTA, and MTAD. However, the use of EDTA (chelating agent) and NaOCl (an organic tissue solvent) in alternative irrigation is found to be more effective for removal of the smear layer from the root canal system.[20] Serper and Calt et al.[22] found that 17% EDTA had a greater demineralizing effect than 10% EDTA.[23,24]

The introduction of MTAD, which consists of an aqueous solution of 3% doxycycline, 4.25% citric acid, and 0.5% polysorbate 80 detergent,[25] represents a clinical effective endodontic irrigant. This biocompatible intracanal irrigant[26] is commercially available as a two-part mix (BioPure MTAD; Dentsply Tulsa, Tulsa, OK). MTAD is more effective in removing the smear layer[27] and also removing the microorganism that resist other endodontic irrigants, intracanal medicament, and provides antimicrobial effect by the effective binding of doxycycline to dental hard tissues.[28] Generally, the irrigation of each canal by 5–10 mL of chelator is for a minimum of 1 min.[29] In the present study, the final irrigation regimen consisting of 40 mL of 17% EDTA, 40 mL of 10% citric acid, and 40 mL of MTAD was chosen.[30] MTAD shows to be very aggressive in its actions; it may weaken the dentinal wall by excessive softening and erosion of the dentin, which could be aggressive by MTAD that remains after drying with paper traces of MTAD is present within dentinal tubules. The use of MTAD in children and pregnant women is avoided because of it deleterious action on dental structures.[31]

The studies conducted by Akhlaghi and Saghiri et al. and Torabinejad et al.[32,33] showed that MTAD was superior to EDTA in removing the smear layer.

The only disadvantage of MTAD is ineffective against C. albicans and its substantivity may be altered when used in conjunction with NaOCl.[34]

CONCLUSION

In this study, 10% citric acid shows the maximum amount demineralization of radicular dentin followed by MTAD and 17% EDTA. When all the subgroups were compared, it was seen that the maximum amount...
of phosphorus liberation was performed by 10% citric acid > MTAD > 17% EDTA at different time intervals. It shows that 10% citric acid followed by MTAD was most effective in demineralization of radicular dentin and 17% EDTA was least effective in comparison to the other two irrigating solutions.

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Conflicts of interest
There are no conflicts of interest.

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