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Original Research

Comparative Evaluation of a New Endodontic Irrigant - Mixture of a Tetracycline Isomer, an Acid, and a Detergent to Remove the Intracanal Smear Layer: A Scanning Electron Microscopic Study

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Abstract:

Background: The most important and demanding aspect of endodontic therapy is considered to be cleaning and shaping. Irrigation is considered a vital adjunct to instrumentation of the root canal for canal debridement. Until date, there is no single solution that simultaneously removes the smear layer and disinfects the entire root canal system. Thus, this *in vitro* study was designed to evaluate the efficiency of a new irrigation solution mixture of a tetracycline isomer, an acid, and a detergent (MTAD) (BioPure, Dentsply) containing a mixture of tetracycline (doxycycline hydrochloride), an acid (citric acid) and a detergent (Tween 80) in comparison with normal saline, 5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) to remove intracanal smear layer.

Materials and Methods: A total of 60 single rooted teeth were irrigated with Saline (Group A), 5% NaOCl (Group B), 17% EDTA (Group C) and MTDA (Group D). The extent of removal of smear layer and erosion was assessed using scanning electron microscope.

Results: Irrigation with 5% NaOCl and MTAD as a final flush produced the cleanest surface with all the dentinal tubules open. No conjugation or erosion of dentinal tubules was noted ($P > 0.05$).

Conclusion: MTAD flush was the most effective debridement regimen in all the three thirds of the canal showing its ability to reach the apex with no conjugation and erosion of dentinal tubules.

Key Words: Ethylenediaminetetraacetic acid, irrigation, MTAD

Introduction

"Endodontics" has stepped into the arena where more and more people are now realizing that saving even an isolated natural tooth is worth the time and effort as there is no substitute for a healthy natural dentition in order to maintain the integrity of the arch, function and esthetics of the masticatory apparatus.

The key objectives of endodontic therapy are cleaning and shaping, obturation of the root canal system in three dimensions and preventing reinfection. Cleaning and shaping are considered to be the most important and most demanding aspect. There is an old age saying in endodontics that is relevant even today "what is taken out of the root canal may be more important than what is put into the root canal."[1]

Irrigation is considered a vital adjunct to instrumentation of the root canal for canal debridement. It has been found that mechanical endodontic instruments provide 90% of canal debridement but cannot accomplish the biologic objectives as irregularities in the canal system prevent complete debridement. Hence, it has been agreed "files shape and irrigants clean."[1] Irrigants can augment mechanical debridement by acting as a physical flush to remove debris, dissolve tissue, and disinfecting the root canal system.[2]

However, in addition to superficial debris, a layer of sludge material forms over the surface of dentinal walls whenever dentin is cut. This layer of debris is called the smear layer, which contains organic and inorganic materials from the pulp and prepared dentin, and also the microorganisms.[3-6] This phenomenon is more evident with the advent of rotary instrumentation of root canal walls. The smear layer may be infected and may protect the bacteria already present in the dentinal tubules. Because of these concerns, removal of the smear layer in infected root canals is advisable to aid penetration of intracanal medications into the dentinal tubules in this teeth.[5]

Violich and Chandler[7] have exhaustively reviewed the smear layer and conclude that the removal of the smear layer aids in complete disinfection of the root canal system allowing better adaptation of filling materials to the canal walls.

An ideal intracanal irrigant should disinfect the dentinal tubules in one visit. It should effectively remove the smear layer, have persistent antimicrobial effect after use and must be biocompatible with a live host tissues.[8]

An ideal smear layer removing agent should eliminate both organic and inorganic phases from all canal surfaces without harmful erosive effects on dentin.[9] Until date, there is no single solution used in endodontics that simultaneously removes the smear layer and disinfects the entire root canal system. Various organic acids, chelating agents, ultrasonic and lasers have been utilized to eliminate the smear layer. Available evidences have shown that these agents and methods alone do not provide complete disinfection of the root canal spaces in all cases when used in a single visit root canal therapy.[8]

The concentration, ideal temperature, frequency of application, and delivery methods for irrigants, along with the time required for these solutions is continuously being investigated.[10] However, less attention has been given to the components of the irrigating solution to achieve the better results. More recently, doxycycline hydrochloride is under study as an irrigant. The usefulness of tetracycline is attributed to their antibacterial and their ability to inhibit mammalian collagenases.[8] The acidic property (pH-2) of doxycycline hydrochloride is probably responsible for the breakdown of both the superficial and intratubular smear layer attached to the dentin surface. The use of the drug offers the additional benefits as it readily attaches to the dentin and is subsequently released without losing its antibacterial activity. This creates a reservoir of active antibacterial property, which releases the agent from the dentin surface in a slow and sustained manner.[11]

A mixture of a tetracycline isomer, an acid, and a detergent (MTAD) has been introduced as a final irrigant, which represents an innovative approach for the simultaneous elimination of inorganic smear layer and disinfection of root canal system.[12] MTAD is a biocompatible material[13] with solubilizing effect on pulp and dentin similar to ethylenediaminetetraacetic acid (EDTA).[12,14,15]

Thus, this *in vitro* study was designed to evaluate the efficiency of a new irrigation solution MTAD (BioPure, Dentsply) containing a mixture of **t**etracycline (doxycycline hydrochloride), an acid (citric acid) and a detergent (Tween 80) in comparison with normal saline, 5% NaOCl and 17% EDTA to remove intracanal smear layer using scanning electron microscope (SEM).

Materials and Methods

Recently extracted non-carious human mandibular and maxillary single rooted teeth obtained from patients 13-60 years old were stored in saline at room temperature. The criteria for selection were length, straightness, and the apical morphology. If the apex was open to over # 20 K-file in diameter, the tooth was rejected and not used in the study. Teeth with average root length of 14-16 mm were selected. A total of 60 teeth were selected and assigned in a random manner into 4 groups of 15 each.

The root surfaces of teeth were debrided and placed in 3% sodium hypochlorite solution for 24 h to remove any remaining organic tissue. The teeth were stored in normal saline till the beginning of the study. After preparing conventional access cavity, the working length of all the teeth was established by passing a no. 10 file to the apical foramen and then reducing the length by 1 mm. The apical portion of the root tip was covered with sticky wax.

Preparation of irrigating solutions

MTAD solution (BioPure) was prepared freshly by mixing the powder (Part A) and liquid (Part B) as supplied by Dentsply. 30-gauge needle with side vent and a blunt distal end was then attached to the syringe for irrigation.

17% EDTA, 5% NaOCl and normal saline were used as supplied by the manufacturer.

Preparation of the root canal

The biomechanical preparation was done using nickel-titanium RACE (FKG) rotary instruments in a gear reduction handpiece (Anthogyr), up to the apical size # 30 with a taper of 0.04. The canals were irrigated with 1 ml of either sterile saline solution or sodium hypochlorite (5%) after use of each instrument, according to group. A volume of 10 ml volume of irrigant was used. The irrigant was delivered with a 30-gauge, 1½ inch needle (ProRinse, Dentsply).

The specimens were then divided into four groups, depending upon irrigant/irrigants used as a final rinse as shown in Table 1.

After completion of BMP till apical size 30, the crowns of all the teeth were removed at the Cementoenamel junction with separating disks with coolant. This was followed by placement of longitudinal grooves on the labial and lingual surfaces using a diamond disk with spray, without penetrating into the canals. Final rinsing of root canals was done with the test solutions in the following manner:

- With the help of 30-gauge Pro Rinse probe, 1 ml of the test solution was delivered in the canal as near as possible to the apex without binding.
- Test solution was left in the canal for 5 min with in-between agitation by # 15 K-file, followed by remaining 4 ml irrigation.

Table 1: Study group.

Group (*n*=15)	Irrigating solution during root canal preparation	Final solution for removal of the smear layer
A	Saline	Saline
B	5% NaOCl	5% NaOCl
C	5% NaOCl	17% EDTA
D	5% NaOCl	MTDA

MTDA: Mixture of a tetracycline isomer, an acid, and a detergent,
EDTA: Ethylenediaminetetraacetic acid

- Final irrigation of root canals was done with 3 ml of distilled water to remove any precipitate that might have formed from the test irrigants and the canals were dried with paper points.

Each tooth was split into two with chisel and mallet stabilizing it in a jig for hammering. One half of the root was discarded and the other half was placed in 2% glutaraldehyde solution for 24 h.

Preparation for SEM study

The specimens were removed from glutaraldehyde solution after 24 h. Three rinses with sodium cacodylate buffered solution (0.1 M, pH 7.2) were done for the fixed specimens and then dehydrated using 30, 50, 70 and 90% of ethyl alcohol by placing them sequentially in each concentration for 10 min. The specimens were then placed in 100% of ethyl alcohol for 8hrs. After dehydration, they were left overnight in the desiccator. Finally, the teeth were coded and mounted on aluminum stubs which were then put into the sputter coater (Gold-Palladium Sputter Coater: JEOL) and glow cleaned for 90 s, followed by sputter coating with gold-palladium alloy under Argon atmosphere. A 20 μm thick film of gold-palladium was coated onto the specimen.

The coded and mounted samples were placed in the vacuum chamber of the SEM. The acceleration voltage was standardized to 7 and 10 K.V with an emission current of 60 μA and width of 20 mm. The angle of tilt and the aperture was adjusted to optimize the quality of photomicrograph. Each sample was micro graphed at these three areas at different magnification and viewed under a SEM (JEOL- JSM).

The photomicrographs taken were qualitatively evaluated blindly and rated for the degree of cleanliness with regard to the presence of debris, smear layer and patency of dentinal tubules on a scale of 1 to 3 where:

1 = No smear layer. Clean and open tubules, the surface of the root canals free of the smear layer.
2 = Moderate smear layer. The surface of the root canals free of the smear layer, but debris found in tubules.
3 = Heavy smear layer. The root canal surface and the tubules covered by the smear layer.

In addition, the degree of erosion of dentinal tubules was scored by the same investigators as follows:

1 = No erosion - Normal appearance and size of all tubules was noted.
2 = Moderate erosion - Erosion of the peritubular dentin was noted.
3 = Severe erosion - Destruction of the intertubular dentin and conjugation of tubules was noted.

Results and Observations

Observations were noted based on the photomicrographs got from the SEM. Inter examiner variability was tested using Kruskal–Wallis test. Mann–Whitney U-test was used for intergroup comparison (Tables 2-5).

Group A - Saline (control): Examination of the surface of root canal walls showed the entire length of the root canals with a heavy smear layer (Figures 1 and 2).

Group B - 5% NaOCl: The surfaces of samples were similarly covered with a heavy layer of debris in the coronal, middle and apical portion of each canal. Dentinal tubules were not visible in Groups A and B (Figure 3).

Group C - 17% EDTA: The surfaces of root canals and the dentinal tubules in the coronal and middle thirds of samples were free of debris. Severe erosion was noted on the root canal surfaces in this group. The surfaces of root canals and the

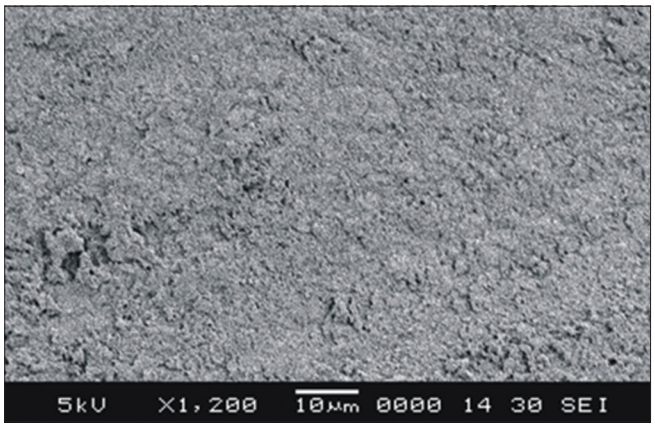

Figure 1: Scanning electron microscope image of saline at middle third (×1200).

Table 2: Comparison between Group AB and CD for smear layer removal.

Canal level	Group	*N*	Mean rank	Sum of ranks	Mann–Whitney U-test	*P* value	Result (at 5% loss)
Cervical	Group A and B	30	45.43	1363	2	0.000	HS
	Group C and D	30	15.56	467			
	Total	60					
Middle	Group A and B	30	45.5	1365	0	0.000	HS
	Group C and D	30	15.5	465			
	Total	60					
Apical	Group A and B	30	40.5	1215	150	0.000	HS
	Group C and D	30	20.5	615			
	Total	60					

HS: Highly significant

Table 3: Comparison between Group C and D for erosion.

Canal level	Group	*N*	Mean rank	Sum of ranks	Mann-Whitney U-test	*P* value	Result (at 5% loss)
Cervical	C (5% NaOCl+17% EDTA)	15	23	345	0	0.000	HS
	D (5% NaOCl+MTAD)	15	8	120			
	Total	30					
Middle	C (5% NaOCI+17% EDTA)	15	23	345	0	0.000	HS
	D (5% NaOCI+MTAD)	15	8	120			
	Total	30					

MTDA: Mixture of a tetracycline isomer, an acid, and a detergent, EDTA: Ethylenediaminetetraacetic acid, HS: Highly significant

Table 4: *P* values for smear layer removal among Groups A to D.

Canal level	Group comparison	*P* value
Coronal	No significant difference between Groups A and B	*P*=1
Coronal	Groups C and D than Groups A and B	*P*=0 (<0.05)
Coronal	No significant difference between Groups C and D	*P*=0.15 (<0.05)
Middle	No significant difference between Groups A and B	*P*=1
Middle	Groups C and D cleaner than Groups A and B	*P*=0 (<0.05)
Middle	Significant difference between Groups C and D	*P*=0.016 (<0.05)
Apical	No significant difference between Groups A and B	*P*=1
Apical	Groups C and D cleaner Than groups A and B	*P*=0 (<0.05)
Apical	No significant difference between Groups C and D	*P*=1

Table 5: *P* values for the amount of erosion Groups C to D.

Canal level	Group comparison	*P* value
Coronal	Group C had more erosion than Group D	*P*<0.05
Middle	Group C had more erosion than Group D	*P*<0.05

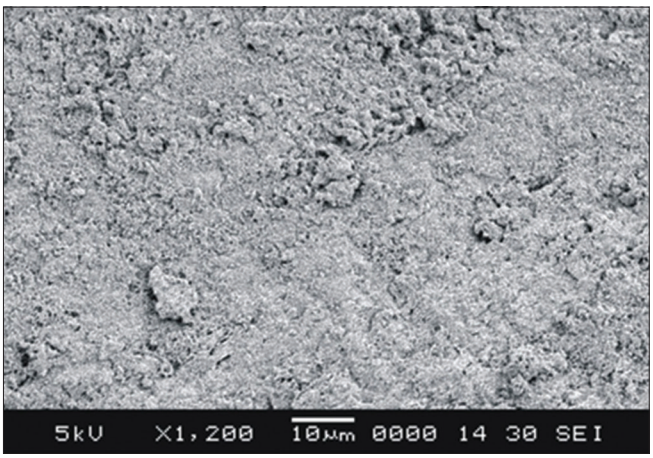

Figure 2: Scanning electron microscope image of saline at apical third showing the smear layer (×1200).

dentinal tubules in the apical third were covered with moderate amounts of debris (Figure 4).

Group D - MTAD: The surfaces of root canals and the dentinal tubules in the coronal, middle and apical thirds of samples were free of debris (Figures 5 and 6).

Discussion

MTAD was selected as an ideal combination in this investigation to determine the effect of this solution as a final rinse for smear layer removal and evaluated for its antibacterial efficiency in comparison with saline, 5% NaOCl and 17% EDTA.

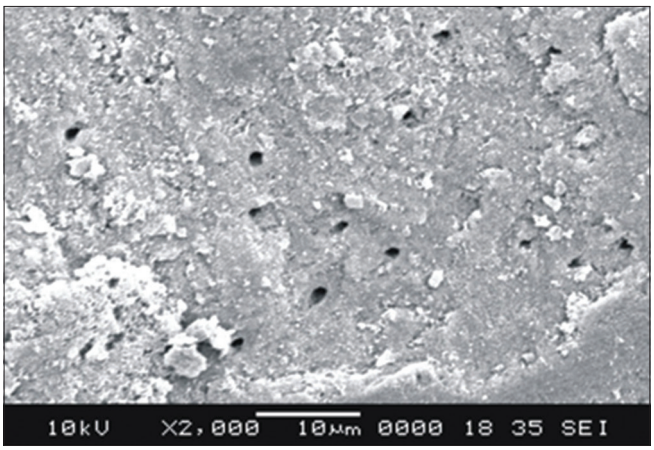

Figure 3: Scanning electron microscope image of NaOCl at coronal third showing the smear layer (×2000).

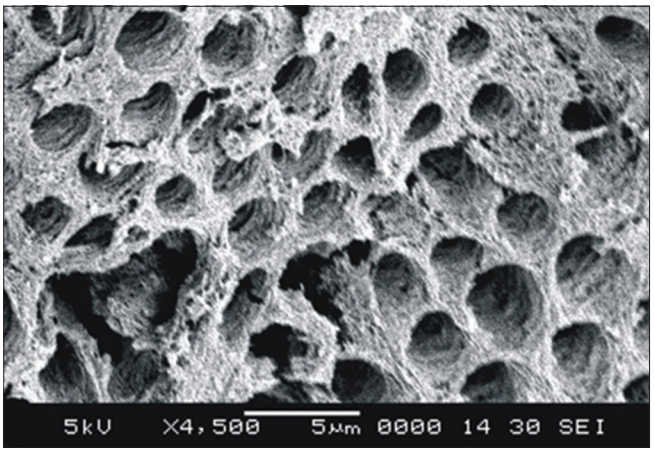

Figure 4: Scanning electron microscope image of ethylenediaminetetraacetic acid at coronal third showing conjugation and erosion of tubules (×2000).

The entire canal length was used to test the effectiveness of the solutions in all segments of the root including the apical third. In many previous studies, the clinical crowns were removed, and the effects of the test solutions at different levels of the root canals were not reported.[16,17] The canals were prepared with RACE rotary nickel-titanium instruments. The technique is an effective method to prepare root canals with rotary instruments. In addition, the use of the rotary files creates a significant amount of smear layer.[18] The apical portion of each canal was enlarged to a size 30-file to allow adequate cleaning and penetration of the solution to the apical third of each root canal.

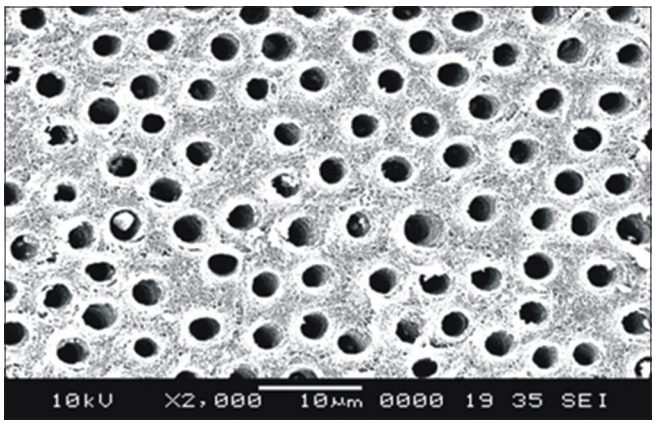

Figure 5: Scanning electron microscope image of mixture of a tetracycline isomer, an acid, and a detergent at coronal third (×2000).

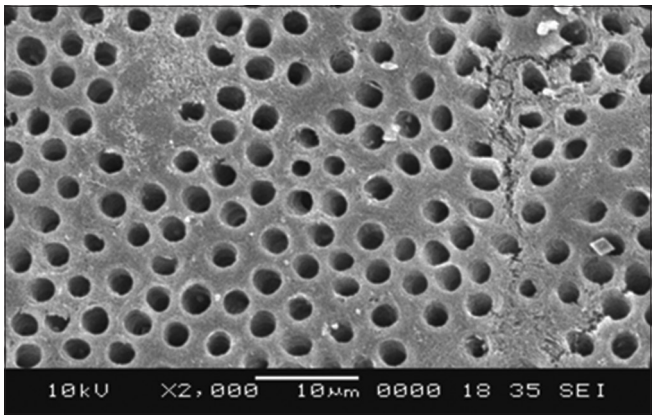

Figure 6: Scanning electron microscope image of mixture of a tetracycline isomer, an acid, and a detergent at middle third (×2000).

Scanning electron microscopy has been used to determine the effectiveness of irrigants to remove the smear layer. It allows an examination of morphologic details of the surfaces of prepared root canal. When examined by the SEM, the smear layer will rarely be discernible on specimens of demineralized teeth because it will be dissolved during the process of demineralization. Undemineralized specimens will appear on electron microscopic examination as a uniform sludge, relatively smooth and featureless.[5,19]

Based on the results of this investigation, Irrigation with 5% NaOCl and MTAD as a final flush produced the cleanest surface with all the dentinal tubules open. In addition, it could be seen that there was no conjugation of dentinal tubules when compared with that of the third group i.e. EDTA group, neither were there any signs of erosion.

Irrigation with 17% EDTA and 5% NaOCl produced a clear surface with occasional strands of isolated superficial debris, but the patent dentinal tubules were clearly seen. Though this irrigating solution efficiently removed the smear layer, there was the conjugation of the dentinal tubules and erosion was seen both at peritubular dentin as well as intertubular dentin. These findings corroborate the results of a recent investigation, which reported a correlation between the erosive property of EDTA and the length of time of dentin exposure to this material.[4,20-22]

From the observations of this study, the efficacy of EDTA and NaOCl regimen were good for coronal and middle, but this combination is less effective in the apical third sections.[20,23] The main disadvantages of the use of EDTA include its destructive effects on coronal and middle thirds of root dentin. In contrast to the destructive effects of 5 min EDTA exposure,[24] we observed no significant dentinal erosion with MTAD.

There was no significant difference in the ability of saline and NaOCl to remove the smear layer from the surfaces of instrumented root canals. The degree of removal of smear layer was dependent on different combinations of irrigating solutions.[3-6,8]

This study is in agreement with the study of Baumgartner *et al*.[22] where, irrigation with normal saline reported a typical amorphous smear layer seen consistently on the instrumented halves and residual superficial pulpal fibers and collagen fibers of the predentin on the uninstrumented halves.

About 5% NaOCl did not show any effect on smear layer proving to be the organic tissue solvent only. Our results are in agreement with previous data showing the ineffectiveness of NaOCl in removing smear layer.[17,21,25,26]

From the observations of this study, the action of MTAD was very effective even in the inaccessible and important apical third, agreeing with the study done by Torabinejad *et al*.,[12] Paul *et al*.[27] However, studies by Lotfi *et al*.[9] concluded that the use of MTAD did not effectively remove the smear layer.

Conclusion

The inferences of the present study are that irrigation with MTAD as final flush results in a remarkably clean surface of the root canal wall. It was the most effective debridement regimen in all the three-third of the canal showing its ability to reach the apex with no conjugation and erosion of dentinal tubules. Thus, MTAD solution is a promising agent for removal of the smear layer from the entire root surface with excellent antibacterial properties.

Further *in vivo* and biocompatibility tests involving MTAD will be necessary to determine whether the results *in vitro* will be validated.

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