Comparative scanning electron microscopy evaluation of Canal Brushing technique, sonic activation, and master apical file for the removal of triple antibiotic paste from root canal (*in vitro* study)

Deepa Ashoksingh Thakur, Sanjay Patil, Vandana Gade, Nitin Jogad, Aparajita Gangrade, Roshan Sinkar

Abstract

Aims: To compare and evaluate the effectiveness of Canal Brushing technique, sonic activation, and master apical file (MAF) for the removal of triple antibiotic paste (TAP) from root canal using scanning electron microscopy (SEM). **Materials and Methods:** Twenty-two single rooted teeth were instrumented with ProTaper up to the size number F2 and dressed with TAP. TAP was removed with Canal Brush technique (Group I, *n*: 6), sonic (EndoActivator) (Group II, *n*: 6), and MAF (Group III, *n*: 6). Four teeth served as positive (*n*: 2) and negative (*n*: 2) controls. The roots were split in the buccolingual direction and prepared for SEM examination (×1000) at coronal, middle, and apical third. Three examiners evaluated the wall cleanliness. **Statistical Analysis:** Statistical analysis was performed by Kruskal–Wallis test and Wilcoxon rank sum test. **Results:** Difference in cleanliness between three groups is statistically significant in cervical region only. Pairwise comparison in cervical region Canal Brush and sonic activation showed more removal of TAP than MAF. **Conclusions:** Canal Brush and sonic activation system showed better result than MAF in the cervical and middle third of canal. In the apical third, none of the techniques showed a better result. None of the techniques showed complete removal of TAP from the canal.

Keywords: Canal Brush, master apical file, sonic activation, triple antibiotic paste

Introduction

Microorganisms have been well-known to play a role in pulpal and periapical diseases. The bacteria associated with primary endodontic infections are mixed but are predominantly Gram-negative anaerobic rods, whereas the bacteria associated with secondary infection comprise only one or a few bacterial species–most important of which is *Enterococcus facecalis*.[1] Eradication of causative microorganisms during root canal treatment procedures help to attain successful results. Because of the complex nature of the root canal system and the presence of many inaccessible areas, a combination of mechanical instrumentation and irrigation is necessary to decrease the amount of bacteria/microorganisms in the root canal system.[2] However, chemomechanical preparation is often not enough, and many bacteria may remain in the root canal system.[3,4] Intracanal medicaments in endodontics have been used for a number of reasons including the elimination or reduction of microorganisms, rendering canal contents inert, prevention of posttreatment pain, and to enhance anesthesia. Calcium hydroxide is the most commonly used intracanal medicament; however, its efficacy toward *E. facecalis* is questionable.[5] Waltimo *et al*. found that calcium hydroxide dressing between appointments did not show the expected effect in disinfecting the root canal system and in treatment outcome.[6] In the recent years, a new concept has been developed, which employs the use of a combination of anti-bacterial drugs (metronidazole, ciprofloxacin, and minocycline) for disinfection of pulpal and periradicular lesions. It has been reported that this mixture can sterilize root dentin.[7]

After disinfection of canal, thorough removal of TAP is essential as it has a detrimental effect on human stem cells in the apical papilla, sealer penetration, and tooth discoloration.[8,9] Studies have been done to assess the

Department of Conservative Dentistry and Endodontics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur, Maharashtra, India

Correspondence: Dr. Deepa Ashoksingh Thakur,
Plot No. 18, Narmada Society, Bhupesh Nagar, Nagpur, Maharashtra, India.
E-mail: deepathakur29788@yahoo.com

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efficacy of irrigation protocols for the removal of triple antibiotic paste (TAP) from root canal, results demonstrated that it was difficult to remove TAP from root canals using irrigating solutions alone. However, the use of ultrasonic agitation with 1% sodium hypochlorite (NaOCl) improved TAP removal.[10]

Hence, the aim of this study is to compare other three techniques namely, Canal Brushing technique, sonic activation, and master apical file (MAF) for the removal of TAP from the root canal.

Materials and Methods

Root canal preparation

A total of 22 freshly extracted, human mandibular premolars with a straight root and a single canal were used in this study. Teeth with caries, internal or external resorption, cracks, and immature apices were excluded. Periapical radiographs were taken in both buccolingual and mesiodistal directions to confirm root canal anatomy.

After removing the tooth crowns, the roots were adjusted to a standardized root length of 14 mm. A size 10 K-file (Mani, Tochigi, Japan) was placed into the canal until the tip of the file became visible at the apical foramen. Working length (WL) was determined by subtracting 1 mm from this measurement for positioning the file. All canals were instrumented with ProTaper (Dentsply, Maillefer, Switzerland) file until F2. At every instrument change, root canals were irrigated with 2 ml of 3% NaOCl using a plastic syringe and a 30 gauge closed-end needle (Irrigation Probe, KerrHawe, Bioggio, Switzerland). Final irrigation was completed using 5 ml of 3% NaOCl and 5 mL 17% ethylenediaminetetraacetic acid. After drying with sterile paper points, TAP was prepared by taking equal portions of (Ciprofloxacin, Metronidazole [IPHARS, Pharmaceutical Company, Solo], and Minocycline [Sigma Aldrich, St. Louis, USA], were mixed with distilled water in a ratio 3:1 and was injected to each canal up to the WL using a lentulo spiral (Mani INC, Japan). Access cavities were temporarily sealed with a cotton pellet and cavit (V-Tempfil). The roots were stored at 37°C in 100% relative humidity for 1 week. The teeth were randomly assigned into three experimental groups ($n = 6$). The remaining teeth served as positive ($n = 2$) and negative ($n = 2$) controls. Two samples without TAPs were used as negative controls. Two samples filled with TAP, but in that removal of paste was not done served as positive control. One week later, temporary restorations were removed, and different techniques were used to remove TAP from the root canals.

Group I

Root canals were cleaned using a medium sized CanalBrush (Roeko CanalBrush™, Coltène, Germany), which was used in a slow-speed handpiece (X-Smart Endodontic motor, Dentsply) running at 600 rpm and advanced to the WL. A circumferential motion was made with the CanalBrush for 30 s. Irrigation was done with 10 ml of 1% NaOCl.

Group II

Sonic activation was delivered for 30 s using the EndoActivator (Advanced Endodontics, Santa Barbara, CA) set at 10,000 cycles per min and a 25/0.02 tip. Irrigation was done with 10 ml of 1% NaOCl.

Group III

Root canals were cleaned using hand instrumentation technique with MAF number F2 ProTaper file (Dentsply, Maillefer, Switzerland) in circumferential motion until number 15K-file could be seen from the apical foramen. Then, irrigation was done with 10 mL 1% NaOCl.

The negative control did not receive TAP material, and the positive control received intracanal dressing, but no subsequent removal was done.

After instrumentation, the roots were grooved vertically on the buccal and lingual surfaces, under water with diamond bur and taking care to avoid touching the root canal. All roots were split in the buccolingual direction using a chisel and mallet, and each sample was divided into three equal parts as apical, middle, and coronal thirds by making small grooves with a sharp knife on the side of the root. The samples were dehydrated by a serious of graded ethanol solutions and then coated with a gold layer, and then evaluated using the scanning electron microscopy (JEOL JSM-6400, Japan) at ×1000 magnification. To standardize the area examined for each sample, the technique described by Paqué *et al.* was used.[11]

Criteria for the degree of TAP removal and cleanliness of the dentinal walls were established by modification of the scoring system of Salgado *et al.*[12] Figure 1 represents the scanning electron microscopy photomicrograph of control groups. similarly Figure 2 represents the scanning electron microscopy photomicrograph of the three experimental groups of all three levels cervical , middle and apical thirds of the root. Three examiners analyzed the Scanning electron

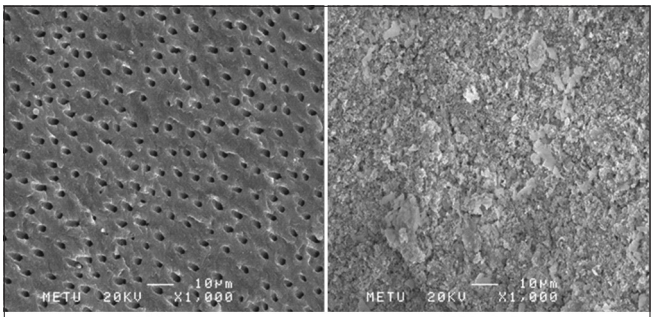

Figure 1: Representative scanning electron microscopy photomicrograph of the control groups

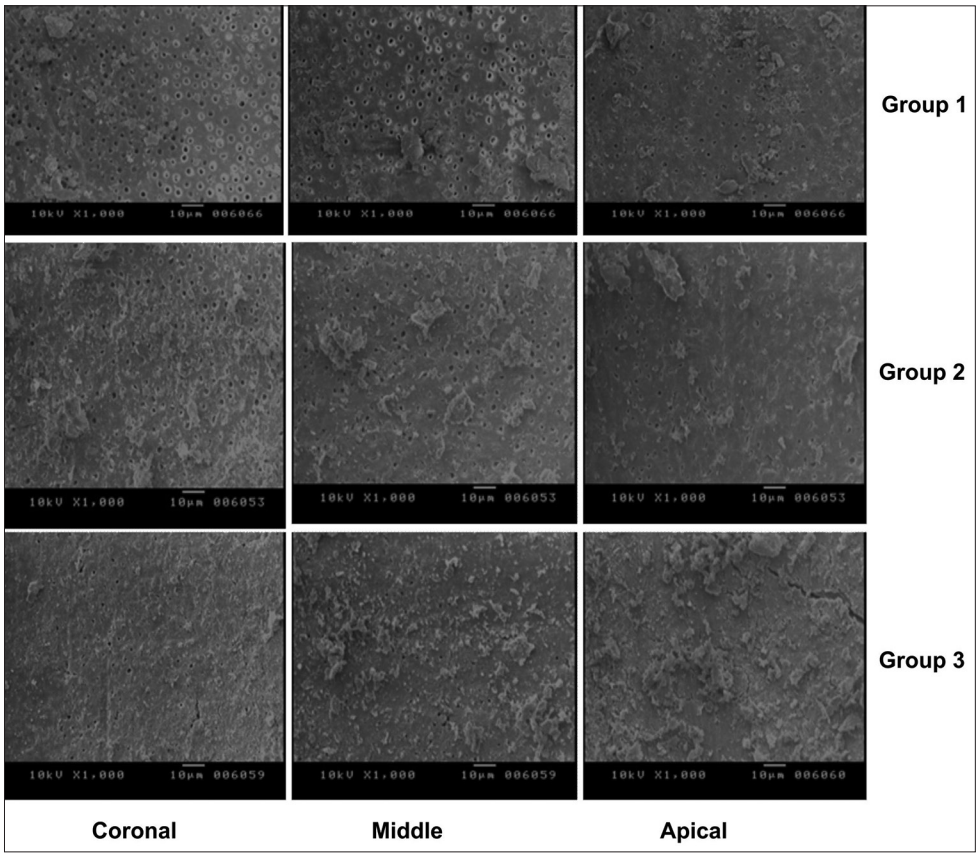

Figure 2: Representative scanning electron microscopy photomicrograph of all thirds of the experimental groups

microscopy photomicrograph, independently in a blind manner. Scores for the TAP removal and cleanliness of the dentinal walls were given using the 4-grade scale.

Criteria for the Degree of Triple Antibiotic Paste Removal and Cleanliness of Dentinal Walls

Score criteria

- 0 = Total cleanliness
- 1 = Good cleanliness (up to 20%)
- 2 = Partial cleanliness (20–60%)
- 3 = No cleanliness (more than 60%).

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences version 11.0 (SPSS Inc.) software.

- *P values obtained based on Kruskal–Wallis test. Test suggests statistically significant difference in the median scores across three groups for cervical region only
- Accordingly, pairwise comparison of median scores among groups for the cervical region was performed using Wilcoxon rank sum test.

Results

Table 1 shows mean median and standard deviation scores of residual TAP at coronal, middle, and apical thirds of root canals. In addition, Table 2 shows pairwise comparison of median scores among groups for cervical region. None of the groups showed complete removal of TAP from the canal walls. Kruskal–Wallis test revealed that statistically significant difference in the median scores across three groups for the cervical region only. Pairwise comparison showed more removal of TAP than MAF with canal and sonic activation in the cervical region suggests that difference between Sonic versus MAF and CanalBrush versus MAF was statistically significant with $P < 0.05$. The mean score for sonic was smaller than that of MAF. Similarly, mean score for Canal Brush was smaller than that of MAF.

The difference between sonic versus Canal Brush is not statistically significant.

Fleiss kappa statistics as a measure of agreement between the observers is given in Table 3. Kappa value indicates that there is fair to moderate agreement between observers in the middle third area in all the three groups.

Discussion

TAP is a well-established antimicrobial agent shown to be highly effective against endodontic pathogens.[13,14] It has been used in the majority of regenerative endodontic procedures.[15] A recent study showed that this medicament

Table 1: Mean, SD and median of scores obtained for smears for samples in each group

Groups/ samples	Mean±SD (median)		
	Cervical	Middle	Apical
Sonic			
1	2.00±0.00 (2)	3.00±0.00 (3)	3.00±0.00 (3)
2	1.33±0.58 (1)	1.67±1.15 (1)	2.00±1.00 (2)
3	1.33±0.58 (1)	2.33±0.58 (2)	2.67±0.58 (3)
4	3.00±0.00 (3)	3.00±0.00 (3)	3.00±0.00 (3)
5	1.67±0.58 (2)	1.33±0.58 (1)	2.67±0.58 (3)
6	1.67±0.58 (2)	1.67±0.58 (2)	2.33±0.58 (2)
Canal Brush			
1	1.33±0.58 (1)	1.67±0.58 (2)	2.33±1.15 (3)
2	1.33±0.58 (1)	1.33±0.58 (1)	2.00±1.00 (2)
3	2.33±0.58 (2)	2.67±0.58 (3)	3.00±0.00 (3)
4	1.33±0.58 (1)	1.00±0.00 (1)	2.00±1.00 (2)
5	1.33±0.58 (1)	1.00±0.00 (1)	3.00±0.00 (3)
6	2.00±0.00 (2)	2.00±0.00 (2)	3.00±0.00 (3)
MAF			
1	2.33±1.15 (3)	2.67±0.58 (3)	3.00±0.00 (3)
2	1.67±0.58 (2)	1.67±1.15 (1)	3.00±0.00 (3)
3	2.67±0.58 (3)	2.67±0.58 (3)	3.00±0.00 (3)
4	2.67±0.58 (3)	2.00±0.00 (2)	3.00±0.00 (3)
5	2.67±0.58 (3)	3.00±0.00 (3)	3.00±0.00 (3)
6	2.67±0.58 (3)	3.00±0.00 (3)	3.00±0.00 (3)
*P**	0.0070 (S)	0.2445 (NS)	0.2969 (NS)

SD: Standard deviation; MAF: Master apical file; *P* – value (Significance)*; NS: Not significant; S: Significant

Table 2: Pairwise comparison of groups for cervical region

Scores	Sonic versus Canal Brush	Sonic versus MAF	Canal Brush versus MAF
Cervical	0.2468	0.0289	0.0049

P: Wilcoxon rank sum test. MAF: Master apical file

Table 3: Fleiss kappa statistics as a measure of agreement between the observers

Group	Position - Fleiss kappa (95% CI)		
	Cervical	Middle	Apical
Sonic	0.2727 (−0.071, 0.616)	0.3143 (−0.0157, 0.6447)	−0.0519 (−0.4458, 0.3419)
Canal Brush	−0.0112 (−0.4125, 0.3901)	0.4316 (0.0669, 0.7962)	−0.0141 (−0.3666, 0.3384)
MAF	−0.1739 (−0.5369, 0.1891)	0.3793 (0.0192, 0.7394)	1.000

CI: Confidence interval; MAF: Master apical file

when used at currently used concentrations has an adverse effect on stem cell survival even after attempts to remove them from the root canal system.[16] Moreover, TAP can directly affect dentin including significant staining because of minocycline,[9] demineralization (possibly because of its very low pH of 3),[17] and reduced microhardness and fracture resistance.[18] Together, these data indicate that TAP has both beneficial antimicrobial efficacy and several potential adverse effects on the microenvironment of the root canal system. Therefore, it is imperative that the TAP be adequately removed from the canal space, once it has served the antimicrobial purpose.

In general, previous studies have demonstrated that several irrigating solutions and irrigation techniques including activation with ultrasonic energy were effective in removing intracanal medicaments.[19,20]

The aim of this study was to determine the efficacy of other various irrigation techniques for the removal of TAP from the root canal.

The result of this study demonstrated that it was difficult to completely remove TAP from root canal. In a study by Berkhoff *et al*., where TAP removal was compared with calcium hydroxide also concluded that that TAP have high diffusion and retention within dentin regardless of removal efforts with different irrigation methods. On the other hand, most of the labeled calcium hydroxide was adequately removed.[21]

Canal Brush showed significantly better result in removing TAP from the cervical third of root canal than sonic and MAF. Canal Brush is highly flexible microbrush is molded entirely from polypropylene and can be used manually with a rotary action. However, the brush was more efficient when operated at 600 rpm in a contra-angle handpiece. Garip *et al.* reported that irrigation and brushing combination were significantly better than irrigation alone in removing the smear layer on the canal walls.[22] It is considered that the use of small and flexible Canal Brush with irrigation solutions removes debris effectively from root canal extensions.

In the middle third, sonic and Canal Brush showed better result in removing TAP than MAF, but difference between two is not statistically significant. Sonic activated devices such as the EndoActivator (Dentsply Tulsa Dental, Tulsa, OK) were recently introduced to improve the irrigation phase. Its design allows for the safe activation of intracanal solutions and could produce vigorous intracanal fluid agitation.[23]

The EndoActivator system has been shown better to irrigate simulated lateral canals at 4.5 and 2 mm from the WL as compared with traditional needle irrigation alone.[24]

Most common method for removing intracanal medicaments is by using MAF and with copious irrigation.[12,25] Nevertheless, canal irregularities may be inaccessible for conventional irrigation procedures, and intracanal medicaments may remain in these extensions.[21]

In the apical third of canal, none of the techniques showed better results. Canal Brush did not remove medicament from the apical third but conversely packed the medicament to the apical part of the canals due to its packing effect.[26] Canal Brush and MAF were ineffective in removing medicament (TAP) from the apical third of canal substanticiate the result of previous study Jain *et al*.[27]

Further study is required to know the reason for EndoActivator group not able to remove TAP from the apical third of the canal.

Conclusion

Within limitations of the study, none of the techniques completely removed TAP from the root canal, but Canal Brush and sonic activation showed less residue in the cervical and middle third of root canal. Thus, remaining medicaments within dentin have the potential to prolong their antibacterial effects but also increase the likelihood of undesirable stem cell toxicity. The concentration and formulation of these drugs must be optimized to provide maximum antimicrobial effect while creating a microenvironment that fosters stem cells proliferation and differentiation. The remaining effects of medicaments on stem cell biology, disinfection, and the clinical outcome in regenerative endodontic procedures require further investigation and warrants careful consideration by the clinicians.

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Conflicts of interest
There are no conflicts of interest.

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