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Comparative evaluation of different irrigation activation methods in removal of modified triple antibiotic paste from root canal system - An ex-vivo study

<sup>1</sup>Dr. Shilpa V, Department of Conservative Dentistry and Endodontics, Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore, Karnataka, India.

<sup>2</sup>Dr. Shiraz Pasha, Department of Conservative Dentistry and Endodontics, Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore, Karnataka, India.

<sup>3</sup>Dr. Muhammed Bilal M, Department of Conservative Dentistry and Endodontics, Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore, Karnataka, India.

**Corresponding Author:** Dr. Muhammed Bilal M, Department of Conservative Dentistry and Endodontics, Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore, Karnataka, India.

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# Abstract

The success of endodontic treatment is related to microbial suppression in the root canal system and periapical region. As introduced by Sato et al and Hoshino et al, the combination of Metronidazole, Ciprofloxacin and Minocycline has been used to disinfect the root canal system in regenerative process. Since, Minocycline is known to cause tooth discoloration, Cefaclor is replaced with Minocycline and is referred to be as modified triple antibiotic paste.

However, the concern with the antibiotic paste is that it may cause bacterial resistance, negatively affects the chemical, physical and mechanical properties of radicular dentin and is detrimental to sealer setting and sealer penetration. For these reasons, complete removal of modified triple antibiotic paste is seen desirable and accordingly, recent physical agitation of irrigants with Sonics, Ultrasonics, Lasers etc have come in the market and claims to be effective in delivering the irrigants to the working length. So there is a need to verify the claims of the manufacturer with proper scientific evidence and to give a consensus as to which activation system is better in removal of modified triple antibiotic paste from root canal space. Therefore, this study was planned to evaluate and compare ex vivo, the efficacy of recently introduced irrigation activation devices XP Endo finisher, Self-Adjusting File System, Endo activator, Laser Activated Irrigation, Passive Ultrasonic Irrigation, and Conventional Irrigation technique on removal of modified triple antibiotic paste from root canal space using Stereomicroscope (30X).

**Keywords:** Modified triple antibiotic paste, Laser, PUI, Endo activator, Stereomicroscope

## Introduction

The ultimate goal of endodontic therapy should be to return the involved teeth to a state of health and function.

Root canal system has a complex anatomy with irregularities, fins, isthmuses and lateral canals that may contain bacteria and necrotic tissue. These microorganisms have been implicated in the pathogenesis and progression of pulp and periapical disease. Thus, Root canal treatment aims to completely eliminate bacteria from the root canal system and prevent reinfection. Although instrumentation procedures have improved considerably over the years, none of the existing techniques can completely clean the root canal system (Hu"lsmann et al. 2005).<sup>[1]</sup> It has been proven that mere mechanical instrumentation is not sufficient in making a bacteria-free area in canal system by self which is predictable considering the complex anatomies of the root canal space. Therefore, additional irrigants and medicaments are required to disinfect the root canal system, to reduce the bacterial load and to promote healing.

In recent years, The Cariology Research Unit of the Niigata University has developed the concept of "Lesion sterilization and tissue repair (LSTR)" therapy which employs the use of a combination of antibacterial drugs for disinfection of oral infectious lesions, including dentinal, pulpal, and peri radicular lesions. Repair of the damaged tissues can be expected if lesions are disinfected.<sup>[2]</sup>

Due to the polymicrobial nature of infected root canal, single empirical antibiotic is insufficient in disinfection of the root canal. As a result, it is essential to use a combination of antibiotics to address the diverse flora encountered. The combination that appears to be most promising consists of Metronidazole, Ciprofloxacin, and Minocycline referred to be as a "Triple Antibiotic Paste" as developed by Hoshini et al and Sato et al.<sup>[2,3]</sup>

Sato et al suggested that Minocycline should be used only for limited periods and attempted to find substitutes for Minocycline in the triple antibiotic paste as a result of the risk of tooth discoloration. They reported that Cefaclor and Fosfomycin are possible alternatives for Minocycline in terms of their antibiotic effectiveness. Thus, Cefaclor is replaced with Minocycline and Sato et al referred it to be as "Modified Triple Antibiotic Paste".<sup>[4]</sup>

There are limited published scientific data to compare new and emerging devices and methods for removal of intracanal medicaments from the canal space. Therefore, this study was planned to evaluate and compare ex vivo, the efficacy of recently introduced irrigation activation devices XP-Endo finisher, Self-Adjusting file system, Endo Activator, laser activated irrigation and Passive ultrasonic irrigation on removal of modified triple antibiotic paste from all the thirds of instrumented root canal using Stereomicroscope (30X).<sup>[5]</sup>

So the aim of this study was to compare the effectiveness of different irrigation activation regimens like XP-Endo finisher, Self-Adjusting File system, Endo activator, Laser activated irrigation system, Passive Ultrasonic Irrigation system and Conventional irrigation techniques in the removal of modified triple antibiotic paste from the root canal space."

### **Materials and Methods**

Seventy mandibular single rooted premolars were cleaned of superficial debris, calculus, tissue tags and was stored in normal saline. Specimens were then decoronated below the Cemento-enamel junction using diamond disk at 12mm from the apex to standardize the length of the roots and x-ray was taken using paralleling angle technique to check for internal anatomy of canals, resorption, caries, fracture and only teeth with straight canals were selected. All the root canals were instrumented with Protaper rotary files till size F3 (size 30). At every instrument change, the root canals were

irrigated with 2mL 3%NaOCl solution. Final irrigation was performed using 5mL 3% NaOCl and 5 mL 17% EDTA.

## **mTAP** Preparation

mTAP was prepared by mixing antibiotic powders compounded in equal portions of mTAP containing Metronidazole, Ciprofloxacin and Cefaclor with distilled water. For the antibiotic pastes, a 1000-mg/mL solution was added to create pasty slurry with a consistency similar to the mixture used clinically. mTAP was then applied in to the canal spaces with a lentulo spiral until the medicament was visible at the apical foramen. Access to the root canals was then temporarily sealed with a cotton pellet and cavit, and teeth were stored at 37° Celsius with 100% humidity for 21days. To simulate clinical conditions, the apices with sealed with hot glue.

#### **Irrigation protocol**

The specimens were then randomly divided into six experimental groups with 10 samples each according to the irrigation regimens used. In 5 teeth, mTAP was not removed (positive controls), and another 5 teeth were not filled with mTAP (negative controls).

Group 1: XP-Endo finisher-Endo finisher file was used with a torque-controlled motor operated at 800rpm and the torque was set at 1Ncm,

Group 2: Self Adjusting File (SAF)- It was operated with a RDT3 handpiece head (Re Dent-Nova, Ra'nana, Israel) at an amplitude of 0.4 mm and 5000 vibrations per minute attached to a special irrigation device VATEA peristaltic pump (Re Dent-Nova) that provided a continuous flow of the irrigation solution at a rate of 5mL/min.

Group 3: Endo activator (EA)- activated using an EA handpiece set at 10,000 cycles per minute with a red (25/04) tip inserted 2mm short of the working length for 1 minute.

Group 4: Laser Activated Irrigation (LAI)- diode laser (980nm, 2W, 200µm tip) was operated for 6 cycles of 10 seconds each.

Group 5: Passive Ultrasonic Irrigation (PUI)- agitated using a size 15 K-file coupled to the file-holding adapter of a Satelec P5 New Tron XS ultrasonic system handpiece (Acteon Group, Merignac, France).

Group 6: Conventional Syringe Irrigation (CI)- A 27gauge side vented needle was introduced 2mm short of working length and irrigated with 10ml of 3% NaOCl for 1min using inside-out motion without any physical agitation meant 16000 A/m or 0.016 A/m.

In all groups, after the final irrigation, 2ml distilled water was used to remove any remaining NaOCl and dried with paper points.

### **Evaluation**

All samples from the six groups were then split longitudinally by creating grooves on the buccal and lingual side without entering the lumen using a diamond disk and chisel. One of the two halves in which the canals were prominently visible was evaluated. Images of the coronal (12mm from apex), middle (8mm from apex), and apical (4mm from apex) thirds of the root canal surfaces were acquired using a digital camera mounted on a stereomicroscope at 30X magnification and were graded from 0-3 (Van Der Sluis et al) by blinded observers as follows:

Score 0 - the canal being empty

Score 1 - mTAP being present in less than half of the canal

Score 2 - mTAP covering more than half of the canal

Score 3 – the canal being completely filled with mTAP

## Results

Images of the coronal (12 mm from apex), middle (8 mm from apex), and apical (4 mm from apex) thirds of the **D** root canal surfaces were acquired for this purpose using a

digital camera mounted on a stereomicroscope at 30X magnification and transferred to the computer. Two calibrating dentists were blinded to the technique used to remove mTAP, and the amount of mTAP remaining in the canal was scored using the following scoring system described by Van Der Sluis et al

Scores	0	1	2	Kruskall-Wallis test	
				Median	p-value
Group 1(XP EF)					
Apical	3	7	-	1.0	0.002*
Middle	3	7	-	1.0	0.011*
Coronal	3	7	-	1.0	0.052*
Group 2(SAF)					
Apical	2	8	-	1.0	0.001*
Middle	6	4	-	0.0	0.005*
Coronal	2	8	-	1.0	0.017*
Group 3(EA)					
Apical	-	4	6	2.0	0.739
Middle	-	7	3	1.0	0.280
Coronal	-	8	2	1.0	0.739
Group 4(LAI)					
Apical	2	8	-	1.0	0.003*
Middle	8	2	-	0.0	<0.001**
Coronal	3	7	-	1.0	0.028*
Group 5(PUI)					
Apical	3	7	-	1.0	0.009*
Middle	3	7	-	1.0	<0.001**
Coronal	3	7	-	1.0	0.0105*
Group 6(CI)					
Apical	-	3	7	2.0	0.615
Middle	-	5	5	1.5	0.189
Coronal	-	7	3	1.0	0.615
The kappa test showed that the interexaminer agreement					

was 96.5% for mTAP medicament removal.

Interexaminer reliability was confirmed using kappa statistics (kappa value >0.94).

Within the limitations of the study none of the groups showed complete removal of mTAP from the root canals. The Kruskal-Wallis test revealed that there were statistically significant differences among all groups (P=0.001). However, Laser Activated Irrigation (LAI) and Self Adjusting File System (SAF) are being very much effective and Conventional Syringe Irrigation (CI) and Endo activator (EA) being the least effective technique in removal of mTAP from the root canal space. At the apical third of the canal, XP-Endo finisher and PUI showed the lowest score values (P=0.001 & P=0.005, respectively), LAI & SAF showed the middle score values and CI & EA showed the highest score values.

At the middle third of the canal, LAI showed the lowest score values, SAF, PUI & XP showed the middle score values and CI & EA showed the highest score values. At the coronal third of the canal, XP, PUI & LAI showed the lowest score values, SAF showed the middle score values and CI & EA showed the highest score values. When the thirds were compared for each group, no

significant difference were found amongst LAI, SAF, XP & PUI groups, over removal of mTAP from the apical, middle and coronal thirds (p=.615).

The SAF (p=.001) and PUI (p=0.009) were significantly efficient for their removal of mTAP at the apical and middle thirds when compared with the coronal third (p <.05). Finally, EF (P=0.002) was significantly efficient at the apical third when compared with the middle and coronal thirds (P < .05).

## Discussion

This ex-vivo study compared the efficacy of different irrigation activation regimens and Conventional syringe irrigation in the removal of mTAP from the apex to the

coronal third of the root canal space. An in vitro closedend canal model was used because it more accurately simulates in vivo conditions. Various methods have been used to investigate the amount of residue on the canal walls, such as the use of Digital Photographs, Stereomicroscopes; Scanning Electron Microscopes, Micro–Computed Tomographic Imaging, and Spiral Computed Tomographic Imaging. In this study, Stereomicroscopy was used, and the remnants of mTAP on the root canal walls were evaluated using a scoring method described by Van Der Sluis et al.<sup>[5]</sup>

Root length was standardized to 12mm after decolonization below CEJ to avoid anatomic variation and to maintain standardization. In all the groups, samples were instrumented with Protaper rotary files till size F3 (size 30) to keep the apical enlargement uniform for all the samples. In the present study, the irrigation regimen and irrigants volumes were standardized in all groups. At every instrument change, the root canals were irrigated with 2mL of 3% NaOCl solution. Final irrigation was done using 5mL of 3% NaOCl and 5 mL of 17% EDTA. Irrigation time was set at 60 seconds. <sup>[6]</sup>

Majority of lateral canals, apical deltas, fins, webs, and transverse anastomoses that are part of the inherent variation in root canal morphology are present in the apical third of the root canal system and also histopathological analysis revealed 77% of the intraarticular biofilm being present in this area only which makes this area of root canal to be considered with significant attention for the success of endodontic treatment.<sup>[7]</sup> Hence, an antibiotic contained within an intracanal medicament must be able to diffuse into these areas to reduce the number of viable bacteria.<sup>[8]</sup>

Due to the polymicrobial nature of infected root canal, single empirical antibiotic is inefficient in disinfection of the root canal. Therefore, it is essential to use

combination of antibiotics to act against all endodontic pathogens and to prevent resistance. Recently Trianti biotic paste containing Ciprofloxacin, Metronidazole and Minocycline has been introduced for lesion sterilization and repair, popularly known as 3-mix paste/ Triple Antibiotic paste.<sup>[9]</sup> Kim et al. (2010) stated that, among the components of the mixture, Minocycline, a derivative of tetracycline, can induce tooth discoloration after longterm oral use. Minocycline is a semisynthetic derivative of tetracycline and is effective against gram-positive and gram-negative bacteria. It binds to calcium ions via chelation to form an insoluble complex that remains in calcifying tissues. Hence, the the minocycline incorporated into the tooth matrix the causes discoloration. Sato et al suggested that minocycline should be used only for limited periods and attempted to find substitutes for minocycline in the triple antibiotic paste as a result of the risk of tooth discoloration. They reported that Cefaclor and Fosfomycin are possible alternatives for minocycline in terms of their antibiotic effectiveness.<sup>[10]</sup> Thus, Minocycline has been discarded from TAP because of its discoloration effect; cefaclor has been included instead, and it is described as modified triple antibiotic paste (mTAP).<sup>[5]</sup> Hence, mTAP was used in the present study.

Sodium hypochlorite (NaOCl) irrigation solutions in the concentration of 1-7% are commonly used for the removal of TAP with traditional syringe irrigation techniques. In the present study 3% NaOCl has been used in between root canal instrumentation. Beside their wide spectrum, non-specific killing effects on all microbes, hypochlorite preparations are sporicidal, viricidal and show far greater tissue dissolving effects on necrotic than on vital tissues.<sup>[11]</sup> However, this is insufficient for complete cleaning of the complex anatomy of the root canal system.

In the present study the efficacy conventional syringe irrigation (CI) technique has been compared with various irrigation activation regimens in the removal of mTAP from the root canal walls20. Wherein, comparing all the thirds of the canal space, the results of the present study showed that the SAF, LAI, XP-Endo finisher and PUI were being more effective than EA and CI in removing mTAP from the root canals.

Different methodologies have been used to assess the amount of medicament remaining. In the present study, the specimens were longitudinally split, and the percentage of residual medicament was calculated from images obtained under magnification of the entire area desired at stereomicroscopy. Images of the coronal -4mm, middle - 4mm and apical - 4 mm of the root canal surfaces were acquired for this purpose using a digital camera mounted on a stereomicroscope at 30X magnification and transferred to the computer.<sup>[5]</sup> This method has been reported as effective in determining the amount of medicament residue and minimized subjectivity in the scoring method based on a scale. The assessment of the remaining mTAP medicament could have been performed using scanning electron microscopy (SEM). However, as the aim was not to evaluate small areas and the opening of dentinal tubules, SEM was not used. [12]

According to the results of the present study, none of the tested techniques (XP-Endo finisher, SAF, LAI, PUI, and CI) completely removed medicament from the canal walls. For the removal of mTAP using CI, intragroup differences among the apical, middle, and coronal thirds of the root canal system were not statistically different. However, the SAF and LAI were significantly efficient for the removal of mTAP at the coronal and middle thirds when compared with the apical third. While, XP-Endo finisher and PUI were superior to CI and EA in removing

mTAP at middle and apical thirds of canal space. CI and EA groups being the least effective technique in removal of mTAP from the instrumented canal space.

Thus, the question of whether these devices are really necessary remains unresolved. There is a need to determine from a practice management perspective how these devices are perceived in terms of their practicality and ease of use. Understanding these fundamental issues is crucial for clinical scientists to improve the design and user-friendliness of future generations of irrigants agitation systems.

## Conclusion

Within the limitations of the study it can be inferred that:

1. None of the groups showed complete removal of mTAP from the canal walls.

2. XP-Endo finisher and Passive Ultrasonic Irrigation removed mTAP medicament better from the apical third of the canal space.

3. Laser and Self-Adjusting File system showed the best result in removing mTAP from the coronal and middle third of the canal space on pairwise comparison.

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