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Edited by Vini Mehta
E-mail: vmehta@statsense.in
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Comparative assessment of penetration depth for various endodontic irrigants in root dentin using confocal laser scanning microscopy

Ankita Mehrotra[1], Jyoti Jain[2,*], Ramesh Chandra[1], Nitika Arora[2], Tarun Pratap Singh[1] & Akanksha Srivastava[1]

[1]Department of Conservative Dentistry and Endodontics, Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow, Uttar Pradesh, India; [2] Department of Conservative Dentistry and Endodontics, King George's Medical University, Lucknow, Uttar Pradesh, India; *Corresponding author

Affiliation URL:
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Author contact:
Ankita Mehrotra - E-mail: ankitabonzai@gmail.com
Jyoti Jain - E-mail: drjyoti82@gmail.com
Ramesh Chandra - E-mail: dr.rameshchandra01@gmail.com
Nitika Arora - E-mail: aroradrnitika@gmail.com
Tarun Pratap Singh - E-mail: tarunyad@gmail.com
Akanksha Srivastava - E-mail: akankshasri1911@gmail.com

Abstract:
Effective root canal disinfection requires irrigant penetration into dentinal tubules, yet comparative tubule penetration of novel organic acids versus conventional solutions remains insufficiently characterized. Therefore, it is of interest to evaluate the 20 single-rooted mandibular premolars prepared to size 35 K-file, randomly allocated into four groups: saline, 0.7% fumaric acid, 7% maleic acid and 18% etidronic acid, all with Rhodamine B dye. Coronal (9 mm), middle (6 mm) and apical (3 mm) sections were analyzed via confocal laser scanning microscopy at 10x magnification; with penetration depth quantified using one-way ANOVA. Fumaric acid (Group II) showed maximal penetration across all root thirds compared to maleic acid, etidronic acid and saline controls. Thus, data shows 0.7% fumaric acid as superior dentinal tubule penetrant, positioning it as promising irrigant for enhanced root canal disinfection efficacy.

Keywords: Confocal laser scanning microscopy (CLSM); irrigants; fumaric acid; dentinal tubules; penetration depth

Background:
The main aim of root canal treatment is to achieve adequate root filling as well as to eradicate bacteria and necrotic pulp through chemical and mechanical preparation **[1, 2]**. The primary source of infection in the root canals is the presence of microbes in the canals. The complex anatomy of the root canal renders it impossible to ensure complete elimination of microorganisms by use of instrumentation methods alone. Microorganisms may penetrate at various levels within the dentinal tubules. *Enterococcus faecalis* is the most common pathogen and its penetration depth may vary between 620 µm and 1483 µm **[3]**. In addition, mechanical instrumentation alone can create a smear layer that coats the root canal walls and removes up to almost 50% of bacteria. The layer of inorganic and organic material that develops on the dentinal walls following mechanical instrumentation is the smear layer, an amorphous and uneven layer with a thickness of 1-5 micron. It has bacteria, necrotic residual pulp tissue, odontoblastic processes and dentinal debris **[4]**. Root canal irrigants are therefore necessary in the removal of the pathogenic microorganisms and enhancing the success of the treatment process **[5].** The most common endodontic irrigant is sodium hypochlorite, which is only able to remove the organic constituents of the smear layer and can penetrate at most 300 µm into the dentinal tubules. Its ability to dissolve in the root canal also decreases due to its low surface tension **[3]**. 17% ethylenediaminetetraacetic acid (EDTA) solution can not satisfy all these demands as yet. Newer irrigants have been tested to determine the penetration depth and efficacy of irrigating solutions in dentinal tubules and lateral canals to enhance the penetration depth and remove the smear layer. The confocal laser scanning microscopy (CLSM) is a very effective technique in measuring dentinal tubule penetration due to its ability to produce consistent and reproducible 3D images and the detailed data it provides at lower magnifications (10x) by using fluorescent irrigants labelled with rhodamine **[2].** Recent *in vitro* evidence has showed that different irrigant activation techniques produce varying penetration depths, with diode laser activation achieving greater penetration in the coronal and apical thirds, while ultrasonic activation showed superior penetration in the middle third **[6].** Therefore, it is of interest to evaluate and compare the penetration depth of different endodontic irrigants into root dentinal tubules using confocal laser scanning microscopy, thereby assessing their potential effectiveness in improving root canal disinfection.

Materials and Methods:
Twenty mandibular premolars with single roots that were removed for orthodontic purposes were selected for this investigation. Teeth with closed apices, no external/internal resorption and no canal calcification were included. The exclusion criteria involved double-rooted premolars and carious premolars. An ultrasonic scaler was used to clean calculus and soft tissues.

Sample preparation:
Following formalin storage, samples were cleaned with regular saline. Next, using a diamond disc with water coolant at low speed, the samples were decoronated below the cementoenamel junction. A size 10 K file was inserted and 1 mm was subtracted from the root canal's overall length to obtain the working length. The step-back technique was used to perform biomechanical preparation for every root canal up to size 35 K file. Following each instrumentation, a 27-gauge needle was used to irrigate 2 ml of 3% sodium hypochlorite; the total irrigant volume utilized was 10 ml per canal.

The experimental solutions used in the study were divided into four groups:

- **[1]** Group I – Saline
- **[2]** Group II – 0.7% fumaric acid
- **[3]** Group III – 7% maleic acid
- **[4]** Group IV – 18% etidronic acid

Rhodamine B dye solution:

A quantity of 0.1 milli mole/0.1% of powdered rhodamine B fluorescent dye was measured. An electronic balance was used to weigh 4.79 mg of rhodamine B, which was then dissolved in 100 ml of experimental solutions to create a 0.1 mol concentration of rhodamine solution.

Sectioning of samples:

Using a diamond disc, two markings were applied at the intersection of the coronal and middle third and the middle and apical third. These markings were separated horizontally. Water was continuously used to cool the teeth at several levels—3 mm, 6 mm and 9 mm—to prevent frictional heat during sectioning. Using a diamond disc series, each segment was reduced apically and coronally to a size of 1 mm. To remove cutting debris, the segment surface was polished using different sandpapers (numbers 500, 700 and 1200, respectively) under running water.

Penetration depth of irrigants using CLSM:

To assess the depth of irrigant penetration, the root canal slices were observed using a confocal laser scanning microscope. Sections were examined at 10X magnifications to ensure accurate viewing of every image. The images were divided into three distinct areas in order to calculate the level of penetration. The calculated mean of the three values was considered the ultimate penetration depth **(Figure 1).**

Data analysis:

The one-way ANOVA test was used for statistical analysis to evaluate data among the four groups. The comparison of penetration depth in all groups at the coronal, middle and apical third levels is displayed. The Statistical Package for Social Sciences (SPSS) and ImageJ software were used in the analysis.

Results:

Significant differences in penetration depth at three sections were found in all four groups according to the results of the ANOVA analysis **(Table 1).** The control group (Group I) did not show any significant changes when compared to the other three experimental groups - Group II, Group III and Group IV. Among all the groups, Group II consistently showed the deepest penetration depths in all three zones, suggesting that this particular material had the highest penetration capabilities.

Table 1: Intergroup comparison of penetration depth

Group	Penetration depth					
	Coronal		**Middle**		**Apical**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Group I	153.28	63.44	81.48	4.19	48.35	11.02
Group II	719.20	92.04	516.27	16.76	272.12	42.54
Group III	523.28	35.55	308.51	5.56	236.16	16.94
Group IV	355.53	38.11	172.15	16.84	95.44	1.95

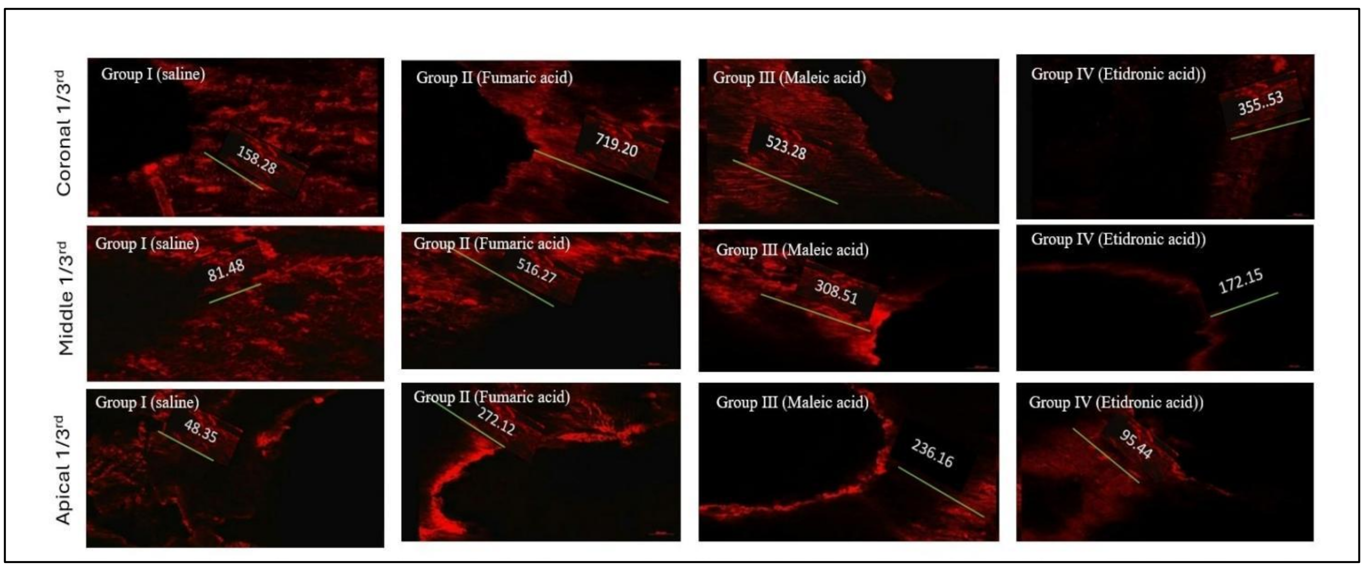

Figure 1: Photomicrographs showing the depth of penetration of saline (Group I), fumaric acid (Group II), maleic acid (Group III) and etidronic acid (Group IV) at the coronal, middle and apical one-third levels using 10 x magnifications

Discussion:

The successful outcome of root canal therapy requires proper biomechanical preparation, disinfection and three-dimensional root canal system obturation. One of the main concerns of endodontic practice is the elimination of pulpal remains, dentinal fillings and bacteria in the root canal system. The adhesion of organic and inorganic dentinal material to the root canal walls during the biomechanical preparation is referred to as smear layer. The smear layer under the SEM typically appears granular, irregular and amorphous **[7, 8].** The thickness of the smear layer is 1-5 um. The smear layer blocks the dentinal tubules, denying the canal irrigants entry to the tubules and harbouring microorganism in the tubules. Bacteria and necrotic tissue in this layer may lead to a break in the seal between the filling material and the canal wall, which may lead to endodontic failure **[5, 9].** To achieve uniformity and prevent any anatomical deviation, straight single canal and single-rooted mandibular premolars were chosen. 27-gauge needle tips were used to irrigate root canals after instrumentation because it penetrates deep into the apical one-third **[5, 10]**. Removal of complete smear layer in this area was still a challenge. This observation supports previous reports that anatomical and histological properties of apical dentin are major constraints to the effectiveness of irrigants. Sclerotic dentin, decreased tubule density and augmented peritubular dentin deposition all limit the diffusion of irrigants and elimination of debris in the apical third **[11]**. Sodium hypochlorite is still the preferred irrigant in dissolving organic tissue, but the failure to dissolve inorganic smear layer components leads to the necessity of using chelating agents. The conventional use of 17% EDTA, despite being clinically proven, has shortcomings due to its neutral pH and high surface tension that decrease its ability to penetrate the small and sclerosed apical tubules. Moreover, EDTA is also very active at neutral pH but as the pH of root canal space rises with time its activity reduces. When compared to organic acids like maleic and fumaric acid, however, they exhibit a greater chelating potential due to their lower pH and reduced molecular size, which allows them to more effectively demineralize intraradicular dentin **[12–14]**. Therefore, to remove the inorganic components of the smear layer, fumaric acid, maleic acid and Etidronic acid were used. Maleic acid harms the intertubular dentin when used in excess of 7%. Etidronic acid, 1-hydroxyethylidine-1, 1-bisphosphonate (HEBP), a weak chelator, is one of the possible alternatives to EDTA. It can be used together with sodium hypochlorite since it does not react with it **[15]**, 0.7% is the maximum concentration of fumaric acid that dissolves in water at room temperature hence 0.7% fumaric acid was chosen. Fumaric acid has been shown to be much more effective than the cis form of maleic acid in removing smear layers. This could be due to the fact that fumaric acid has two carboxylic groups which are in a three dimensional opposite orientation. When one of the protons (H+) is stripped off these carboxylic groups, they are able to bind with an increased amount of Ca++ present in intraradicular dentin, creating a strong intramolecular H-bond with the adjacent remaining carboxyl group. Therefore, the increased efficacy of fumaric acid can be explained by its molecular structure, where oppositely oriented carboxylic groups enable the greater binding with calcium and intramolecular hydrogen bonding leading to a more effective chelation in addition, the reduced molecular size of fumaric and maleic acids enables deeper penetration into the dentinal tubules compared to etidronic acid **[16]**. Although with a low chelating strength, etidronic acid (HEBP) has a unique clinical benefit, since it can be used in combination with sodium hypochlorite without chemical interference. This continuous chelation method does not allow the build-up of smear layer early and allows organic and inorganic debridement during instrumentation. This kind of approach can also help to enhance the cleanliness of canals before the final rinse and it is a time-saving option compared to the conventional sequential irrigation procedures **[4, 15]**. However, the apical third was still the most resistant area to the removal of the smear layer despite the use of advanced irrigants in the current study. This observation is in line with the current literature that characterizes the apical third as a microbiological refuge. The physical barrier to exchange of irrigant is a combination of decreased canal diameter, tubular sclerosis and complicated apical anatomy. Hence, the chemical efficacy is not enough and irrigant activation methods (like passive ultrasonic irrigation or photoacoustic streaming induced by photons) are needed to improve the fluid dynamics and break biofilms in this critical area **[4, 17]**. According to the above study, the apical most region has the least penetration of the irrigant. Some reasons commonly attributed to the same are – due to the sclerotic dentin in the apical region, fewer patent dentinal tubules are present, also, more peritubular dentin is visible and the architecture of the apical region renders it difficult to access and remove debris and the smear layer with irrigants. The increased size of the dentin in the coronal region of the canal and lack of complex anatomical structures could be the cause of the increased penetration observed in the coronal third and middle third levels in the present study.

Conclusion

Fumaric acid showed the greatest penetration depth across the coronal, middle, and apical regions, with other materials showing varying degrees of penetration. The superior penetration of fumaric and maleic acids compared with EDTA supports their potential as low-pH, low-surface-tension organic acids for effective smear-layer removal. These findings suggest that the physicochemical limitations of EDTA warrant consideration of newer irrigant alternatives with greater penetration and demineralization potential.

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