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Comparative evaluation of smear layer removal by EDTA, Apple Cider Vinegar and Fumaric acid when used as irrigant - an Invitro Atomic Force Microscopic study

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Abstract

Introduction: Various types of irrigants and irrigating techniques have been used to remove smear layer from the root canal system. However, none of them has completely proven their efficacy and efficiency clinically. This lead to the discovery of the new endodontic irrigants and combination of irrigating solutions.

Aim: The aim of this study was to assess the efficacy of various smear layer removing irrigating agents such as EDTA, Apple Cider Vinegar and Fumaric acid using Atomic Force Microscopy (AFM).

Materials and Methods: Thirty single-rooted mandibular premolars were decoronated to a standard length of 13 mm and enlarged to MTwo rotary file 20/6 with irrigation of 1 mL 3% NaOCl. Samples were randomly divided into

3 groups according to the final irrigating solution used for 1 min: 17% EDTA solution (group 1), Apple Cider Vinegar (group 2) and Fumaric Acid (group 3). All the samples sectioned longitudinally and subjected to Atomic Force Microscopy (AFM) analysis to study the smear layer removal and nano structural changes. Statistical analysis was done using ANOVA and Tukey Test.

Results: Fumaric acid demonstrated significantly higher smear layer removal efficiency compared to the other tested irrigants with significant difference between the groups. ($p<0.005$)

Conclusion: it have been concluded that Fumaric acid enabled greater smear layer removal and promoted more surface roughness compared with Apple Cider Vinegar and EDTA.

Keywords: smear layer, EDTA, Apple Cider Vinegar, Fumaric acid, Atomic Force Microscopy (AFM), surface roughness.

Introduction

The success of endodontic treatment depends on cleaning and shaping of root canal system. In endodontic therapy, during biomechanical preparation, an amorphous, granular and irregular layer known as the smear layer formed on dentin walls. Biomechanical preparation in conjugation with certain irrigants may able to remove smear layer, tissue debris, necrotic pulpal remnants, bacterial byproducts etc. Smear layer occludes the tubules and decrease the penetrating ability of endodontic irrigants, intracanal medicaments and sealers into lateral canals and dentinal tubules. Use of irrigating agents can clean and disinfect the root canal to the removal of microbial agents. The literature reminds us combining the irrigants with each other may lead to removal of both organic and inorganic substances of smear layer from dentinal tubules, which enhances the sealing ability of root dentin[13].

Chelating agents or Demineralizing agents in root canal irrigation used to remove the inorganic smear layer. The most commonly used chelating agent is ethylenediaminetetraacetic acid (EDTA) which is biocompatible and chelates the root dentin by protein denaturation at neutral pH [17].

Apple Cider Vinegar is capable of removing the smear layer and has the antimicrobial action. Apple Cider Vinegar constitutes of acetic acid, malic acid, lactic acid, formic acid and citric acid[1].

Fumaric acid is a naturally occurring organic acid. It was first isolated from the plant Fumaria officinalis, Fumaric acid is also known as (E)-2- butenedioic acid or trans-1, 2-ethylenedicarboxylic acid[18]. The fumarate esters have cardio protective, chemoprotective, antiulcerative effects and effective in immunomodulation in psoriatric patients[9, 20]. Hence, a newer irrigating solution used in the study to remove the smear layer from the root canal system.

Application of Atomic Force Microscopy (AFM) in dental research mainly deals with dentin affected by caries, hybrid layer analysis, and dentine roughness. It has minimal sample preparation, non-destructive, and three-dimentional view of sample at nanometric scale[5, 6]. For the longitudinal observation of entire dentin surface for smear layer removal efficacy, atomic force microscopy (AFM) is the technique of choice. The purpose of this study was to evaluate the efficacy of EDTA, Apple Cider Vinegar, and Fumaric acid as final irrigant in smear layer removal by using Atomic Force Microscopy (AFM).

Materials and Methods

Thirty permanent intact single canal mandibular premolar teeth were decoronated to a length of 13 mm and biomechanical preparation done upto 20/6 using MTwo rotary file system (VDW, Munich, Germany) with the standardized working length of 1 mm short of apex. Irrigation with 1 mL of 3% sodium hypochlorite

(PREVEST DENPRO HYPOSOL , India) done for all samples .Canals were dried with paper points to receive final irrigation and divided into 3 groups with 10 samples:

Group 1: 17% EDTA (EDTA DeSmear, Ahmadabad, India)

Group 2: Apple Cider Vinegar {St.Botanica, India}

Group 3: 0.7% Fumaric acid (aksharchemicals, GUJRAT, India) .prepared by taking 0.7gm of Fumaric acid power and mixed with 100ml of distilled water}.

Each group received 5 ml of their respective chelating solution for 1 min.

Longitudinal grooves were made on the buccolingual surfaces using a diamond disc without penetrating the canal, then split into two halves. One half from each sample is taken and External surface of sample was mounted on acrylic resin block, denuded the cementum layer with help of cast trimmer to make surface flat, and subjected to AFM analysis, as Atomic force microscope requires an absolute flat surface for analysis.

Results

The samples were tested under atomic force microscope The results of AFM images were subjected to statistical analysis using (SPSS Inc. Released 2007(α = 0.05) by means of Chi-square tests. Roughness parameters from AFM subjected to statistical analysis by means of ANOVA. Multiple comparisons between groups have done by Tukey's honest significant difference analysis.

Figures 1-3 are representative images of samples treated with EDTA, Apple Cider Vinegar and Fumaric acid which shows the smear layer removal efficiency of test samples by evaluating the surface roughness parameters under atomic force microscopy. Table 1, Graphs 1 & 2 shows roughness parameters: The roughness average (Ra), Rq (roughness quoitent) and root mean square (R_{max}) parameters, which belong to the class of amplitude parameters quantifying the properties of technical surfaces. Evaluation of surface roughness parameters using atomic force microscopy showed the topographic irregularities and nanostructural changes at nanometric scale, which directly resembles the penetrating ability of dentin and smear layer removal. Table 1 shows the Ra, Rq, and Rmax value of both EDTA and Apple cider vinegar are lower than Fumaric acid group and is statistically significant. Table 2 shows Tukey's significant difference analysis in which multiple comparisons between and within the groups of Ra ,Rq and Rmax values. This indicates that the roughness average produced by Fumaric acid on tooth surface is higher.

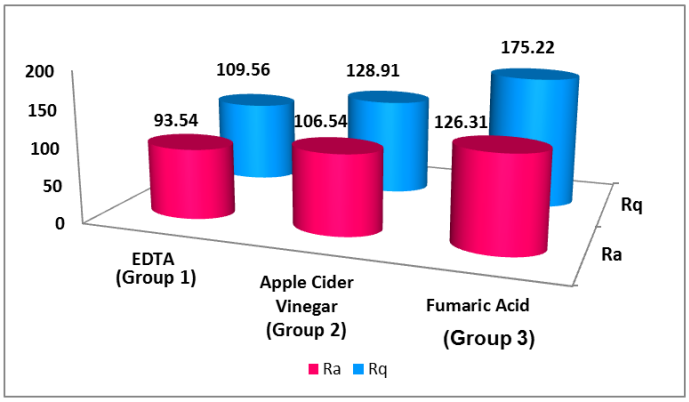
		N	Mean	Std. Deviation	Std. Error
Ra	EDTA	10	93.540	4.1326	1.3068
	APPLE CIDER VINEGAR	10	106.540	6.1475	1.9440
	FUMARIC ACID	10	126.310	9.4695	2.9945
	Total	30	108.797	15.2526	2.7847
Rq	EDTA	10	109.560	4.5162	1.4281
	APPLE CIDER VINEGAR	10	128.910	3.5794	1.1319
	FUMARIC ACID	10	175.220	7.7849	2.4618
	Total	30	137.897	28.5342	5.2096
Rmax	EDTA	10	1159.71	56.7749	17.9538
	APPLE CIDER VINEGAR	10	1556.28	24.3694	7.7063
	FUMARIC ACID	10	1915.41	37.3393	11.8077
	Total	30	1543.80	316.4813	57.7813

P<0.005

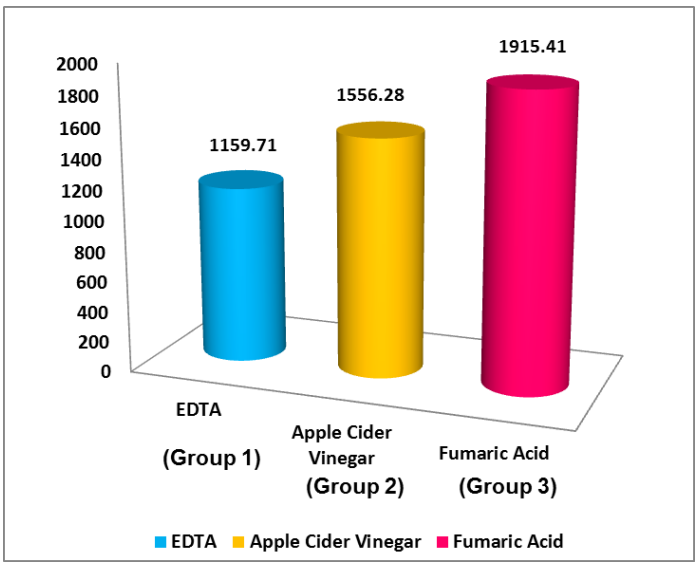
Table 1: Comparison of mean roughness parameters of three groups

		Sum of Squares	df	Mean Square
Ra	Between Groups	5445.753	2	2722.876
	Within Groups	1300.877	27	48.181
	Total	6746.630	29	
Rq	Between Groups	22767.581	2	11383.790
	Within Groups	844.309	27	31.271
	Total	23611.890	29	
Rmax	Between Groups	2857748.706	2	1428874.353
	Within Groups	46903.294	27	1737.159
	Total	2904652.000	29	

Table 2: Tukey's significant difference analysis in which multiple comparisons between and within the groups



Graph 1: Showing Roughness parameters (Ra and Rq) of all test samples.



Graph 2: Showing Rmax values of all test samples

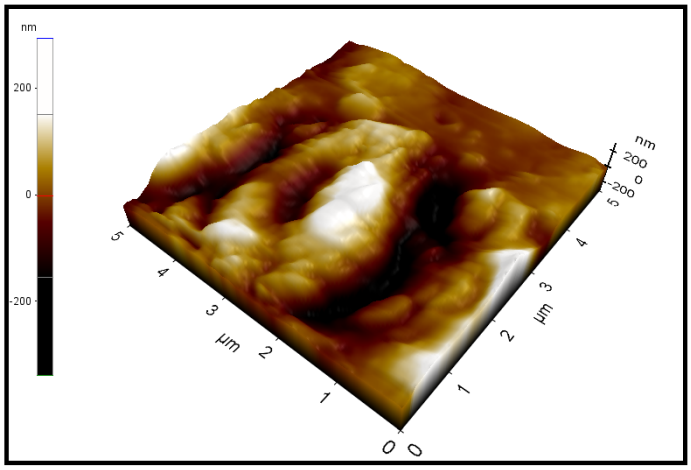


Figure 1: showing 3D image sample treated with EDTA

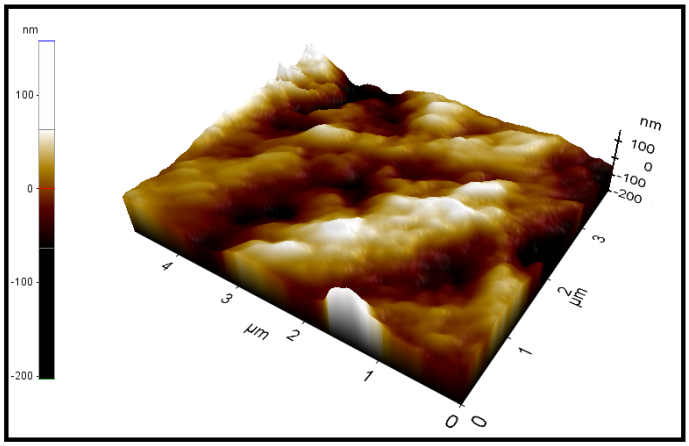


Figure 2: Showing 3D image of sample treated with Apple Cider Vineger

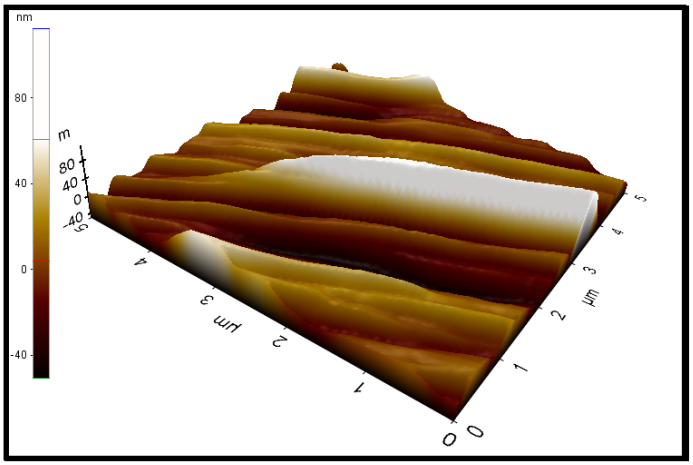


Figure 3: showing 3D image sample treated with Fumaric Acid

Discussion

Straight single-rooted and single canal mandibular premolars were selected with root length of approximately 13mm in order to avoid anatomic variation and to maintain standardization. Standardization was confirmed using radiograph. Standardized crown down technique followed for preparation of root canal reaching full working length, which facilitates penetration of irrigants to the apical third. This technique produces a greater reduction in bacterial count and facilitates removal of dentinal debris.

In the present study, Atomic Force Microscopy used which has an AFM liquid scanner to work directly with testing samples even in liquid medium[6.] It overcomes the drawbacks created by dehydration of samples in stereomicroscopy and scanning electron microscopy

Chelating agent remove the smear layer that is created during biomechanical preparation. Most of the manufacturers recommend chelating agents as a lubricating agent even during biomechanical preparation, which improves the efficiency of the instruments. Chelating agent efficiency depends on application time, pH, concentration and amount of the solution. Highly concentrated solutions applied for a long period, cause roughness of dentin surface[10].

EDTA had limited antibacterial activity. It seems that the antibacterial activity of EDTA is due to the chelation of cations from the outer membrane of bacteria produced a zone of bacterial growth inhibition [12]. In the present study, EDTA showed low surface roughness values due to high surface tension, EDTA was not able to remove smear layer effectively. EDTA does not dependent on a high hydrogen ion concentration for decalcification and is effective only at a neutral pH. The exchange of Ca^{2+} ions from dentin by H^{+} results in decrease in pH [12, 14]. Long time exposure to root dentin causes erosion, and decrease in dentin microhardness[13].Hence, the efficacy of EDTA decreases over time because of the decrease in pH, as neutral EDTA solution reduces the mineral and noncollagenous proteins (NCPs) component of dentin. Thus, EDTA not only removes calcium ions but also calcium bonded to NCPs. The degree of decalcification of EDTA in the apical third of the root canal system decreases as the content of NCPs in this area is low and the dentin is sclerosed. Hence, EDTA may not have such a pronounced action on sclerosed dentin in the apical third[16].

In the present study, Apple Cider Vinegar shows high roughness paramaters than EDTA due to its composition, which contains acetic acid, maleic acid, lactic acid, formic acid and citric acid. Malic acid is responsible for the therapeutic property of the solution[1]; it decreases the dentin microhardness by its strong acidic pH and demineralization capacity. pH of Apple Cider Vinegar used in this study was around three and it could cause damage on the root dentin walls due to the action of H+ ions present[2]. The more the concentration of H+ ions the more efficient the attack of the acid would be. It believed that adsorption, ionic exchange and chelation are responsible for the elimination of smear layer[10]. Apple

Cider Vinegar has a remarkable medicinal potential due to its high mineral content (potassium, phosphorus, magnesium, sulfur, calcium, fluoride and silicon), and contains other elements, such as pectin, beta-carotene, enzymes and amino acids, which attack free radicals that affect the immune system[11,29]. Apple Cider Vinegar has some anti-inflammatory activity, which is an important characteristic for an endodontic irrigating solution and plays a beneficial role in periapical repair process[28]. In addition to the biocompatibility, apple vinegar has bactericidal activity against E. faecalis[11, 29].

Fumaric acid used in this study produced highest roughness parameters due to pH reduction from the initial value 3.6 to 3.4–3.3 and have the interesting property of enhancing their hydrogen-bonding capability by forming isomers on changes in their physical environment, such as heat, light etc. It partially dissociate into H+ cations and RCOO− anions in neutral aqueous solution[22]. Fumaric acid has the property to modulate cytokine production in immune cells and increase the amount of reactive oxygen species (oxidative stress) in it[23]Fumaric acid is one of the important compounds in a lot of fruits and herbs, and it is a main substance in the tricarboxylic acid (TCA) cycle and has a low molecular weight[24]. It is also a key intermediate in organic acids biosynthesis, and forms interesting one-, two- and three-dimensional supramolecular architectures as adducts with various amines.. It showed the highest inhibitory activity against S. aureus, Streptococcus and Campylobacter jejun[24]. Fumaric acid macromers also used in minimally invasive tissue engineering for a variety of tissue types[25].

Conclusion

Within the limitation of this study, it have been concluded that Fumaric acid has better smear layer removing property than Apple Cider Vinegar and 17% EDTA and can be efficiently used as a irrigating solution. Further studies in vitro as well as in vivo needed with larger sample size and with different clinical situations for its use.

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