Comparison of smear layer removal ability and the effect on root dentin strength of garlic extract and EDTA used as final irrigants – An in vitro study

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Abstract

Aim: To compare smear layer removal ability and evaluation of compressive strength of root dentin using various concentrations of garlic extract and 17% EDTA solutions.

Materials and Methodology: One hundred and twenty human mandibular first premolars were decoronated to a root length of 17mm. They were cleaned and shaped upto 40k size file using saline as an irrigant throughout the instrumentation. The specimens were equally divided into 4 groups according to final irrigant used for both smear layer and compressive strength evaluation; group 1- 17% EDTA, group 2 – 30mg/ml garlic solution, group 3 – 10mg/ml garlic solution, group 4 – saline. Samples were split longitudinally and examined under microscope for smear layer evaluation at coronal, middle and apical levels. For compressive strength evaluation, specimens were cut to obtain 6mm cylinders and placed under universal testing machine at a cross head speed of 1mm/min.

Results: 17% EDTA had a lowest mean range of smear layer scores compared to other groups. Coronal sections recorded lowest mean rank of smear layer scores when compared to apical region scores. Compressive strength values were lowest for EDTA group.

Keywords: Garlic extract, Compressive strength, Smear layer, EDTA.

Introduction

Chemo-mechanical preparation is one of the important factors for successful endodontic treatment, which is achieved by the instruments and effective irrigating solutions.[18] The use of irrigating solution is to dissolve organic and necrotic tissue and to prevent packing of dentin chips in apical region, removes debris & smear layer.[5]

Smear layer is composed of inorganic tissue, remnants of odontoblastic process, bacteria and organic tissue. According to the hypothesis proposed by Cengiz et al. (1990), penetration of smear material into dentinal tubules might be caused by capillary action as a result of adhesives forces between tubules and smear material. Typically the texture of the smear material in the tubules is granular or particulate.[14]

Allium sativum commonly known as garlic is one of the most extensively researched medicinal plants. It contains 33 sulfur compounds such as ALIIN, ALLICIN, ajoene, allylpropl diallyl trisulfide, sallylcysteine, vinyldithiines. 13 amino acids, vitamins and minerals. It has various pharmacological properties such as anti – bacterial, anti-fungal, anti-viral, anti-mutagenic, anti-oxidant, anti-cancer, anti-aging, immune - modulatory effect, and also tissue dissolving capacity. Allicin, a general name for alk(en)yl thiosulfinates, is well- known as an unstable volatile organosulfur compound produced by garlic.[8,9]

Human dentin is composed by approximately 70% of inorganic material, 20% of organic material and 10% of water. 90% of the organic matter is collagen, which plays a major mechanical role in dentin. Depletion of the organic phase after root canal irrigation may cause changes in the mechanical properties such as compressive strength.

Ethylene diamine tetra acetic acid (EDTA) is commonly used irrigating solution in root canal therapy. According to NYGARD-OSTBY it chemically softens root canal dentin and allows instrumentation of calcified narrow canals by a process called chelation. Chelates are stable complexes of metal ions with organic substances as a result of ring shaped bonds.[2]

Hence, the aim of the present study was to evaluate the smear layer removal ability and their effect on root dentin compressive strength using various concentrations of garlic extract solution and 17% EDTA solution used as final irrigant.

Materials and Methodology

One hundred and twenty freshly extracted single rooted human mandibular first premolars were taken and equally distributed for smear layer and compressive strength evaluation (60 each). Samples were divided into 4 groups according to the final irrigant used.

Group 1: 17%EDTA solution
Group 2: 30mg/ml of garlic extract solution
Group 3: 10mg/ml of garlic extract solution
Group 4: saline

Smear layer groups were sub-divided based on coronal third, middle third, apical third of root canal.

A. Garlic extract preparation:

Fresh garlic cloves of medium size are dehydrated and dried in the oven at 140°c then ground into a fine powder. 60gms of garlic powder is diluted in 100ml of distilled water in a beaker and placed in soxhlet

apparatus. This solution is then subjected to repeated cycles until a homogenous thick extract is obtained. This extract is transferred into a petri dish and stored in an incubator 60°c. Later this dried extract is scrapped for the experimental purpose.

B. Specimen preparation:

All the 120 teeth were cleaned from debris. Access cavity was prepared using high speed airotor. Specimens were decoronated at CEJ using micromotor with diamond disc, working length was established using radiographs and instrumentation upto 40k file. Saline is used as irrigant for this complete procedure.

C. For smear layer evaluation:

60 specimens were divided into 4 groups (n=15). Longitudinal grooves were made on the buccolingual surfaces on each root by using a diamond disk at low speed without penetrating the canal, the roots were then split in two halves with a chisel. Each root was color coded specimens were secured on metal stubs, desiccated, sputter coated with gold, and examined under SEM at X1000 magnification. Then samples were observed under scanning electron microscope at coronal, middle and apical thirds. Scoring criteria for smear layer evaluation is as follows:

Score 1: No smear layer on the surface of root canals, all tubules were clean and open.

Score 2: No smear layer on the surface of root canals, but tubules contained debris.

Score 3: Smear layer contained tubules and debris

D. For compressive strength evaluation:

60 specimen were irrigated using final irrigants for 30minutes and incubated at 37°c.6mm dimension specimen was placed under universal testing machine at a cross head speed of 1mm/min.

Statistical analysis

For smear layer analyses, differences within groups (between the coronal, Middle and Apical levels) were analyzed by the Wilcoxon signed-rank test; differences among groups (at coronal, middle and apical levels) were analyzed by the Kruskal–Wallis test, followed by pair wise comparison by the adjusted Mann–Whitney U test. Compressive Strength data were analyzed by the Kolmogorov–Smirnov and Shapiro-Wilk test to determine the normality of the distribution, followed by analysis of variance (ANOVA) and the Tukey HSD test. A p value less than 0.05 was considered statistically significant. All analysis was done using SPSS 17.0 ver.

Results

For smear layer groups

Table 1: Descriptive table for smear layer scores

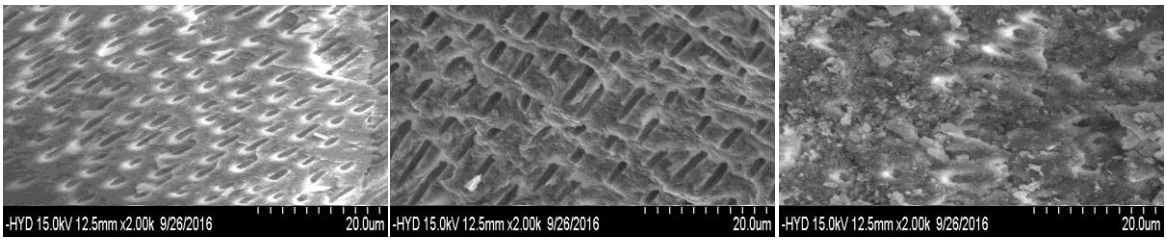
Group	Coronal			Middle			Apical		
	Median	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**
Group 1 (EDTA)	1	1.20	0.41	1	1.27	0.46	2	2.27	0.46
Group 2 (Garlic extract 30mg/ml)	1	1.27	0.46	1	1.33	0.49	3	2.67	0.49
Group 3 (Garlic extract 10mg/ml)	1	1.33	0.49	1	1.40	0.51	3	2.60	0.51
Group 4 (Saline)	3	2.67	0.62	3	2.87	0.35	3	2.93	0.26
P value #	<0.001**			<0.001**			0.003*		

#Kruskal-Wallis Test; @Mann-Whitney Test; $Wilcoxon Signed Rank Test; NS; p > 0.05; Not significant; *p<0.05; Significant;**p<0.001; highly significant

Smear layer scores (Table 1) were lower level for 17% EDTA (1.20). Group 2 showed almost similar mean values of smear layer compared to group 1. Group 3 and 4 had highest mean values 1.33 and 2.67 respectively.

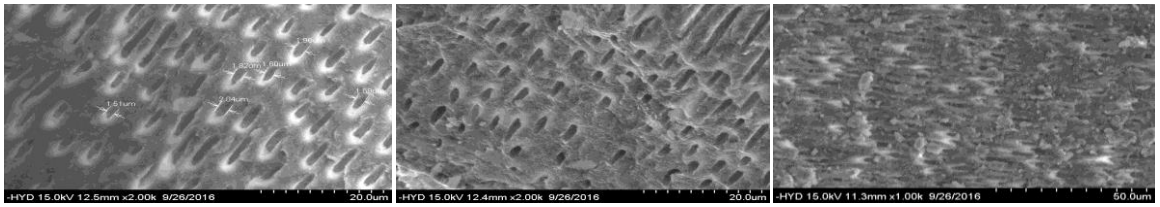
In intra group comparison coronal third had lowest mean values followed by middle and apical third regions.

SEM images of smear layer for 4groups at coronal, middle and apical regions are as follows:



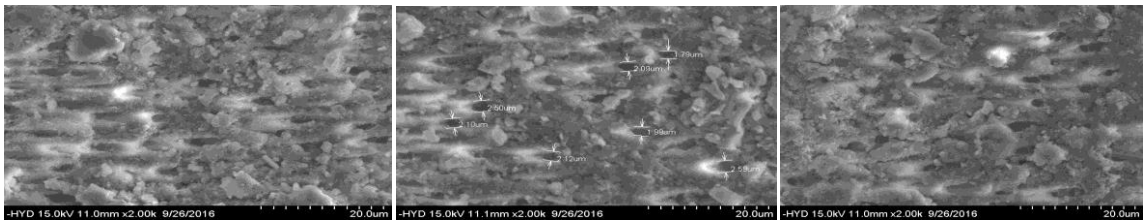
Scanning electron micrograph of dentine surface showing open dentinal tubules (1000x)

Fig. 1: Group 1 (17% EDTA)



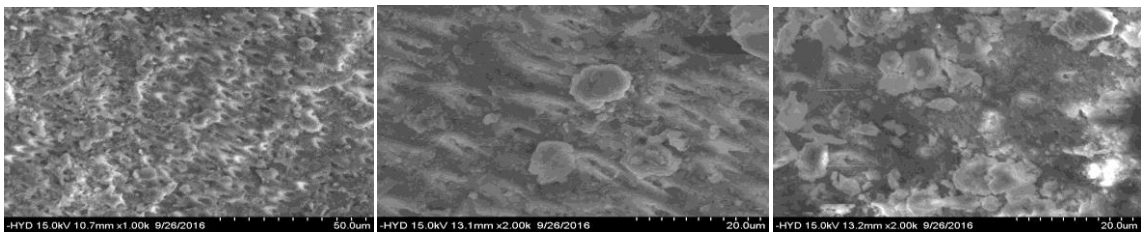
Scanning electron micrograph of dentine surface showing dentinal tubules covered with least amount of debris (1000x)

Fig. 2: Group 2 (30% garlic solution)



Scanning electron micrograph of dentine surface showing dentinal tubules covered with good amount of debris(1000x)

Fig. 3: Group 3 (10% garlic solution)



Scanning electron micrograph of dentine surface showing dentinal tubule which are closed and covered with debris (1000x)

Fig. 4: Group 4 (saline)

Table 2: Descriptive table for compressive strength

					95% Confidence Interval for Mean			
	N	**Mean**	**STD. Deviation**	**STD. Error**	**Lower bound**	**Upper bound**	**Minimum**	**Maximum**
1.EDTA	15	18.67	2.158	.557	17.47	19.87	15	22
2.Garlic Extract 30mg/ml	15	20.72	1.717	.443	19.77	21.67	17	23
3.Garlic Extract 10mg/ml	15	20.65	1.224	.316	19.98	21.33	19	23
4.Saline	15	20.65	1.260	.325	19.98	21.38	19	24
Total	60	20.18	1.820	.235	19.71	20.65	15	24

There was no significant statistical difference in compressive strength values between the groups. Group 1 (17% EDTA) with mean value 18.67 showed lowest value for compressive strength compared to other groups. Group 3 and Group 4 had highest mean values.

Discussion

The success of root canal therapy depends on the method and quality of instrumentation, irrigation, disinfection, and three-dimensional obturation of root canal. Irrigation is presently the best method for removal of tissue remnants and dentin debris during. Removal of inorganic content from the root dentin causes alterations on dentin surface which further effects the mechanical properties of the dentin.

Presence of smear layer prevents penetration of sealers and endodontic materials into dentinal tubules which in turn causes apical micro-leakage. Removing smear layer throughout the root canal is essential for success of endodontic treatment.[2]

CALT and SERPER demonstrated that 10 mL irrigation with 17% EDTA for 1 minute was effective in removal of smear layer, but a 10-minute application caused excessive peritubular and intertubular dentinal erosion.[12]

17% EDTA has been proved to have better smear layer removing capacity but because of its chelating property there may be alterations in microstructure of root dentin. Garlic being a natural product with great medicinal values has been chosen for this study. Alliin on rupture releases Allicin by the enzymatic activity of ALLINASE. The allicin helps in degradation of organic and necrotic content within the root canal and thus reduces the amount of smear layer occluding the dentinal tubules.

Higher concentrations of garlic extract solution (30mg/ml) garlic solution being more stable was effective in removal of smear layer compared to lower concentration (10mg/ml) garlic solution as the latter was least stable at optimal pH.[2] 30 mg/ml of garlic extract solution due to its higher concentration has got more staining property compared to its lower concentration 10mg/ml.

At room temperature, ALLICIN in water could be stored for 5 days without obvious degradation. Higher concentrations of ALLICIN in solution were somewhat more stable than low concentrations. The half-life of ALLICIN ranged from 10 to 17 days at optimal pH 5–6. Allicin being the main component of garlic is stable at 25-37°c. But during root canal instrumentation it is postulated that there is temperature rise above the normal body temperature i.e. 37 degrees. At this temperature allicin component of garlic degrades so it is not able to form calcium chelates in the root dentin. Hence there is no reduction in the compressive strength of root dentin.[14]

17% EDTA is a popular chelating agent that reacts with calcium ions in dentin and forms soluble calcium chelates. The chelation reaction theoretically alters the calcium-phosphorus ratio of hydroxyapatite and the microstructure. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentin to a depth of 20–30 μm in 5 min. The decalcifying process is self-limiting, because the chelator is used up.[17]

Increasing contact time and concentration of EDTA from 10% to 17% as well as a pH of 7.5 versus pH 9.0 has been shown to increase dentin demineralization which further affects its compressive strength .The chelating action of EDTA solution induces an adverse softening potential on the calcified components of dentin and consequently a reduction in the compressive strength.[4]

Conclusion

Within the limitations of the present study, 17% EDTA had maximum efficiency of removing smear layer in coronal and middle third of root canal followed by 30mg/ml garlic solution. 30mg/ml garlic solution was equally effective in removing smear layer when compared to EDTA. Compressive strength was least affected by the irrigants used. Garlic extract solution can be considered as an alternative irrigant because of its anti-oxidant, tissue dissolving, anti-bacterial properties and also being less cytotoxic.

References

1. J.H. Kinney et al., The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature Crit Rev Oral Biol Med 14(1):13-29(2003).
2. D. R. Violich et al., The smear layer in endodontics – a review 21 July 2009.
3. Ali Saghiri et al., The effect of morinda citrofolia juice as an endodontic irrigant on smear layer and microhardness of root canal dentin oral science international 10(2013)53.
4. Giezem demiray et al., Comparison of smear layer and debris removal abilities and the effects on dentinal microhardness of 5% and 17% EDTA solutions used as final irrigants: An in vitro study. Acta odontology 2016,33 (2).
5. Torabinejad et al, A new solution for the removal of the smear layer. J Endod, 2003;29:170-175.
6. Nikhilet et al., Effect of phytic acid, ethylenediaminetetra acetic acid, and chitosan solutions on micro-hardness of the human radicular dentin Journal of Conservative Dentistry, Mar-Apr 2016,Vol 19,Issue 2.
7. Amagase et al, Intake of garlic and its bioactive components. J Nutr, 2003;131 (3s) 955S–62S.
8. Banerjee et al, Garlic as an Antioxidant: The good, the bad and the ugly. Phytotherapy Research, 2001;17(2):97–106.
9. Morteza et al, Burn caused by topical application of crushed garlic cloves on skin: A case report, trends in life sciences, 2014.
10. Harini et al, Garlic: It's role In oral and systemic health, NUJHS, 2013;3:(4),17-22.
11. Ozgur et al., Effects of different irrigantion solutions on root dentine micro hardness, smear layer removal and erosion. Australian endodontic journal 2013,39.
12. Aashih Handa et al., Influence of root canal irrigants on dental tissues: A review of literature. Indian journal of dental sciences sep 2013,3.

13. Gebreselema et al, Medicinal values of garlic: A review, IJMM, 2013;5(9),401-408.
14. Influence of pH, concentration and light on stability of allicin in garlic aqueous extract as measured by UPLC J Sci Food Agric 2015;95:1838–1844.
15. Dechichi P et al., Smear layer*:* a brief review of general concepts. Part I. Characteristics, compounds, structure, bacteria and sealing RFO UPF 2006;11(2):96-99.
16. De-Deus. G et al., Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. International Endodontic Journal, 39,401–407,2006.
17. Kökçü et al., Comparison of the smear layer-and debris-removal abilities and the effects on dentinal microhardness of 5% and 17% EDTA solutions used as final irrigants: *in vitro* study Acta Odontol Turc 2016;33(2):63.
18. Patil R et al., Effect of endodontic irrigating solutions on the micro-hardness and roughness of root canal dentin: An *in vitro* study Indian Journal of Dental Research, 22(1),2011.

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