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Comparative Evaluation of the Effect of Antioxidants on Dentinal Penetration of an Endodontic Sealer- An Invitro Sem Study

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Abstract

Background And Objective: The goal of thorough canal obturation can be achieved by removing the root canal debris and contaminates by irrigants and thereby achieving high adaptability of the filling materials.The aim of this study is to evaluate the effect 10% Tannic acid and 10% Ellagic acid on the dentinal tubular penetration of an endodontic sealer on sodium hypochlorite-treated root canal dentin.

Materials And Methods: Forty human premolars were decoronated to attain 16-mm root length and divided into four groups of 10 teeth each. Biomechanical preparation was done with rotary instruments. Group I specimens were irrigated with saline and 17% ethylenediaminetetraacetic acid (EDTA). Specimens from groups II, III and IV were irrigated with 5.25% sodium hypochlorite and 17% EDTA. Specimens from groups III and IV underwent additional irrigation with antioxidants– 10% Tannic acid and 10% Ellagic acid respectively. Following obturation with AH Plus sealer, scanning electron microscope (SEM) analysis was done to note the maximum dentinal tubular penetration at the coronal , middle and apical thirds of each specimen. The data were statistically analyzed using Independent 'T' test, one-way analysis of variance (ANOVA) and Tukeys HSD Post hoc test.

Results: Maximum dentinal tubular penetration of AH Plus sealer was obtained following irrigation with 10% Ellagic acid compared with 10% Tannic acid in the coronal third of the root canal than middle third and apical third of the root canal system.

Conclusion: 10% Ellagic acid was superior among the antioxidant irrigants compared with 10% Tannic acid that enabled the increased dentinal tubular penetration of endodontic sealer.

Keywords: Ellagic acid, SEM, Sodium hypochlorite, Tannic acid.

Introduction

The most important step in an endodontic treatment is to eliminate the micro-organism from the root canal system, which can be achieved by the combination of the appropriate instruments and effective irrigants during the root canal treatment. However, these methods are not successful if employed alone 1 due to the extremely complex anatomy of the root canal pulp space. Most commonly used root canal irrigants includes acids (citric and phosphoric), chelating agent (ethylene diaminetetraacetic acid EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative agents (hydrogen peroxide and Gly-Oxide), local anesthetic solutions, and normal saline 2

Use of irrigants before obturation serves to lubricate the dentinal wall, flush out debris, dissolve organic and inorganic components of the smear layer to clean dentine surface, and improve the bonding ability of resin-based sealers. It has been shown that the use of these endodontic irrigants may alter the chemical composition of the dentin surface, dentin permeability, wettability, or extent of collagen degradation, thus altering the interaction of the dentin with the resin-based sealer 3

Sodium hypochlorite (NaOCl), because of its antimicrobial properties and tissue-dissolving capabilities, has been used as the irrigant of choice for cleaning root canals in endodontic therapy 4 According to Maria et al, NaOCl has to be combined with EDTA to remove organic and inorganic debris that is mainly smear layer . The smear layer removal is important because may be infected or it can prevent access to the dentinal tubules, which may contain bacteria and their by-products and will also affect the the ability of sealer to penetrate the dentinal tubules and adhere properly. . 5, 6, 7

According the studies, it is thought that NaOCl leads to oxidation of some component in the dentin matrix which leads to the formation protein-derived radicals which in turn effect the mechanical properties of dentin, such as elastic modulus, flexural strength, and microhardness and also reported after irrigation of root canals with 5% sodium hypochlorite, which can also contribute to a decrease in the micromechanical interaction between adhesive resins and NaOCl-treated dentin. 8,9,10

The compromised bond strengths to NaOCl-treated dentin could be restored by the use antioxidant solution before the adhesive procedure because it can interact with the by-products of NaOCl, resulting in neutralization and reversal of the oxidizing effect of the NaOCl-treated dentin surface.11 Various antioxidant used are vitamin E, flavonoids, catechins, gallic acid derivatives, salicylic acid derivatives, cinnamic acid derivatives chlorogenic acid, resveratrol, folate, curcumin, caffeine, anthocyanins and tannins which are examples of polyphenolic natural antioxidants derived from plant sources where as Non-phenolic secondary metabolites such as melatonin, carotenoids, retinal, thiols, jasmonic acid, ei-cosapentaenoic acid, ascopyrones and allicin that show excellent antioxidant activity.12

The benefit of antioxidants may increase the bond strength of root canal filling materials producing a better seal of the root canal space .With this view point the present study was undertaken to observe the enhancement of dentinal tubular penetration of NaOCl treated root canal dentin there by increasing the bondstrength using 10% tannic acid and 10% ellagic acid as an antioxidant..

Materials and Methods

Tooth Preparation

A total of 40 single rooted teeth recently extracted for orthodontic purpose with straight canal was used in this study. Care was taken not to select teeth that had cracks, caries, root fracture, resorption defects and open apices which were confirmed under dental operating microscope. Using gracey curettes, the hard deposits on the teeth followed by soft deposits were removed by soaking teeth in 5.25 % NaOCl for 20 minutes, followed by rinsing with saline. Then they were stored in 0.1% thymol until the experimental use. The teeth were decoronated using diamond saw.The root length was measured and standardised to 16mm. The apex of each sample was sealed with sticky wax to prevent extrusion of the experimental irrigants.

A 10 size K file was inserted into the canal to get patency to the apical foramen. Working length was determined 1 mm short of apex at 15mm which was confirmed by radiograph. Cleaning and shaping was done by using Protaper nickel-titanium rotary instruments (Dentsply, Germany) to size #F3, 6% taper, at the working length. Irrigation with 3 mL of 2.5% sodium hypochlorite was performed between each file upto 30/0.06% size. The roots were then randomly divided into four groups (n = 10) according to the final irrigation regimen.

Group I: 5 ml of saline 0.9% (control) (NIRLIFE, GUJARAT) + 17% EDTA

Group II: 5 mL of 5.25% sodium hypochlorite (Novo Dental Products Pvt. Ltd., Mumbai, Maharashtra, India) + 17% EDTA (Fischer Scientific, MUMBAI)

Group III: 5 mL of 5.25% sodium hypochlorite (Novo Dental Products Pvt. Ltd., Mumbai, Maharashtra, India) + 17% EDTA (Fischer Scientific, MUMBAI) + 5ml 25% tannic acid (Sigma-Aldrich, Bangalore, Karnataka, India)

GROUP IV : 5 mL of 5.25% sodium hypochlorite (Novo Dental Products Pvt. Ltd., Mumbai, Maharashtra, India) + 17% EDTA (Fischer Scientific, MUMBAI) + 5ml 10% ellagic acd (Sigma-Aldrich, Bangalore, Karnataka, India)

Irrigation Protocol for All the Experimental Groups

For group I (negative control), irrigation was done with saline (Nirlife, Nirma Ltd., Ahmedabad, Gujarat, India) and 17% ethylenediaminetetraacetic acid (EDTA) (Desmear, Anabond Stedman Pharma Research Ltd., Chennai, Tamil Nadu, India). For groups II (positive control), III, IV the irrigation protocol followed was 5 mL of 5.25% sodium hypochlorite (Novo Dental Products Pvt. Ltd., Mumbai, Maharashtra, India) between the change of each instrument and 5 mL of 17% EDTA for 1 min after biomechanical preparation. Then flushing of the canals were done using distilled water to remove the remaining irrigant.Root canals of groups III, IV were further irrigated with freshly prepared solutions of 10% tannic acid(Group III), and 10% ellagic acid (Group IV) , respectively, for 10 min. After the irrigation of the prepared solution, the canals were flushed with distilled water to flush the remaining irrigants.

10% tannic acid (Sigma-Aldrich, Bangalore, Karnataka, India) were obtained by mixing 10 g of powder of the respective antioxidants in 100 mL of distilled water. 10% ellagic acid (Sigma-Aldrich, Bangalore, Karnataka, India) were also prepared in the same way as that of tannic acid

Following irrigation, all the canals of the specimens were dried with paper points (Dentsply, Chemin du Verger, Ballaigues, Maillefer, Switzerland) and then AH Plus sealer was introduced into the canals using lentilospiral instrument. The canals in all the groups were obturated with gutta percha obturating material using single cone technique. Radiographs were taken to assess the quality of obturation. The canal orifices were sealed with cavit (3M ESPE) and were stored in 100% relative humidity at 37°C for 24 h to ensure a complete set of the obturating material.

After 24 hours, the specimens were stored in bottles filled with 1.5 ml distilled water for 2 days. Each of the specimens was sectioned in the bucco-lingual direction with the help of a safe-sided cutting disc under copious irrigation with distilled water. The sectioned tooth which retained most of the obturation material was selected for observing under a scanning electron microscope (LEOVP435, Cambridge, UK). The sectioned parts were soaked in 15% EDTA solution for 10 minutes, followed by soaking in 3% NaOCl solution for 10 minutes, and then washed thoroughly with distilled water.

Scanning Electron Microscopic Evaluation

Specimen was dehydrated and silver sputtered for SEM evaluation at the cervical, middle, and apical thirds. A SEM (NO. S-2400, Hitachi, Omeshi, Tokyo, Japan) was used at 500x magnification. The samples were evaluated for dentinal tubular penetration of sealer at three levels – coronal, middle, and apical third of the root canal . The maximum tubular penetration at each level was deterimined in micrometers (µm). At each level, five points were marked corresponding to the maximum penetration. The mean of the five readings was taken at each level and tabulated.

Statistical Analysis

Data entry was done in Microsoft Excel. The values obtained were statistically analyzed using computer software Statistical Package for the Social Sciences (SPSS) (16.0) (SPSS Inc, Chicago, USA). The data were expressed with the mean and standard deviation. One-way analysis of variance (ANOVA) was applied for statistical analysis. Post hoc test followed by Dunnett's test was used to find the statistical significance between the groups. P value less than 0.05 (P < 0.05) was considered to be statistically significant at 95% confidence interval.

Results

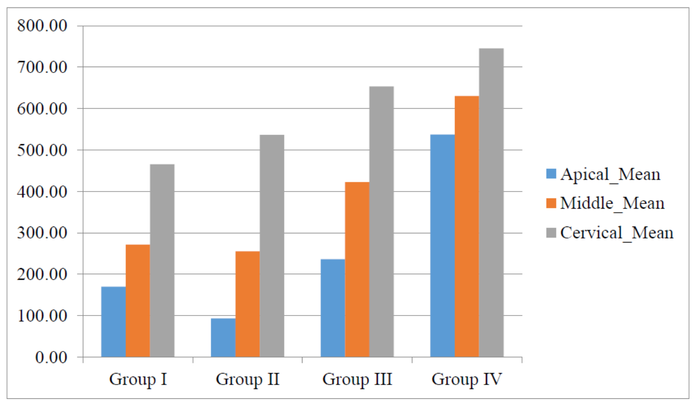
Table 01: Comparison of mean dentinal penetration depth (in µm) between 04 groups

Comparison of mean dentinal penetration depth (in µm) between 04 groups using One-way ANOVA test

Groups	N	CERVICAL (Mean ±SD)	MIDDLE (Mean±SD)	APICAL (Mean±SD)	P-Value
Group I	10	465.38±9.09	272.01±9.16	57.65±8.25	<0.05*
Group II	10	536.60± 6.61	255.82±3.83	93.73±6.74	
Group III	10	653.25±4.35	422.75±9.87	170±5.38	
Group IV	10	744.86±3.35	630.63±15.69	236.82±3.96	

The results of the present study showed maximum depth of the sealer penetration was observed in Group IV (10% Ellagic acid) in the the cervical third(744.86±3.35) followed by middle third (630.63±15.69) and less in apical region(236.82±3.96)compared with all the other 3 experimental groups . Minimum tubular penetration is observed in Group I: control group (saline +17% EDTA) at all levels: coronal, middle and apical which is statistically significant (p, 0.05). The mean value of Group 1 (middle) was not statistically significant compared to mean value of Group II (middle) ($p<0.05$). The mean value of Group II (cervical) was not statistically significant compared to mean value of Group IV (middle) ($p<0.05$).

Graph 01: Graphical Representation Depth of Dentinal Tubular Penetration of Sealers at Different Levels



Graph 01 shows that maximum dentinal tubular penetration of sealers was observed in Group IV in all the levels of the root canal ,ie, coronal ,middle ,apical third of the root canal. Minimum dentinal tubular penetration was observed in Group I in all the levels of the root canal ,ie, coronal ,middle ,apical third of the root canal.

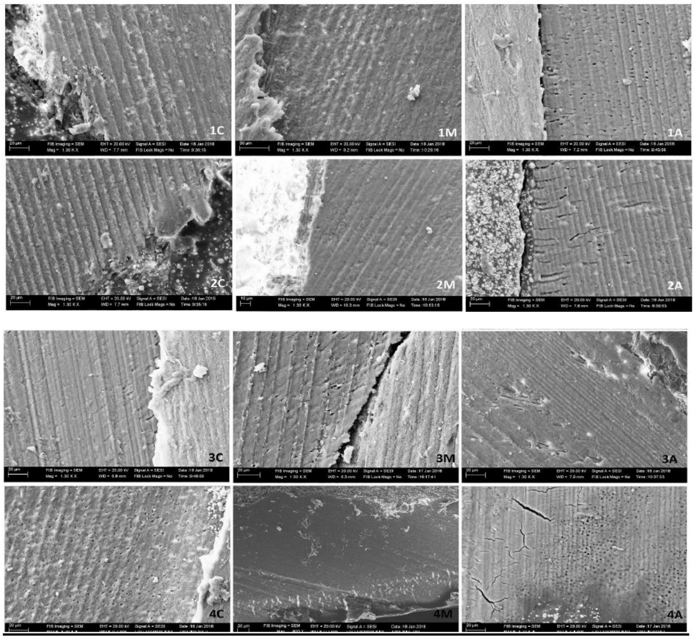


Figure 01: Scanning electron microscope (SEM) images of the cervical, middle, and apical thirds of the root (1.3x): 1a, 1b, 1c (saline + 17% EDTA irrigation), 2a, 2b, 2c (5.25% NaOCl + 17% EDTA irrigation), 3a, 3b, 3c (5.25% NaOCl + 17% EDTA + 10% tannic acid irrigation), 4a, 4b, 4 c (5.25% NaOCl + 17% EDTA + 10% Ellagic acid irrigation).

Discussion

A successful endodontic treatment requires good shaping, removal of infected tissues, and three dimensional obturation of the canal. Proper use of irrigation agents and instruments are the key to success of root canal treatment. 13 According to the study done by Berutti et al. solutions that is used as irrigation agents could penetrate about 130 µm into the internal surfaces of root dentin. Bacteria may be present in root dentinal tubules upto 1000-µm depths. 14 To achieve proper antibacterial effectiveness, irrigating agents should remove organic and inorganic tissues in root canals, open dentinal tubules, and penetrate into the root canal system. Sodium hypochlorite, (NaOCl), and ethylene diamine tetra-acetic acid (EDTA) solution are commonly used endodontic irrigants among the different root canal irrigants for removal of smear layer.15 NaOCl is the routinely used endodontic irrigant with excellent antibacterial property which is capable of dissolving necrotic tissue, vital pulp tissue and the organic components of dentin and biofilms with concentration ranging from 0.5% to 5.25% . According to several studies, it has been been suggested that the irrigation with NaOCl affects the dentin bond strength. Weston et al states that NaOCl removes any exposed organic matrix or soft tissue from the dentin and leaves a mineralized surface less receptive to bonding with resin cement. It is likely that NaOCl acts to oxidize a component in the dentinal matrix that interferes with free radical propogation at the resin– dentin interface, leading to lower bond strength.16

The possible cause for the reduction in bond strength has been also attributed due to the presence of residual irrigants and/or their products that can easily diffuse into the dentin via dentinal tubules. These residual chemicals may contaminate the dentin surface and interfere with the penetration of resin adhesive into the dentin or the polymerization of resin monomer.17 Based on the results of the studies that were evaluated, NaOCl may adversely affect the sealing ability and adhesion of dental materials, such as resin based cements and root canal sealers, to dentine.18

The adverse effect of NaOCl on dentin bond strength can be reversed by the use of natural antioxidants such as ascorbic acid, sodium ascorbate, rosmarinic acid 19 , green tea extracts 20 , and proanthocyanidin 21. These antioxidants facilitate to increase the bond strength to NaOCl treated dentin thereby stabilizing the resin-dentin interface due to their antioxidative capacities. Antioxidants also remove the residual of sodium hypochlorite diffused into the dentinal tubules by redox reaction. Thus antioxidants may increase the dentinal tubular penetration of endodontic sealers and thus the bond strength. The present study was conducted to evaluate the effect of antioxidants such as 10% ellagic acid and 10% tannic acid increased the dentinal tubular penetration of an endodontic sealer thereby enhanced the bondstrength.

This is in accordance with the various studies on (Pratiet ,1992; Sabbak and Hassanin,1998 ; Natsu et al,1999 ; Okamot et al ,1991; Tomiyana et al 2004) with regard to tannic acid ; it is used in dentistry as a desensitizing agent, astringent and surface treatment for smear layer removal . Tannic acid is a naturally occurring collagen cross-linking agent consisting of a complex mixture of polygalloyl glucose esters.22

A study done by Bedran Russo et al 22 states that 25% tanic acid solution was used as a root canal irrigant after normal cleansing with hydrogen peroxide and sodium hypochlorite. This would appear to have a desirable effect for greater penetration of the root canal sealant after the completion of root canal preparation.

A study done by Ebrahimi et al on the effect of tannic acid on bond strength of etch and rinse and self-etch adhesive systems in dentin of primary teeth states that tannic acid could bond by its hydroxyl group with amide (NH) of collagen through hydrogen bonds. This bond would increase stability of the collagen fibers and elevate their resistance against dentin matrix metalloproteinases (MMPs) 23 . Various natural as well as synthetic cross-linking agents such as glutaraldehyde, tannic acid, PA, genipin, and cocoa seed extract have been used to strengthen the hybrid layer and have shown positive results in improving the bond strength to significant levels according to the study done by Bharti et al. 24

A study conducted by Sevinc Askerbeyli et al on the effect of tannic acid irrigation on microhardness of root canal dentin and bond strength of epoxy resin based sealer showed that tannic acid increased dentin microhardness and the bond strength of the epoxy resin based sealer to root canal dentin . Collagen cross-linking ability of TA is most likely to be responsible for higher bond strength values, considering the bonding ability of the epoxy resin based sealers to dentin collagen. 25

Ellagic Acid is a poly phenolic compound present in green tea and other natural sources like pomegranate fruit , strawberries, blackberries, raspberries, walnuts and bark of eucalyptus. Ellagic acid has been found to have antioxidants, anti carcinogenic, antifibrosis, antiplasmodial activity and chemopreventive activity 26. In vitro assay of a fermented pomegranate juice extract which contains ellagic acid and a cold pressed seed oil extract found the antioxidant capacity of both are superior to red wine and similar to green tea extract. No toxic effects were seen after the repeated consumption of the polyphenol antioxidant punicalgin in rats, which were confirmed by histopathological analysis of their organs.27.

A study done by Payal et al on Punica granatum peel(Pomegrate peel) and Emblica officinalis fruit extract solutions which had tannin, ellagic acid and glucose had satisfactory cleansing action, which was comparable and better fuction than the standard 17% EDTA solution.28

According to Badole et al , Chebulinic acid, tannic acid and ellagic acid are responsible for the anti bacterial action of terminalia Chebula which is used as a herbal irrigant used in endodontics. The antioxidant property of phenolic compounds is attributed to their ability to absorb and neutralize free radicals. 29 Hence this approves the superior antioxidant property of ellagic acid when used as an irrigant .

A 10% concentration of the two antioxidants was used to irrigate the root canals of the respective groups for a time period of 10 min. This is in accordance with the done study by Bedran-Russo et al. who demonstrated that 10% tannic acid irrigation for 10 min can improve the elastic modulus and bond strength of demineralized dentin22 . After sectioning of the roots, the specimens were cleaned with 15% EDTA and 3% NaOCl to remove the smear layer produced during sectioning.

The sections of the root were examined using scanning electron microscope as it allows highly detailed observation of the dentinal tubules and the sealer. It allows accurate measurement of penetration depths and it does not require any dye incorporation

The results of the present study showed that the maximum depth of the sealer penetration was better in the coronal thirds followed by the middle third, and least in the apical third in all experimental groups . This is in accordance with the study done by Balguerie et al 30 and Thota et al 31 . This may be attributed to the better removal of the smear layer in coronal thirds than in apical thirds of root canals. Also the apical root canal contains less dentinal tubules with smaller diameter and also , the apical portion of roots shows a pronounced variation in structure. The teeth specimens treated with antioxidants showed maximum tubular penetration in Group IV(10% Ellagic acid) followed by Group III(10% tannic acid) at cerical ,middle and apical regions of the root canal. This is in accordance with the study done by Christopher, et al in which antioxidants showed deeper tubular penetration of Resilon and Real Seal SE at the cervical, middle, and apical thirds of the root canal dentin. 32

As the antioxidant solutions such as 10% tannic acid and 10% ellagic acid are used as an irrigants in this *in vitro* study which cannot absolutely mimic the *in vivo* conditions of the oral cavity. Secondary tests and clinical trials are the ultimate in deciding the outcome of *in vitro* studies. Hence, further clinical trials are necessary to substantiate the results and findings.

Conclusion

Within the limitations of this in vitro study it was observed that among the antioxidant irrigants, 10% Ellagic acid was superior in enhancing the dentinal tubular penetration of AH Plus sealer on sodium hypochlorite-treated root canal dentin compared to 10% Tannic acid thereby creating a better adaptation, good penetration and adhesion. Further studies are required to support this concept of dentinal tubular penetration of AH Plus sealer.

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