ORIGINAL RESEARCH PAPER

Volume-8 | Issue-4 | April-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# EFFECT OF PAPAIN SOLUTION ON SMEAR LAYER REMOVAL A SCANNING ELECTRON MICROSCOPE STUDY

Science

**Arsha D V**

**Shiji Dinakaran*** *Corresponding Author

**Soumya S L**

**Ancy A**

**Karthik jayan**

**Gayathri P**

## ABSTRACT

The aim of the study was to evaluate the efficacy of liquid papain (10%) to remove the smear layer from the root canal wall at various levels, using scanning electron microscopy. Forty five premolars were divided into three groups (n =15). The canals were prepared upto protaper F3. In group1, canals were irrigated with normal saline, in group 2 canals with 5%NaOCl and in group 3 with 10% papain during instrumentation and 17% EDTA as a final irrigant. Smear layer removal was evaluated by Gutmann's scoring system using scanning electron microscopy (SEM). Statistical analysis using Oneway ANOVA showed significant difference in the mean score value for the three experimental groups ($p<0.05$). From the study, it can be concluded that Group 2 (5% NaOCl and 17% EDTA) and Group 3 (10% papain and 17% EDTA) have the ability to remove smear layer at three levels (apical, middle and cervical third).

## KEYWORDS

EDTA; NaOCl; Papain; Scanning Electron Microscopy

## INTRODUCTION

Following root canal instrumentation a layer of organic and inorganic materials accumulate on the root canal walls, known as smear layer. It has 1–5 µm thickness and extends into dentinal tubule as smear plug (1). This smear layer is responsible for harboring remnants of tissues along with bacteria and their by-products. Studies have shown that presence of microorganism in the smear layer prevents the canal from being completely disinfected (2). The smear layer may interfere with the penetration of medicaments used for intracanal disinfection (3). Also it interferes with penetration and adhesion of root canal sealer into the dentinal tubules during obturation (4,5). Therefore complete elimination of smear layer improves disinfection of the root canal and adaptation of root canal filling. Studies have found that removal of the smear layer minimizes both coronal and apical leakage regardless of the type of sealer used (6,7). An ideal smear layer removing agent should be able to remove both organic and inorganic debris from all canal surfaces without causing dentinal erosion(8).

Different solutions have been utilised for removal of the smear layer. NaOCl is the most frequently used endodontic irrigant because of its excellent antimicrobial activity and pulp dissolution property(9). The use of sodium hypochlorite in patients requires caution due to its cytotoxicity. Another major drawback is its inability to remove the smear layer completely (10). Extrusion of NaOCl into periapical tissues can cause severe pain, oedema and necrosis of the tisssues. NaOCl allergy also has been reported(11).

A commonly used chelating agent for smear layer removal is 17% ethylene diamine tetra acetic acid. Studies have reported that single and combined use of EDTA with NaOCl decreases the microhardness of the root canal dentine significantly(12). EDTA should be used with caution inside root canals because it may weaken dentine and thereby increase the risk of creating a perforation during mechanical root canal instrumentation (13). Studies have demonstrated that EDTA had no measurable antibacterial effect as a root canal irrigant(14).

Liquid papain is a solution of proteolytic plant enzyme extracted from unripe fruits of papaya tree (15). The enzyme is able to break down organic molecules made of amino acids such as proteins, short chain peptides, aminoacid ester and amide(16). It has been used in medicine for debriding chronic wounds and burns which exhibit necrotic areas (17). In dentistry, papain in gelform (papacarie) has been used for chemomechanical caries removal(18). Papain is biocompatible and also a potent natural antioxidant(19).

The aim of this study was to evaluate the efficacy of liquid papain(10%) to remove smear layer from the root canal wall at various levels, using scanning electron microscopy.

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation

Samples were 45 single and straight rooted human premolars extracted for orthodontic purpose. The teeth were washed in distilled water to remove the blood and stored in distilled water until the beginning of the study. The teeth were decoronated at the level of CEJ, using a diamond disk with straight handpiece and the root lengths were standardized to 15mm. The patency of each root canal was checked with10 size k file.

The samples were randomly divided into three groups.
Group 1: Samples in which only normal saline was used as root canal irrigant during instrumentation, 2.5 ml between each file and 5ml as a final irrigant for 2 minutes. It was considered as a control group.

Group 2: Samples in which 5% NaOCl was used as an irrigant during instrumentation. 2.5ml between each file and 5ml 17% EDTA as a final irrigant for 2 minutes, then washed with 5 ml of normal saline.

Group 3: Samples in which 10% papain was used as the root canal irrigant during instrumentation, 2.5 ml of liquid papain between each file and 5 ml of 17% EDTA for 2 minutes as a final irrigant, then washed with 5 ml of normal saline.

### 2.2. Preparation of the irrigant solution

- The experimental solution was prepared by dissolving 5gms of powdered form of enzyme papain in 50 ml of distilled water to produce 10% papain solution. The solution was freshly prepared before use.

### 2.3. Root canal preparation

The samples were closed from the apical area with sticky wax. The samples were prepared using protaper NiTi rotary file system starting from Sx, S1, S2, F1, F2, F3 at 300 rpm and 2 as torque in a rotary endodontic handpiece (Xsmart, Dentsply). Each file was inserted 3 times in a canal.

The irrigation was performed by endodontic syringe and 30 gauge side vented needle, inserted upto 2 mm short of the apex. The speed of irrigation in each file was 1ml per 5 seconds. Each canal was irrigated with 5ml of normal saline as final irrigant and dried with sterile endodontic paper point size F3.

### 2.4. SEM sample preparation

After the root canal preparation, a sterile paper point was kept inside the canal and coronal orifice was closed with modeling wax to protect the prepared canal from being contaminated by dentinal chips or debris during the splitting process(20). Each root was grooved buccally and lingually without penetrating the canal wall using a diamond disc with straight handpiece. The root was split gently into two halves with the aid of a chisel. The optimum half of each root was used for SEM examination for apical, middle and cervical third. The specimens were dehydrated in a water / ethanol mixture; the ethanol content was increased step by step in 70, 80 and 96% for 24 hours and three times in 100% ethanol for 24 hours(21). Then the specimens were dried in a desiccator for 24 hours.

The specimens were left to dry overnight, mounted on copper stubs, coated with gold, and examined and photographed using a scanning electron microscope (TESCAN VEGA 3 LMU high-performance, Variable Pressure Analytical SEM with LAB6) at an accelerating voltage of 15 KV at low vacuum. The examination was performed at several areas of each third, such as cervical, middle and apical third.

Smear layer removal was evaluated using the four-point scoring system reported by Gutmann. The scoring criteria are given below(22).

1. Little or no smear layer, covering less than 25% of the specimen, most of the tubules visible and patent
2. Little to moderate or patchy amount of smear layer, covering between 25 and 50% of the specimen, many tubules visible and patent
3. Moderate amount of scattered or aggregated smear layer covering between 50 and 75% of the specimen, no tubule visible or patent
4. Heavy smear layer covering over75% of the specimen, no tubule orifices visible or patent

## RESULTS

In the present study, for Group 1 in which normal saline was used as the sole endodontic irrigant, the SEM image showed presence of heavy smear layer throughout the entire length of root canal(Table/ Figure- 1: A, B, C). The area is devoid of any patent dentinal tubules indicating that normal saline has no ability to remove smear layer. There was no significant difference in smear layer removal between three levels of root canal, such as cervical 3rd, middle 3rd and apical 3rd

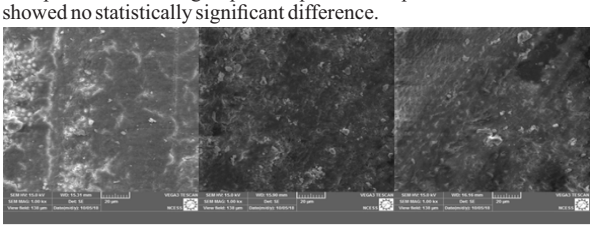
For Group 2, in which EDTA and NaOCl were used as the endodontic irrigants, there was complete removal of the smear layer at all the three levels of root canal. Most of the dentinal tubules were clearly opened and tubule diameter was larger than that of papain groups. It was also seen that smear plugs (extending part into dentinal tubules) were completely removed in the clean areas of dentin (Table/Figure -2: A, B, C).

For Group 3, where 10% papain and EDTA were used, the SEM images showed partial removal of smear layer at the three levels of prepared canal (Table/Figure 3: A, B, C) In the cervical third and middle third there was more removal of smear layer and more number of patent dentinal tubules compared to the apical third.

The mean values of the scores obtained for the three groups at the three levels of root canal such as cervical third, middle third and apical third are given in (Table/Figure-4 ) Statistical analysis using Oneway ANOVA showed there was significant difference in the mean value obtained for the three groups. Since the values were based on the cleaning capacity, it is presumed that lower the mean score value, greater is the cleaning capability.

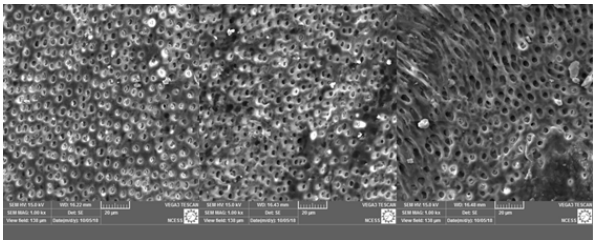
Tukeys post hoc test –simultaneous comparison method applied showed there was statistically significant difference between Group 1 & Group 2 and also Group 1 & Group 3 ( Table/Figure- 5).

Comparison between groups Group 2 & Group 3 at the three levels showed no statistically significant difference.



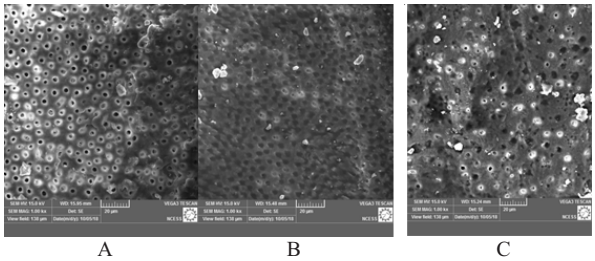
A B C

Table/Figure1: A cervical third, B middle third, C apical third) SEM images of Group 1 (normal saline) reveals heavy smear layer covering dentine surface, all images at X 1000



A B C

Table/Figure-2; A cervical third, B middle third, C apical third) SEM images of Group 2 (5% NaOCl & 17% EDTA) showing complete removal of smear layer with patent dentinal tubules, all images at X 1000



A B C

Table/Figure-3; A cervical third, B middle third, C apical third) SEM image of the Group 3 (10% papain + 17% EDTA) showing partial removal of smear layer and different patent dentinal tubules with remnants of smear, all the images at X 1000

**Table -4: Mean score ± SD of smear layer in different groups, * p<.0.05**

| Area Examined | N | Group 1 (normal saline) | Group 2 (NaOCl & EDTA) | Group 3 (10%papain & EDTA) | F | P |
|---|---|---|---|---|---|---|
| Cervical3rd | 15 | 3.9±0.35 | 1.1±0.26 | 1.1±0.35 | 365.48 | 0.000* |
| Middle 3rd | 15 | 4±0.00 | 1.1±0.41 | 1.1±0.49 | 274.14 | 0.000* |
| Apical 3rd | 15 | 4±0.00 | 1.2±0.35 | 1.3±0.74 | 163.56 | 0.000* |

**Table-5 : Tukeys Post hoc test - simultaneous comparison values,*statistically significant**

| Area Examined | Group 1 & Group 2 (Experimental error) | Group 1 & Group 3 (Experimental error) | Group 2 & Group 3 (Experimental error) | Standard error | p |
|---|---|---|---|---|---|
| Cervical 3rd | 23.69* | 23.13* | 0.56 | 2.43 | 0.000 |
| Middle 3rd | 20.75* | 19.77* | 0.99 | 2.43 | 0.000 |
| Apical 3rd | 16.54* | 14.61* | 1.92 | 2.43 | 0.000 |

## DISCUSSION

For the complete removal of smear layer from the root canal an irrigant should be able to remove both its organic and inorganic componens(23). None of the presently available endodontic irrigants are able to satisfy this requirement. So irrigants are always used in combination. In the present study an attempt was made to use a plant enzyme papain for its ability to remove the organic component of smear layer.

In the present study new irrigant combination used consisted of a plant enzyme 'papain' and an inorganic chelator 'EDTA'. Papain shows extensive proteolytic activity towards proteins, shortchain peptides, amino acid esters and amide. It shows antibacterial activity against *Streptococcus mutans*. The minimum inhibitory concentration of papain (MIC) against *Streptococcus mutans* was found to be7.5% and the minimum bactericidal concentration (MBC) was 15% (24). So in this study a concentration of 10% liquid papain was used for a time interval of 2 minutes.

Inside the oral cavity teeth are located within sockets with a normal physiological negative pressure. To simulate this condition in this study all the roots were closed at the apex with sticky wax during canal preparation. Also all the teeth of three experimental groups were standardized to the same apical diameter and identical sequence of files were used for canal preparation.

In the present study removal of inorganic component of smear layer was aided by EDTA. There are 4 hydroxyl groups which is present in EDTA, to which $Ca^{2+}$ attached to produce the chelating effect.

According to Amon *et al* proteolytic activity of papain is enhanced by the presence of heavy metal chelating agent(25). The use of 30 gauge side vented needle in this study aids better transportation of irrigant to the apical and middle third of canal. studies had shown that rotary files produce significant amount of smear layer than manual instrumentation(26). This may be the reason why smear layer removal is more difficult from rotary instrumented canals.

Another factor which influences smear layer removal is agitation of irrigant. Any such agitation was avoided in this study as it affects the result. So any removal of smear layer obtained in this study can be due to the chemical action of irrigant alone.

The removal of smear layer was better in cervical and middle third of the root (Group 2 & Group 3) possibly due to larger canal diameter than the apical third of root canal(8). Due to which the volume and velocity of irrigant is more in this region. Also the numbers of dentinal tubules are lesser in apical third than cervical and middle third of the canal.

Similar results were obtained in a study conducted by Bolhari *et al* which states that 17% EDTA is a more effective chelating agent in removing smear layer from the root canals than an herbal solution (citrus aurantifolia extracts) which was not able to remove the smear layer completely(27). These results gives a coincidence with results of Uroz-Torres and Saito *et al* they reported that more complete smear layer removal was at the cervical and middle third level than at the apical level of root(28).

## CONCLUSION

Within the limitations of the present study, it can be concluded that Group 2 (5% NaOCl and 17% EDTA) and Group 3 (10% papain and 17% EDTA) have the ability to remove smear layer at three levels (apical, middle and cervical third). On intergroup comparison Group 2 (5% NaOCl and 17% EDTA) showed more significant removal than Group 3. Also the apical area of root canal showed anatomical limitation during irrigation in all three study groups. Further studies should be carried out to use papain as a potential irrigant in combination with other agents and also regarding other properties of papain so that it could be developed as an alternate non cytotoxic natural irrigant for endodontic purposes.

## REFERENCES


1. Mccomb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. Vol 1, No 7, July 1975:238-42
2. Elio Berutti, MD, DDS RM. Penetration Ability of Different Irrigants into Dentinal Tubules. J Enoodontics. 23(12):725–7.
3. Orstavik D, Disinfection HM, Haapasalo DM. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990; 6: 142-149.
4. White RR, Goldman M, Lin PS. The Influence of the Smeared Layer Upon Dentinal Tubule Penetration by Plastic Filling Materials, J Endod. 1984;558–62.
5. Gençolu N, Samani S, Günday M. Dentinal wall adaptation of thermoplasticized gutta-percha in the absence or presence of smear layer: A scanning electron microscopic study. J Endod. 1993;19(11):558–62.
6. Çobankara FK, Adanir N, Belli S. Evaluation of the influence of smear layer on the apical and coronal sealing ability of two sealers. J Endod. 2004;30(6):406–9.
7. Arash Shahravan, , Ali-Akbar Haghdoost, , Alireza Adl, Effect of Smear Layer on Sealing Ability of Canal Obturation : A Systematic Review and Meta-analysis. J Endod 2007;33(2):96–105.
8. Zakarea NAA, Mohamad TH, Taqa AA, Chumbley S, Al-Juaid S, Balto H. A Newly Prepared Solution for the Removal of the Smear Layer. Int J Dent Sci Res. 2014;2(1):19–26.
9. Yamada RS, Armas A, Goldman M, Lin PS. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3. J Endod. 1983;9(4):137–42.
10. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of Antimicrobial Efficacy of Herbal Alternatives (Triphala and Green Tea Polyphenols), MTAD, and 5% Sodium Hypochlorite against Enterococcus faecalis Biofilm Formed on Tooth Substrate: An In Vitro Study. J Endod 2010;36(1):83–6.
11. Çalişkan Mk, Türkün M, Alper S. Allergy to sodium hypochlorite during root canal therapy: a case report. Int Endod J. 1994;27(3):163–7.
12. Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;104(3):418–24.
13. Shaheen Venghat MH and CS. Irrigants used in endodontics. Int Curr Microbiol Appl Sci. (2014) 3(3): 126-132
14. Morgental RD, Singh A, Sappal H, Kopper PMP, Vier-Pelisser FV, Peters OA. Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants. J Endod. 2013;39(3):406–10.
15. Amri E, Mamboya F. Papain, a plant enzyme of biological importance: A review. Am J Biochem Biotechnol. 2012;8(2):99–104.
16. Uhlig. H, York. WN. Industrial Enzymes and Their Applications. J. Am. Chem. Soc., 1998, 120 (35), pp 9118–9118
17. Vijay C, Bhandari CPS, Rajagopalan BS, Mukherjee. Enzymatic debridement of large burn wounds with papain e urea : medical journal armed cesdia. 2012;9:4–10.
18. Motta L, Bussadori S, Campanelli A, Silva A, Alfaya T, Godoy C, et al. Efficacy of Papacarie® in reduction of residual bacteria in deciduous teeth: a randomized, controlled clinical trial. Clinics 2014;69(5):319-322
19. Silva CRD, Oliveira MBN, Motta ES, Almeida GSD, Varanda LL, Pádula M De, et al. Genotoxic and cytotoxic safety evaluation of papain using in vitro assays. J Biomed Biotechnol. 2010;2010:197898
20. Peeters HH, Suardita K. Efficacy of smear layer removal at the root tip by using ethylenediaminetetraacetic acid and erbium, chromium: Yttrium, scandium, gallium garnet laser. J Endod; 2011;37(11):1585–9.
21. Janda R. Preparation of extracted natural human teeth for SEM investigations. Biomaterials. 1995;16(3):209–17.
22. Gutmann Jl, Saunders Wp, Nguyen L, Guo Iy, Saunders Em. Ultrasonic root end preparation Part 1. SEM analysis. Int Endod J. 1994;27(6):318–24.
23. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface Tension Comparison of Four Common Root Canal Irrigants and Two New Irrigants Containing Antibiotic. J Endod. 2006;32(11):1091–3.
24. Sasmita IS, Satari MH, Chairulfattah A, Hilmanto D. Antibacterial Activity Of Papain Against Streptococcus Mutans. Int J Dev Res. 2014;4(10):2075–7.
25. Ming CC, Bono A, Krishnaiah D, Han TS. Effects of Ionic and Non-Ionic Surfactants on Papain Activity. Vol. 12, Borneo Science. 2002. p. 71–7.
26. Ahlquist M., Henningsson O., Hultenby K. OJ. The effectiveness of manual and rotary techniques in the cleaning of root canals: a scanning electron microscopy study. Internal Endo J. 2001 Oct; 34(7):533-7.
27. Sharifian MR, Bolhari B, Aminsobhani M, Tavakolian P, Esfehani HRM. Assessing the efficacy of citrus aurantifolia extract on smear layer removal with scanning electron microscope. Iran Endod J. 2012;7(2):88–97.
28. Uroz-Torres D, González-Rodríguez MP, Ferrer-Luque CM. Effectiveness of the EndoActivator System in Removing the Smear Layer after Root Canal Instrumentation. J Endod. 2010;36(2):308–11.