Comparative evaluation of efficacy of ethylenediaminetetraacetic acid, maleic acid, and dimercaptosuccinic acid against the combination of these with sodium hypochlorite for removal of smear layer: An *in vitro* scanning electron microscope study

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Abstract

Context: The effect of dimercaptosuccinic acid (DMSA) and maleic acid (MA) when used alone on smear layer has been evaluated with mixed results, but their effect when combined with sodium hypochlorite (NaOCl) has not been studied. **Aim:** To compare the effectiveness of ethylenediaminetetraacetic acid, MA, and DMSA against the combination of these with NaOCl in the removal of smear layer.

Settings and Design:

$n = 4pq/L^2$

q = 1 – p

p = Incident rate

L = Allowable error

Methods: One hundred and forty extracted, anterior teeth were radiovisiographically assessed. Access preparation was done; apical patency was established. Cleaning and shaping was accomplished using step-back technique. The specimens were randomly allocated as per the final irrigation protocol. After final irrigation, teeth were prepared for scanning electron microscope analysis, and the middle and apical thirds of radicular dentin were evaluated at ×1000 for evaluation of severity of occlusion of dentinal tubules with smear layer. **Statistical Analysis**: The data were statistically analyzed using the Student's *t*-test and kappa test. **Results:** For combined irrigation, 10% DMSA + NaOCl was significantly better than all other groups both in the middle third and the apical third. It was more effective in the middle third than at apical third. **Conclusion:** Ten percent DMSA in combination with NaOCl removes the smear layer more effectively at both the middle and apical third.

Keywords: Dimercaptosuccinic acid, maleic acid, smear layer, sodium hypochlorite

Introduction

The main objectives of root canal therapy are cleaning, shaping, and three dimensional obturation of the root canal system to prevent reinfection.[1] For attaining a fluid-tight seal, the basic requirement is that the endodontic filling material should completely obliterate the root canal system. However, during biomechanical preparation, there is the formation of smear layer. This smear layer consists of primarily the inorganic particles and some organic materials in the form of necrotic, viable pulp tissue, odontoblastic processes, bacteria, and blood cells.[2]

The clinical implications of smear layer are very controversial. Those favoring its retention, emphasize the fact that it plugs the dentinal tubules and reduces permeability of dentin to bacteria and bacterial products.[3] Various materials and techniques have been reported with wide variations

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in their efficacy regarding removal of the intracanal smear layer. The most widely used chemical for this purpose is ethylenediaminetetraacetic acid (EDTA) in different formulations.[4] They have been reported to consistently produce canals with patent dentinal tubules. However, studies have shown that EDTA is incapable of removing the organic component of the smear layer from instrumented root canal walls.

Yamada (1983). found that when used alone sodium hypochlorite (NaOCl) may have little or no effect on the smear layer.[5] Furthermore, NaOCl has cytotoxic effect if injected into the periapical tissues, has foul smell and taste, and can cause allergic reactions.[6] Hence, the search for an effectively safe irrigating solution is still on.

Van Meerbeek (1992).[7] reported maleic acid (MA) for its ability to remove the smear layer. Recently,[3] a new chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), has shown promising results when used as an irrigant. It removes the smear layer and widens the dentinal tubules as reported by Hottel (1999).[8] The purpose of this *in vitro* study was to evaluate the efficiency of EDTA, MA, and DMSA against the combination of these with NaOCl in removal of smear layer on the root canals of extracted human teeth, using scanning electron microscope (SEM).

Methods

By assuming the probability of success as well as failure equal to 0.5, i.e., standard difference for the success or failure of the above procedure on dentally extracted teeth to achieve more than 85% of power, the sample size required for above procedure using Altmans nomogram is 140.

One hundred and forty recently extracted single-rooted, permanent, anterior human teeth were selected for the study. Extracted teeth with crown fractures, restorations, severe attrition, and previous history of root canal treatment were excluded from the study. The age, sex, and reasons for extraction were not noted. All the teeth were stored in 0.9% saline solution. Specimens were assessed radiovisiographically, and both mesiodistal and buccolingual images were taken. Access cavity preparation was accomplished with air rotor handpiece using a number four round carbide bur. After preparation of access cavity, a number 10 K-file was placed in the canal until it was visible at the apical foramen. The working length was established by subtracting 1 mm from the apical patency length. The apical portion of the root tip of all sample teeth was covered with sticky wax.

Cleaning and shaping was accomplished using the conventional step-back technique. The apical portion was enlarged to number 40 K-file and irrigated with NaOCl solution after each file. Following number 40 K-file, the canal was stepped back with sequential larger files until number 60 K-file and the working length was reduced by 1 mm after each large file. Coronal third was enlarged using gates Glidden Drills from sizes 2 to 5. The samples were randomly divided into seven groups of 20 teeth each. Of these, one group was taken as control group. The samples of each group were color coded at the apical third.

MA LR (cis-butenedioic acid) was obtained in powdered form from SD Fine Chem Ltd., Tarapur, India. Seven percent MA irrigating solution was prepared by mixing 7 g MA powder in 100 ml distilled water. In the present study, DMSA ($C_4H_6O_4S_2$) was procured in powder form from Chemsworth, SEZ, Enterprise, Surat, India. Ten percent DMSA solution was prepared by adding 70 µl of 10N NaOH solution (taken with micropipette) to 100 mg of DMSA powder (Fluka). Distilled water was added to make 1 ml of solution. The pH was adjusted to 7.0 using pH paper (Mylar Scientific Sales, Nashik, India) indicator. For preparation of 10N NaOH solution, 20 g of sodium hydroxide pellets (Thermo Fisher Scientific Ltd., Mumbai) were dissolved in 50 ml of distilled water in glass jars. Thorough mixing of the solution was done by centrifuging it on ultrasonicator (Model DTC 503). Electronic Weighing Machine (Essae DS-852J) was used for accurately measuring 100 mg of DMSA powder. For homogeneous mixing of the NaOH solution, ultrasonicator was used. Final irrigation was carried out as per the individual group protocol as enumerated in Table 1.

After completing the irrigation, the canals were dried with absorbent paper points. A longitudinal groove was prepared on the facial and palatal surfaces of all samples using diamond

Table 1: Final irrigation protocol

Groups	Irrigation procedures followed
I	5 ml of distilled water followed by normal saline (1 ml for 5 min) followed by 5 ml distilled water
II	5 ml distilled water followed by 17% EDTA (1 ml for 5 min) followed by 5 ml distilled water
III	3 ml distilled water followed by 17% EDTA (1 ml for 5 min) followed by distilled water 3 ml followed by NaOCl (1 ml for 5 min) followed by 3 ml distilled water
IV	5 ml distilled water followed by 7% maleic acid (1 ml for 5 min) followed by 5 ml distilled water
V	3 ml distilled water followed by 7% maleic acid (1 ml for 5 min) followed by 3 ml distilled water followed by NaOCl (1 ml for 5 min) followed by 3 ml distilled water
VI	5 ml distilled water followed by 10% DMSA (1 ml for 5 min) followed by 5 ml distilled water
VII	3 ml distilled water followed by 10% DMSA (1 ml for 5 min) followed by 3 ml distilled water followed by NaOCl (1 ml for 5 min) followed by 3 ml distilled water

DMSA: Dimercaptosuccinic acid; NaOCl: Sodium hypochlorite; EDTA: Ethylenediaminetetraacetic acid

disc. Care was taken not to penetrate the canal with disc. The samples were then splitted into two halves using a mallet and chisel. The samples were then dehydrated by placing them sequentially in 50%, 75%, and 100% ethyl alcohol for total 8 h (2 h 40 min each sample). The samples were mounted on aluminum stubs using an adhesive tape. The samples were gold sputtered in a vacuum chamber. The samples were examined at ×1000 magnification under SEM [Figure 1]. Only the middle and apical third images were taken. Scoring of each sample at both the middle and apical third was done according to the scoring criteria [Table 2].

Results

Individual score was allotted to each sample, for middle third and apical third separately at ×1000. The data were subjected to statistical analysis using SPSS for Windows 12.0 software package (SPSS Inc., Chicago, IL, USA). The maximum score that was allotted to each individual sample was three which meant all dentinal tubules were obliterated with smear layer. The minimum score that was allotted to each individual sample was 0 which meant all dentinal tubules open and no smear layer. The scoring was done by three independent investigators who were unaware of the experimental groups [Table 3].

Tooth specimens from Group 5 (MA + NaOCl) and Group 7 (DMSA + NaOCl) demonstrated statistically superior results as compared to other experimental groups; comparatively cleaner canals which were significantly free of debris, and smear layer was seen. Group 7 depicted the best outcome in terms of smear layer removal which was followed by specimens of Group 5. Except for Group 3, all experimental groups using NaOCl in combination with the primary irrigants demonstrated comparatively better results emphasizing the importance of NaOCl in final irrigation protocol. In all the experimental groups, better smear layer removal was observed in middle third of the root canal system as compared to apical third.

Table 2: Scoring criteria for smear layer evaluation

Score	Smear layer evaluation
0	No smear layer; all dentinal tubules open
1	Minimum smear layer; >50% of dentinal tubules visible
2	Moderate smear layer; <50% of dentinal tubules open
3	Heavy smear layer; outline of dentinal tubules obliterated

Discussion

Smear layer has become a critical factor in root canal therapy since its inception as reported by McComb and Smith (1975). Among all the different methods for removal of smear layer, NaOCl and EDTA solution are most commonly used for removal of smear layer. However, NaOCl is not able to dissolve the inorganic components of smear layer.

Recent studies[2] showed promising results with MA and DMSA owing to their ability to remove the smear layer. In the present study, we tried to compare the efficacy of 7% MA and 10% DMSA used individually as root canal irrigants with the conventional chelating agent 17% EDTA. The results of the present study showed that 10% DMSA is more effective than 17% EDTA in smear layer removal from both the middle and apical third of the canal.

A new chelating agent, DMSA, which is a chemical derivative of dimercaprol was used as an irrigant. Compared with traditional chelating agents, DMSA has relatively low toxicity.[9] It contains two sulfhydryl (-SH) groups and has been shown to be an effective chelator of toxic metal mainly lead and arsenic. Few major advantages of DMSA in medical fields include its low toxicity, oral administration, and no redistribution of metal from one organ to another.[10] Saline was used instead of fixative for storage to avoid any possible "fixing" effect on the pulp or dentin that might alter the result of canal preparation.[11] The apical portion was enlarged up to 40 size K-file. Current evidence-based literature reveals that larger apical preparation produces a greater reduction in remaining bacteria. Apical part of root tip was sealed with sticky wax to simulate closed apical system.[12] Distilled water was used after each irrigating solution, to flush out the residual irrigants.[3] Irrigation regimen as described by Bogra *et al*.[3] was followed, i.e., in experimental groups involving NaOCl combination, first, 3 ml of distilled water was taken

Table 3: Unpaired *t*-test for comparison of Group 2 with others at apical third at×1000

Group	Mean	SD	SE	*t*	*P*	Remark	Percentage change
2	1.8	0.6325	-	-	-	-	-
3	1.8	1.0328	0.383	0.000	1.00	NS	-
4	1.5	0.8498	0.335	0.896	0.382	NS	-
5	1.0	0.9428	0.359	2.228	0.039	S	44.44
6	1.7	0.8232	0.328	0.305	0.764	NS	-
7	0.9	0.5677	0.2687	3.349	0.004	S	50.00

SD: Standard deviation; SE: Standard error; NS: Not significant; S: Significant

to flush out any remnants of cutting debris, whereas in experimental groups without NaOCl combination, flushing was done using 5 ml of distilled water. This was so done because the total amount of fluid taken for irrigating each sample should come to 11 ml. This was applied to all groups since volume of irrigant is also an important variable.[13] Once the flushing with distilled water was done, then the respective irrigating solution was tested.

Specimens were evaluated at ×1000 magnification for evaluation of removal of smear layer. The objectives of the study were two-fold with respect to removal of smear layer at middle third and apical third at ×1000. These were: (1) To find out which primary irrigant is better when combined with NaOCl (2) to find which individual irrigant is better as compared to others.

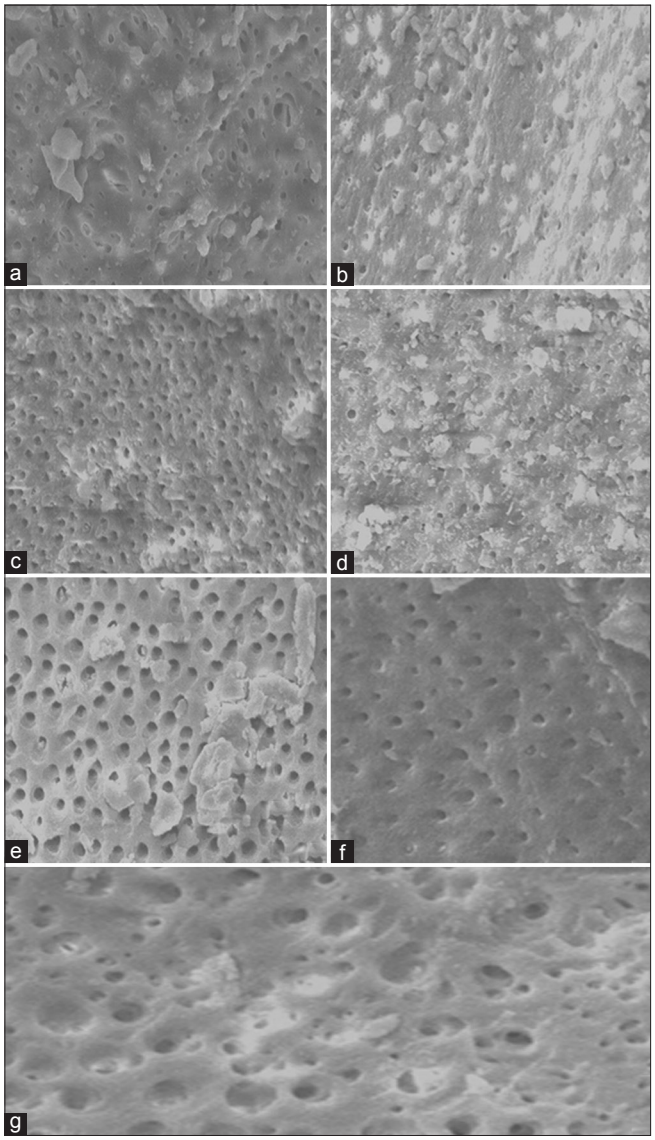
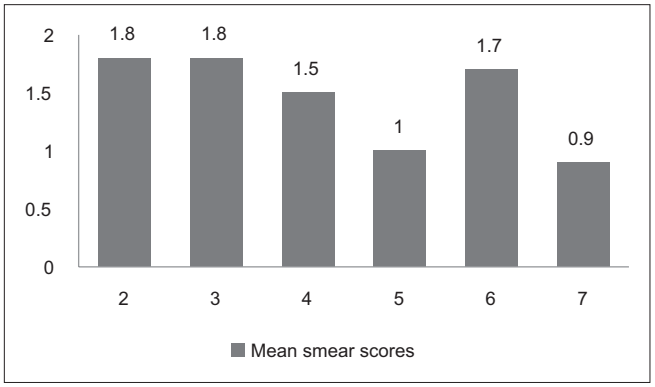


Figure 1: (a) Normal saline, (b) ethylenediaminetetraacetic acid, (c) ethylenediaminetetraacetic acid + sodium hypochlorite, (d) maleic acid, (e) maleic acid + sodium hypochlorite, (f) dimercaptosuccinic acid, (g) dimercaptosuccinic acid + sodium hypochlorite

Seven percent MA resulted in greater smear layer removal from the apical third as compared to other individual irrigants (17% EDTA and 10% DMSA) [Graph 1]. These results support the findings of Ballal (2009),[14] who found increase smear layer removal with 7% MA than 17% EDTA at the apical third. However, when combined with NaOCl, it was found to be less effective than 10% DMSA + NaOCl combination, but more effective than 17% EDTA-NaOCl in removing smear layer in the present study.

Because EDTA is a chelating agent, it is not dependent on a high hydrogen ion concentration to accomplish decalcification and is effective at a neutral pH. The exchange of calcium from dentin by hydrogen results in a subsequent decrease in pH. Hence, the efficacy of EDTA decreases over time because of the decrease in pH.[15] Thus, overall, it was observed that among combination groups, DMSA + NaOCl was most effective in smear layer removal, both at middle third and apical third. Vasiliadis *et al*.[16] and Paque *et al*.[17] reported that dentin in the apical third of the root canal is sclerosed. Hence, EDTA may not have such a pronounced action on sclerosed dentin in the apical third. When 0.9% saline was compared to all other groups, at middle third and apical third, saline showed high mean score values. This suggests that 0.9% saline has no effect on smear layer removal. This is in accordance with the results of the study done by Carvalho *et al*.[18] Among all individual irrigating solutions, 7% MA has greater smear layer removing ability. Hence, it was anticipated that 7% MA + NaOCl combination should result in greater debris and smear layer removal as compared to 10% DMSA + NaOCl, but on the contrary, 7% MA + NaOCl removed lesser debris and smear layer as compared to 10% DMSA + NaOCl. This is explained by the fact when MA reacts with NaOCl, the available chlorine content decreases which resulted in a decrease in the efficiency of removal of organic component from the smear layer.[19]



Graph 1: Mean smear scores of different groups. (2) Ethylenediaminetetraacetic acid, (3) ethylenediaminetetraacetic acid + sodium hypochlorite, (4) Maleic acid; (5) maleic acid + sodium hypochlorite; (6) dimercaptosuccinic acid, (7) dimercaptosuccinic acid + sodium hypochlorite

However, since limited studies have been done on DMSA, hence more studies have to be done both *in vitro* and *in vivo* conditions to substantiate its use. Further studies using digital image analysis can be conducted to evaluate whether a similar effect of DMSA and MA can be obtained in multirooted teeth with curved canals. Further research needs to be done to study the tissue-dissolving action of 10% DMSA, its toxicity, adverse effects, and its action *in vivo* conditions before it can be incorporated into clinical use. Furthermore, to further substantiate the findings of this study, the smear layer can be seen at higher magnification above ×1000. Within the limitations of this study, it can be said that 10% DMSA + NaOCl and 7% MA are both effective and can be a promising alternative to 17% EDTA + NaOCl.

Conclusion

Based on the results of the present study, following conclusions can be made:

Regarding combined groups (i.e Irrigant + NaOCl):

- Ten percent DMSA + NaOCl removes the smear layer more effectively as compared to all other groups (Maleic acid + NaOCl, EDTA + NaOCl, Saline alone) in both the apical and middle third. But, its smear layer removing capacity was less in the apical third as compared to middle third.
- Seven percent MA was most effective for removal of smear layer when used alone.

Clinical implications

The most commonly followed standard irrigation regime used in the clinical practice includes NaOCl along with EDTA, but our present research highlighted the fact that the experimental irrigating regime of DMSA and NaOCl is better in terms of smear layer removal than the conventionally used ones.

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Conflicts of interest

There are no conflicts of interest.

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