Original Article

Evaluation and Comparison of Smear Layer Removal Potency of Three Different Irrigation Regimes – A Stereomicroscopic and Scanning Electron Microscopic Study

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Abstract

Background: Irrigation plays a pivotal role in pulp therapy owing to its flushing action and removal of the smear layer which if present prevents penetration of medicaments and sealers into the dentinal tubules. **Aim:** This study aims to evaluate and compare the smear layer removal potency of three commercial Irrigating agents. **Materials and Methods:** Single rooted 30 teeth were selected, decoronated, and randomly divided into three groups according to the irrigation regime to be used (Group I − 5% sodium hypochlorite [NaOCl] + ethylenediaminetetraacetic acid [EDTA], Group II – Chloraxid Gel + EDTA, Group III – Tween Kleen + 3% NaOCl). After recommended biomechanical preparation and irrigation, teeth were stained with 1% methylene blue dye for 24 h and sectioned in 2 halves. Stereomicroscopy was used to assess dye penetration and scanning electron microscope (SEM) analysis was done to detect dentinal surface changes. Data were analyzed using one-way ANOVA test and Tukey honestly significant difference test by statistical software SPSS version 20.0. **Results:** The highest dye penetration exhibiting smear layer removal for the apical third was seen in Group II (44.78) and for the middle third in Group I (64.73) which was statistically significant. SEM analysis showed maximum dentinal tubules visibility and patency in Group I and minimum in Group III. **Conclusion:** Newer irrigating materials (1-hydroxyethylidene-1, 1-bisphosphonate) exhibited weak potency for smear layer removal. The sequential use of gold standard NaOCl + EDTA gave satisfactory results, however, in apical third NaOCl Gel + EDTA was found to be more effective. Therefore, it can be prudent to use NaOCl Gels as a safer alternative to conventional means.

Keywords: 1-hydroxyethylidene-1, 1-bisphosphonate, ethylenediaminetetraacetic acid, irrigation, scanning electron microscope, smear layer, sodium hypochlorite gel

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Introduction

Deciduous teeth play an equally vital role as permanent teeth for the harmonious development of occlusion, maintenance of arch length, mastication, and speech. The caries development is a rapid process in deciduous teeth causing damage to the pulpal tissues due to contamination by microorganisms and their released toxins.[1] To increase their retention in the oral cavity, pulp therapy is a necessity which is the process of removal of bacteria and infected dentin chemo-mechanically. However, in primary teeth, it gets a bit complex because of the varied anatomy of the roots. Primary teeth have bizarre internal geometry with perplexing features such as ribbon-shaped canals, internal connections, horizontal anastomoses, and accessory canals which makes the use of additional irrigating solutions a necessity.[2]

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A pivotal factor that influences the success of pulp therapy is smear layer. It is an irregular amorphous granular layer that is formed while cleaning and shaping the canals, covering the radicular dentin, and occluding the orifices of dentinal tubules. It decreases the penetration of irrigants or obturating materials into the canals by 25%–49%. The smear layer contains both organic (debris from pulpal and bacterial tissues) and inorganic (dentinal chips and debris) components and together

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they prevent the ingress of intracanal disinfecting agents and sealers into blocked dentinal tubules. Therefore, it is crucial to eliminate this layer to achieve a hermetic seal.

Irrigation plays a key role in pulp therapy as it expedites the removal of microorganisms, loose necrotic tissues, or infected dentin from the root canals through flushing action. The contaminated tissue is thus prevented from being pushed deeper into the canal space or extruded periapically. Irrigation aided mechanical instrumentation helps to keep the canal wall lubricated while simultaneously facilitating easy instrumentation within the canal space.[3] The choice of an irrigant thus is a crucial factor and should take into consideration the differences among the dentin substrata. The dilemma arises while treating a primary tooth due to physiological root resorption which might allow the apical extrusion of the solution causing severe pain and possible damage to the succedaneous tooth. Therefore, it is preferable to use a nonirritating solution in deciduous teeth.[4]

The success of any irrigation agent relies on various attributes such as its mechanism of action, its property to contact the tissues, root canal structures, restorative material, and most vitally its penetration depth in the main canal and smaller accessory canals.[5]

Numerous irrigants are available such as normal saline, sodium hypochlorite (NaOCl), chlorhexidine digluconate, citric acid, maleic acid, Mixture of Tetracycline, acid and detergent (MTAD), 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP), tetra clean, aqueous ozone, Qmix, ethanol, and several herbal irrigants. The irrigants used in this study are Chloraxid gel and Tween Kleen. Chloraxid acid gel is a NaOCl irrigant in a gel form and its consistency makes it easier to control its flow in the canals. Another agent that is used is a single-step irrigation material-Tween Keen which is a mild chelating agent HEBP with short-term compatibility with NaOCl. It is antibacterial and has a definite proteolytic action.

Despite the availability of numerous irrigating agents, there still lacks a need for a single potent solution that can accomplish the need to eliminate the smear layer's organic and inorganic constituents. Thus, this study was undertaken with the purpose to determine the efficacy of three different commercially available irrigants to remove the smear layer from the dentinal tubules by evaluating them under stereomicroscope and scanning electron microscope (SEM).

Materials and Methods

The proposed study was conducted as an *ex vivo* study with prior permission and consent obtained from the ethical committee of the institution. Thirty single-rooted teeth extracted due to orthodontic or periodontal conditions were selected for the study and the presence of single canals was confirmed with the radiographs.

The exclusion criteria involved:

1. Teeth extracted due to any pathological cause
2. Teeth with carious involvement in crown/root surfaces
3. Teeth having external root resorption
4. Teeth with open apices
5. Teeth with a history of root canal treatment/restorations.

The selected teeth were thoroughly cleaned to remove debris and calculus and stored in distilled water. Further, they were decoronated using a slicing disc (1 mm) to standardize the root canal length of 14 mm. The position of the apical foramen and the patency of the canal were established with No 10 K file (Mani, Japan). For working length estimation, the file was inserted in the canal and working length was considered to be 1 mm short of the length of the file when the tip was visible beyond the apex.

Random allocation of sample teeth was done into three groups according to the irrigating regime and sequence to be used [Table 1]. Before preparing the root canal, the apical ends were blocked using wax to prevent the escape of the irrigants periapically. Biomechanical preparation was done using Protaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) till the apical working length increasing the canal size as per sequence till the F2 file. After using each file and before preceding to the next file, the root canals were irrigated with the assigned group irrigant in the predecided sequence and time.

The teeth were then coated with transparent nail polish lacquer to prevent penetration of dye from any surface cracks.

Table 1: Irrigation agents and their sequence

GROUP	AGENTS	SEQUENCE
Group I (*n*=10)	5% NaOCl solution (Eusol Solution, JL Morison India Ltd) + 17% EDTA (RC Prep, Prime Dental)	First, the root canal was irrigated with 3 ml of 5% NaOCl with a 30G side vented needle and left in the canal for 20 s. Between each subsequent file, 17% EDTA was used as a chelating agent
Group II (*n*=10)	5.25% NaOCl Gel without surfactant (Chloraxid Gel, Cerkamed) + 17% EDTA (RC Prep, Prime Dental)	The root canal was filled with Chloraxid gel with the applicator tip till the apical end and kept for 20 s. The canal was then flushed alternately with 17% EDTA
Group III (*n*=10)	HEBP (Tween Kleen, Maarc Dental) + 3% NaOCl (Eusol Solution, JL Morison India Ltd)	Tween Kleen solution was freshly prepared according to the manufacturer's instruction by dispensing 2 capsules into 10 ml of 3% NaOCl solution. The freshly prepared solution was used in the canals for 20 s and the instrumentation was done without the use of EDTA. The canals were flushed with 5 ml of solution between each sized file

EDTA: Ethylenediaminetetraacetic acid, NaOCl: Sodium hypochlorite, HEBP: 1-hydroxyethylidene-1, 1-bisphosphonate

Staining was done with 1% methylene blue dye by immersing the teeth in the solution for 24 h to detect the amount of dye penetration caused by the removal of the smear layer. The teeth were then split longitudinally into two halves to be viewed under stereomicroscope and SEM. The stained samples were analyzed under stereomicroscope at ×4 magnification to determine the area of dye penetration in apical one-third and the middle one-third of the canal [Figure 1a-c].

ImageJ software was used to measure the specific area of dye penetration in all the specimens [Figure 2].

SEM analysis was done to determine the surface changes on the root dentin. The specimen to be analyzed were mounted on aluminum stubs and coated with 25 u of Gold Palladium by a process known as sputtering. They were then viewed under SEM at × 5000 magnification. Photomicrographs were obtained from the middle third of each sample and evaluated for the dentinal surface changes [Figures 3-5].

Statistical analysis

The data were assessed with IBM SPSS 20 (SPSS20.0, SPSS Inc., Chicago, IL, USA). Statistical analysis was done using one-way ANOVA test and Tukey honestly significant difference test. For all statistical analyses, the $P < 0.05$ was considered statistically significant.

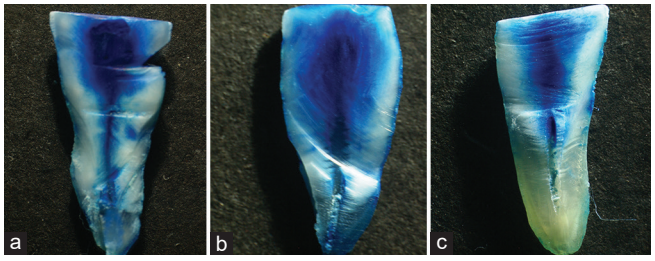

Figure 1: (a) Sectioned tooth samples showing dye penetration in the middle and apical regions (Group I). (b) Sectioned tooth samples showing dye penetration in the middle and apical regions (Group II). (c) Sectioned tooth samples showing dye penetration in the middle and apical regions (Group III)

Results

All 30 teeth were divided into three groups comprising of 20 sections each.

The depth (measured in microns) of dye penetration in the middle third and the apical third was assessed in all the groups.

Stereomicroscope findings of dye penetration

Middle third of the canal

Group I (mean 64.73) showed the highest amount of dye penetration indicating maximum smear layer removal, second being Group II (mean 61.03), and least dye penetration was seen in Group III (mean 42.41). The results were statistically significant ($P = 0.001$) [Figure 6].

Apical third of the canal

Statistically significant result ($P = 0.00$) was found with maximum dye penetration seen in Group II (mean 44.78) followed by Group I (mean 41.68) and least being Group III (mean 34.88) [Figure 7].

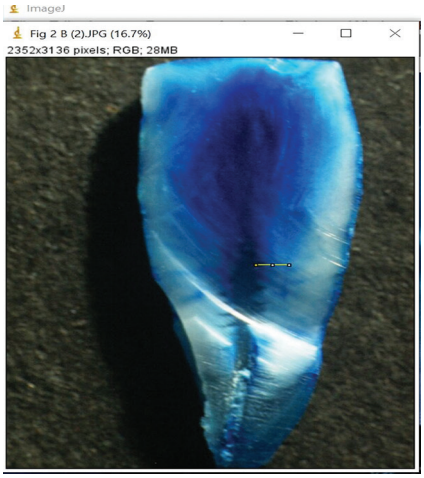

Figure 2: Image analysis of stained section captured in stereomicroscope and analyzed in Image J software

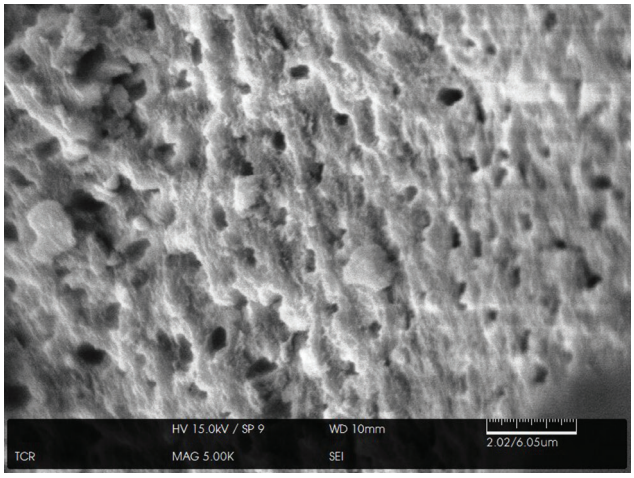

Figure 3: Scanning electron microscope photomicrograph of Group I

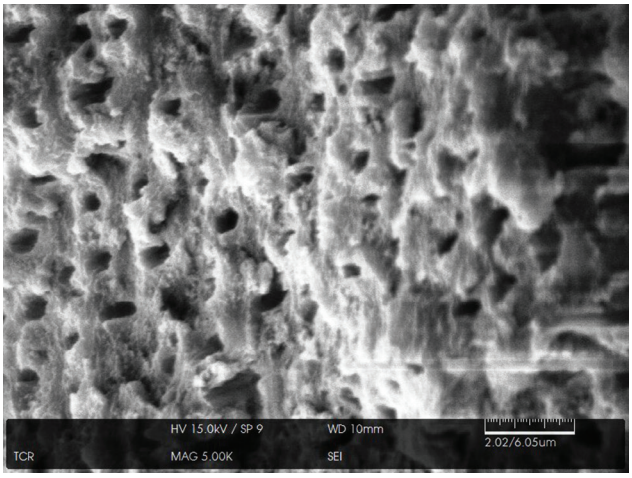

Figure 4: Scanning electron microscope photomicrograph of Group II

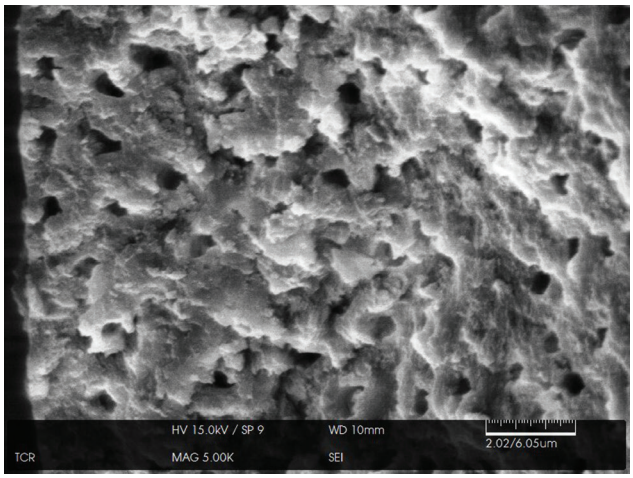

Figure 5: Scanning electron microscope photomicrograph of Group III

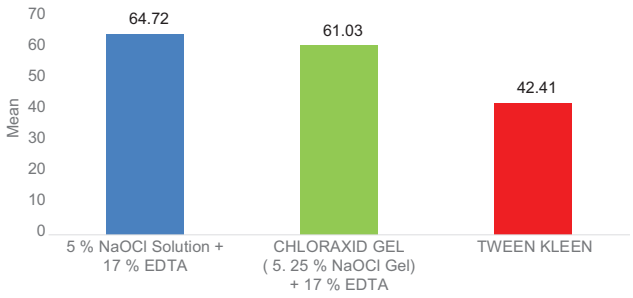

Figure 6: Graph demonstrating smear layer removal in the middle third region (*P*-value=0.000 calculated using one-way ANOVA test)

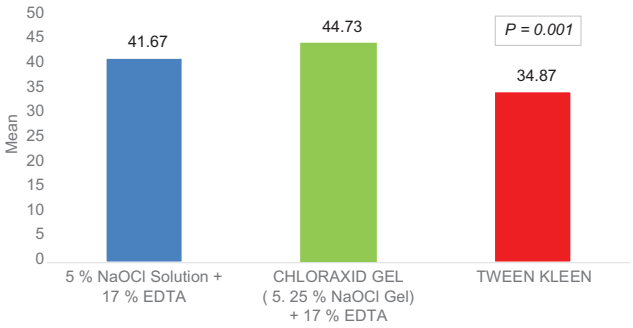

Figure 7: Graph demonstrating smear layer removal in the apical third region (*P*- value=0.001 calculated using one-way ANOVA test)

Group I and II exhibited significant differences for smear layer removal in the middle third and apical third when compared with Group III. However, no significant difference was found between Groups I and II in both the regions [Table 2].

Dentin patency as seen under scanning electron microscope

SEM was done to assess the changes in the dentinal surface and effect of the irrigation regimes on the smear layer and dentinal tubules.

- Group I – Showed the minimum amount of smear layer covering the dentinal tubules. Maximum number of tubules were visible and patent when compared to the other two groups [Figure 3]
- Group II – Showed little or patchy amount of smear layer covering the dentinal tubules. Many of the dentinal tubules were visible and patent [Figure 4]
- Group III – Showed a moderate amount of scattered aggregated smear layer covering dentinal tubules indicating minimum tubule visibility and patency [Figure 5].

Discussion

To measure the penetration extent and depth of any irrigating solution *in vivo* is not possible due to practical limitations. Ideally, an irrigating agent should primarily possess the property to dissolve the smear layer or prevent its formation in the first place. Concurrently, it should have broad antimicrobial activity, should disinfect the canal, and dissolve the necrotic tissues without altering the physical characteristics of the tooth.[6] Since a single irrigant cannot comply with all these optimal requirements, various studies have reported using a specific sequence or combination of two or more irrigation solutions for desired results.

One of the most commonly used irrigants in pulp therapies with potent antimicrobial activity is NaOCl. It can kill most of the pathogenic microorganisms in direct contact and remove the necrotic and loose organic tissue as well. Commercially, it is available in a concentration ranging from 0.5% to 6%. The most effective concentration is reported to be is 5.25% at 40 min.[7] Irrigation using 1.3% and 2.5% NaOCl for this same time interval is ineffective in removing *Enterococcus faecalis* from infected dentin.[8] Although it lacks the capability to remove the smear layer completely, it disturbs the organic portion of the smear layer facilitating its removal with subsequent use of ethylenediaminetetraacetic acid (EDTA). In a study done by Ghorbanzadeh *et al*., it was reported that irrigation done with a 5% NaOCl solution along with 15% EDTA was the most effective way to disturb and remove the smear layer.[9] Hence, in the present study, NaOCl solution was considered as the conventional and gold standard agent to compare with the experimental groups.

Even though being considered as near to an ideal irrigant, NaOCl has marked adverse effects if extruded beyond the apical foramen. The operator needs to be extremely careful while applying pressure or binding the needle tip in the canal during irrigation. The canal if blocked by the needle tip presents no escape route for the additional irrigant causing it to accumulate in large volumes in the periapical tissues. It causes severe pain and irritation in the periapical tissues (Farook *et al.* 2014) and might also hinder the survival and differentiation potential of the stem cells of the apical papilla. This leads to impaired periapical repair and regeneration of pulpal tissues (Faria *et al.* 2019).[10]

In teeth with open apices, incomplete root formation, or with root resorption which is commonly encountered in primary

Table 2: Intergroup comparison done using the *post hoc* Tukey test

Intergroup comparison	Mean difference	Standard error	*P*
Smear layer removal in apical 3rd			
Group I			
Group II	−3.10	2.643	0.474
Group III	6.80	2.643	0.033
Group II			
Group I	3.10	2.643	0.474
Group III	9.90	2.643	0.001
Group III			
Group I	−6.80	2.643	0.033
Group II	−9.90	2.643	0.001
Smear layer removal in middle 3rd			
Group I			
Group II	3.69	3.070	0.456
Group III	22.32	3.070	0.000
Group II			
Group I	−3.69	3.070	0.456
Group III	18.62	3.070	0.000
Group III			
Group I	−22.32	3.070	0.000
Group II	−18.62	3.070	0.000

teeth, it is a likely possibility for NaOCl solution to extrude apically. To overcome this issue, NaOCl gel can be used as a safer alternative for such teeth. Chloraxid acid Gel is a newer commercially available gel-based NaOCl irrigant used to disinfect the canals and also for smear layer removal. The Gel is prepared as a water-soluble agent by adding 5.25% NaOCl to a vehicle such as polypropylene and polyethylene glycol. It can be coated on the file as per requirement thus it is easy to use as a lubricant in limited quantity. The viscosity of the gel makes its handling easier in comparison with the liquid form thus minimizing the chances of being pushed beyond the apex. It can easily be flushed out with saline as a final irrigant.[11]

Studies have shown that NaOCl in gel form has similar effects such as solution on the smear layer and dentin surface. Due to its consistency, it gets retained for a longer duration in the apical third region leading to deeper penetration in the tubules. The increased contact time with the root canal dentin also influences the penetration depth and simultaneously reduces the chances of apical extrusion of the agent. This might explain the maximum efficacy of Chloraxid acid Gel among tested irrigants in the apical third region. Our results are in agreement with the study by Zand *et al*., wherein NaOCl gel was as effective as NaOCl solution along with EDTA in smear layer removal in all three parts of root canal walls.[11] Crumpton *et al*. stated that 5.25% NaOCl when used after 1 min of 17% EDTA aids in dislodging the entire smear layer. However, when the application time of any strong chelating agent such as EDTA exceeds greater than a minute with a quantity over 1 ml, clinically significant erosion can be seen.[12]

Few of the newer irrigating materials have gained popularity as a potential alternative to EDTA or citric acid. One such agent is HEBP also known as etidronic acid or etidronate. It shows no short-term reactivity when added to NaOCl and is used as a single step irrigant. HEBP is a weak chelating agent that attacks less dentin surface than other commonly used chelators, such as EDTA. HEBP when mixed with NaOCl, has a combination pH of 11.86 which makes the survival of *E. faecalis* bacteria in the oral cavity difficult as it cannot survive beyond pH of 11.5. HEBP has a property to reduce the action of NaOCl solution after 1 h of their mixture. However, when freshly prepared, both the agents remain active. According to the manufacturer's guidelines, HEBP solutions need approximately 300 s to remove the smear layer completely from the dentinal surface. Since the steps are reduced with this irrigant it can be time-saving and hence advantageous especially for children. However, because of its soft chelation action, the efficacy of removing the smear layer was found to be inferior when compared with the sequential use of NaOCl and EDTA in the present study which is in accordance to study conducted by Girard *et al*., where he stated that HEBP was compatible with NaOCl and showed better calcium-binding but was unable to significantly inhibit the formation of a smear layer on apical root canal walls.[13]

Several studies have reported the presence of bacteria and their penetration into the dentinal tubules and on the dentinal surfaces. Siqueira *et al*. (2002) observed bacterial cells were present till a depth of 300 μ in the tubules whereas Schafer *et al*. (2005) reported up to the depth of 0.25 mm. The bacteria which cannot be removed even after proper debridement of the canal become residual and are responsible for the failure of the treatment.[9]

In the present study, SEM analyses for all three groups were done and dentinal surface changes were observed in the middle third region. The results demonstrated that smear layer removal was more thorough and effective in the middle third region when compared to the apical third. The possible explanation for this might be the dentinal tubules which are larger in diameter in the middle third allows the irrigating solution to enter the tubules in higher volumes. Thus, it improves wetting efficiency and better flow of the solution pushing the smear layer out of the tubules.[14] However, results of this study are not in agreement with the study done by Faria *et al*.(2019) where the author reported that solutions provide a better disinfection than gels.[10]

The limitation of this study is the lack of use of the specific rating scale for the amount of the smear layer. Thus, the quantitative assessment of the smear portion cannot be done like in the study where Guttman Rating scale has been used.

Conclusion

Within the restraints of the present study, it can be stated that:

- Standard and conventional protocols for irrigation bring about efficient elimination of the smear layer and greater depth of penetration of the irrigating agent

- Newer single-step irrigating agent (HEBP) exhibited weak potency in the removal of smear layers in both, middle and apical third
- It can be prudent to use NaOCl Gels as a safer alternative to the conventional NaOCl solution in teeth where there are higher chances of extrusion of irrigant due to resorption or open apices.

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Conflicts of interest

There are no conflicts of interest.

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