Removal of the root canal smear layer using Carisolv III and sodium hypochlorite

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Abstract
The present study investigated the effectiveness of a Carisolv III + 0.5% sodium hypochlorite (NaOCl)-based root canal irrigant for smear layer removal.

Forty maxillary incisors were randomly divided into 4 groups (n = 10 per group). The canals in group A (experimental) were prepared with 0.5% NaOCl, and Carisolv III and 0.5% NaOCl was used for the final washing; groups B and C (positive controls) used 2% and 5.25% NaOCl, respectively; and group D (negative control) used phosphate-buffered saline (PBS). Ethylenediaminetetraacetic acid (EDTA) was used for all of the groups. A 5-point scoring scale and scanning electron microscopy were used to evaluate the effectiveness of the irrigants. The canals were consistently cleaner in the coronal and middle thirds than in the apical thirds (P < .05).

For cleaning the root canals, 5.25% NaOCl was more effective than 2% NaOCl, 0.5% NaOCl + Carisolv III, and phosphate-buffered saline, respectively (P < .05). The 2% NaOCl solution showed similar results to 0.5% NaOCl + Carisolv III (P > .05). The combination of 5.25% NaOCl and 17% EDTA remains the most effective irrigant for removal of the root canal smear layer.

A combination of Carisolv III + 0.5% NaOCl (with 17% EDTA) showed a cleaning ability similar to that of 2% NaOCl (with 17% EDTA).

Abbreviations: EDTA = ethylenediaminetetraacetic acid, NaOCl = sodium hypochlorite, PBS = phosphate-buffered saline, SEM = scanning electron microscopy.

Keywords: Carisolv III, endodontic, irrigant, scanning electron microscopy, sodium hypochlorite

1. Introduction
Microorganisms and their associated metabolites play an essential role in the development of pulpsitis and periapical diseases. The key goals of endodontic treatment include elimination of microbial infections, removal of necrotic pulp tissues from the root canal system, and establishing apical sealing to prevent recurrence. The mechanical preparation of a root canal results in a large amount of smear layer (thickness of ∼2–5 μm) formation mixed with inorganic calcified tissues, organic matrix, and dentinal debris.

Although the smear layer has not been directly connected to the outcome of root canal treatment, incomplete canal debridement may lead to endodontic failure. Smear layer formation leads to a number of unfavorable consequences such as blocking the surface of dentinal tubules as well as the penetration of irrigants, medications, and filling materials into the dentinal tubules.

In endodontics, there is a great emphasis on removal of the smear layer and using intra-canal irrigants. Sodium hypochlorite (NaOCl) at concentrations of 0.5% to 5.25% is the most widely recommended endodontic irrigant due to its excellent ability to dissolve tissues and its antimicrobial properties. It has been shown that the use of 17% ethylenediaminetetraacetic acid (EDTA) and 5.25% NaOCl during root canal preparation enhances the removal of the smear layer; however, it also results in excessive demineralization of the dentinal tubules and a damaged surface morphology. Similarly, higher concentrations of NaOCl solution (≥5.25%) may lead to localized irritation and inflammation of the periapical tissues.

Alternatively, 17% EDTA is an organic acid that acts as a chelator to bind the calcium ions within hydroxyapatite. The EDTA solution can completely remove the inorganic components from the smear layer and open dentinal tubules within 1 minute. However, a prolonged treatment (>10 minute) may lead to erosion of the intertubular and peritubular dentin.

Antimicrobial agents such as chlorhexidine have a strong broad-spectrum activity and effectiveness against the majority of endodontic pathogens, especially Enterococcus faecalis. However, chlorhexidine lacks the ability to dissolve tissue and cannot effectively remove the root canal smear layer.
irrigants such as phosphate-buffered saline (PBS) and hydrogen peroxide can be applied with mechanical cleaning, they are rarely used due to their lack of antimicrobial activity and their inability to remove the smear layer.\[10\]

The Carisolv III system consists of Carisolv III gel and Carisolv III tools, which are designed to maximize the protection of healthy dental tissues and remove carious debris\[11,12\]. Carisolv I and II, which contain NaOCl and 3 different amino acids (glutamic acid, leucine, and lysine), have been used to remove the smear layer in immature ovine incisors in previous studies.\[13,14\]

However, the potential problems of tooth discoloration and a long waiting time have been reported. The main components of the Carisolv III gel are chloretamine T, papain, sodium chloride, erythritol, and carboxymethyl cellulose; this mixture has shown a high efficiency of caries removal. Moreover, Carisolv III is a colorless gel, so it does not cause tooth discoloration. Therefore, the aim of the present study was to evaluate the effectiveness of the Carisolv III system in the removal of the root canal smear layer during the final wash, compared with NaOCl irrigation.

2. Materials and methods

2.1. Materials

This experimental study was performed on caries-free maxillary incisors (n=40; 10 per group) extracted due to periodontal conditions. The present study included 98 patients (46 women and 52 men, aged 40-60 years) attending the Department of Cariology and Endodontology, Qingdao Stomatological Hospital, China, between January 2017 and January 2018. We conducted this study in May 2018. To calculate the sample size and power of the tests, PASS 15.0 software was used, as described previously.\[15\] To achieve a power of 0.9 and a significance level of 0.05, the sample size for each group was determined to be a minimum of 4. The extracted teeth were examined carefully to ensure that they met the following criteria: fully developed teeth with a completely closed apex, length of the teeth ranged from 20 to 25 mm. Any teeth treated endodontically or presenting with dysplasia, calcification, or root resorption were excluded. The Institutional Ethics Research Committee (Qingdao Stomatological Hospital, Qingdao, Shandong, China) approved the design of this study, and an informed written consent to participate was obtained from all patients. All teeth were numbered according to the patient’s information and were cleaned and stored in 0.9% normal saline until further experimentation.

The irrigation regents used in the present study included Carisolv III (MediTeam, Goteborg, Sweden), NaOCl (Chloarex, Durham, UK), EDTA gel and solution (17%) (Chloarex, Durham, UK), and PBS (Fresenius Kabi, Warrington, UK). The instruments used in the present study included K-type files (Dentsply-Maillefer, Ballaigues, Switzerland) and 27-gauge Monoject endodontic needles (Ultradent, South Jordan, UT).

All maxillary incisors were divided randomly into 4 groups (n=10 for each group), according to the irrigation protocol during the initial root canal preparation and the final wash (after completion of the root canal preparation) (Table 1). Group A was the experimental group, groups B and C were positive controls, and group D was the negative control.

2.2. Specimen preparation

To yield root specimens of uniform length (~1.2 mm), the teeth were decoronated at the level of the cementoenamel junction using a high-speed bur under a water-cooled diamond disk (Fig. 1). Following the length measurement, the apices were sealed with sticky wax to prevent extrusion of the irrigants through the apical foramen and to simulate the closed-end system.

Pulp tissue remnants were removed from each root canal with fine, barbed broaches (Maillefer, Ballaigues, Switzerland) before the biomechanical preparations. An ISO815 K-type file was inserted into the root canal until it was visible at the apical foramen, and the working length of each root canal was established at 1 mm from the apical foramen. All canals were treated using a traditional step-back technique up to a coronal size of ISO#60 and an apical size up to ISO#40K file. The canals were prepared using 17% EDTA gel and irrigated with 2 mL of different irrigants between each filing (Table 1). The total irrigation time during instrumentation was 15 minute.

During the final wash, the canals were irrigated as detailed in Table 1, and irrigation with 2 mL of distilled water was performed between each irrigant treatment. The total irrigation time was 5 minute. Subsequently, the canal was rinsed with 2 mL of distilled water. The irrigants were carefully introduced into the canals using a 27-gauge Monoject endodontic needle attached to a Luer-Loc syringe. The needle was inserted into the apical limit of the root, and the canal was back-filled with irrigant until it was full to the brim. Finally, the teeth were rinsed free of agents with PBS and transferred to the fixative (2.5% glutaraldehyde).

2.3. Scanning electron microscopy (SEM) and debris scoring

All roots were removed from the fixative, and gutta-percha cones were inserted into the root canals. The objective was to avoid any intrusion of the cutting disc into the canals, which would pollute the samples by splattering cutting debris into the root canal system. The roots were then split into 2 halves with a hammer and a microtome blade (Fig. 1). For each root, the half containing the most visible prepared parts were used in the study, returned to fresh fixative solution (2.5% glutaraldehyde), and incubated overnight at 4°C. The specimens were rinsed with sterile water and sequentially dehydrated using a gradient of ethanol (30%, 50%, 70%, 80%, 90%, and 100%, v/v) at 15-minute intervals. The dehydrated specimens were transferred to a critical point

| Table 1 |
| Description of the various study groups and the corresponding irrigation treatments. |
| Group | Preparation | Final washing |
| A (experimental group) | 17% EDTA gel + 0.5% NaOCl | Carisolv III + 0.5% NaOCl + 17% EDTA solution |
| B (positive control) | 17% EDTA gel + 2% NaOCl | 2% NaOCl + 17% EDTA solution |
| C (positive control) | 17% EDTA gel + 5.25% NaOCl | 5.25% NaOCl + 17% EDTA solution |
| D (negative control) | 17% EDTA gel + PBS | PBS + 17% EDTA solution |

EDTA = ethylenediaminetetraacetic acid; NaOCl = sodium hypochlorite; PBS = phosphate-buffered saline.
dryer (Tsousimis Autosamdri-815 Series A, USA) with absolute alcohol as the intermediate fluid and liquid CO2 as the transition fluid. Following mounting and gold sputter coating (EikoIB-3 ion sputter coater, Japan), the surface morphology of the specimens was analyzed using SEM (Vega3 Twscan, Czech Republic, 15 kV, WD: 16 mm). At the observation stage, the canal walls (Fig. 1) in the apical, middle, and coronal thirds were examined, and photomicrographs of representative areas were taken at 2000 x magnification.

The quantitative scoring of the canal wall debris was evaluated using the protocol described by Hülsmann et al., as follows:

1. no smear layer and open dentinal tubules;
2. a small amount of smear layer and open dentinal tubules;
3. a thin smear layer and partially open dentinal tubules;
4. partial covering of dentinal tubules with a thick smear layer; and
5. full covering of dentinal tubules with a thick smear layer.

2.4. Statistical analysis

All statistical analyses were performed with SPSS 19.0 software (IBM-SPSS Inc., Chicago, IL). First, the full set of samples was independently and blindly evaluated by 2 observers, and Cohen K scores were calculated to determine the inter-examiner reliability. Second, the debris scores for different irrigants were analyzed by the nonparametric Kruskal-Wallis test and the Mann-Whitney rank sum test for pairwise comparisons. The level of statistical significance was set at P < .05.

3. Results

The kappa value for inter-observer agreement (0.88) showed the reliability of the evaluation procedure and the measurements performed by the 2 observers. In terms of the root canal sections, in group A, the coronal thirds (2.63 ± 0.490) and middle thirds (3.23 ± 0.430) were cleaned significantly (P < .05) better than the apical thirds (4.17 ± 0.379) (Table 2). A similar trend was observed for groups B, C, and D; the coronal thirds and middle thirds were cleaned significantly (P < .05) better than the apical thirds (Table 2). The positive control (group C) was significantly (P < .05) more effective than all of the other irrigants (groups A, B, and D), showing that 5.25% NaOCl has a better capability to remove almost all canal debris (scores ranging from 1 to 3) and to open the dentinal tubules (Fig. 2).

The experimental (Carisolv III + 0.5% NaOCl) group (group A) removed the root canal wall debris as effectively as group B (P > .05), with both groups showing a significant effectiveness compared to the negative control group (PBS, group D) (P < .05). Although groups A and B removed the canal debris (scores of 2–4, Figs. 3–4), group C was the most effective treatment. The negative control group (group D) that used only PBS irrigation was the least effective in removing debris and showed the highest debris scores (scores of 4–5). Therefore, the amount of open dentinal tubules was the lowest with PBS treatment (Fig. 5). All SEM images showed that the opening of dentinal tubules was the

| Group          | Apical third | Middle third | Coronal third |
|----------------|--------------|--------------|---------------|
| Carisolv II + 0.5% NaOCl | 4.17 ± 0.379  | 3.23 ± 0.430  | 2.63 ± 0.490  |
| 2% NaOCl      | 4.13 ± 0.346  | 3.17 ± 0.379  | 2.69 ± 0.408  |
| 5.25% NaOCl   | 2.70 ± 0.466  | 2.20 ± 0.407  | 1.53 ± 0.507  |
| PBS           | 4.90 ± 0.305  | 4.43 ± 0.504  | 3.53 ± 0.507  |

NaOCl = sodium hypochlorite; PBS = phosphate-buffered saline.
lowest in the apical third, compared to the middle and coronal thirds of the root canal.

4. Discussion

The present study investigated the efficacy of the Carisolv III system in removing the smear layer in the final washing of a root canal. The effectiveness of the Carisolv III system was also compared with that of NaOCl irrigation for the removal of the smear layer in extracted teeth. For this purpose, a 5-point scale was used to score the removal of canal wall debris in the apical, middle, and coronal thirds of roots. The morphological changes as well as the level of dentinal tubule opening were assessed by SEM. Our results showed that a combination of 5.25% NaOCl...
and 17% EDTA was the most effective irrigant for removal of the root canal smear layer; while the combination of 0.5% NaOCl and Carisolv III was as effective as 2% NaOCl irrigation.

The presence of a smear layer during root canal preparation was first described by McComb et al., followed by various studies reporting the adherence of the smear layer to the root canal surface and its embedment into the dentinal tubules as well as the lateral and accessory canals. The present study confirmed that the use of distilled water or mechanical preparation alone (negative control group) is not effective for complete elimination of the smear layer. Root canal irrigation is an important way to reduce the presence of microorganisms and the smear layer from root canals. The effect of irrigation is determined by its ability to dissolve tissue and demineralize dental tubules as well as its antibacterial effects. Although a variety of root canal irrigants, including acids, decalcification agents, proteolytic enzymes, alkaline solutions, oxidants, and normal saline, have been used, there is no known irrigant with ideal characteristics. The most commonly used irrigant, 5.25% NaOCl, is considered as the optimal irrigant for its broad-spectrum antibacterial effects and its capability to dissolve necrotic tissue debris. In addition, the combination of 5.25% NaOCl and 17% EDTA gel for root canal preparation effectively removes the smear layer. However, there are a number of associated issues such as excessive demineralization of dental tubules and destruction of intertubular dentin. Although 0.5% NaOCl has no cytotoxicity to living tissues, the use of a higher concentration of NaOCl increases the cytotoxicity and inflammatory response in periapical tissues. Due to the complex nature and clinical significance, the safe and effective removal of the smear layer from the root canal has become a hot topic for researchers in recent years.

The present study revealed that 5.25% NaOCl is remarkably effective in cleaning debris from a root canal. It has been reported previously that NaOCl alone does not remove the smear layer debris fully due to the presence of inorganic components in the root canal smear layer. Therefore, the chelating agent 17% EDTA was used to remove the inorganic components during canal irrigation. So, to avoid any cytotoxicity, a low concentration of NaOCl (0.5%) was used. Additionally, Carisolv (I and II) gels, comprised of a mixture of 0.5% NaOCl and 3 amino acids (lysine, leucine, and glutamic acid), have been widely used in the treatment of carious lesions. Carisolv has a proven capability of smear layer removal and carious dentinal tubule exposure, hence facilitating a clean surface and chimeric effects for resin.

In terms of endodontic treatment, Carisolv III may have similar effects and is likely to strengthen the adhesion between obturation materials (e.g., gutta-percha, sealants) and root canal walls; it is believed that a clean surface as well as a better adhesion and seal can prevent microleakage and reinfection. On the other hand, the presence of the smear layer may interfere with the adhesion of sealing materials to a clean surface, thus compromising the apical seal. Using the Carisolv system as a root canal irrigant can remove the smear layer selectively, without damaging the healthy dentin.

Al-Kilani et al. have reported that the Carisolv system is more effective in cleaning and removing the root smear layer, compared to PBS. Another study by Rahman et al. found that the Carisolv system cleans the pulp debris from the walls of immature root canals as effectively as 1% NaOCl. Currently, Carisolv is well known for its efficiency in the removal of the root smear layer. Based on the above information, the present study investigated the Carisolv III system, which is mainly comprised of chloramine T and papain. This mixture does not color dental tissues, which is the main concern with the use of Carisolv I and II. In addition, chloramine T releases NaOCl progressively, leading to the chlorination of active chloride ions. Meanwhile, papain decomposes the collagen fibers present in the disintegrated infected dentin. Due to the specificity of this enzyme, there is no harmful effect on healthy dentin. Its apparent weak activity to living pulp tissues suggests that it can be tolerated by periapical tissues. The SEM data from the present study showed that the combination of Carisolv III and 0.5% NaOCl was as effective as 2% NaOCl in removing the smear layer from the root canals, suggesting a synergistic effect of the combination. Using such a combination can effectively reduce the concentration of NaOCl and the corresponding unwanted effects. The experimental procedure in the present study was conducted in 2 steps: preparation and final washing. In the final wash, Carisolv III gel was introduced into the prepared root canal. Carisolv III is available in gel form; therefore, it can only be applied in prepared canals in the final wash.

Interestingly, we found that the opening of dentinal tubules was less conspicuous in the apical third, compared to the middle and coronal thirds of the root. Such differences may be attributed to a number of factors. First, as Carisolv III is in the gel state, it is viscous and may not flow to the narrow apical end. Second, even assuming the absence of any obstructing debris, injection of the irrigant into the coronal part of the canal may not penetrate to the

Figure 5. Representative scanning electron microscopy images showing the surface morphology following phosphate-buffered saline irrigation (group D), which showed the least removal of canal debris and almost no opening of the dentinal tubules. There was no noticeable difference in the coronal (A, score of 4), middle (B, score of 5), and apical thirds (C, score of 5) of the root canals (scale bar = 20 μm).
apical part unless the needle is jammed into the canal\[17\]. Under normal circumstances, the fluid dynamics in a root canal lead to a “stagnation plane,” hence hindering fluid penetration\[38\].

The main strength of the present study was that we used human incisors extracted due to severe periodontitis, thus approximating clinical practice. Previously, similar studies\[13,14\] have been reported using animal teeth. The combination of Carisolv III and 0.5% NaOCl showed a synergistic ability to remove the smear layer, and it can be used as a beneficial adjunct in the final cleaning of root canals.

Currently, the Carisolv III system is available in a gel form. The delivery of Carisolv III in solution may enhance its wetting ability and cleaning capability to remove the root canal smear layer more effectively. In the present study, we did not explore the associated factors that may affect its effectiveness, for example, ultrasonication, degree of freshness, and tooth-associated factors (such as root canal morphology, endodontic microbiology). These factors can potentially influence the performance of root canal irrigants; hence, further studies are required to address the influence of such factors on the effectiveness of Carisolv III for smear layer removal.

In terms of sample observation and scoring methods, SEM and blind evaluation have been used widely in previous studies.\[19,20\] Although new technologies such as environmental SEM, atomic force microscopy, and co-site optical microscopy have been developed recently, they have their own shortcomings.\[21\] Therefore, further research is needed to develop and test new methodological approaches to assess smear layer removal.

With the use of EDTA in all groups, the present study concluded that 5.25% NaOCl remains the most effective irrigant for removal of the root canal smear layer. The combination of 0.5% NaOCl and Carisolv III showed a synergistic effect, and the cleaning ability was similar to that of 2% NaOCl. The use of Carisolv III with an even lower concentration of NaOCl (0.5%) showed a better effectiveness for cleaning the smear layer, compared to PBS.

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Author contributions

Di Wu and Ling-xiang Wang performed most of the investigation, analyzed the data, and wrote the manuscript. Yong-zhen Ma, Jing-Jia, and Da-shan Wang provided instrument and reagent assistance; Bing-chang Xin and De-gang Sun contributed to data interpretation and analysis. All of the authors have read and approved the manuscript.

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