# Scanning Electron Microscopic Investigation of the Effectiveness of Phosphoric Acid in Smear Layer Removal When Compared with EDTA and Citric Acid

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## Abstract

**Introduction:** The smear layer adheres to dentinal surface, thus occluding the dentinal tubules. Because this layer disfavors the penetration of irrigant solutions and root canal fillings, it should be removed. The aim of this study was to compare the effectiveness of 37% phosphoric acid with that of 17% EDTA and 10% citric acid in the removal of smear layer. **Materials and Methods:** Fifty-two maxillary single-rooted human canines were accessed and instrumented. Between each instrument used, the canals were irrigated with sodium hypochlorite. After instrumentation, the teeth were irrigated with distilled water and then divided into groups according to the time and substances employed. The substances used were 17% EDTA, 10% citric acid, and 37% phosphoric acid solution and gel. The experimental time periods were of 30 seconds, 1 minute, and 3 minutes. The samples were prepared and observed by means of scanning electron microscopy. Three photomicrographs (2,000×) were recorded for each sample regarding the apical, middle, and cervical thirds. A score system was used to evaluate the images. **Results:** None of the substances analyzed in this study was effective for removing the smear layer at 30 seconds. In the 1-minute period, the phosphoric acid solution showed better results than the other substances evaluated. In the 3-minute period, all the substances worked well in the middle and cervical thirds although phosphoric acid solution showed excellent results even in the apical third. **Conclusions:** These findings point toward the possibility that phosphoric acid solution could be a promising agent for smear layer removal. *(J Endod 2011;37:255–258)*

## Key Words

Citric acid, EDTA, endodontics, phosphoric acid, smear layer

During the cleaning and shaping of the root canal system, dentin chips are created by instrument action. These chips associated with organic materials, microorganisms, and irrigant solutions form the so-called smear layer. This layer adheres to the dentinal surface and occludes the dentinal tubules (1, 2).

Many researchers believe that the smear layer should be removed. This layer contains bacteria and necrotic tissue (3). It forms a barrier between the filling material and sound dentin that inhibits the penetration of irrigants into dentinal tubules, increases microleakage with commonly used sealers, and decreases the bond strength of resin based materials (4–10).

Some chemical agents such as EDTA solutions at concentrations ranging from 15 to 17%, citric acid (5%-50%), and phosphoric acid (5%-37%), therefore, are used to remove this layer (11). Despite the relevant literature available concerning the effect of these agents on the smear layer removal, the small number of studies with similar methodologies and comparable time intervals and concentrations limits the ability to make valid comparisons between these treatments, especially when considering the use of phosphoric acid. This chemical agent has been extensively used to remove the smear layer from coronal dentin (12–14), and only a few studies have analyzed its performance in root dentin (15–17). Therefore, the aim of this study was to compare the effectiveness of 37% phosphoric acid with that of 17% EDTA and 10% citric acid in removing the smear layer by means of scanning electron microscopy (SEM).

## Materials and Methods

### Smear Layer Production and Irrigation Protocols

This study was approved by the Ethics Committee of the Federal University of Rio de Janeiro. Fifty-two single-rooted maxillary human canines, extracted because of periodontal or prosthetic reasons, were used. The teeth were randomly selected from known patients. All patients signed an informed consent document to take part of this research. Their age ranged from 45 to 73 years old. The teeth with straight roots, mature root apex, and similar anatomic characteristics were selected for this study. The teeth were accessed by using #1558 carbide burs (Kg Sorensen, São Paulo, SP, Brazil). The teeth were shaped by using a K3 NiTi rotary system (SybronEndo, Orange, CA). The sequence used was the following: 25/.06, followed by a sequence of Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) from 1 to 5 to prepare the middle-cervical third. The K3 sequence used in the apical third was 15/.04, 20/.02, 20/.04, 25/.04, 20/.06 and 25/.06. All files achieved both working length in the apex. Between files, the

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**TABLE 1.** Irrigation Protocols by Group Description

| Group | Irrigant Solution | Time |
|---|---|---|
| G1 | 17% EDTA | 30 seconds |
| G2 | 17% EDTA | 1 minute |
| G3 | 17% EDTA | 3 minutes |
| G4 | 10% citric acid | 30 seconds |
| G5 | 10% citric acid | 1 minute |
| G6 | 10% citric acid | 3 minutes |
| G7 | 37% phosphoric acid solution | 30 seconds |
| G8 | 37% phosphoric acid solution | 1 minute |
| G9 | 37% phosphoric acid solution | 3 minutes |
| G10 | 37% phosphoric acid gel | 30 seconds |
| G11 | 37% phosphoric acid gel | 1 minute |
| G12 | 37% phosphoric acid gel | 3 minutes |
| G13 | Control–distilled water | 3 minutes |

canals were irrigated with 1 mL of sodium hypochlorite. After instrumentation, the teeth were irrigated with 5 mL of distilled water. All teeth had their apexes sealed with utility wax (Technew, Rio de Janeiro, RJ, Brazil) to prevent the flow through them. Then, the teeth were randomly divided into 13 groups of four teeth each according to the time and substances used.

The substances used were 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil), 10% citric acid (Formulativa, Rio de Janeiro, RJ, Brazil), 37% phosphoric acid solution (COPPE, Rio de Janeiro, RJ, Brazil), and 37% phosphoric acid gel (Condac, Joinville, SC, Brazil). The irrigation protocols and experimental time periods used in this study are described in Table 1, and 1 mL of substance was used without replacement.

## Scanning Electron Microscopy

After the removal of the smear layer, all teeth were irrigated again with 5 mL distilled water and dried with medium-sized paper points (Endopoints, Paraiba do Sul, RJ, Brazil). Finally, two longitudinal grooves were prepared on both buccal and lingual surfaces by using a diamond disc without penetrating the canal. The roots were then split into two halves with a hammer and chisel. For each root, the half containing the most visible part of the apex was used for study.

The samples were coated with gold and analyzed with a scanning electron microscope (JSM 6460 LV; JEOL, Tokyo, Japan). All samples were numbered, and the images were performed without knowledge of the group tested. First, a scan of all samples was made at 30× magnification for each group. Then, the most representative area of each third of each tooth was selected and magnified at 100×. Each 100× image was scanned, and the three most representative areas were magnified at 2,000×. For example, if the image of 100× showed 70% of the surface covered with smear layer, two images with smear layer and one without were selected. Therefore, three images of each third were obtained for each tooth, providing nine images per tooth and 36 images per group ($n = 4$). In the end, each group had 12 images for the three thirds.

## SEM Evaluation

To evaluate the degree of smear layer removal, the scoring system described by Takeda et al (16) was used but with modifications. Briefly, score 1 = no smear layer, with all tubules cleaned and opened; score 2 = few areas covered by smear layer, with most tubules cleaned and opened; score 3 = smear layer covering almost all the surface, with few tubules opened; and score 4 = smear layer covering all the surfaces. It was a blinded evaluation performed by three independent observers.

## Statistical Analysis

Intraexaminer and interexaminer reliability for the SEM evaluation was verified by Kappa test. Data were analyzed using Kruskal-Wallis and Mann-Whitney $U$ tests ($p < 0.05$).

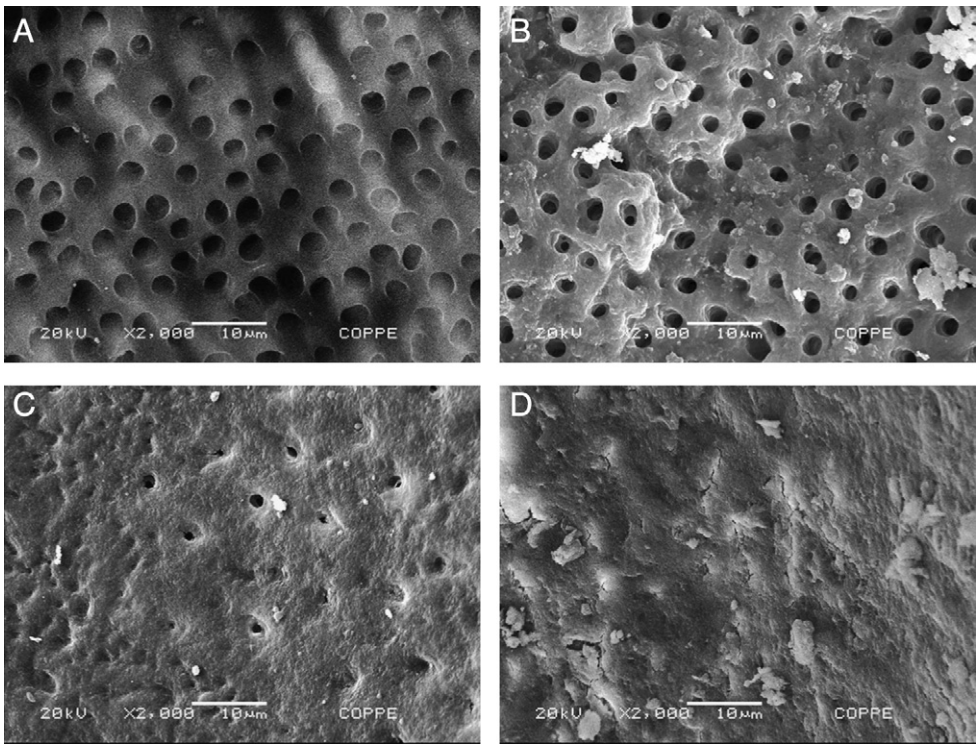


**Figure 1.** Representative photomicrographs of the scoring system used to analyze the SEM results. (*A*) Score 1: no smear layer, with all tubules cleaned and opened. (*B*) Score 2: few areas covered by smear layer, with most tubules cleaned and opened. (*C*) Score 3: smear layer covering almost all the surface, with few tubules opened. (*D*) Score 4: smear layer covering all the surface.

**TABLE 2.** Mean and Standard Deviation (SD) Values of Smear Layer Scores

| | 30 seconds (mean scores ± SD) | | | 1 minute (mean scores ± SD) | | | 3 minutes (mean scores ± SD) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Apical | Middle | Cervical | Apical | Middle | Cervical | Apical | Middle | Cervical |
| 17% EDTA | $4.0 \pm 0.0^{B,a}$ | $3.7 \pm 0.5^{B,a}$ | $3.5 \pm 0.6^{B,a}$ | $3.7 \pm 0.5^{B,b}$ | $2.7 \pm 1.0^{B,b}$ | $2.3 \pm 0.4^{B,a}$ | $2.0 \pm 0.0^{B,b}$ | $1.5 \pm 0.6^{A,b}$ | $1.0 \pm 0.0^{A,a}$ |
| 10% citric acid | $2.8 \pm 1.1^{A,a}$ | $1.7 \pm 0.6^{A,a}$ | $1.2 \pm 0.5^{A,a}$ | $2.9 \pm 0.7^{B,b}$ | $2.0 \pm 0.0^{B,a}$ | $2.0 \pm 0.5^{B,a}$ | $2.0 \pm 0.5^{B,b}$ | $1.4 \pm 0.5^{A,b}$ | $1.2 \pm 0.3^{A,a}$ |
| 37% phosphoric acid solution | $3.1 \pm 1.1^{A,b}$ | $1.6 \pm 0.9^{A,a}$ | $1.3 \pm 0.7^{A,a}$ | $1.5 \pm 0.6^{A,a}$ | $1.0 \pm 0.0^{A,a}$ | $1.0 \pm 0.0^{A,a}$ | $1.0 \pm 0.0^{A,a}$ | $1.0 \pm 0.0^{A,a}$ | $1.0 \pm 0.0^{A,a}$ |
| 37% phosphoric acid gel | $3.0 \pm 0.0^{A,a}$ | $2.5 \pm 0.6^{A,a}$ | $2.5 \pm 0.6^{B,a}$ | $2.8 \pm 1.0^{B,a}$ | $2.0 \pm 0.0^{B,a}$ | $1.5 \pm 0.6^{A,a}$ | $2.9 \pm 0.5^{C,b}$ | $1.6 \pm 0.4^{A,a}$ | $1.3 \pm 1.1^{A,a}$ |
| Control | $4.0 \pm 0.0^{B,a}$ | $3.7 \pm 0.5^{B,a}$ | $3.5 \pm 0.6^{B,a}$ | $4.0 \pm 0.0^{C,a}$ | $3.7 \pm 0.5^{C,a}$ | $3.5 \pm 0.6^{C,a}$ | $4.0 \pm 0.0^{D,a}$ | $3.7 \pm 0.5^{B,a}$ | $3.5 \pm 0.6^{B,a}$ |

The superscript capital letters (A, B, C, and D) indicate, in each column, values statistically significant ($p < 0.05$). The superscript lowercase letters (a and b) indicate, in the row, values statistically significant ($p < 0.05$) in the thirds in the same time and solution.

## Results

The Kappa test showed good agreement between observers, with values of 0.9 or above. Figure 1 shows representative images of the scores. The results of the smear layer scores for each group are listed in Table 2.

At 30 seconds, citric acid solution, phosphoric acid solution, and phosphoric acid gel were more effective than EDTA and control group for the apical and middle thirds. In the cervical third, citric acid and phosphoric acid solution were significantly more effective than phosphoric acid gel, EDTA, and the control group. By evaluating the action of the solution in the different thirds, no significant difference was observed when EDTA, citric acid, and phosphoric acid gel were used. The use of phosphoric acid was more effective in the cervical and middle thirds than in the apical third.

At 1 minute, the control group showed the worst results compared with the experimental ones. The phosphoric acid solution was more effective than EDTA, citric acid, and phosphoric acid gel in the apical and middle thirds. In the cervical third, the phosphoric acid solution was significantly better than citric acid and EDTA, and no statistical difference was observed between phosphoric acid solution and gel. With regard to the action of the same solution in different thirds, EDTA showed better activity in cervical third than in middle and apical thirds. The citric acid was shown to be more effective in the cervical and middle thirds than in the apical third. The use of phosphoric acid solution and gel did not show difference between the thirds.

At 3 minutes, phosphoric acid solution was the most effective chemical agent used in the apical third, followed by citric acid, EDTA, and phosphoric acid gel. In the middle and cervical thirds, no significant differences were observed. Again, the control group showed the worst results. By comparing the same solutions in different thirds, EDTA and citric acid were more effective in the cervical third than in the middle and apical thirds. The phosphoric acid gel was more efficient in the cervical and middle thirds than in the apical third. Phosphoric acid solution did not show significant difference between the thirds. When the phosphoric acid gel was used in all periods of time, it was possible to verify in some samples the persistence of a residual layer of this substance. Regarding the dentinal integrity, all substances generated some degree of erosion in the cervical and middle thirds for irrigation at 1 minute or longer.

## Discussion

It is noteworthy that the literature describes a variety of chemicals with a broad range of concentrations and different irrigation regimens to remove the smear layer. This study used EDTA, a well-known chelating agent widely used to remove inorganic components of the smear layer (18, 19), citric acid, a weak organic acid with relatively low cytotoxicity used as an aqueous acidic solution (20, 21); and finally, phosphoric acid, a strong acid routinely used in dentistry to remove the smear layer and smear plugs formed during coronal cavity preparations (22). Although some studies on the ability of phosphoric acid in removing smear layer from root canals are available in the literature, the concentrations used are rather low (below 5% and 24%) compared with the ones used to remove the smear layer from coronal dentin. In addition, there is no consensus on the ideal time of irrigation (7, 16, 17). Therefore, the present study has compared the action of 37% phosphoric acid with well-established solutions, such as 17% EDTA and 10% citric acid at experimental periods of time in which these chemicals are known to be effective. As far as we are concerned, there is no study in the literature comparing EDTA, citric acid, and phosphoric acid at the same concentrations as those used in the present study.

The lowest time period used here was 30 seconds, which has been suggested by the manufacturer as being the ideal time for optimal action of phosphoric acid. However, EDTA resulted in lower performance comparable to the ones obtained with the control, which means that this solution was not able to remove the smear layer in 30 seconds. This finding is in accordance with other studies assessing the use of EDTA for 1 minute, showing that it did not work well in this period of time (23). On the other hand, 37% phosphoric acid solution and 10% citric acid were more effective than 17% EDTA in removing the smear layer in all thirds.

The use of phosphoric acid solution for 1 minute was more effective than citric acid, EDTA, and phosphoric acid gel in the middle and apical thirds. In the cervical third, phosphoric acid solution and gel were more effective than citric acid and EDTA. Khedmati and Shohouhinejad (24) evaluated smear layer removal using 17% EDTA and 10% citric acid and found that these solutions were equally efficient and more effective in the cervical and middle thirds than in the apical third. These data are partially in agreement with the present study, which found that EDTA and citric acid were equally efficient, but in the present study the EDTA was more effective in the cervical third than in the middle and apical thirds.

At 3 minutes, phosphoric acid solution was the most effective chemical used in the apical third, followed by citric acid and EDTA, and finally by phosphoric acid gel. In the middle and cervical thirds, no significant differences among the substances were observed. An interesting finding was that phosphoric acid solution was very effective in removing the smear layer of the apical third at 1 and 3 minutes compared with EDTA and citric acid. Also, dentinal erosion was not found in the apical third when phosphoric acid solution was used. Di Lenarda et al (20), using 15% EDTA and 19% citric acid to remove the smear layer, have shown that citric acid was better than EDTA in the apical third when used for 3 minutes. The differences from our findings may be caused by the different concentrations of citric acid and EDTA used. Our findings are in accordance with Pérez-Heredia et al (17), who used 15% EDTA and 15% citric acid and found better results for cervical and middle thirds compared with apical third.

Regarding the dentinal erosion, in our study, the use of 37% phosphoric acid showed that dentin erosion was related to the exposure time. At 30 seconds, it was noted only in the cervical third. However, at 1 minute or longer, the erosion was present in the middle and cervical thirds, in the same degree, in both periods of time. No evidence of dentinal erosion was found in the apical third. Our results are in accordance with Ayad (22), who observed erosion of coronal dentin after 10 seconds of application of 32% phosphoric acid.

Comparing the degree of dentinal erosion of the three tested solutions, it was noted that after 1 minute or longer, all substances behaved equally in the middle and cervical thirds, exhibiting no sort of erosion in the apical third. Torabinejad et al (25) observed that the use of 17% EDTA in association with NaOCl for 1 minute or longer leads to dentinal erosion although it presented a greater cleanness of the apical third.

The use of a high concentration of phosphoric acid may carry a higher risk of cytotoxicity, especially when used in the apical third of the root canal. Therefore, the use of gel might be preferred than the liquid form although no study evaluating this effect in the periapical tissue was found in the literature. In the present study, although the phosphoric acid gel has shown good results, it was possible to verify the persistence of a residual layer of this substance in some samples, mainly in the apical third. A final wash with 5 mL distilled water was not able to remove the gel present mainly in apical area.

In conclusion, none of the substances analyzed in this study was effective for removal of the smear layer in 30 seconds. At 3 minutes, all the substances worked well in the middle and cervical thirds, with phosphoric acid solution exhibiting excellent results even in the apical third. These findings point toward the possibility that phosphoric acid solution may be a promising agent for smear layer removal. Further studies are needed to evaluate the depth of demineralization caused by phosphoric acid, its influence on adhesion, and cytotoxicity of this solution in order to enable this substance to be used routinely in endodontics.

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