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

Maíra Prado, DDS, MSc, Heloisa Gusman, DDS, MSc, PhD,† Brenda P.F.A. Gomes, DDS, MSc, PhD,‡ and Renata A. Simão, DSc, MSc, PhD**

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

From the *Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; †Department of Endodontics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; ‡Department of Restorative Dentistry, Endodontics Division, State University of Campinas-UNICAMP, Piracicaba, SP, Brazil.

Supported by the Brazilian agencies FAPERJ and CNPq.

Address requests for reprints to Dr Brenda P.F.A. Gomes, Department of Restorative Dentistry, Piracicaba Dental School State University of Campinas, Avenida Limeira, 901, Piracicaba, SP, Brazil 13414-018. E-mail address: bpgomes@fop.unicamp.br

0099-2399/$ - see front matter

doi:10.1016/j.joen.2010.11.011

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 ($p < 0.05$).

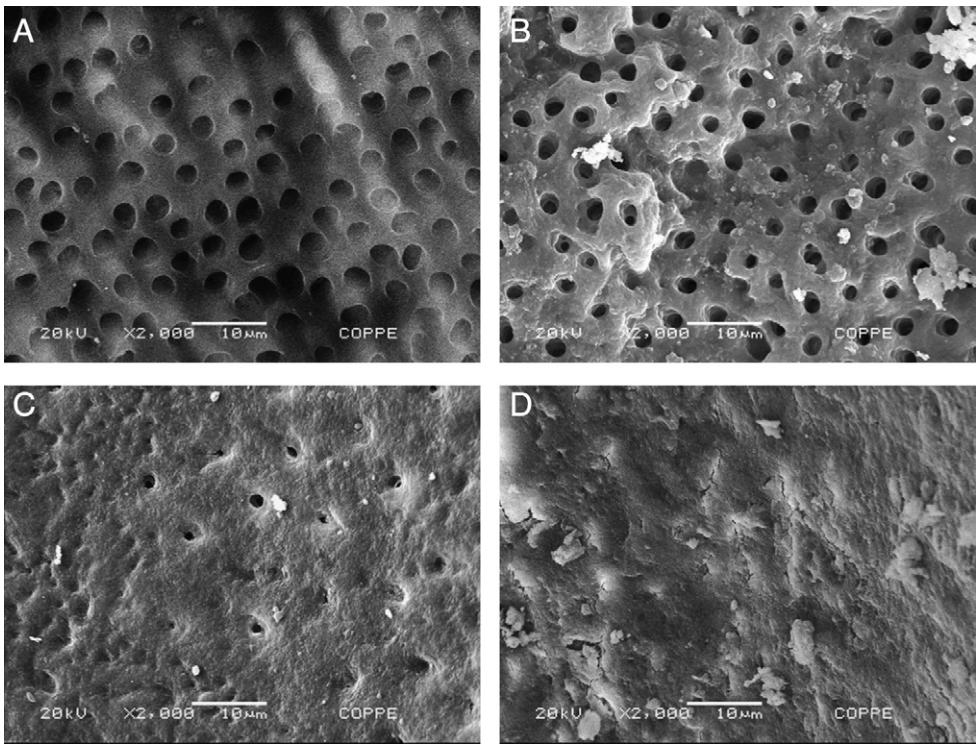

Figure 1. Representative photomicrographs of the scoring system used to analyze the SEM results. (*A*) Score 1: no smear layer, with all tubules cleaned and opened. (*B*) Score 2: few areas covered by smear layer, with most tubules cleaned and opened. (*C*) Score 3: smear layer covering almost all the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