

# Longitudinal Co-site Optical Microscopy Study on the Chelating Ability of Etidronate and EDTA Using a Comparative Single-tooth Model

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## Abstract

In the present study the smear layer dissolution kinetics of 18% etidronate (HEBP), 9% HEBP, and 17% ethylenediaminetetraacetic acid (EDTA) on human dentin were quantitatively and longitudinally analyzed by using a single-tooth comparative model. Coronal dentin disks were prepared from 3 maxillary human molars. A standardized smear layer was produced on the pulpal side of each disk. The smear layer-covered surface was divided into 3 similar areas. Each of these was then exposed to 1 of the 3 irrigants under investigation, whereas the others were covered with adhesive tape. Co-site image sequences of the areas under investigation were obtained after several cumulative demineralization times. Sixteen images were obtained from each dentin area of each tooth for each experimental time at 1000× magnification. An image processing and analysis sequence measured sets of images, providing data of area fraction for thousands of tubules over time and allowing us to quantitatively follow the effect of the chelating substances. The Kruskal-Wallis H test and Dunn multiple comparison test were used to analyze the data. Overall, it can be concluded that the demineralization kinetics promoted by both 9% HEBP and 18% HEBP were significantly slower than those of 17% EDTA ( $P < .05$ ). In addition, the single-tooth model is advantageous over the first co-site optical microscopy dentin assessments when different chelator solutions are compared. (*J Endod* 2008;34:71–75)

## Key Words

Chelators, co-site optical microscopy, dentin, EDTA, HEBP, single-tooth model

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In endodontic practice, combinations of decalcifying agents and sodium hypochlorite have been recommended to chemically clean the root canal system. This chemical cleansing procedure involves the dissolution of organic pulp remnants and the organic-inorganic smear layer on root dentin. A variety of decalcifying agents have been used to dissolve the smear layer, which is a side effect of mechanical root canal preparation (1). Nowadays, the chelating agents ethylenediaminetetraacetic acid (EDTA) and citric acid are probably the most frequently used chemicals for that purpose (2, 3). However, alternative chemicals to remove the smear layer have been suggested (4–8). One recently raised issue regarding the use of EDTA or citric acid is that these agents strongly react with sodium hypochlorite, thus rendering the latter agent ineffective (7, 9, 10). Consequently, 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP), also known as etidronic acid or etidronate, has been proposed as a potential alternative to EDTA or citric acid because this agent shows no short-term reactivity with sodium hypochlorite (7). HEBP is nontoxic and has been systematically applied to treat bone diseases (11). Furthermore, like EDTA, it is a chelator commonly used as an adjunct in household and personal care products such as soaps (12).

Co-site optical microscopy (CSOM) was recently introduced (13) and represents an efficient method for direct comparison of the smear-reducing ability of irrigating solutions used in endodontics. The accuracy and reproducibility of CSOM have been verified previously (13); the method proved to be fast, robust, and reproducible. Moreover, CSOM provides quantitative data linked to the longitudinal observation of the dentinal substrate changes.

The present work aimed to assess, both longitudinally and quantitatively (CSOM and digital image analysis), the efficacy of HEBP in reducing the smear layer on standardized human dentin specimens by using a single-tooth model. Seventeen percent EDTA was used as a reference solution to compare the results. The tested null hypotheses were (1) that there is no difference between the chelating abilities of HEBP and EDTA and (2) that there is no correlation between the HEBP concentration and its chelating ability.

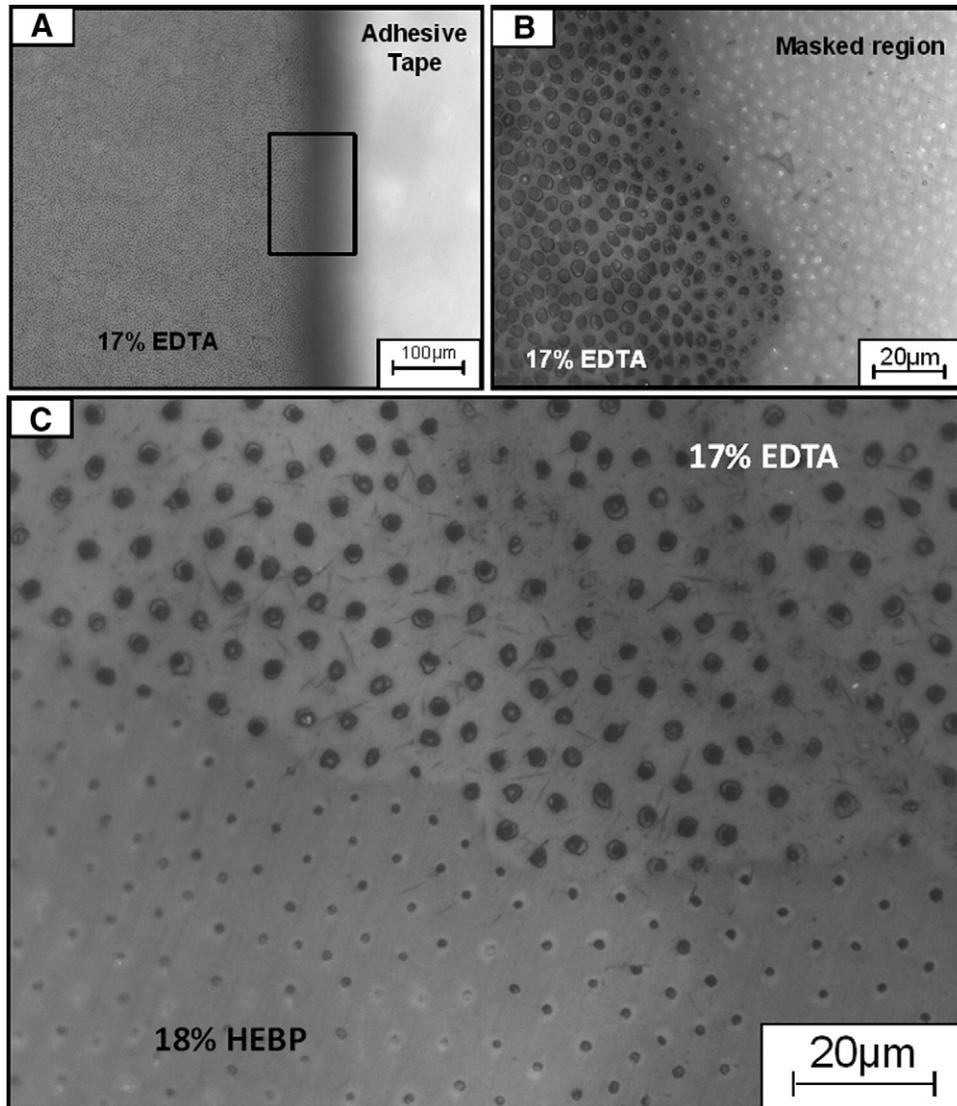
## Materials and Methods

### Specimen Selection and Dentin Disk Preparation

Three unerupted third molars, recently extracted surgically, were kept in 0.2% sodium azide at 4°C for no longer than 7 days. The teeth were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the Institutional Review Board of the Nucleus of Collective Health Studies, Rio de Janeiro State University, Brazil.

Dentin disks  $3 \pm 0.3$  mm thick were cut from the crown's middle third above the root canal. A standard polishing procedure with SiC paper (200, 300, 400, and 600 grit) followed by 3  $\mu$ m diamond paste was used on the pulpal surface of each disk to produce a standardized smear layer (1,13).

To minimize the influence of the variability of human dentin when comparing different chelators, a single-tooth approach was followed. The central dentin area of each disk was divided into 3 equal areas, each to be exposed to 1 of the 3 different chelators. Adhesive tape (Scotch; 3M, Sumaré, SP, Brazil) was used to



**Figure 1.** Control images to check the reliability of the masking procedure. (A) Low magnification image of the interface region between masked and unmasked dentin after 600 seconds of EDTA etching. (B) Higher magnification image of the same region (framed area) after removal of the masking tape. The boundary between etched and unetched regions is clear, proving that the tape was efficient to avoid etching the masked region. (C) Another interface between 2 regions etched with EDTA and HEBP, respectively. Again, a well-defined boundary is visible.

mask the areas assigned to the 2 other solutions during the irrigation procedure for each of the 3 solutions.

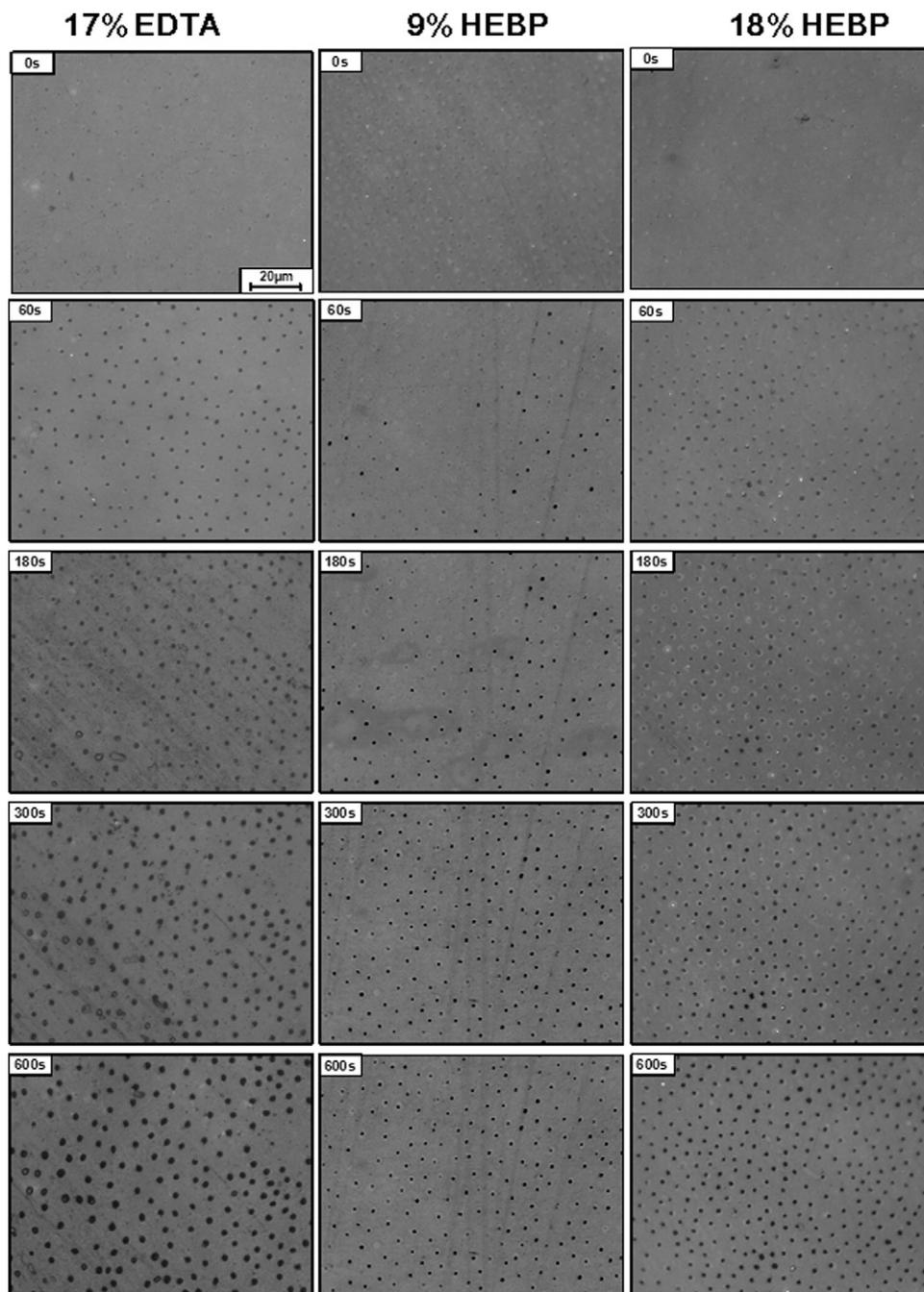
The 17% EDTA solution was bought from a commercial source (Formula & Ação Ltda, São Paulo, SP, Brazil). HEBP solutions were freshly prepared by the graduate laboratory of Rio de Janeiro State University. HEBP powder (Zschimmer & Schwarz Mohsdorf GmbH & Co KG, Burgstädt, Germany) was mixed with bi-distilled water to w/v concentrations of 9% and 18%.

**Experimental Procedure (Co-site Microscopy) and Image Analysis**

The experiments were performed in an Axioplan 2 Imaging motorized microscope (Carl Zeiss Vision GmbH, Hallbergmoos, Germany) controlled by a special routine implemented under the AxioVision 4.5 software (Carl Zeiss Vision). An Epiplan 100× HD objective lens was used coupled to a 1300 × 1030 pixels Axiocam HR digital camera (Carl Zeiss), leading to a total magnification of approximately 1000× and a resolution of 0.1 µm/pixel.

In the co-site microscopy experiment a special holder allowed application of the chelating solutions without removing the dentin specimen from the microscope. A motorized specimen stage was used to automatically acquire 16 image fields at specific x-y positions of a given specimen for several cumulative demineralization times (60, 180, 300, and 600 seconds). Thus it was possible to follow the same fields with high reproducibility of the x-y positions and autofocus, allowing the observation of the effect of demineralization in the very same regions. The details of the procedure have been described earlier (13).

A previously developed image analysis routine (13, 14) was used to enhance image contrast and discriminate (15, 16) and measure open dentin tubules in each acquired image. Then the ratio between the total area of open tubules and the area of the full image field, the area fraction (AF), was measured. All steps were implemented as a macro routine under the KS400 3.0 software (Carl Zeiss Vision). During this longitudinal evaluation, each specimen served as its own control.



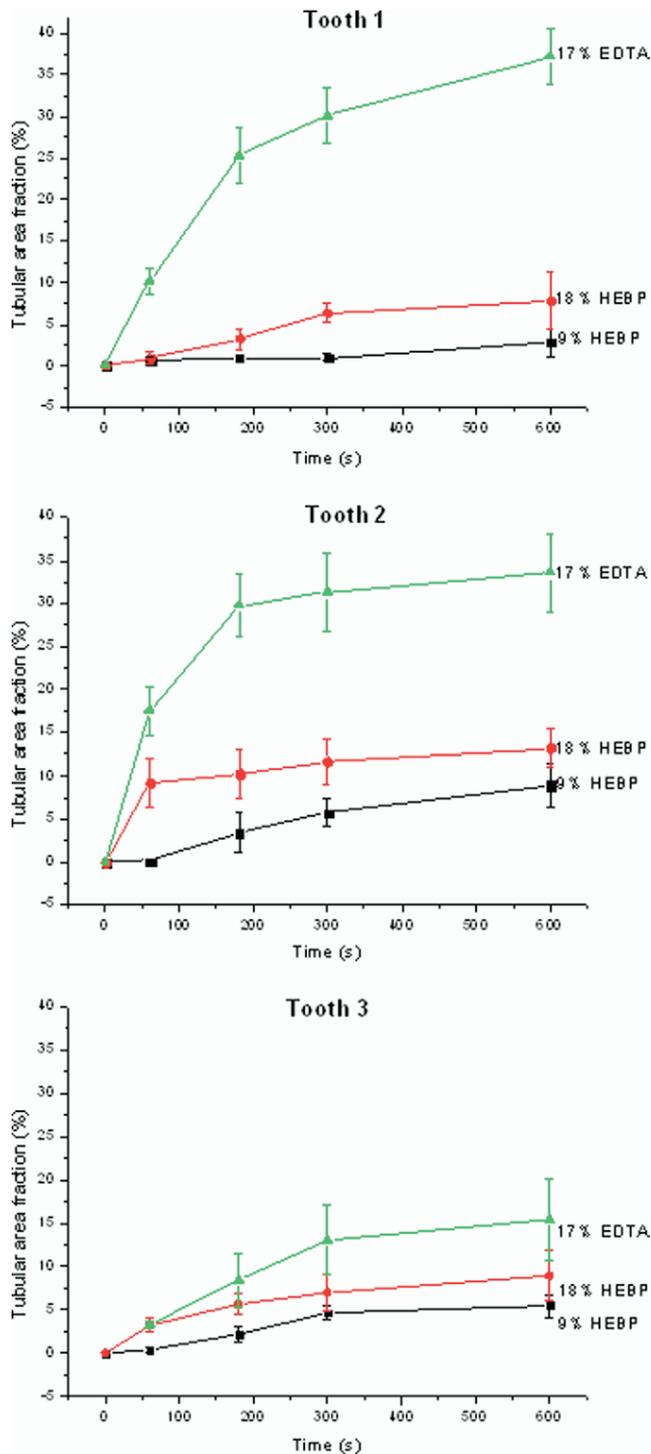
**Figure 2.** Surface changes of dentin regions during demineralization with each chelator. Columns show the evolution of demineralization over time for 17% EDTA, 9% HEBP, and 18% HEBP (from left). In each column, an image field at a specific x-y position of a specimen is shown for 4 cumulative demineralization times. The claim of high reproducibility of x-y positions is confirmed by these figures because almost the exact same dentin features are visible for all times.

### Data Presentation and Analysis

Data are presented as tubule AF in percent of the whole dentin area (13). The preliminary analysis of the raw pooled data from the experimental groups did not show a normal distribution (Kolmogorov-Smirnov test). Further statistical analysis was performed with Kruskal-Wallis H test. Where differences were found, Dunn multiple comparison test was further used to isolate the differences, and the level of significance was set at  $P < .05$ . SPSS 11.0 (for Windows, Version 11.0; SPSS Inc, Chicago, IL) was used as analytical tool.

### Results

To verify the reliability of the masking procedure, 3 test images were acquired, as shown in Fig. 1. The image montages in Fig. 2 show the time evolution of the demineralization process. On the basis of these images the following observations were made: (1) Overall, EDTA specimens were completely smear-free after 60 seconds of etching followed by an enlargement of the dentinal tubules over time. (2) HEBP specimens in both concentrations were completely smear-free only after 300 seconds of etching.



**Figure 3.** Time evolution of the open tubule AF for each solution per tooth. Data points are the average of 16 measurements. Error bars indicate standard deviations.

The graphs in Fig. 3 show the increase of AF of open tubules against time for each tooth. Each point in the graph corresponds to the mean value of AF for 16 image fields per specimen for each solution.

On the basis of the present data and statistical comparison the following observations were made: (1) Seventeen percent EDTA uncovered a significantly larger AF than 9% HEBP at all experimental times ( $P < 0.05$ ). (2) Eighteen percent HEBP was less effective than 17%

EDTA at all experimental times ( $P < .05$ ) except for tooth 3 at 60 seconds ( $P > .05$ ). (3) Eighteen percent HEBP was more effective than 9% HEBP at all experimental times except for tooth 1 at 60 seconds ( $P > .05$ ). (4) The demineralization kinetics promoted by 17% EDTA were faster than those for both concentrations of HEBP.

### Discussion

The current data showed that EDTA is a more powerful agent in removing the smear layer than HEBP is. Consequently, the null hypothesis was rejected. This is in agreement with earlier results regarding the higher chelating efficiency of EDTA compared with HEBP (7). On the other hand, Baumgartner and Mader (17) reported that the sequential use of EDTA and NaOCl caused a progressive dissolution of dentin at the expense of peritubular and intertubular areas. The erosive effects of EDTA have also been reported in other studies (18, 19).

Because of their erosive effects, there is a debate on the ideal application of chelating agents. There is uncertainty at this point as to whether strong or weak decalcifying agents should be used in conjunction with chemomechanical root canal preparation. Strong agents completely remove the smear layer but bear the disadvantage that they attack the dentin and thus affect its mechanical integrity (20, 21). Consequently, a moderate decalcifying effect might represent a good choice in case the prevention of dentin is desired. Strong chelators such as EDTA and citric acid are recommended by some authors after instrumentation of the root canal system. They suggested that if used in conjunction with shaping instruments, these agents could cause preparation errors (22). Furthermore, EDTA and citric acid interfere with the organic tissue dissolution properties and antimicrobial efficacy of sodium hypochlorite (7, 10). In contrast, HEBP could probably be used during instrumentation because it shows no short-term interference with sodium hypochlorite (10). This approach could prevent the formation of a smear layer with accumulated debris. This would differ from the current concept in which a smear layer is first created and then removed. Because HEBP is a relatively weak chelator as shown in the current study, the creation of preparation errors might be less than with EDTA. Further studies should be addressed before any conclusive statements can be made.

The accuracy and reproducibility of the method used in this investigation have been verified previously, and it proved to be fast, robust, and reproducible (13). Moreover, the method provides quantitative data linked to the longitudinal observation of the dentinal substrate changes. There appear to be few reports in the literature involving longitudinal and quantitative analysis of the process of dentin demineralization. Atomic absorption spectroscopy analysis (18) and microhardness tests (20, 21) provide quantitative data of the demineralization process but do not offer the possibility of observing the evolution of this course of action. The processing and analysis sequence used here was fully automatic and allowed an unbiased measurement process.

Use of the same dentin substrate as done in the current study appears advantageous over the first CSOM dentin assessments (13). Dentin morphologic variations were controlled; thus the variance when different teeth are used in comparative assessments was reduced (23).

The goal of the present work was restricted to a direct longitudinal and quantitative comparison of the chelating ability of EDTA and HEBP. The application of these results to the clinical situation is not straightforward. One of the limitations of the current method is that the chelator solution was applied to a flat horizontal dentin surface, which is different from the clinical situation in which the contact between the chelating substance and the dentin surface is affected by the vertical position of the teeth and the intrinsic anatomic variability of the root canal system.

In conclusion, the current results showed different efficacies for the 2 chelating agents tested, which might affect their best mode of clinical application. EDTA is a strong chelator that quickly removes the smear layer but that might also affect underlying sound dentin structure. HEBP, on the other hand, is a weaker chelator that probably should be administered in conjunction with sodium hypochlorite during the whole instrumentation process. Future studies should aim at providing a better understanding of the mechanism of chelator-induced dentin destruction and its effect on the adaptation and sealing ability of root fillings as well as its possible influence on root strength.

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