

Dentin Demineralization When Subjected to BioPure MTAD: A Longitudinal and Quantitative Assessment

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Abstract

In the present study, the demineralizing ability of BioPure MTAD (Dentsply/Tulsa, Tulsa, OK), 17% EDTA, and 5% citric acid on radicular dentin was quantitatively and longitudinally analyzed. 3 mm thick disks were obtained at the root cervical third from 9 maxillary human molars, and a standardized smear layer was produced. Cosite images of the dentin surface were obtained after several cumulative demineralization times. Sixteen images were obtained in each dentin sample 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. Thus, it was possible to follow the demineralization phenomenon and quantitatively analyze the effect of the various substances. The nonparametric Kruskal-Wallis H-test was used to analyze the data. Based on the present results, it can be concluded that the demineralization kinetics promoted by both 5% CA and BioPure MTAD was significantly faster than by 17% EDTA. (*J Endod* 2007;33:1364–1368)

Key Words

Chelators, cosite microscopy, demineralization, dentin, digital image analysis

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0099-2399/\$0 - see front matter

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doi:10.1016/j.joen.2007.07.009

The two targets of root canal instrumentation are, first, to clean and shape the root canal system and, second, to allow the placement of a hermetic filling (1). To date, the cleaning of the root canal system is linked to bacterial control and the dissolution of organic pulpal tissue. Moreover, a smear-free dentin seems desirable (1). Combinations of decalcifying agents (generally organic acids) and sodium hypochlorite (NaOCl) have been recommended because no single irrigator is capable of providing the conditions mentioned earlier (3, 4).

With the aim of improving the root dentin cleanness and disinfection, Torabinejad et al (5) developed a new solution, the so-called BioPure MTAD (Dentsply/Tulsa, Tulsa, OK), which contains a mixture of a tetracycline isomer, citric acid, and a detergent. BioPure MTAD represents an innovative approach for the simultaneous removal of smear layer and disinfection of the root canal system. BioPure MTAD is commercially available as a two-part set that is mixed on demand. In recent times, many reports have been focused on BioPure MTAD properties. To date, an English PubMed search for “BioPure MTAD” produced more than 20 papers published since 2003.

BioPure MTAD is a biocompatible material (6) and has similar solubilizing effects on pulp and dentin to those of EDTA (5, 7, 8). A major difference between BioPure MTAD and EDTA is a high binding affinity to dentin of the doxycycline present in BioPure MTAD that allows for an extended antibacterial effect. Torabinejad et al (9) reported that BioPure MTAD possessed superior bactericidal activity compared with NaOCl or REDTA when tested against *Enterococcus faecalis*. However, Baumgartner et al (10) reported that the combination of 1.3% NaOCl/BioPure MTAD left nearly 50% of the canals contaminated with *E. faecalis*.

In addition to these desirable properties, a few experiments have shown that BioPure MTAD is an effective solution for the removal of the smear layer (5, 7). According to a recent study (11), a longer period of time is required for bacteria to penetrate when either EDTA or MTAD is used for smear layer removal.

In addition, the microstructural dentin changes promoted by BioPure MTAD do not seem to cause significant damage to the dentin matrix when used as a final rinse in conjunction with low concentrations of NaOCl (6). Unlike EDTA and citric acid (12, 13, 14), minimal erosion of intraradicular dentin has been reported when NaOCl and Biopure MTAD were used in an equivalent sequence (7). For optimal removal of endodontic smear layers and to avoid inadvertent erosion of the intraradicular dentin, a revised clinical protocol has been proposed that involves the use of an initial rinse with 1.3% NaOCl for a period of 20 minutes followed by the use of BioPure MTAD as the final rinse for a cumulative period of 5 minutes (5, 7).

So far, two SEM investigations conducted by Torabinejad et al (5, 7) have represented the most important source of information concerning the demineralizing ability of BioPure MTAD. In those studies, prepared root sections were examined under scanning electron microscopy in a long-established approach in which the smear-removal ability was quantified by a scoring model. However, Gulabivala et al (15), Hülsmann et al (16), and De-Deus et al (14, 17) have pointed out that the main factor leading to the lack of conclusions regarding the smear layer removal is the qualitative and nonreproducible character of most studies. In addition, the magnifications used in the SEM differ widely; in some studies, such data are not presented at all or different magnifications were used during the investigation. A certain observer bias may occur in the SEM when working with higher magnifications because only a small area of the root canal wall can be observed. This area may be adjusted on the screen by chance or be

selected by the SEM operator. It is a common finding that most SEM operators tend to select clean canal areas with open dentinal tubules rather than areas with large bulk of debris or smear layer (15, 16).

Moreover, SEM investigations do not allow longitudinal observations of the dentinal morphology. In this kind of study, the status of the dentinal surface before the application of the chelator is unknown because the evaluation is not performed in the same sample. In an important article, Peters et al (18), using a high-quality microcomputed tomography method, showed that the amount of uninstrumented canal areas after preparation is 35% or more of their total. So, it is possible to conclude that uninstrumented dentinal walls are smear free, and this fact highlights the importance of a longitudinal character in smear-removal investigations, in which the status of the dentinal surface is well known before the initial application of the chelator.

It is worth mentioning that atomic force microscopy (AFM) has been used for longitudinal evaluation of the dentin demineralization process (14, 19). This approach showed relevant advantages such as the observation of the process in near real time as the samples were immersed in the chelating substance during observation. However, limitations caused by specific characteristics of AFM precluded obtaining accurate quantitative results. Watari (20) used AFM to obtain quantitative results regarding relief measurements such as roughness.

Cosite optical microscopy (CSOM) was recently introduced (17), and it represents an efficient method for direct comparison of the demineralizing power of the solutions used in Endodontics. The accuracy and reproducibility of CSOM have been verified previously (17), and it proved to be fast and robust. Moreover, the method provides quantitative data linked to the longitudinal observation of the dentinal substrate changes.

Hence, the present work aimed to assess, both longitudinally and quantitatively, the demineralizing ability of BioPure MTAD through CSOM and digital image analysis. Both 17% EDTA and 5% citric acid were used as reference solutions to compare the results. The null hypothesis tested was that there is no difference in demineralization of dentin irrigated with either BioPure MTAD or EDTA or 5% citric acid.

Materials and Methods

Specimen Selection and Preparation

This study was revised and approved by the Ethics Committee, Nucleus of Collective Health Studies, Rio de Janeiro State University, Rio de Janeiro, Brazil. Nine maxillary human molars were selected from the tooth bank of Rio de Janeiro State University. Each specimen was mounted in an epoxy resin cylinder (Arazyn 1.0; Ara Química, Sao Paulo, Brazil) to facilitate manipulation and improve the metallographic preparation. Following a protocol described previously (21), dentin discs approximately 3 ± 0.3 mm thick were cut at the middle third level of the crowns but above the root canal using a low-speed saw (Isomet; Buhler, Ltd, Lake Bluff, NY) with a diamond disc ($\varnothing 125$ mm \times 0.35 mm \times 12.7 mm, 33°C), with continuous water irrigation in order to prevent overheating. The dentin surfaces were carefully inspected to ensure that they were free of coronal enamel or pulpal exposures.

The decision to perform the present evaluation on crown dentin instead of on intraradicular dentin was based on a pilot study in which the difficulty to obtain useful disks from intraradicular dentin was determined. In addition, crown dentin can provide more homogeneous than intraradicular dentin (22), and this represents a desirable feature in comparative assessments.

A standard metallographic procedure (grinding with SiC paper [200, 300, 400, and 600] grits and 3 μ m diamond paste) was used on the pulpal surface of the tooth to prepare them for the experimental process and to produce a standardized smear layer (14, 17, 19, 23).

At this point, the samples were randomly divided into 3 groups according to the chelating agent used as follows ($n = 3$ per group): (1) G1: BioPure MTAD, (2) G2: 17% EDTA (pH 7.7), and (3) G3: 5% citric acid (pH 2.0). EDTA and citric acid were freshly prepared by the manufacturer (Formula & Ação Ltda., Sao Paulo, Brazil).

Experimental Procedure (Cosite Microscopy)

The experiments were developed in an Axioplan 2 Imaging motorized microscope (Carl Zeiss Vision GmbH, Hallbergmoos, Germany). An Epiplan 100 \times HD objective lens was used coupled to a 1300 \times 1030 pixels Axiocam HR digital camera (Carl Zeiss), leading to a total magnification of approximately 1000 \times , and a resolution of 0.1 μ m/pixel.

In the cosite microscopy experiment, a special holder allowed application of the chelating solutions without removing the dentin sample from the microscope. A motorized specimen stage was used to automatically acquire 16 image fields at specific x-y positions of a given sample for several cumulative demineralization times (0, 15, 30, 60, 180, and 300 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. A pilot test was performed for the first methodologic article (17) in which a number of 16 image fields per tooth was defined. This decision was based on the following criteria: (1) the available dentin area and (2) the amount of useful data obtained per image. The details of the procedure were described by De-Deus et al (17). The complete image acquisition sequence was controlled by a special routine implemented under the AxiVision 4.5 software (Carl Zeiss Vision).

Image Analysis

A previously developed image analysis routine (17) was used to enhance image contrast, discriminate (17, 19, 24), 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 so-called area fraction (AF), was measured. The change of AF over demineralization time was used to quantify the process. The routine was applied without operator influence to the vast majority of the images acquired for different samples at different times. All steps were implemented as a macroroutine under the KS400 3.0 software (Carl Zeiss Vision). The initial images (0 seconds) with the standardized smear layer were not analyzed because of the low contrast of the tubules covered by the smear layer, which prevented their discrimination. As an overtime evaluation, each specimen served as its own control.

Data Presentation and Analysis

Data are presented as tubule AF in percentage of the whole dentin area (17). The preliminary analysis of the pooled data from the experimental groups (SPSS for Windows, Version 8.0; SPSS Inc, Chicago, IL) did not show normal distribution (Kolmogorov-Smirnov test). Further statistical analysis was performed with nonparametric test methods using the Kruskal-Wallis H-test with Bonferroni correction to compare the AF values between the groups at the respective experimental times. The AF and the time were used as factors and the level of significance was set at $p < 0.05$. Origin 6.0 (Microcal Software, Inc, Northampton, MA) was also used as analytic tool.

Results

The columns of the image montage in Figure 1 show the evolution of demineralization over time for 17% EDTA, Biopure MTAD, and 5% CA. In each column, an image field at a specific x-y position of a sample is shown for 5 cumulative demineralization times.

Figure 2 shows the increase of AF of open tubules against time for each group. Based on the present data the following statements can be

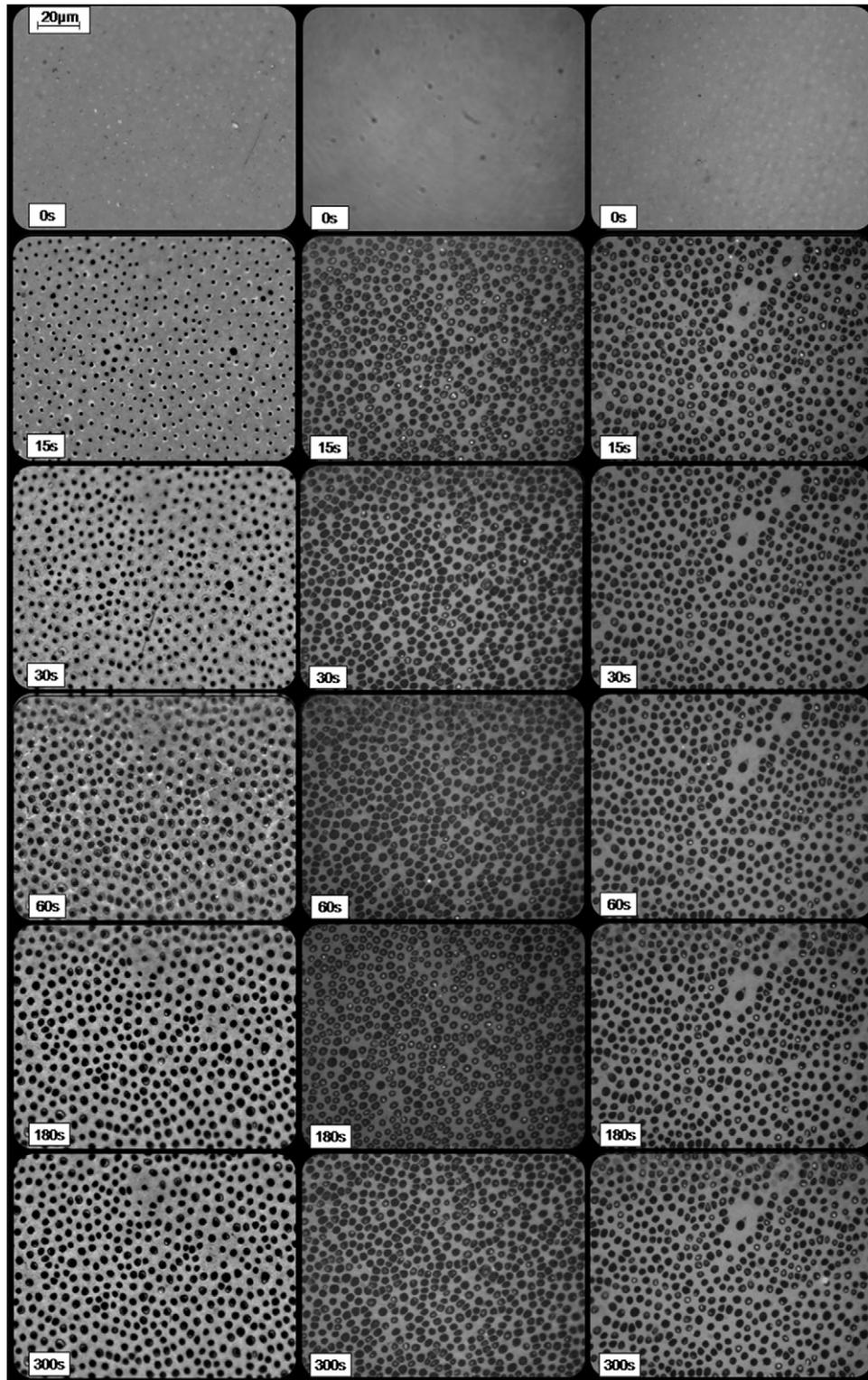


Figure 1. Time evolution of a given dentin region during demineralization with each chelator. The columns show the evolution of demineralization over time for 17% EDTA, 5% CA, and BioPure MTAD (from left).

made: (1) generally, all images were smear free after $\approx 15/30$ seconds of etching and one can observe the enlargement of the dentinal tubules, as expected in the typical evolution of demineralization; (2) BioPure MTAD and 5% citric acid were more effective than 17% EDTA for all experimental times ($p < 0.05$); (3) BioPure MTAD was more effective than 5% citric acid at both 15 seconds and 30 seconds ($p < 0.05$),

whereas 5% citric acid was more effective than BioPure MTAD at both 180 seconds and 300 seconds; (4) there is a clear saturation on the demineralizing ability of BioPure MTAD after 30 seconds; (5) there is a trend of saturation on the demineralizing ability of 5% citric acid after 30 seconds; and (6) the demineralization kinetics promoted by both 5% citric acid and BioPure MTAD was clearly faster than for 17% EDTA.

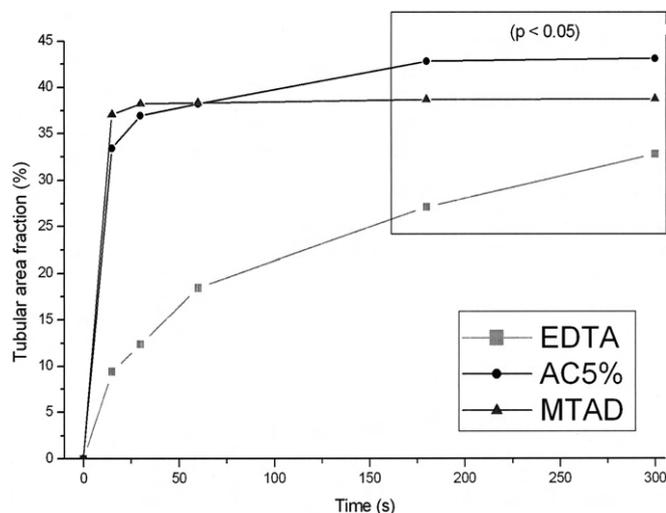


Figure 2. Time evolution of the open tubule AF. Data points are the average of 48 measurements from 3 different samples. The frame highlights the experimental times for which all the solutions were statistically different.

Discussion

Based on the present results, the null hypothesis tested was plainly rejected. It can be straightforwardly observed in the graph of Figure 2 that under the same experimental conditions, both BioPure MTAD and 5% CA were more effective than 17% EDTA at all experimental times.

Regarding the data obtained, two interesting points can be drawn: (1) the fast and sharp increase of the tubular area fraction promoted by BioPure MTAD and 5% CA and (2) the full saturation of the demineralizing ability of BioPure MTAD after 30 seconds.

The first point, the faster demineralization kinetics of BioPure MTAD and 5% CA, can be viewed as positive from a clinical point of view because smear removal is fast and efficient. The faster effect of CA has been reported in a few earlier studies. Recently, De-Deus et al (14) using a longitudinal observation method (AFM) found that CA had the strongest time-effect relationship when compared with EDTA and EDTAC. However, to the best of the authors' knowledge, this faster effect has not been reported for BioPure MTAD so far. However, because of the current concern regarding erosive effects stemming from the association of chelators with NaOCl, one cannot state that the fastest or most powerful solution is the best indicated for smear layer removal.

The second point, the self-limiting effect of BioPure MTAD, suggests a novelty in relation to its well-known properties. The self-limiting effect of EDTA has already been shown (13, 14). Once all the chelating ions have reacted with the calcium ions of the dentin, equilibrium is established and demineralization stops. However, the few reports about the smear-removal ability of BioPure MTAD do not point out a self-limiting effect. Considering the concerns mentioned earlier regarding erosive effects, a solution that presents a self-limiting effect associated to efficiency is very attractive.

The consequences of dentin matrix destruction remains undefined although Park et al (25) speculated that an increased coronal leakage in samples treated with EDTA compared with those treated with BioPure MTAD might be caused by the erosive property of EDTA and the length of dentin exposure to this solution.

Future research should try to answer the following question: what is the best clinical indication? Either a very strong chelator that removes completely the smear layer but that might also destroy the dentin matrix

or a relatively weaker chelator with the opposite behavior? The answer requires a better understanding of the mechanism of dentin matrix destruction and its effects on the adaptation of the filling and achieved sealing as well as a possible influence on root strength.

The erosive effects of EDTA and citric acid have been reported in several studies (12, 13, 14, 26, 27). On the other hand, the manufacturer (28) and Torabinejad et al (7) reported that, unlike the use of EDTA as the final rinse, minimal erosion of intraradicular dentin has been reported when NaOCl and BioPure MTAD were used in a similar sequence. The citric acid and doxycycline present in BioPure MTAD are responsible for its chelating ability. However, De-Deus et al (14) reported that CA led to strong erosive effects.

Possibly, some kind of chemical interaction between the components of BioPure MTAD is responsible for the self-limiting effect. Torabinejad et al (7) reported that apparently BioPure MTAD reacts with the dentin surface differently when compared with CA or EDTA, and these findings are in agreement with the current results.

The use of BioPure MTAD with doxycycline affinity to dentin may lead to a different effect on the dentin structure when compared with the 17% EDTA and 5% CA (29). Krause et al (30) point out that in the BioPure MTAD preparation, CA may serve to remove the smear layer, thus allowing doxycycline to penetrate the dentinal tubules and exert an antimicrobial effect. However, some studies have shown that both doxycycline and CA have acid etching ability (31, 32).

Analyzing the present results, it is apparent that 5% CA and BioPure MTAD have comparable effects. So, despite its self-limiting effect, it is not clear that BioPure MTAD caused minimal dentin erosion. More investigation is necessary to clarify whether the clinical protocol for the use of BioPure MTAD really prevents dentin erosion. Transmission electron microscopy can be considered the ideal method to assess the effects of the irrigation protocols on the dentin matrix (33).

This project is a part of a larger study comparing the demineralization power of the demineralizing agents available in current endodontic practice. In the present study, BioPure MTAD was evaluated through CSOM and digital image analysis. The method provides quantitative data linked to the longitudinal observation of the dentinal substrate changes.

Because the goal of the present work was restricted to a direct longitudinal and quantitative assessment of the chelating ability of BioPure MTAD, the application of these results to the clinical situation is not straightforward. The limitations of the present result are directly linked to the requirements of the light optical microscopy techniques. The chelator solution was applied to a flat horizontal dentin surface, 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 anatomical variability of the root canal system. Moreover, in the present method, a rinsing procedure was not performed and this is also not in line with the clinical situation. However, for a direct comparison of the chelating ability of the solutions the present methodological model proved to be fast, robust, reliable and statistically sound. Moreover, the method provides longitudinal information about the dentin morphology and does not depend on the traditional descriptive scores of SEM studies.

Under the conditions of the present ex vivo evaluation, the following conclusions can be drawn: (1) the demineralization kinetics promoted by both 5% citric acid and BioPure MTAD was clearly faster than for 17% EDTA, and (2) CSOM represents a powerful approach to compare directly longitudinally and quantitatively the ability of the demineralizing solutions.

Acknowledgments

The authors wish to thank Dr. Carlos Cleber Garcia for his essential assistance.

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