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Smear layer dissolution by peracetic acid of low concentration

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Abstract

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Aim To test the effect of a noncaustic concentration of peracetic acid (PAA) in a standardized smear layer model.

Methodology The smear layer dissolution kinetics of 0.5% PAA on human dentine were compared to those of 2.25% PAA and 17% ethylenediaminetetraacetic acid (EDTA) solutions. Coronal dentine discs were prepared from six human maxillary molars. A standardized smear layer was produced on the pulpal side of each disc. The smear layer–covered surface was divided into three similar areas and then exposed to one of the three solutions tested. Co-site image sequences (around 40, 500×) of the specific areas were obtained after four cumulative demineralisation times (15, 30, 60 and 180 s). An image processing and analysis sequence measured sets of images, providing data of area fraction (AF, dentine-free area in % of total analysis area). A general linear model for repeated measures was used to verify the influence of time and solution type over the change in AF from baseline (ΔAF).

Results Overall, EDTA and 2.25% PAA produced higher ΔAF values than the 0.5% PAA solution ($P < 0.05$). No significant difference was observed in ΔAF between 15 s and 30 s ($P > 0.05$). After 60 s of etching, all tested solutions produced similar ΔAF ($P > 0.05$), whereas at 180 s, ΔAF of both EDTA and 2.25% PAA continued to increase ($P > 0.05$).

Conclusions After 60 s of contact, the 0.5% PAA solution dissolved smear layer as well as 2.25% PAA and 17% EDTA.

Keywords: co-site microscopy, dentine, peracetic acid, smear layer.

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Introduction

Despite a lack of strong evidence, removal of the smear layer that is created during mechanical root canal instrumentation (McComb & Smith 1975) is considered to be important (Torabinejad *et al.* 2002). This iatrogenic layer consists of a mixture of organic and inorganic debris (Sen *et al.* 1995). The organic portion is dissolved by sodium hypochlorite, the main endodontic irrigant (Zehnder 2006). To remove the inorganic portion of the smear layer, a decalcifying agent is used, which can be either a chelator or an acid. Currently, all the products on the dental market sold to dissolve smear layer are based on ethylenediaminetetraacetic acid (EDTA) or citric acid. In theory, however, any biologically acceptable compound able to dissolve hydroxyapatite could be used for this specific purpose. For the sake of simplicity and to expedite root canal disinfection and debridement, it may be best to employ either a chemical that is compatible with sodium hypochlorite (Zehnder *et al.* 2005) or a counterpart that has a strong antimicrobial effect.

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Neither EDTA nor citric acid has strong antimicrobial properties (Zehnder *et al.* 2005, Bryce *et al.* 2009). Therefore, it has been proposed to use peracetic acid (PAA) instead of these 'classical' decalcifying agents to dissolve the smear layer and concomitantly continue to disinfect the root canal system (Lottanti *et al.* 2009).

Peracetic acid solutions are amongst the strongest disinfectants known, with antibacterial, sporicidal, antifungal and antiviral properties (McDonnell & Russell 1999). They have been used in the former German Democratic Republic as single endodontic irrigants (Kühlfluck & Klammt 1980). Nowadays, PAA-related products are used mainly for veterinary purposes and for water treatment. In aqueous solution, PAA is in equilibrium with hydrogen peroxide, acetic acid and acetylhydroperoxide. It is the acetic acid content that is probably responsible for the smear layer dissolution. Acetic acid forms complexes with calcium, which are easily soluble in water. As has been shown, a 2.25% PAA solution has an effect on the smear layer, and the root canal wall that is comparable to that of 17% EDTA (Lottanti *et al.* 2009). However, 2.25% PAA may be caustic when in contact with oral mucosa, and thus, it may be better to use a lower concentration (Kühlfluck & Klammt 1980). It is not known how the concentration of PAA affects its clearance of the smear layer. Furthermore, a direct comparison of the smear layer dissolution kinetics of PAA and EDTA has not been performed. Thus, the goal of the present investigation was to study the effect of the exposure time and concentration of PAA on removal of the smear layer. A standard 17% EDTA solution was used as a reference for comparison.

Materials and methods

Specimen selection and dentine disc preparation

Six 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 (Ethics Committee).

Dentine discs approximately 3 ± 0.3 mm thick were cut from the middle third of the crowns above the pulp chamber (n = 6). A standard procedure [polishing with SiC paper (200, 300, 400, 600) grits and 3 μm diamond paste] was employed on the pulpal surface of each disc, to prepare them for the experimental process and to produce a standardized smear layer (De-Deus *et al.* 2006, 2007).

Co-site microscopy

Seventeen per cent EDTA solution was bought from a common commercial source (Formula & Ação Ltda., São Paulo, SP, Brazil). PAA solutions were freshly prepared. According to the manufacturer (Kesla Pharma Wolfen GmbH, Greppin, Germany), the original commercial solution (Uterofertil; Kesla Pharma Wolfen GmbH) contained 4.5% (wt/vol) PAA, 3.5% acetic acid and 7.3% hydrogen peroxide. It was diluted with bi-distilled water resulting in 2.25% and 0.5% PAA solutions.

To minimize the influence of the variability of human dentine when comparing different solutions, a single-tooth approach was followed (De-Deus *et al.* 2008a,b). The central dentine area of each tooth was divided into three similar sections delineated by holes in an adhesive tape, 0.85 mm in diameter each. Each analysis area was exposed to 1 mL of the tested solutions for four cumulative experimental times (15, 30, 60 and 180 s). After each experimental time, the demineralising process was interrupted with 5 mL of bi-distilled water.

The experiment was performed with an Axioplan 2 Imaging motorized microscope (Carl Zeiss Vision Gmbh, Hallbergmoos, Germany) controlled by AxioVision 4.5 software (Carl Zeiss Vision). An Epiplan 50× HD objective lens was used coupled to a 1300 × 1030 pixels Axiocam HR digital camera (Carl Zeiss), resulting in a total magnification of approximately 500×, and a resolution of 0.21 μm pixel^{-1}.

The microscopic mosaic method was used to produce a single high-resolution image, composed of smaller images, which covered the full analysis area. Pre-defined mosaic settings controlled the microscopic motorized specimen stage to perform an automatic acquirement of 40 small images, at specific x–y positions, so as to cover the whole analysis area. This procedure was followed for four cumulative demineralisation times (15, 30, 60 and 180 s). A previously developed image analysis routine (De-Deus *et al.* 2007, 2008a) was used to enhance image contrast, discriminate and measure open dentine tubules in each acquired image. The final product of the image analysis routine is the ratio between the total dentine-free area (open and eroded tubules) and the full analysis area – this ratio was termed area fraction (AF – %). During the evaluation over time, each specimen served as its own control.

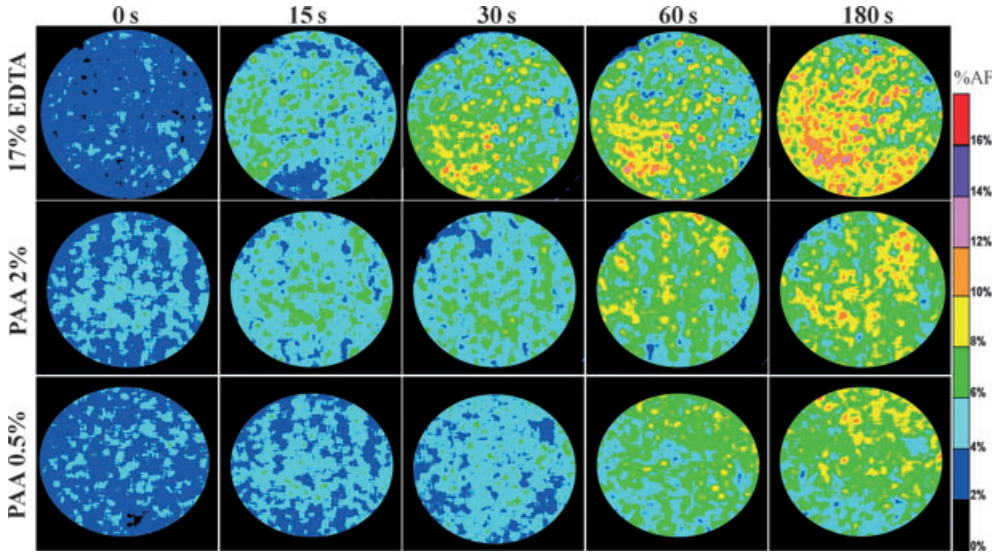

Figure 1 Dentine mapping highlighting the differences per tubule area fraction (AF) for each tested solution.

Dentine mapping

An automatic image analysis routine was developed to convert mean AF for each analysis area into false colours. A low-pass filter was used on the binary image, transforming it into a blurred grayscale image. Subsequently, a colour scale table was used to convert grayscale values into false colours (Fig. 1).

Data presentation and analysis

Considering that the AF of the full analysis area at the baseline (time-point = 0 s) was not similar amongst the experimental teeth because of the natural differences in the morphology of the dentine substrate, the AF obtained at each time-point was deducted from the respective AF at the baseline. Consequently, the difference in AF compared to baseline (ΔAF) was used to express the effect of the solutions over the analysis area.

Considering the clustered nature of the data, a General Linear Model for repeated measures (GLMrep, SPSS 17.0; SPSS Inc., Chicago, IL, USA) was used to verify the influence of time and solutions over the ΔAF. Time was considered as the repeated factor, solutions the grouping factor. Bonferroni correction and Tukey tests were applied for multiple comparisons amongst time-points and solutions, respectively. Mauchly's Sphericity was used to verify the equality of the variances of the differences between levels of the repeated measures factor. The alpha-type error was set at 0.05.

Results

The results of GLMrep indicated that sphericity was not assumed (Mauchly's sphericity, $P < 0.05$); thus, the Greenhouse–Geisser test was used to correct for violations of sphericity. The variation in time significantly influenced the ΔAF of solutions (GLMrep, $P < 0.05$). There was no significant difference in ΔAF between 15 and 30 s ($P > 0.05$), whilst ΔAF was significantly different from these time-points at 60 and 180 s ($P < 0.05$). A significant difference was also found between time-points 60 and 180 s ($P < 0.05$). The descriptive analysis of the data is displayed in Table 1 as ΔAF.

Table 1 Mean and standard deviations (±SD) of area fraction from baseline (ΔAF) in % for the tested solutions at each time-point

	Time-point (s)			
Solutions	15	30	60	180
0.5% peracetic acid (PAA)	0.19 (±0.40)	0.32 (±0.44)	0.71 (±0.87)	0.80 (±0.88)
2.25% PAA	0.30 (±0.37)	0.51 (±0.65)	0.80 (±0.92)	2.30 (±2.33)
Ethylenediaminetetraacetic acid (EDTA)	0.64 (±0.81)	0.9 (±0.67)	1.06 (±0.84)	2.18 (±1.60)

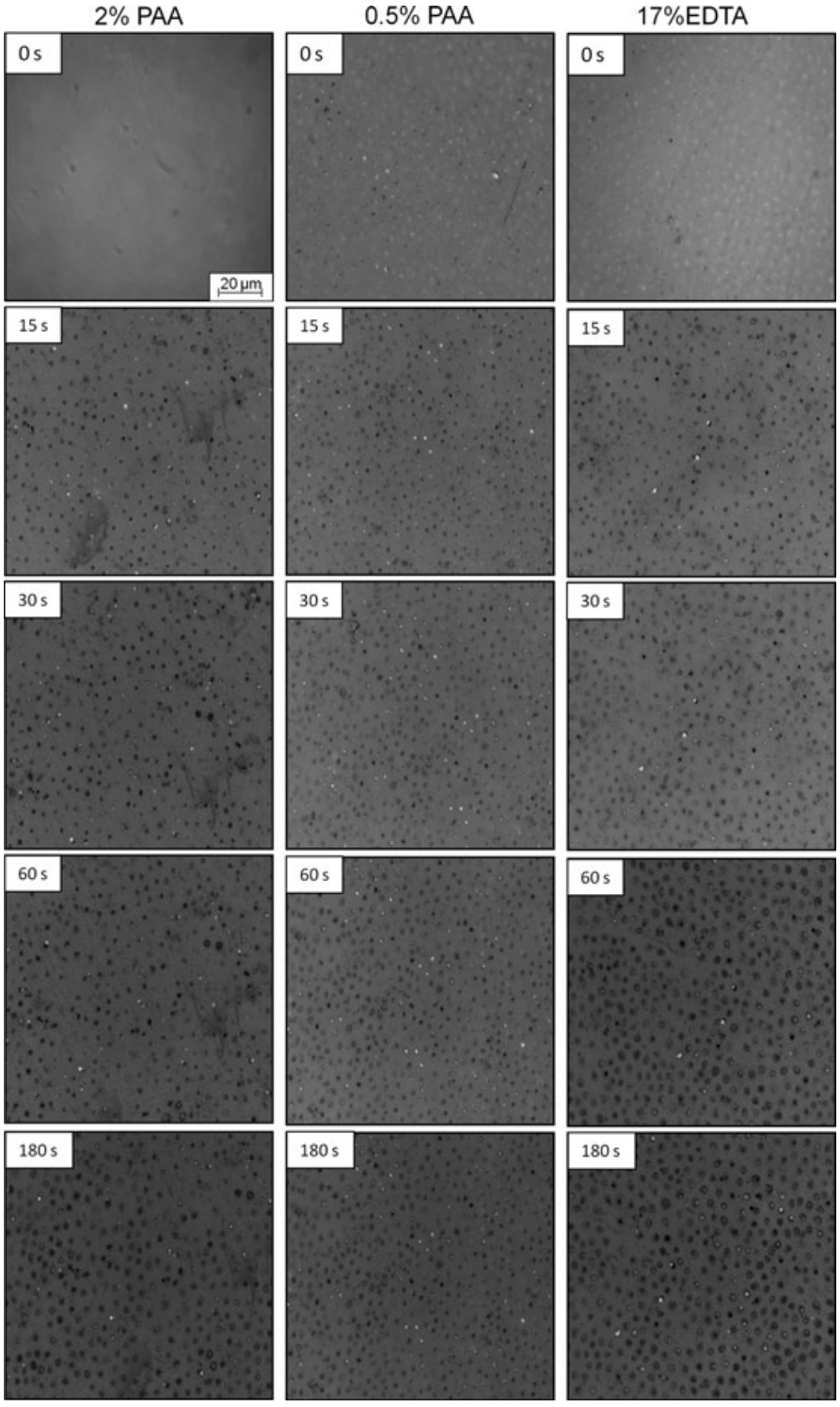

Figure 2 Surface changes of dentine regions during demineralisation with each solution. The columns show the evolution of demineralisation over time for 2% peracetic acid (PAA), 0.5% PAA and 17% ethylenediaminetetraacetic acid (from left). In each column, an image field at a specific x–y position of a specimen is shown for four cumulative demineralisation times. The claim of high reproducibility of x–y positions is confirmed by these figures as almost the exact same dentine features are visible for all times.

The type of solution significantly influenced the ΔAF (GLMrep, $P < 0.05$). Tukey *post hoc* test detected a significant difference in ΔAF amongst the solutions in the following order: 0.5% PAA < 2.25% PAA < 17% EDTA ($P < 0.05$).

The interaction time–solutions was also significant (GLMrep, $P < 0.05$), indicating that the ΔAF differently varied along time according to the solution that was applied. From time-point 15 s to time-point 30 s, solutions behaved differently ($P < 0.05$). The same was seen for the time-point 60–180 s ($P < 0.05$), whereas from time-point 30 to 60 s, solutions had a similar effect on ΔAF ($P > 0.05$).

Qualitatively, all tested solutions produced increased demineralisation over time and similar AF after 60 s of etching (Fig. 1). Typical differences in dentine morphology over the experimental times are depicted in Fig. 2.

Discussion

The current study showed that PAA solutions, according to their concentration, can dissolve an experimental smear layer as quickly as a standard 17% EDTA solution does. After 60 s of contact, the 0.5% PAA solution dissolved smear layer as well as 2.25% PAA and 17% EDTA.

The advantages and disadvantages of the current method have been discussed in detail previously (De-Deus *et al.* 2007, 2008a, Reis *et al.* 2008, De-Deus *et al.* 2008b). The application of these results to the clinical situation is not straightforward. The goal of the present work was restricted to a direct comparison of the ability of PAA and EDTA to remove a standardized smear layer. One of the limitations of the current method is that the solutions were applied to a flat horizontal dentine surface and exposed to gravitational force without agitation, different from the clinical situation, in which the contact between the demineralising agent and the dentine surface is affected by the vertical position of the teeth and the intrinsic anatomical variability of the root canal system. On the other hand, several experimental parameters are better controlled in the current method, such as the amount of available solution, contact surface area and contact time.

The current findings on PAA are in line with the quantitative results described by Reis *et al.* (2008) in which a direct relation between chelator concentration and the increase in the AF was shown. Moreover, it should be noted that both PAA concentrations tested in the present study were able to remove the experimental smear layer to the same extend as EDTA after 60 s. The use of low concentrate PAA solutions appears clinically attractive, as concentration is linked to tissue irritation. In a clinical trial, a solution containing 0.5% PAA did not irritate oral mucosa, whilst more concentrated solutions did (Kühlfluck & Klammt 1980). Lottanti *et al.* (2009) used scanning electron microscopy, including energy-dispersive X-ray analysis, to assess the extent 17% EDTA, 2.25% PAA and 9% etidronic acid removed the smear layer and demineralized the root canal wall. The authors concluded that all tested protocols resulted in clean canal walls. However, as was observed in root cross sections, the effect on the root canal dentine differed between PAA and EDTA. This was in line with a study on simulated dentine caries, which showed a clear difference in mineral profiles obtained with acetic acid versus EDTA, with a gradual demineralisation caused by acetic acid opposed to the total surface demineralisation by EDTA (Kawasaki *et al.* 2000). Apparently, the high ΔAF caused by the standard EDTA solution and 2.25% PAA at 180 s in this study is explained by erosion of dentine (Figs 1 and 2). However, it must be cautioned that dentine erosion cannot be directly followed by the current methodology. Diluted EDTA solutions may have a different effect under the current conditions. Depending on the type of root filling and sealer, demineralisation patterns may well be important. Total demineralisation and a collapsed collagen network are generally not considered advantageous if the goal is to infiltrate the dentine with a resin to produce a so-called hybrid layer (García-Godoy *et al.* 2005, Tay *et al.* 2006).

It is worthwhile underlining that PAA use could be clinically advantageous over the use EDTA if it could optimize the disinfection of the root canal space. Intracanal disinfection achieved with PAA in comparison with other decalcifying agents needs to be investigated in future studies.

Conclusion

The current results suggest that a noncaustic concentration of PAA could be sufficient to dissolve endodontic smear layers.

Acknowledgement

The authors deny any conflicts of interest.

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