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Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer

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Abstract

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Aim To evaluate the effects of ethylenediaminetetraacetic (EDTA), etidronic (EA) and peracetic acid (PA) when used in conjunction with sodium hypochlorite (NaOCl) as root canal irrigants on calcium eluted from canals, smear layer, and root dentine demineralization after instrumentation/irrigation.

Methodology Single-rooted human premolars were irrigated as follows (n = 12 per group): (1) 1% NaOCl during instrumentation, deionized water after instrumentation, (2) 1% NaOCl during, 17% EDTA after instrumentation, (3) a 1 : 1-mixture of 2% NaOCl and 18% EA during and after instrumentation, and (4) 1% NaOCl during, 2.25% PA after instrumentation. Irrigant volumes and contact times were 10 mL/15 min during and 5 mL/3 min after instrumentation. The evaluated outcomes were eluted calcium by atomic absorption spectroscopy, smear-covered areas by scanning electron microscopy in secondary electron mode and apparent canal wall decalcifications on root transsections in backscatter mode. For the smear layer analysis, sclerotic dentine was taken into consideration. Results were compared using appropriate parametric and nonparametric tests, alpha = 0.05.

Results The statistical comparison of the protocols regarding calcium elution revealed that protocol (1) yielded less calcium than (3), which yielded less than protocols (2) and (4). Most of the instrumented canal walls treated with one of the decalcifying agents were free of smear layer. Protocols (1) and (3) caused no decalcification of root dentine, whilst (2) and (4) showed substance typical demineralization patterns.

Conclusions The decalcifying agents under investigation were all able to remove or prevent a smear layer. However, they eroded the dentine wall differently.

Keywords: EDTA, etidronic acid, peracetic acid, smear layer.

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Introduction

Root canal irrigation is important for proper debridement of infected root canals. Ideally, all microorganisms, necrotic tissue remnants, and the smear layer that is created during mechanical canal instrumentation should be removed. The last issue, however, is not based on evidence. Current clinical concepts rely on sodium hypochlorite for necrotic soft tissue management and infection control (Naenni *et al.* 2004, Vianna *et al.* 2006). When canals are rinsed with hypochlorite during preparation, instrumented root dentine walls are more or less free of organic remnants, but covered with inorganic shavings forming a smear layer (Lester & Boyde 1977, Koskinen *et al.* 1980). Depending on the materials used for root filling, it is not clear how much of this inorganic smear layer should be removed

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(De-Deus *et al.* 2008a, Saleh *et al.* 2008). Specifically, when strong chelators are employed to completely remove the smear layer, the decalcification of the root canal wall is a side effect that could have a negative impact on canal sealability (De-Deus *et al.* 2008a). Yet, decalcification of root dentine by endodontic irrigants has received little attention in the dental literature (Garcia-Godoy *et al.* 2005). Moreover, when an attempt is made to remove the smear layer, the use of chemicals in conjunction with sodium hypochlorite is not straightforward, as hypochlorite reacts with most moieties that can be oxidized (Zehnder 2006). Consequently, it has been recommended that a sodium hypochlorite irrigant be used during instrumentation of the root canal to prolong disinfection and tissue dissolution time, and then a chelator solution be administered to clean the canal system of inorganic debris. Finally, sodium hypochlorite or another antiseptic can be applied to optimize disinfection (Yamada *et al.* 1983).

In theory, there are two ways to simplify this protocol: i) to use a chelator that does not interfere with sodium hypochlorite or ii) to use a chelator with a strong disinfecting capacity as final irrigant. Etidronic acid (also known as 1-hydroxyethylidene-1, 1-bisphosphonate or HEBP) is a biocompatible chelator that can be used in combination with sodium hypochlorite without short-term loss of the desired properties of either compounds (Girard *et al.* 2005, Zehnder *et al.* 2005b). This could have the advantage that a sodium hypochlorite-etidronic acid combination could be used as a single irrigant during and after instrumentation so that a smear layer is never created. However, the chelating capacity of etidronic acid is relatively weak (De-Deus *et al.* 2008b), and it is not known whether its use results in root canals that are as clean as counterparts irrigated with NaOCl followed by EDTA. As for the second option, peracetic acid is a strong candidate to be used as a final irrigant. This peroxygen is sporicidal, bactericidal, virucidal and fungicidal at low concentrations of less than 0.5%, even in the presence of protein (Lensing & Oei 1985). It decomposes to safe by-products, acetic acid and oxygen. Peracetic acid actually does not exist in pure form in aqueous solution, but occurs in equilibrium with hydrogen peroxide, acetic acid and acetylhydroperoxide. The fact that acetic acid is liberated from/present in a peracetic acid solution poses the possibility that a peracetic acid solution could be used after instrumentation to dissolve the smear layer and provide a thorough disinfection of the root canal system pre-treated with NaOCl. Acetic acid is a weak chelator that forms water-soluble complexes with calcium (Martell & Motekaitis 1992). Interestingly, irrigants containing peracetic acid were used throughout Eastern Europe and the former eastern block to disinfect root canal systems (Kühlfluck & Klammt 1980). However, with recent political changes, their potential usefulness in endodontics has somehow been forgotten.

When comparing different irrigating regimens on the smear layer that is created during instrumentation, the canal walls of fractured root specimens have traditionally been inspected using scanning electron microscopy (Cameron 1983). This method, however, is prone to bias, because it largely compares the amount of open dentinal tubules between groups. Dentine is a heterogeneous structure. In addition, it undergoes changes during ageing. First in the apical and later in middle and coronal root aspects, tubules become sclerotic (Vasiliadis *et al.* 1983a,b). It is possible, that in the studies on root canal smear layer that have been published so far, smear layer may not have been differentiated from sclerotic dentine.

It was the goal of this study to compare the following irrigating protocols on extracted human premolars: 1% NaOCl throughout instrumentation followed by water (negative control); 1% NaOCl throughout instrumentation followed by 17% EDTA (gold standard, positive control); a fresh 1 : 1 mixture of 2% NaOCl and 18% etidronic acid throughout instrumentation and as final irrigant; or 1% NaOCl throughout instrumentation followed by 2.25% peracetic acid. The outcome variables evaluated were: calcium eluted from the canal system, smear layer on instrumented canal walls and dentine decalcification observed on root transsections. A new way to calculate smear layer taking dentine sclerosis into consideration is presented.

Materials and methods

Solutions

The 1% (wt/vol) and 2% NaOCl as well as the 17% EDTA solutions were bought from a commercial source (Kantonsapotheke, Zürich, Switzerland), as was the peracetic acid solution (Uterofertil, Kesla Pharma Wolfen GmbH, Greppin, Germany). According to the manufacturer, this solution contained 4.5% (wt/vol) peracetic acid, 3.5% acetic acid and 7.3% hydrogen peroxide. It was diluted 1 : 1 with deionized water, resulting in a 2.25% peracetic acid solution. The 18% (saturated) etidronic acid solution was prepared from

HEBP salt (Cublen K8514P, Zschimmer & Schwarz, Burgstädt, Germany) in deionized water. Using a calibrated pH meter (827 pH Lab, Metrohm, Herisau, Switzerland), the pH values of these solutions was determined.

All solutions were stored at 5 °C in air-tight dark containers between experiments. On experimental days, the solutions were taken from the refrigerator and stored for 60 min at room temperature prior to being used. A fresh 1 : 1 mixture of 2% NaOCl and 18% etidronic acid was prepared immediately before the experiments, resulting in a solution that contained both 1% NaOCl and 9% etidronic acid (Zehnder *et al.* 2005b).

Tooth selection and preparation

A total of 51 single-rooted premolars from the department's collection of extracted teeth were used for this study. Teeth had been stored in 0.1% thymol solution at 5 °C for no more than 1 year. Teeth were selected based on their appearance suggesting one single root canal, which was verified using digital radiography (Digora, Soredex, Helsinki, Finland). The crowns of all teeth were shortened to a full root length of 12 mm as measured from the apex using a laboratory hand piece and a diamond-coated microsaw. To prevent calcium from being eluted from outer root surfaces, these were covered with nail varnish.

Three premolars were used as positive controls for the canal wall decalcification analysis (see below).

Simulated clinical procedures

A step-down procedure was performed with Gates–Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland). Canals were instrumented using ProFile instruments (Dentsply Maillefer) in a crown-down manner, so that finally a size 45, 0.04 taper instrument reached working length, which was set at 11 mm for all teeth. Using a random sequence generator (http://www.random.org), teeth were allocated to four experimental ($n = 12$) and one positive control group ($n = 3$) for root canal wall decalcification (see below). Teeth were irrigated as follows: group 1: 1% NaOCl during instrumentation, deionized water after instrumentation; group 2: 1% NaOCl during, 17% EDTA after instrumentation; group 3: the 1 : 1-mixture of 2% NaOCl and 18% etidronic acid during and after instrumentation; group 4: 1% NaOCl during, 2.25% peracetic acid after instrumentation. The total irrigation time during instrumentation was 15 min, the volume was 10 mL. After instrumentation, 5 mL of the final irrigant was administered 1 mm from working length over 3 min. Subsequently, the canal was rinsed with 5 mL of deionized water.

In the positive control group for the root canal wall demineralization analysis, the canal was irrigated during instrumentation as described before, and subsequently, 10 mL of 17% EDTA was administered over 30 min.

Atomic absorption spectroscopy

The 20 mL of total eluate per specimen were collected in individual glass vials (Duran, Schott, Mainz, Germany). Each time after instrumentation and irrigation of one specimen per group, the eluates were centrifuged (Z320, BHG Hermle, Wehringen, Germany) at $4000 \times g$ for 10 min. Subsequently, 10 mL of the supernatant was transferred to a polypropylene tube (TPP, Trasadingen, Switzerland) with a lid and stored at −20 °C until further analysis.

Once all the eluates had been collected, they were thawed and then analysed for their calcium content using an atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, Norwalk, CT, USA) with an air-acetylene flame. Measurements were performed in duplicates. Each eluate was measured against a standard series of Ca^{2+}. Phosphate was masked with strontium chloride. Results are expressed as ppm Ca^{2+} in the eluate.

Electron microscopy

Longitudinal grooves, which did not penetrate into the canal, were placed in the facial and lingual surfaces of the roots to facilitate and guide their fracture. Roots were fractured along these grooves by dipping them in water and then in liquid nitrogen. Root sections were dehydrated using an ascending ethanol series up to 100%.

Root transsections

One root half was infiltrated and finally embedded in ascending concentrations of an isobornyl methacrylate resin (Technovit 7200 VLC, EXAKT, Norderstedt, Germany). Resin blocks polymerized with white and blue light were cut to expose root transsections in the coronal, middle and apical root third in each specimen using a diamond-coated saw (Isomet, Bühler, Switzerland) under water cooling. Root sections were mounted on individual stubs and raw polished using 1200 and

then 2400 grit silicon paper. Thereafter, specimens were polished using diamond pastes of particle sizes down to 0.5 μm. The exposed surfaces were carbon-coated in an electron-beam evaporator (BAL-TEC MED 020, Baltec Union, Balzers, Liechtenstein). These specimens were examined in the scanning electron microscope (Supra 50 VP, Zeiss, Oberkochen, Germany) equipped with an annular mono-crystal scintillation type (YAG) backscatter detector in backscatter mode at an accelerating voltage of 11 kV and 9 to 12 mm working distance. Digital images were taken at magnifications of 100 to 500× (overviews) followed by 2000× for the detailed views at a resolution of 1024 × 768 pixels. For control reasons, the specimens that were exposed to EDTA for 30 min were used to compare images obtained in backscatter mode to counterparts from the same specimen obtained by energy-dispersive x-ray (EDX) analysis sampled over 300 min (Fig. 1). Because EDX analysis takes half a working day per specimen and the dark areas in the backscattered electron micrographs exactly matched the decalcified areas, backscatter images were used to compare apparent decalcifications between groups. High-resolution images taken from the whole canal wall area (2000× magnification, typically eight per specimen) were analysed by one observer. Furthermore, the length of sclerotic dentine per total canal wall length on each specimen was determined using an image analysis software (IMAGEJ, rsbweb.nih.gov/ij/).

Smear layer on canal wall

The opposing half of each root was prepared for the smear layer analysis by mounting it on a stub and gold-sputtering (BAL-TEC SCD 030, Baltec Union). Prepared root canal surfaces (i.e. areas without calcospherites) were observed in a scanning electron microscope (Supra 50 VP, Zeiss) equipped with an SE (secondary electron) detector, and three photomicrographs were taken at 1000× magnification from typical areas of the coronal, middle, and apical root canal thirds at an accelerating voltage of 10 kV and a working distance of 7 to 9 mm. For the analysis of the smear layer, canal wall images were compared with the pattern of sclerotic dentine from the opposing root half (i.e. the corresponding root transsection specimen). This made it possible to discern between smear-covered and sclerotic areas (Fig. 2). The IMAGEJ software was used to calculate the area that was covered with a smear layer in percent of the whole image. Mean values of the three images per specimen were used for further calculations.

Data presentation and analysis

Data pertaining to calcium eluted from root canals (ppm) were compared between groups using one-way analysis of variance (ANOVA). Because of their normal distribution, these data are presented as means and standard deviations. Sclerotic dentine per canal wall and smear score comparisons were done using Kruskal–Wallis analysis of variance followed by the Mann–Whitney *U* test. These values are presented as medians and ranges. Bonferroni's correction for multiple testing was applied for all individual comparisons. The alpha-type error was set at 0.05.

Results

The pH values of the solutions used in this study were: 1% NaOCl: 12.0; 17% EDTA: 8.0; 18% etidronic acid: 10.5; the 1 : 1 mixture of 18% etidronic acid with 2% NaOCl; 10.5, 2.25% peracetic acid: 2.5.

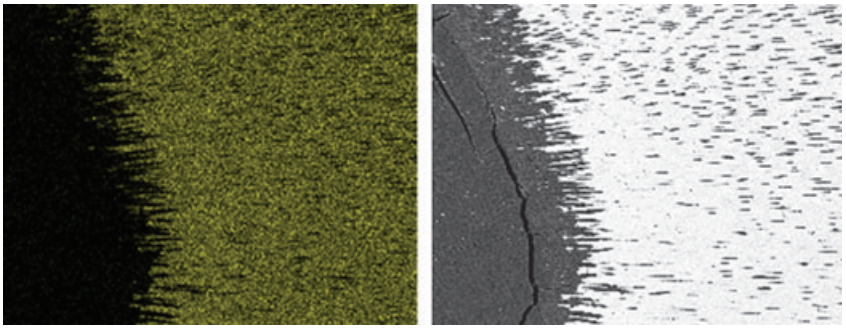

Figure 1 Comparison between images from a sample treated for 30 min with ethylenediaminetetraacetic acid (EDTA) (positive demineralization control) obtained by energy-dispersive x-ray (EDX) analysis (left, calcium in yellow) and in backscatter mode (right). The decalcification reached 50–100 μm into the dentine and followed the tubules.

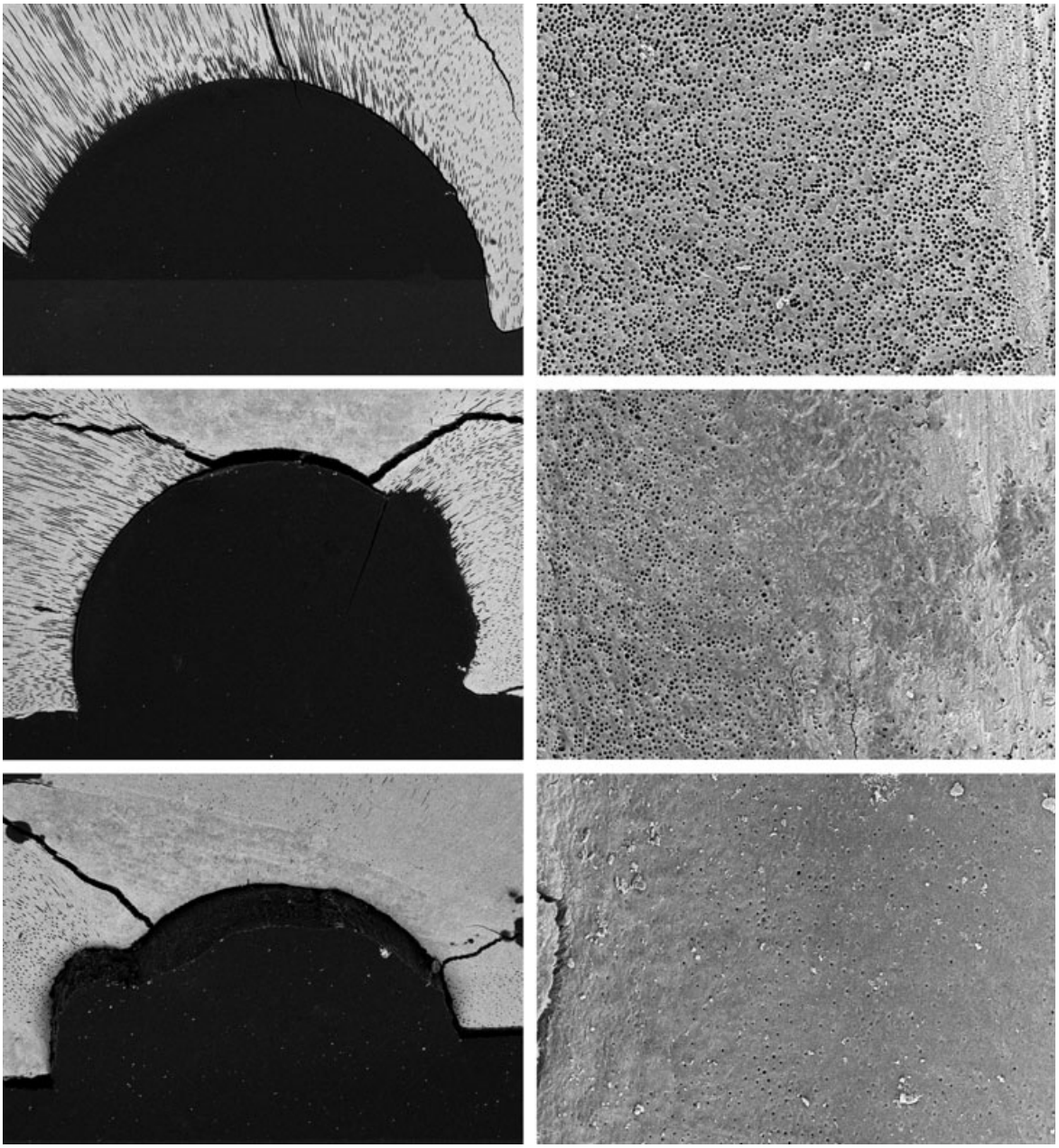

Figure 2 Root transsections (left) and corresponding canal walls from the opposing root segment (right) from the coronal, middle and apical root third (top to bottom) of a typical specimen rinsed with ethylenediaminetetraacetic acid (EDTA). Note that despite the fact that there is no smear layer, the tubules appear 'occluded' in middle and apical root sections because they are sclerotic and hence not visible. Sclerotic areas have a typical surface structure and show few tubular openings. This is in contrast to smear-covered instrumented areas that are covered with a homogenous layer (not shown).

Irrigation with the sodium hypochlorite irrigant during instrumentation followed by water as a final rinse hardly eluted any calcium from the canal system: 1 ± 1 ppm (mean ± SD). By contrast, the protocols with EDTA and peracetic acid dissolved 62 ± 13 and 57 ± 10 ppm respectively (P between these two groups >0.05). The irrigating protocol with the 2% NaOCl/18% etidronic acid mixture resulted in 32 ± 14 ppm calcium in the eluate, which was significantly ($P < 0.05$) less than with the other two decalcifying

agents, but significantly more than with the NaOCl/water treatment.

The median portion of sclerotic dentine per instrumented canal wall length was 37% (range: 0%–100%), 49% (range: 0%–100%) and 69% (range: 0%–100%) in coronal, middle and apical root thirds respectively. Coronal and middle root thirds did not differ significantly regarding the percentage of sclerotic dentine, whilst the apical root third had significantly more sclerotic areas compared to coronal and middle aspects ($P < 0.05$).

All the protocols with a decalcifying agent resulted in statistically similar reduction of smear-covered dentine, whilst the specimens irrigated with NaOCl and then with water were almost completely covered with a smear layer (Table 1). In coronal root areas, EDTA removed slightly more smear layer than peracetic acid ($P < 0.05$), whilst in middle root and apical root areas, no differences between EDTA, etidronic and peracetic acid protocols were detected. There were no differences in the amount of smear layer between coronal, middle and apical root aspects (Kruskal–Wallis, $P = 0.95$).

Apparent decalcifications were not observed in specimens irrigated with sodium hypochlorite and then water. Few if any demineralizations were seen in the NaOCl/etidronic acid-treated specimens. Specimens irrigated with EDTA for 3 min as a final rinse showed typical decalcification patterns: the tubules were enlarged at their canal wall opening, and the decalcification followed the tubular walls up to 20 μm into the dentine. EDTA decalcifications showed no gradual increase in mineral, but rather 1–5 μm of complete decalcification into the intertubular dentine with a sharp demarcation between demineralized and unaffected dentine (Fig. 3). By contrast, peracetic acid decalcifications typically showed a gradual mineral increase from the exposed canal wall into the root dentine, with fewer decalcified areas into the tubules.

Table 1 Smear-covered instrumented canal wall area in % of total area (median values, ranges) in coronal and middle root thirds according to the irrigating protocol (during instrumentation–final rinse)

Treatment	Coronal third	Middle third	Apical third
NaOCl – Water	97 (55–100)[a]	100 (60–100)[a]	100 (100–100)[a]
NaOCl – EDTA	0 (0–1)[b]	0 (0–55)[b]	0 (0–41)[b]
NaOCl & EA–NaOCl & EA	0 (0–54)[b,c]	0 (0–35)[b]	8 (0–20)[b]
NaOCl – PA	4 (0–29)[c]	0 (0–4)[b]	0 (0–0)[b]

NaOCl: sodium hypochlorite, EDTA: ethylenediaminetetraacetic acid, EA: etidronic acid, PA: peracetic acid. Data sets that share an identical superscript letter in a column did not differ significantly at the 5% level from each other (Kruskal–Wallis, Mann–Whitney *U* test, Bonferroni correction).

Discussion

The current study showed that the smear layer could be reduced in instrumented root canals by a protocol employing either etidronic or peracetic acid to a similar extent as with the conventional EDTA treatment. Both protocols could be clinically advantageous over the use of EDTA. An etidronic acid/sodium hypochlorite mixture could be administered as the sole irrigant. Alternatively, peracetic acid could be left in the canal after instrumentation for combined disinfection and smear layer dissolution.

Whilst many articles on the appearance of root dentine walls after different irrigating protocols in the scanning electron microscope have been published, surprisingly few studies have addressed what is actually happening to the underlying dentine (Garcia-Godoy

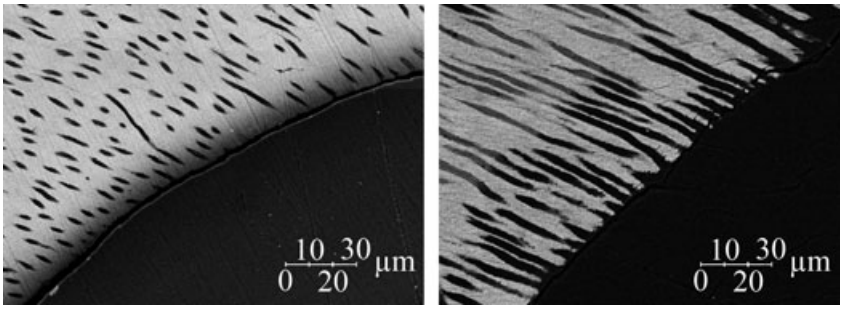

Figure 3 Backscatter images of typical root transsections rinsed with 1% NaOCl throughout instrumentation followed by 2.25% peracetic acid (left) and 1% NaOCl followed by 17% ethylenediaminetetraacetic acid (EDTA) (right). Note how the pattern of the demineralization in the EDTA-treated specimen differs from that in the peracetic acid group.

et al. 2005, Marending *et al.* 2007). To the best of our knowledge, this would be the first investigation to specifically assess the effects of root canal irrigation with decalcifying agents on the appearance of root dentine transsections. Furthermore, the amount and distribution of sclerotic dentine in the studied tooth material was taken into consideration. The results reported here regarding sclerotic dentine in the different root thirds are in line with published observations; tubular sclerosis is most pronounced in the apical area (Vasiliadis *et al.* 1983a). Using a split-root analysis as was done here, the amount of smear layer on root canal walls could be assessed with more certainty compared to conventional investigations that relied solely on the amount of open tubules in a given canal wall area. This was possible because in single-rooted teeth, tubular sclerosis around the main canal is symmetrical (Vasiliadis *et al.* 1983a). Based on the current data, it may be surmised that previous reports on smear layer in the apical root area were prone to a systematic error. In other words, it may not be the case that there is more smear layer in apical areas than in coronal or mid-root counterparts, although this has been reported in most studies.

The backscatter analysis that was performed to screen for apparent decalcifications in root dentine walls can be regarded as sound. Many studies have used a similar methodology to investigate dentinal caries (Angker *et al.* 2004). A backscatter detector identifies high-energy electrons whilst the EDX detector can spot specific elements. As indicated in the Material and methods section, however, EDX analysis takes too long to screen such a large study material. Furthermore, the backscatter images show more contrast than the corresponding EDX micrographs (see Fig. 1) and apparent decalcifications are easier to detect.

The current study was performed in a laboratory environment, and direct clinical conclusions should therefore not be drawn from this report. The main concern regarding the use of any new compound clinically is the question of its potential side effects. Etidronic acid is biocompatible and is used as an additive in various personal care products such as soaps (Licata 1993). It is also used in swimming pools because of its compatibility with hypochlorite to prevent stains from metal ions. However, etidronic acid is a bisphosphonate, and the systemic administration of bisphosphonates has been linked to osteonecroses of the jaws (Migliorati *et al.* 2005). On the other hand, no such reports have appeared related to etidronate, only to other bisphosphonates with a much higher capacity to inhibit osteoclast function (Krueger *et al.* 2007). Peracetic acid is relatively cytotoxic (Sagripanti & Bonifacino 2000). Nevertheless, it is considered to be an alternative to sodium hypochlorite for drinking water disinfection (Marabini *et al.* 2006). Moreover, despite its ubiquitous use in Eastern Europe in the 1980s, no adverse effects have been reported other than the acidic smell that permeated the dental office. In the current study, a 2.25% peracetic acid solution was used, which is probably as caustic as a hypochlorite solution of the same concentration. In the only published clinical study on root canal disinfection with a peracetic acid irrigant, a solution containing 0.4% peracetic acid (1% Wofasteril) was used, based on tests that were previously made on oral mucosa (Kühlfluck & Klammt 1980). Consequently, it may be necessary to investigate the effect of lower concentrations of peracetic acid on the outcome variables studied here. In this study, a protocol with 3 min of peracetic acid treatment was used, based on the observation that this should be enough for the EDTA to dissolve the smear layer (Saito *et al.* 2008). However, low concentrations of peracetic acid could be left in the root canal system for extended times. It has been suggested to leave an iodine solution in the canal system for 10 min as an 'intra-appointment dressing' (Kvist *et al.* 2004). Conceivably, this could be done with peracetic acid. Whilst the current data look promising, more research is necessary to further investigate the possible usefulness of peracetic acid preparations in endodontics. Studies regarding the antimicrobial effectiveness under the specific conditions of the root canal system should be performed to further evaluate irrigation protocols with either etidronic or peracetic acid.

Calcium ppm contained in EDTA and etidronic acid eluates as determined by AAS were comparable to published material (Zehnder *et al.* 2005a,b). Because of the acidity of peracetic acid, the calcium stays in solution and does not reprecipitate (Gubler *et al.* 2008). This could explain that despite the weak chelating power of this agent, similar amounts of calcium were eluted from the root canal as compared to EDTA, which is a much stronger chelator but can only be in solution at a slightly alkaline pH. Apparently, the decalcification kinetics differ between EDTA and acetic acid. The results regarding the mineral profiles after EDTA and peracetic acid treatment presented here are in line with a published report on chemically induced artificial caries in dentine (Kawasaki *et al.* 2000). EDTA left no mineral on the outer half of the lesion, whilst acetic acid produced a more gradual demineralization

pattern. The consequences of these differences on the sealability of root canals with the different types of root filling materials that are on the market remain to be investigated. As has already been shown, the minimal demineralization promoted by the sodium hypochlorite/etidronic acid treatment may result in a better bond of resin-based materials to the canal wall (De-Deus *et al.* 2008a).

Conclusions

- Irrigation protocols employing 1% NaOCl and then 17% EDTA, 1% NaOCl and then 2.25% peracetic acid, or a combined solution containing 1% NaOCl and 9% etidronic acid left similar amounts of smear layer on instrumented root canal walls.
- Three minutes of 17% EDTA caused complete, 3 min of 2.25% peracetic acid a gradual demineralization of the first few micrometers of root dentine that were exposed to the irrigant, whilst a combined NaOCl/etidronic acid irrigant during canal instrumentation and as a final rinse did not decalcify the canal walls.
- Irrigating protocols employing either etidronic or peracetic acid showed potential to replace the conventional treatment with EDTA.

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