

Interaction between EDTA and Sodium Hypochlorite: A Nuclear Magnetic Resonance Analysis

Nicola Maria Grande, DDS,* Gianluca Plotino, DDS,* Alessandro Falanga, DDS,* Massimo Pomponi,[†] and Francesco Somma, MD, DDS*

Abstract

Recent studies detected erosion of the dentinal walls following the use of EDTA as a final flush. Several authors have studied degradation of EDTA and it appears to be caused by an oxidation reaction. The objective of this paper was to verify through nuclear magnetic resonance (NMR) analysis if the oxidizing property of sodium hypochlorite inactivates EDTA. Solutions of sodium hypochlorite and EDTA were analyzed. EDTA tracing and the appearance of new signals indicative of by-products of the reaction, were studied at different time intervals with a NMR analysis. The tracings of NMR analysis confirmed that the reaction between sodium hypochlorite and EDTA lead to a very slow but progressive degradation of this compound. Mindful of the limitations of an in vitro study, the results of this study nevertheless demonstrated that a final flush with sodium hypochlorite cannot limit the chelating effects of EDTA in a clinically realistic time period. (*J Endod* 2006;32:460–464)

Key Words

Cleaning, EDTA, nuclear magnetic resonance (NMR), sodium hypochlorite

From the *Department of Endodontics, [†]Department of Biochemistry and Clinical Biochemistry Catholic University of Sacred Heart, Rome, Italy.

Address requests for reprints to Dr. Gianluca Plotino, Via Eleonora Duse, 22, 00197 Rome, Italy. E-mail address: gplotino@fastwebnet.it.

0099-2399/\$0 - see front matter

Copyright © 2006 by the American Association of Endodontists.

doi:10.1016/j.joen.2005.08.007

A number of studies have shown that mechanical instrumentation alone is not able to reduce the microbial population and leave dentinal surfaces free of a smear layer (1–4). Davis et al. (2) have shown that, even in well-shaped canals, there are remnants of pulpal tissue and inorganic debris, especially in those areas with which the instruments do not come in contact. Peters et al. (3) showed that the instruments regardless of the instrumentation technique never touch 35% or more of the root canal surface. Goldman et al. (4) gave evidence that in canals that were treated without irrigation, the amount of residual tissue was much more than in canals in which irrigants were used.

Several studies have shown that the use of a combination of sodium hypochlorite (2.5-5%) and EDTA (10-17%) is particularly effective in the removal of organic and inorganic debris (5–7). Sodium hypochlorite (NaOCl) is known for its antibacterial properties (8) and its ability to dissolve organic components (9); EDTA is a Ca²⁺ chelating agent and therefore capable of removing the smear layer (1, 4, 7, 10–12). The smear layer adheres to the canal walls and occludes the dentinal tubules (smear plugs). This negates the ability of medications to penetrate into deeper tissues (6–8, 13), and prevents the filling material from optimally adhering to canal walls (14–16). Most authors consider the removal of the smear layer important because it may be infected or it can prevent access to the dentinal tubules, which may contain bacteria and their by-products (1, 6, 10, 17).

EDTA can be used as a final flush to open up the dentinal tubules thus allowing an increasing number of lateral canals to be filled (18). Recent research, however, seems to demonstrate an excessively aggressive effect on the canal walls that could cause too much erosion and degradation of the peri-tubular and inter-tubular dentin (7, 10, 11, 19–21) and subsequently an alteration of its mechanical properties (22, 23). It remains unclear, however, whether the erosion of the parietal dentin and the joining orifices of the dentinal tubules have a negative effects on the long-term success of endodontic therapy (21). It has been suggested that these effects could create more difficulties in the adaptation of the root filling materials to the canal walls (24).

The erosion of the dentinal walls could therefore be a result of the prolonged irrigation with EDTA as well as the fact that it remains active inside the endodontic space even after irrigation has finished. Even though the amount of EDTA that is left inside the root canals at the end of irrigation is minimal and its properties seem to be self-limiting (24, 25), one cannot exclude the possibility that some damage may occur to the tubular dentin. Some studies have shown that until the self-limiting effects take place, the extent of demineralization depends on the length of time that the chelating agents are used (24). To prevent the chelating effects of EDTA to continue to act against the mineralized portion of the radicular dentin, it is important to ensure that its action is fully neutralized before proceeding with obturation. A final flush with sodium hypochlorite has been recommended to rinse out EDTA (6, 26). Moreover, Goldman et al. (5) have shown that when used alone, EDTA removes the inorganic portion and leaves an organic layer intact in the tubules. It has been stated that the alternating use of sodium hypochlorite and a chelating agent during root canal preparation removes also the organic portion of the smear layer (6, 7).

This final rinse with sodium hypochlorite may act to further sterilize the root canal system, as its penetration inside the dentinal tubules is promoted by the complete removal of the smear layer (27). However, it would appear that this effect is limited to the dentinal layer immediately adjacent to the canal lumen. In deeper regions of dentin, this effect is insignificant or highly time dependent (28). Furthermore, given the oxi-

dizing properties of sodium hypochlorite, in addition to rinsing out the chelating solution, it could also reduce its chemical activity. Inactivation of EDTA would appear to be achieved through an oxidation reaction (29), which limits the progressive demineralization and could therefore prevent a further weakening of the inorganic structure of the tooth. The degradation of the EDTA molecule has been studied by several authors (29–32). This process brings about the formation of intermediate by-products such as glioxilic acid and ethylenediaminotriacetic acid, both of which are biocompatible and not very aggressive towards the dentinal components. Neither one seems able to damage the periapical cells in the event of their extrusion from the apex that should nonetheless be studiously avoided (12).

The reducing action of NaOCl on EDTA could influence the combined use of these irrigating solutions during root canal therapy. If degradation of EDTA would be immediate, its effect should be limited sooner.

Nuclear magnetic resonance (NMR) spectroscopy is based on the disturbance of the energy levels of the nuclei influenced by a strong magnetic field. Some nuclei have a certain spin that can be described as the rotation of the nuclei around an axis. A particular nuclear magnetic momentum can be associated to each nucleus. When one of these nuclei is inserted in a homogenous external magnetic field, there is a magnetic interaction between its magnetic momentum and the magnetic field itself. NMR utilizes this interaction to allow the sample to absorb a known wavelength of electromagnetic radiation. The use of NMR facilitates the recording of signals that indicate the progress of the oxidation of the original sample.

The aim of this study was to elucidate the chemical interactions between EDTA and sodium hypochlorite and to analyze the oxidation reaction utilizing NMR.

Materials and Methods

EDTA powder (SIGMA, St. Louis, MO) and 5% sodium hypochlorite solution (OGNA Laboratori Farm, Milan, Italy) were analyzed. All substances used in this study were analytically pure. The test solutions were kept at room temperature and away from direct light at all times. During the experiment the chemical reaction was monitored with reagents mixed inside a NMR tube. Solutions of EDTA (without the addition of NaOCl in the test tube) remained stable for many days under the same experimental conditions of temperature and partial oxygen pressure.

The oxidation process of EDTA was monitored by following the loss of nuclear magnetic signals relative to both the protons (^1H) and the ^{13}C isotopes of EDTA. Optimal conditions to record the NMR signal was obtained utilizing 40 mg of EDTA dissolved in 0.7 ml deuterated water (D_2O). The spectrum of the ^1H -300 MHz proton and the spectrum of ^{13}C -75.5 MHz carbon were obtained using a Gemini spectrometer (Varian SpA, Leini, Torino, Italy).

The calculation of the number of moles (n) in a certain substance was derived using the following formula:

$$n = \frac{g}{Mr} \quad (1)$$

in which g represents the grams and Mr the relative mass of a compound. The number of moles (n) of EDTA in 40 mg is equal to 0.096 mmoles.

This data was important because it allowed the calculation of the quantity of sodium hypochlorite that needed to be added to the EDTA for oxidation to take place. The interaction between sodium hypochlorite and EDTA involves the transfer of electrons (e^-) from one atom to another. The complete oxidation of a molecular group $-\text{CH}_2-\text{COO}^-$

requires 1.5 equivalent molecules of sodium hypochlorite. Because the EDTA molecule is made up of four $-\text{CH}_2-\text{COO}^-$ groups, the relationship would be 1:6 and therefore, to completely inactivate the 0.096 mmoles of EDTA, a quantity of sodium hypochlorite containing a number of mmoles six times greater (0.576 mmoles) needed to be added. Utilizing the same formula as before, it was determined that the number of moles (n) in 0.1 ml of 5% NaOCl would have to be 0.067 mmoles. Therefore, 0.86 ml of 5% NaOCl was added to the 0.7 ml of EDTA to guarantee its complete deactivation, at least on a theoretical basis.

The peaks in the EDTA graphs as well as the emergence of new signals related to the by-products of its interaction with NaOCl were analyzed with NMR at different time intervals, 1.5 min, 4.5 min, 7 min, 11 min, 16 min, 25 min, 50 min, 80 min, 120 min.

Results

The graphs obtained from the NMR analysis confirmed that the reactions between NaOCl and EDTA caused the oxidation of the latter which lead to its progressive deactivation, however, they also showed that this reaction was extremely slow (Fig. 1). The peaks of EDTA and its by-products are detailed in Fig. 2. The kinetics of this oxidation was not immediate, rather under the conditions of this *in vitro* experiment they were observed at the different time intervals at which the peaks were analyzed. It was incomplete, even after 120 min. Table 1 presents both the frequency in Hz [frequency/(300 MHz) = chemical shift = part per million (ppm)] and the intensity (in arbitrary units). It demonstrates that the oxidation of EDTA by sodium hypochlorite is complex and requires time. In addition, the kinetic oxidation of both peak 1 (= $\text{N}-\text{CH}_2-\text{C}(=\text{O})\text{OR}$) and peak 2 (= $\text{N}-\text{CH}_2-\text{CH}_2-\text{N}=\text{}$) progresses at a different rate. This indicates the progressive formation of by-products of the reaction between the two chemicals. The continuous evolution of the signals relative to the EDTA protons indicate that during the first 7 min of the reaction between the two compounds no significant changes in the peaks were recorded, between 7 and 11 min some as a result of the formation of the by-products of the reaction were noted. This increased during the following time intervals 11 to 16, 16 to 25, 25 to 50, and 50 to 80 min (Fig. 1). These signals indicate the progression of the oxidation reaction of the chelating agent (EDTA). At the end of the experiment, the spectra indicated that the reaction between NaOCl and EDTA caused the formation of unknown by-products. Therefore the possible action of the EDTA by-products on both Ca and Mg ions is also unknown.

Discussion

EDTA can potentially modify dentin and therefore its physical and mechanical properties through excessively demineralization of the inorganic portion. This has been demonstrated in various studies by means of images of dentinal walls, which showed noticeable signs of erosion (7, 10, 11, 19–23). Radicular dentinal structures represent the substrate for a root canal filling. The frequency with which different authors have found these alterations as well as the need to ensure a hermetic seal, has lead to the search for solutions, which preserve tooth structure.

In a recent study, Cobankara et al. (33) showed that the presence of the smear layer could negatively influence both coronal and apical leakage of root canal treated teeth obturated with different methods and materials. This could be explained by the fact that complete removal of the smear layer allows sealers to penetrate into the dentinal tubules as recently was demonstrated by Kokkas et al. (16). In this study the authors showed, that when the smear layer was not removed, no penetration of the sealers into the dentinal tubules could be achieved (14, 15). However, it has been hypothesized that excessive demineralization of dentin could create more difficulties in the adaptation of the root-

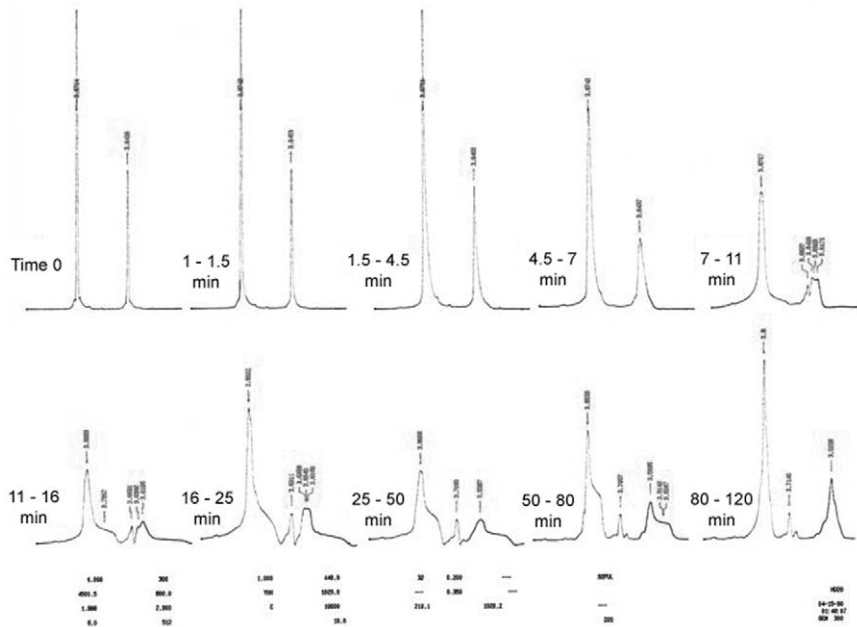


Figure 1. NMR analysis graph of the oxidation reaction occurring between NaOCl and EDTA—at the different time intervals (Time 0, 1.5 min, 4.5 min, 7 min, 11 min, 16 min, 25 min, 50 min, 80 min, 120 min)—in the test tube. The spectrum shows how the signals relative to the EDTA protons change at different times.

filling materials to the canal walls. Park et al. (34) suggested that an increased coronal leakage in samples treated with EDTA compared with those treated with MTAD might be caused by the erosive property of EDTA and the length of dentin exposure to this solution. It has been hypothesized that the effects of EDTA within the canal are a function of contact time (23, 35) because its self-limiting action is known to be completed within 7 h (24). Crumpton et al. (35) have demonstrated that the volume of irrigation does not influence the quality of smear layer removal, since this is a function of contact time. In this study 1 ml of EDTA with a contact time of 1 min was just as effective as 10 ml.

The results obtained in the present study lead to certain questions regarding the combined use of NaOCl and EDTA. The experiment determined that the oxidation of the EDTA molecule is not immediate, but progressive over time. In particular, the use of NMR accurately demonstrated the slow oxidation of the chelating agent. The frequency of the radiation necessary for the absorption of energy depends on both the

type of nucleus (e.g. ¹H or ¹³C) as well as its chemical surrounding. Identical nuclei in different molecules absorb energy differently. This is why the oxidation of EDTA, which involves a change in the molecular structure, can easily be identified with NMR.

The fact that the quantity of NaOCl utilized did not completely oxidize all of the EDTA, even though theoretically it would have been sufficient, may be explained by the fact that in a solution the oxidation follows a statistical criteria. Therefore, it is reasonable to expect to find a certain number of molecules completely oxidized, another not oxidized at all, as well as other possible intermediate combinations. Furthermore, if the oxidation process does not exclusively involve EDTA, but also some of its by-products, then the quantity of molecules actually oxidized reduces even more. Table 1, though indicative of the oxidation

TABLE 1. 300 MHz (D₂O/H₂O) ¹H NMR data for the oxidation of EDTA in the presence of NaOCl. Time vs. observed frequency* and intensity† corresponding to both peak 1‡ and peak 2§||.

| Time (min) | Frequency (Hz) | | Intensity | |
|------------|----------------|---------|-----------|--------|
| | Peak 1 | Peak 2 | Peak 1 | Peak 2 |
| 0 | 1161.72 | 1093.04 | 199.66 | 89.56 |
| 1 | 1162.92 | 1094.41 | 199.66 | 90.65 |
| 1.0–1.5 | 1162.54 | 1093.65 | 199.66 | 89.99 |
| 1.5–2.5 | 1162.27 | 1093.65 | 199.66 | 86.68 |
| 2.5–4.5 | 1162.82 | 1094.14 | 199.66 | 79.20 |
| 4.5–7.0 | 1162.54 | 1093.38 | 128.66 | 43.96 |
| 7.0–11.0 | 1161.50 | 1089.96 | 70.48 | 13.62 |
| 11.0–16.0 | 1160.95 | 1091.88 | 46.87 | 9.39 |
| 16.0–25.0 | 1164.95 | 1089.26 | 64.824 | 15.9 |
| 25.0–50.0 | 1165.98 | 1076.01 | — | — |
| 50.0–80.0 | 1156.28 | 1071.13 | 53.27 | 18.29 |
| 80.0–120.0 | 1147.71 | 1057.06 | 86.91 | 29.04 |

* (Hz; frequency/(300 MHz) = chemical shift)

† (in arbitrary units)

‡ (=N-CH₂-C(=O)OR)

§ (=N-CH₂-CH₂-N=)

||Oxidation started at about 2.5 min (25° C).

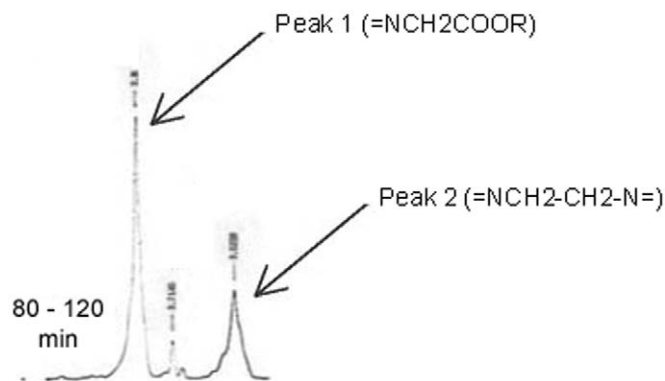


Figure 2. Detail of Fig. 1. The peaks relative to the different chemical products are specified. The smaller peak between peak 1 and peak 2 is because of oxidation of EDTA. The presence in the spectrum of both the decreasing and the widening of EDTA peaks, as well as the emergence of new signals, demonstrate the oxidation progress of the chelating agent.

References

kinetics, does not allow any characterization of the oxidation by-products, and therefore the effect of their action on both the inorganic (Ca and Mg ions) and organic matrix is still unknown.

Of importance to consider is not that deactivation occurs, but that it is extremely slow. This implies that active EDTA is present inside the endodontic space, which action is time dependent and not influenced by volume (35). This may also provide an explanation for the SEM images that show erosion of the dentinal walls and the presence of micro fractures of the intertubular septi.

The results of this study are in agreement with previous investigations in which the effect of the presence of sodium hypochlorite on the chelating ability of EDTA was tested with a calcium titration method, using murexide as an indicator (26) or a Ni-dimethylglyoxime titration method, flame spectrometry and measurements of dentin microhardness (36).

The self-limiting properties of EDTA demonstrate that its action goes on until all of its cationic receptors are saturated with Ca^{2+} ions and therefore demineralization terminates when this equilibrium has been reached (24, 25). Perez et al. (37) observed how the self-limiting effect of EDTA is determined by changes in pH that occur during the demineralization process. Because the pH decreases as the process progresses, both the quantity of the demineralized dentin as well as the dissolved one decrease with time. However, not all authors agree on the self-limiting property of EDTA. For instance Patterson (21) does not come agree with this characteristic finding.

A final flush with NaOCl has been questioned in several studies that have shown that dentinal erosion is more evident when irrigation with EDTA is followed by a final flush with NaOCl (7, 20, 38). This finding suggests that using NaOCl after a flush with EDTA could further increase dentinal erosion. It was hypothesized that this could occur because the factor that limits the dissolution of dentin by EDTA appears to be the organic matrix of the dentin itself as it accumulates on the surface of the canal wall while it gets progressively demineralized, thereby avoiding further dissolution. Morphological findings suggest that EDTA may decalcify peritubular dentin during the early stages of the final irrigation and that the subsequent use of NaOCl dissolves the exposed organic matrix (20, 38). These complementary effects may result in a deeper erosion of the dentinal wall (20). To limit this phenomenon, new irrigating solutions and protocols have been proposed (MTAD) and have been reported in the literature (34, 39). Other authors proposed to reduce the EDTA concentration and the duration of exposure. Nakashima and Terata (40) reported that an alkaline 3% EDTA solution with adjusted pH 9.0 allowed smear layer removal without excessive demineralization and that it had the same effect as 15% EDTA.

Conclusions

Recognizing the inherent limitations of an in vitro experiment, the results of this study suggest that there appears to be no indication for a final flush with NaOCl for the purpose of utilizing its oxidation properties to deactivate the action of EDTA, at least not for time periods that are clinically realistic and acceptable.

Thus, irrigation with NaOCl for the purpose of mechanically rinsing out the EDTA solution does not seem sufficient. It demonstrated an aggressive action with root canal dentin and may accelerate erosion of the radicular substrate, which is needed to facilitate a matrix for the best possible three-dimensional root canal filling.

Acknowledgments

The authors wish to express their gratitude to Dr. Kenneth S. Serota, DDS, and Professor Emeritus Cornelis H. Pameijer, DMD, PhD, for their help in the preparation of this manuscript.

- McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod* 1975;1:238–42.
- Davis SR, Brayton SM, Goldman M. The morphology of the prepared root canal: a study utilizing infectable silicone. *Oral Surg Oral Med Oral Pathol* 1972;34:642–8.
- Peters OA, Schonberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. *Int Endod J* 2001;34:221–30.
- Goldman L, Goldman M, Kronman J, Lin P. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol* 1985;52:197–204.
- Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study. Part 2. *J Endod* 1982;8:487–92.
- Yamada R, Armas A, Goldman M, Lin P. A scanning electron microscopic comparison of a high-volume final flush with several irrigation solutions. Part 3. *J Endod* 1983;9:137–42.
- Baumgartner J, Mader C. A scanning electron microscopic evaluation of four root canal irrigation regimens. *J Endod* 1987;13:147–57.
- Bystrom A, Sunqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985;18:35–40.
- Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *J Endod* 1978;4:60–4.
- Torabinejad M, Handysides R, Ali Khademi A, Bakland LK. Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;94:658–66.
- Hottel T, El-Refai N, Jones J. A comparison of the effects of three chelating agents on the root canals of extracted human teeth. *J Endod* 1999;25:716–7.
- Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indication for their use. *Int Endod J* 2003;36:810–30.
- White R, Goldman M, Lin P. The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. *J Endod* 1984;10:558–62.
- Pallares A, Faus V, Glickman GN. The adaptation of mechanically softened gutta-percha to the canal walls in the presence or absence of smear layer: a scanning electron microscopic study. *Int Endod J* 1995;28:266–9.
- Kouvas V, Liolios E, Vassiliadis L, Parissis-Messimeris S, Boutsoukias A. Influence of smear layer depth of penetration of three endodontic sealers: a SEM study. *Endod Dent Traumatol* 1998;14:191–5.
- Kokkas AB, Boutsoukias A, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. *J Endod* 2004;30:100–2.
- Williams S, Goldman M. Penetrability of the smeared layer by a strain of *Proteus vulgaris*. *J Endod* 1985;11:385–8.
- Villegas JC, Yoshioka T, Kobayashi C, Suda H. Obturation of accessory canals after four different final irrigation regimens. *J Endod* 2002;28:534–6.
- Torabinejad M, Al Khademi A, Babagoli J, et al. A new solution for the removal of the smear layer. *J Endod* 2003;29:170–5.
- Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. *Int Endod J* 2002;35:934–9.
- Patterson S. In vivo and in vitro studies of the effect of the disodium salt of ethylenediamine tetraacetate on human dentine and its endodontic implications. *Oral Surg Oral Med Oral Pathol* 1963;16:83–103.
- Cruz-Filho A, Sousa-Neto M, Saquy P, Pecora J. Evaluation of the effect of EDTAC, CDTA, and EGTA on radicular dentin microhardness. *J Endod* 2001;27:183–4.
- Calt S, Serper A. Time dependent effects of EDTA on dentin structures. *J Endod* 2002;28:17–9.
- Seidberg B, Schilder H. An evaluation of EDTA in endodontics. *Oral Surg Oral Med Oral Pathol* 1974;37:609–20.
- Von der Fehr F, Nygaard-Ostby B. Effect of EDTAC and sulfuric acid on root canal dentine. *Oral Surg Oral Med Oral Pathol* 1963;16:199–205.
- Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. *Int Endod J* 2003;36:411–7.
- Buck R, Eleazer PD, Staat RH. In vitro disinfection of dentinal tubules by various endodontics irrigants. *J Endod* 1999;25:786–8.
- Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. *Int Endod J* 1998;31:8–14.
- Nortemann B. Biodegradation of EDTA. *Appl Microbiol Biotechnol* 1999;51:751–9.
- Henneken L, Nortemann B, Hempel DC. Influence of physiological conditions on EDTA degradation. *Appl Microbiol Biotechnol* 1995;44:190–7.
- Kluner T, Hempel DC, Nortemann B. Metabolism of EDTA and its metal chelates by whole cells and cell-free extracts of strain BNC1. *Appl Microbiol Biotechnol* 1998;49:194–201.
- Nortemann B. Total degradation of EDTA by mixed cultures and a bacterial isolate. *Appl Environ Microbiol* 1992;58:671–6.

33. Cobankara FK, Adanr N, Belli S. Evaluation of the influence of smear layer on the apical and coronal sealing ability of two sealers. *J Endod* 2004;30:406–9.
34. Park DS, Torabinejad M, Shabahang S. The effect of MTAD on the coronal leakage of obturated root canals. *J Endod* 2004;30:890–2.
35. Crumpton BJ, Goodell GG, McClanahan SB. Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. *J Endod* 2005;31:536–8.
36. Saquy PC, Maia Campos G, Sousa Neto MD, Guimaraes LF, Pecora JD. Evaluation of chelating action of EDTA in association with Dakin's solution. *Braz Dent J* 1994;5:65–70.
37. Perez VC, Cardenas MSM, Planells US. The possible role of pH changes during EDTA demineralization of teeth. *Oral Surg Oral Med Oral Pathol* 1989;68:220–2.
38. Apostolopoulos AX, Buonocore MG. Comparative dissolution rates of enamel, dentin and bone. I. Effect of the organic matter. *J Dent Res* 1966;45:1093–100.
39. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. *J Endod* 2003;29:170–5.
40. Nakashima K, Terata R. Effect of pH modified EDTA solution to the properties of dentin. *J Endod* 2005;31:47–9.