Clin Oral Invest (2008) 12:319–324
DOI 10.1007/s00784-008-0197-5

ORIGINAL ARTICLE

Highly concentrated EDTA gel improves cleaning efficiency of root canal preparation in vitro

P. Putzer · L. Hoy · H. Günay

Received: 11 September 2007 / Accepted: 4 March 2008 / Published online: 16 April 2008
© Springer-Verlag 2008

Abstract Debris and smear layer, as a product of mechanical root canal instrumentation, reduce the effectiveness of pharmacological substances to prevent post-treatment diseases and impair direct contact of filling materials with a clean dentinal surface. The aim of this in vitro study was to investigate the presence and localization of debris and smear layer via scanning electron microscope analysis after standardized root canal preparation with different chelating agents. Dentin surfaces received treatment with: (1) 15% ethylenediaminetetraacetic acid (EDTA), (2) 18.6% EDTA (3) and 24% EDTA or without any demineralizing chemicals as control. Forty vertically split human premolars were sputtered and divided into coronal, middle, and apical sections, followed by a randomized, blinded score evaluation using five scores. Pairwise comparisons of all treatment groups against a control group have been performed by Mann–Whitney *U* test and the Kruskal–Wallis test. Debris grades showed no significant difference between the three regions of the root canals, except for 18.6% EDTA in the central third. Smear layer and smear plug removal was concentration-dependent. Removal of the smear layer in the three areas showed that there was a statistically significant difference between all parts when using 18.6% and 24% EDTA concentrations compared with the control. The best smear layer removal in the apical region was observed using a 24% EDTA gel as chelating agent and lubricant. The usage of EDTA gel ≥18.6% presented a better cleaning regime when compared to the control group.

Keywords Root canal preparation · EDTA · Chelating agents · Debris · Smear layer

P. Putzer (✉) · H. Günay
Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School
Carl-Neuberg-Str.1
30625 Hannover, Germany
e-mail: Putzer.Philip@mh-hannover.de

L. Hoy
Institute of Biometrics, Hannover Medical School
Hannover, Germany

Introduction

Chemomechanical cleaning and shaping of root canals is essential to achieve a sufficient disinfection of the infected endodontium. Variations concerning its morphology [13] point out the importance of chemicals to support cleaning of recessus, isthmus, lateral canals, and apical ramifications. Ethylenediaminetetraacetic acid (EDTA) as an additive supports cleanliness of peripulpal dentin by demineralizing the superficial, irregular smear layer and smear plugs. Different types of EDTA preparations have gained popularity because almost all manufacturers of nickel–titanium (NiTi) instruments recommend the use of lubricants during rotary root canal preparation. Horizontal calcifications in the orifice region are often caused by biofilm-induced defects in the marginal region, class-V fillings, or after prosthetic therapy. Obliterated pulp-dentin units are difficult to open, but achieving full working length is obligatory to restore the tooth. In endodontology, EDTA is used to open calcified canals, to eliminate potentially infected smear layer [1, 5], and to reduce possible microleakage [8]. EDTA is a unique molecule that has six potential sites for binding

metal ions. EDTA forms stable complexes with Ca ions, demineralizes the root canal surface, and therefore in some cases simplifies root canal preparation. The ability of EDTA to bind bivalent metals with high affinity is used in different areas of medicine, biochemistry, and chemistry in vivo and in vitro. Dental and bony tissues of animal studies are for instance decalcified in EDTA [34]. In endodontics, time- and dose-dependent effects of EDTA have been reported [17], and after a working time of 24–48 h demineralization of circumpulpal dentin is limited to a distinct deepness of approximately 20–50 μm [26]. Smear layer removal is achieved with different compositions. EDTA preparations in either liquid, paste-type [17], or gel application forms [4] are available. Different instrumentation and application techniques of EDTA preparations as well as irrigation protocols have been described, and an improvement of root canal cleanliness was found in several studies [8, 17, 28, 32, 40], but a complete removal of the smear layer in the apical part of human roots with its ramifications is still a continuous problem.

The purpose of the present in vitro study was to determine the intracanal cleaning efficiency after standardized root canal preparation with 24% EDTA gel.

Materials and methods

Forty caries-free, freshly extracted, single-rooted human premolars with one straight root canal were stored at 4°C in 0.2% chlorhexidine solution prior to our investigations. The teeth had been extracted as a part of routine treatment at the Department of Oral and Maxillofacial Surgery, Hannover Medical School. Patients' consents had been obtained according to the guidelines of the Medical School for the use of human samples in research. Occlusal portions of the premolars were removed to reach a standardized root length of 16 mm with a working length of 15 mm. Apical patency was checked using a #10 K-Flexofile® (Dentsply-Maillefer, Ballaigues, Suisse). Radiographs were taken of each canal to verify its position and anatomy. Teeth were then randomly divided into one control and three test groups, each comprising ten human premolars. Teeth were incubated for 1 h at 37°C (Nunc, Wiesbaden, Germany) before instrumentation, and Mtwo® instruments were used as rotary NiTi instruments (VDW, Munich, Germany). The Mtwo® files were used in a careful brushing working motion. Root canal preparation took place in the hand of the operator, supported by a KS head-worn loupe (Zeiss, Aalen, Germany). The cleaning technique started with an initial opening of the canal orifice using #10 and #15 Mtwo® files. Enlargement and shaping of the root canal was performed with Mtwo® files #20, #25, #30, #35, and #40. Chelator preparations were applied directly to files and were used for rotary canal preparation with each file twice, using FileCare® (FC), File-EZE® (FE), and PrefGel® (PG). Table 1 lists the components, coding, and manufacturers of the chelating agents. Irrigation with 3 ml (NaOCl 2.5%) was performed after use of each instrument using a syringe (Braun, Melsungen, Germany) and #27 gauge needle (Ultradent Products, South Jordan, USA) which was inserted as far as possible into the prepared root without binding. The prepared canals were checked using an M715 stereomicroscope (Leica, Bensheim, Germany) and loaded under the microscope with the EDTA preparations using "skini syringes" and "capillary tips" (Ultradent Products). Complete wetting of the cavity was ensured with a #10 K-Flexofile, and the root was conditioned for 2 min. A final rinsing with 5 ml of physiological saline solution finished instrumentation. The controls were neither prepared with a chelating agent nor filled with a chelator afterwards but were only irrigated with NaOCl and saline solution. The time needed for the procedure was recorded for each sample. Thereafter, teeth were closed with sticky wax and split longitudinally, and coronal, middle, and apical thirds were marked with a length of 5 mm. After that samples were mounted on aluminum plates for scanning electron microscope (SEM) analysis and sputter-coated with Au-Pd (Balzers SCD 004, Oestrich-Winkel, Germany). Fractured root canals were examined using an SEM (S-2300, Hitachi Tokyo, Japan) at a ×30 magnification for debris and ×500 magnification for the smear layer at an impressed voltage of 20 kV. Calibration with reference to the scoring system of the SEM evaluations was performed ahead of the examination. Roots were coded, blinded, and randomized between all experimental groups by the SEM operator before evaluation using a numerical scale and ten pre-

Table 1 Compositions, coding, and manufacturers of EDTA lubricants tested

Materials	Coding	Components	Manufacturer
FileCare®	FC	15% EDTA and 10% urea peroxide in aqueous solution, pH 6.0	VDW
File-Eze®	FE	18.6% EDTA in aqueous solution, pH approximately 10.3	Ultradent Products
Pref-Gel®	PG	24% EDTA and 2.75% carboxymethyl cellulose, pH 6.5–7.2	Biora AB, Malmö, Sweden

selected squares of a grid as described by Hülsmann et al. [15]. The following scheme was used:

Debris (dentine chips, pulp remnants, and particles loosely attached to the canal wall):

Score 1: clean canal wall, only very few debris particles
Score 2: few small conglomerations
Score 3: many conglomerations; less debris than 50% of the canal wall covered
Score 4: more than 50% of the canal wall covered
Score 5: complete or nearly complete covering of the canal wall by debris

Smear layer (dentine particles, remnants of vital or necrotic pulp tissue, bacterial components, and retained irrigant):

Score 1: no smear layer, orifice of dentinal tubules patent
Score 2: small amount of smear layer, some open dentinal tubules
Score 3: homogenous smear layer along almost the entire canal wall, only very few open dentinal tubules
Score 4: the entire root canal wall covered with a homogenous smear layer, no open dentinal tubules
Score 5: a thick, homogenous smear layer covering the entire root canal wall

The data for scoring debris and smear layer were recorded separately and analyzed statistically. All statistical computations were performed with the program SPSS 15 (SPSS, Chicago IL, USA).

Pairwise comparisons of all treatment groups against a control group have been performed by Mann–Whitney *U* test. Additionally, Kruskal–Wallis test has been performed to analyze the total sample.

Results

The standardized technique of root canal cleaning and shaping was applied with an interval of EDTA conditioning, excluding the controls. Time needed to complete the procedure averaged 11 min 47 s (±0.53 s), while the treatment time for the three groups analyzed was between 10 min 30 s and 13 min 50 s.

Cleaning efficiency scores obtained for debris and smear layer removal are shown in Figs. 1 and 2. Scores for debris did not differ significantly between the analyzed groups or the controls, except for the middle area of 18.6% EDTA ($p=0.025$) by means of Kruskal–Wallis test. Similar amounts of debris were detected in all groups depending on the distance between the orifice and the apex. Particles loosely attached to the canal wall persisted in the canal

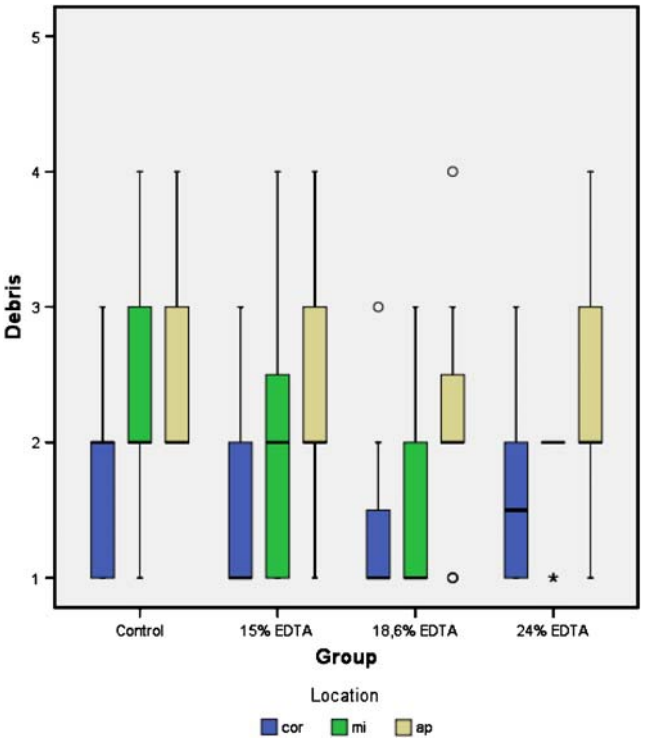

Fig. 1 Box blots of debris removal for the individual canal thirds using lubricants with 15%, 18.6%, and 24% EDTA during and for 2 min after root canal preparation. Irrigation was performed with 2.5% NaOCl, and a final rinse of 5 ml saline solution finished instrumentation

system with no regard to the type of EDTA preparation (Fig. 1). In general, the use of EDTA additives at concentrations of 18.6% and 24% EDTA provided a significantly reduced smear layer ($p<0.001$) by means of Kruskal–Wallis as compared with the controls. FC with 15% EDTA removed the smear layer in the coronal and the middle parts, but removal of the smear layer in the apical third appeared not to be significant ($p>0.05$) by means of Kruskal–Wallis as compared to the controls (Fig. 2). Scores for smear layer decreased continuously with increasing concentrations of EDTA lubricants. Parts of the individual canal thirds were freed from the irregular smear layer, showing clean canal walls when FE and PG were used during and after root canal preparation (Fig. 3).

Discussion

Advancements in cleaning and shaping root canals have played an important role in the development of endodontic therapy. Only one study has reported results of 24% EDTA application in the root canal system [9] and none in combination with Mtwo® files. Mtwo NiTi instruments have been introduced recently, and in vitro studies documented their cyclic fatigue resistance [11] and their preservation of the original canal anatomy [35], and according to a recent in vitro study, these instruments

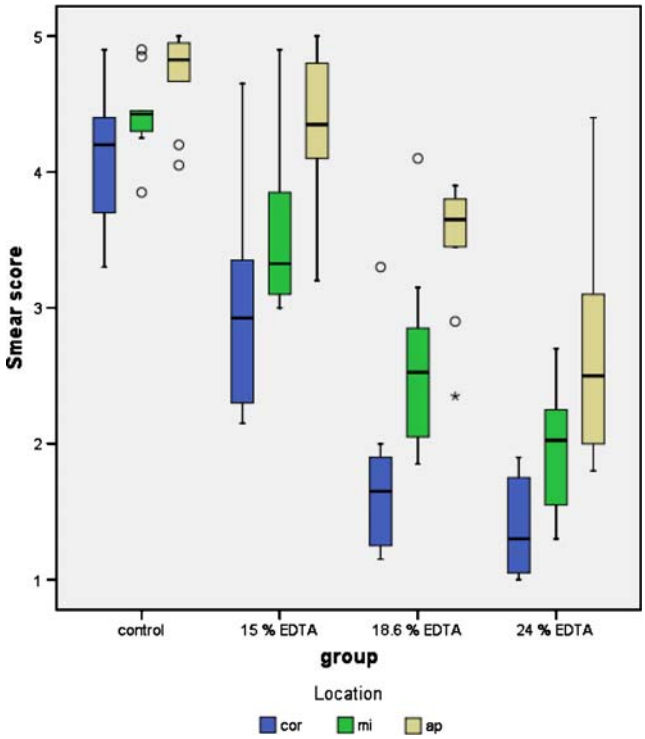

Fig. 2 Box blots of smear layer removal for the individual canal thirds using lubricants with 15%, 18.6%, and 24% EDTA during and for 2 min after root canal preparation. Irrigation was performed with 2.5% NaOCl and a with final flush of 5 ml saline solution

warrant an effective shaping and cleaning of the main root canal [30].

In the present study, the cleaning efficiency of a highly concentrated EDTA preparation was evaluated in comparison to other EDTA lubricants that are commercially available. Debris scores showed no significant differences within the groups analyzed, except for the middle part of roots cleaned with 18.6% EDTA. Sufficient dissolution of the smear layer was achieved in the coronal and middle areas of the roots when using 18.6% and 24% EDTA preparations. All EDTA preparations failed to clean the complete surface of the apical region. FE and PG sufficiently cleaned the coronal and middle areas of the samples, resulting in approximately more than two scores better as compared to the controls. Only slightly better scores of PG as compared to FE were achieved with 18.6% EDTA. The chemomechanical cleaning strategy with 24% EDTA resulted in a small amount of smear layer remaining in the middle part of the canal walls with a mean score (±standard deviation) of 1.97 (±0.45) and a tendency to a very thin homogenous smear layer in the apical third with a mean score of 2.74 (±0.90), respectively. The observation that the cleaning efficiency of the smear layer in the apical region was less than in the middle and coronal thirds is in agreement with other in vitro studies [16, 17, 29, 37, 39]. It has been reported that irrigation with liquid 17% EDTA compared to ultrasonically activated irrigation resulted in the same tendency of smear layer reduction apical < middle < coronal [21]. Similar results of canal cleanliness in the apical, middle, and coronal parts of the dentinal matrix were achieved by Crumpton et al. [7] using different volumes of 1, 3, and 10 ml of liquid 17% EDTA. In a recent study, irrigation with liquid 17% EDTA in comparison to 24% EDTA gel-supported root canal preparation of human canine teeth showed no significant differences in smear layer removal [9].

EDTA lubricants are well documented and commonly used in endodontics [23, 32, 40] to remove the smear layer attached to dentinal matrix and to minimize the risk of instrument fracture [2]. Analysis of demineralizing effects of paste-type 15% EDTA showed a reduction of weight loss and microhardness, but it remains unclear whether this has any clinical significance [16]. A certain improvement of

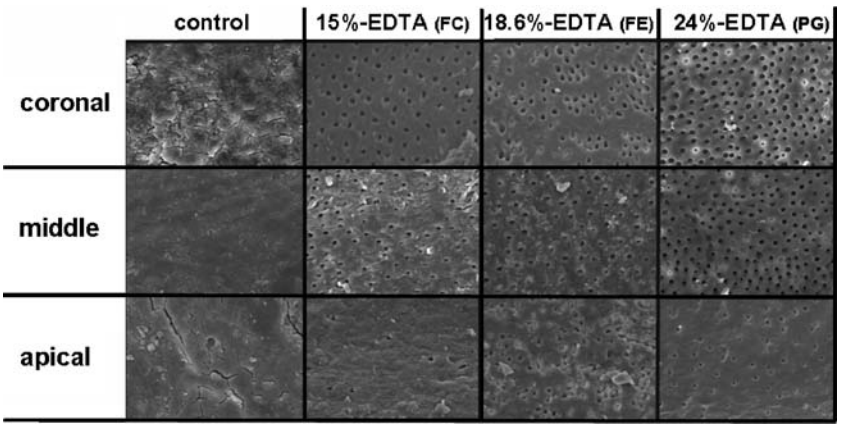

Fig. 3 Smear layer analysis of EDTA lubricants used during and for 2 min after root canal preparation. Effects of the different lubricants, FC, FE, and PG, as well as untreated control canals are shown by examples for the coronal, middle, and apical canal thirds. Magnification ×1000

disinfection via antimicrobial actions against *Enterococcus faecalis* and *Candida albicans* cultures was found [12]. Resistant pathologic microorganisms with high virulence, however, can survive and multiply in ramifications of the apex [24] or in remaining smear layer [38], resulting in post-treatment diseases [25, 38]. Chronic inflammation of the pulp can therefore result in permanent infection of peritubular and intertubular dentin [6]; pain or apical parodontitis are detectable, and retreatment strategies become necessary [22]. EDTA with its moderate antibacterial and fungicide properties [12] in combination with its main function to remove attached smear layer contributes to a better chemomechanical regime [21, 40]. PG is a dentin conditioner based on EDTA as a demineralizing agent in gel condition. The substance is an integral part of regenerative therapy in periodontology. In comparison to other strategies, very good results are achieved when PG is applied on root surfaces that have been cleaned and afterwards treated with emdogain [31]. After instrumentation with EDTA, we left an additional dose of EDTA in the root canal for 2 min such as recommended for PG by the manufacturer.

Contact of EDTA with the periapical tissue cannot be excluded during endodontic instrumentation, but neither liquid nor gel application forms will reach a toxic level in the human body compared to concentrations used in therapy of heavy metal intoxication [36]. The risk of periapical tissue irritation or damage can be reduced by using a material with thixotropic character rather than a liquid substance. Cytotoxic effects were found in vitro in several cell culture studies [18, 20, 28]. On the other hand, 15% EDTA solution showed no clinically relevant damage to human periapical tissues [26], and 24% EDTA is commonly used as a conditioner for root surfaces in periodontology [30]. Several investigations found that EDTA pretreatment leaves the fibrillar structure of dentin unaltered [3, 10, 14, 19]. Intracanal pretreatment with 24% EDTA gel and the application of modern adhesive materials could achieve a linkage with dentinal tubuli or collagen fibrils or form a hybridized smear layer in order to achieve an impermeable apical seal. In a recent study, 0.1 M EDTA improved resin–dentin bond strength of a total-etch adhesive when challenged with 10% NaOCl in comparison to pretreatment with phosphoric acid using a total-etch adhesive or a self-etching adhesive [27]. Since there is still controversy about whether to remove or not remove intracanal smear layer in endodontics [33], further research is necessary to prove the benefit of pharmacologically active substances in restoring non-contaminated tooth structures so as to prevent post-treatment diseases. To achieve a fundamental control of intracanal biofilm, better irrigation regimes have to be established, resulting in smear-free surfaces for a tight seal in the apical region.

Conclusion

Within the limitations of this in vitro investigation, we conclude that:

–All tested EDTA lubricants showed similar cleaning efficiencies regarding debris removal.
–FE and PG presented an improved cleaning efficiency with a significant better dissolution of the smear layer when compared to the control group.

Acknowledgements The authors wish to thank MTA U. Bergmann for her assistance during SEM analysis.

References

1. Aktener BO, Bilkay U (1993) Smear layer removal with different concentrations of EDTA–ethylenediamine mixtures. J Endod 19:228–231
2. Anderson DN, Joyce AP, Roberts S, Runner R (2006) A comparative photoelastic stress analysis of internal root stresses between RC Prep and saline when applied to the Profile/GT rotary instrumentation system. J Endod 32:222–224
3. Blomlof JP, Blomlof LB, Cederlund AL, Hultenby KR, Lindskog SF (1999) A new concept for etching in restorative dentistry? Int J Periodontics Restorative Dent 19:30–35
4. Bóveda C, Fajardo M, Millán B (1999) Root canal treatment of an invaginated maxillary lateral incisor with a C-shaped canal. Quintessence Int 30:707–711
5. Brännstrom M (1984) Smear layer: pathological and treatment considerations. Oper Dent Suppl 3:35–42
6. Bystrom A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 89:321–328
7. Crumpton BJ, Goodell GG, McClanahan SB (2005) Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. J Endod 31:536–538
8. Czonstkowsky M, Wilson EG, Holstein FA (1990) The smear layer in endodontics. Dent Clin North Am 34:13–25
9. Dotto SR, Travassos RM, de Oliveira MP, de Lima Machado E, Martins L (2007) Evaluation of ethylendiamintetraacetic acid (EDTA) solution and gel for smear layer removal. Aust Endod J 33:1–4
10. Fuentes V, Ceballos L, Osorio R, Toledano M, Carvalho RM, Pashley DH (2004) Tensile strength and microhardness of treated human dentin. Dent Mater 20:522–529
11. Grande NM, Plotino G, Pecci R, Bedini R, Malagnino VA, Somma F (2006) Cyclic fatigue resistance and three-dimensional analysis of instruments from two nickel–titanium rotary systems. Int Endod J 39:755–763
12. Grawehr M, Sener B, Waltimo T, Zehnder M (2003) Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J 36:411–417
13. Hannig C, Krieger E, Dullin C, Merten HA, Attin T, Grabbe E, Heidrich G (2007) Volumetry of human molars with flat panel-based volume CT in vitro. Clin Oral Investig 10:253–257
14. Habelitz S, Balooch M, Marshall SJ, Balooch G, Marshall GW Jr. (2002) In situ atomic force microscopy of partially demineralized human dentin collagen fibrils. J Struct Biol 138:227–236
15. Hülsmann M, Rummelin C, Schäfers F (1997) Root canal cleanliness after preparation with different endodontic handpieces

and hand instruments: a comparative SEM investigation. J Endod 23:301–306
16. Hülsmann M, Heckendorff M, Schäfers F (2002) Comparative in-vitro evaluation of three chelator pastes. Int Endod J 35:668–679
17. Hülsmann M, Heckendorff M, Lennon A (2003) Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 36:810–830
18. Koulaouzidou EA, Margelos J, Beltes P, Kortsaris AH (1999) Cytotoxic effects of different concentrations of neutral and alkaline EDTA solutions used as root canal irrigants. J Endod 25:21–23
19. Lindemann RA, Hume WR, Wolcott RB (1985) Dentin permeability and pulpal response to EDTA. J Prosthet Dent 53:341–343
20. Masillamoni CR, Kettering JD, Torabinejad M (1981) The biocompatibility of some root canal medicaments and irrigants. Int Endod J 14(2):115–120
21. Mayer BE, Peters OA, Barbakow F (2002) Effects of rotary instruments and ultrasonic irrigation on debris and smear layer scores: a scanning electron microscopic study. Int Endod J 35 (7):582–589
22. Martos J, Gastal MT, Sommer L, Lund RG, Del-Pino FA, Osinaga PW (2006) Dissolving efficacy of organic solvents on root canal sealers. Clin Oral Investig 10:50–54
23. McComb D, Smith DC (1975) A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1:238–242
24. Nair PN, Sjogren U, Krey G, Kahnberg KE, Sundqvist G (1990) Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 16:580–588
25. Nissan R, Segal H, Pashley D, Stevens R, Trowbridge H (1995) Ability of bacterial endotoxin to diffuse through human dentin. J Endod 21:62–64
26. Nygaard-Østby B (1957) Chelation in root canal therapy: ethylenediaminetetraacetic acid for cleansing and widening of root canals. Odontologisk Tidskrift 65:3–11
27. Osorio R, Erhardt MC, Pimenta LA, Osorio E, Toledano M (2005) EDTA treatment improves resin–dentin bonds' resistance to degradation. J Dent Res 84:736–740
28. Sceiza MF, Daniel RL, Santos EM, Jaeger MM (2001) Cytotoxic effects of 10% citric acid and EDTA–T used as root canal irrigants: an in vitro analysis. J Endod 27:741–743
29. Schäfer E, Vlassis M (2004) Comparative investigation of two rotary nickel–titanium instruments: ProTaper versus RaCe. Part 2. Cleaning effectiveness and shaping ability in severely curved root canals of extracted teeth. Int Endod J 37:239–248
30. Schäfer E, Erler M, Dammaschke T (2006) Comparative study on the shaping ability and cleaning efficiency of rotary Mtwo instruments. Part 2. Cleaning effectiveness and shaping ability in severely curved root canals of extracted teeth. Int Endod J 39:203–212
31. Sculean A, Schwarz F, Becker J, Brecx M (2007) The application of an enamel matrix protein derivative (Emdogain) in regenerative periodontal therapy: a review. Med Princ Pract 16:167–180
32. Serper A, Calt S (2002) The demineralizing effects of EDTA at different concentrations and pH. J Endod 28:501–502
33. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F (2007) Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod 33:96–105
34. Schwarz F, Stratul SL, Herten M, Beck B, Becker J, Sculean A (2006) Effect of an oily calcium hydroxide suspension (Osteoinductal) on healing of intrabony periodontal defects. A pilot study in dogs. Clin Oral Investig 10:29–34
35. Veltri M, Mollo A, Mantovani L, Pini P, Balleri P, Grandini S (2005) A comparative study of Endoflare-Hero Shaper and Mtwo NiTi instruments in the preparation of curved root canals. Int Endod J 38:610–616
36. Wellhöhner H (1997) Allgemeine und systematische Pharmakologie und Toxikologie. Springer, Berlin, Germany
37. Wu MK, Wesselink PR (1995) Efficacy of three techniques in cleaning the apical portion of curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 79:492–496
38. Wu MK, Dummer PM, Wesselink PR (2006) Consequences of and strategies to deal with residual post-treatment root canal infection. Int Endod J 39:343–356
39. Zehnder M, Lehnert B, Schonenberger K, Waltimo T (2003) Spüllösungen und medikamentöse Einlagen in der Endodontie. Schweiz Monatsschr Zahnmed 113:756–763
40. Zehnder M (2006) Root canal irrigants. J Endod 32:389–398

Copyright of Clinical Oral Investigations is the property of Springer Science & Business Media B.V. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.