

Irrigation in endodontics

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IN BRIEF

- Highlights the importance of irrigation in endodontics.
- Provides an overview of solutions used in the irrigation of the root canal.
- Outlines old and new equipment for irrigation.

Irrigation is a key part of successful root canal treatment. It has several important functions, which may vary according to the irrigant used: it reduces friction between the instrument and dentine, improves the cutting effectiveness of the files, dissolves tissue, cools the file and tooth, and furthermore, it has a washing effect and an antimicrobial/antibiofilm effect. Irrigation is also the only way to impact those areas of the root canal wall not touched by mechanical instrumentation. Sodium hypochlorite is the main irrigating solution used to dissolve organic matter and kill microbes effectively. High concentration sodium hypochlorite (NaOCl) has a better effect than 1 and 2% solutions. Ethylenediaminetetraacetic acid (EDTA) is needed as a final rinse to remove the smear layer. Sterile water or saline may be used between these two main irrigants, however, they must not be the only solutions used. The apical root canal imposes a special challenge to irrigation as the balance between safety and effectiveness is particularly important in this area. Different means of delivery are used for root canal irrigation, from traditional syringe-needle delivery to various machine-driven systems, including automatic pumps and sonic or ultrasonic energy.

INTRODUCTION

Irrigation is a key part of successful root canal treatment as it fulfils several important mechanical, chemical and (micro) biological functions. Irrigation is also the only way to impact those areas of the root canal wall that are not touched by mechanical instrumentation. Much of the research on endodontic irrigation has focused on the effect of irrigation on the smear layer.¹⁻⁴ However, smear layer removal can be accomplished relatively easily when correct protocols are followed. A bigger challenge for irrigation may be the areas untouched by the files, such as fins, isthmuses and large lateral canals.⁵ Also, large areas in the oval and flat canals may remain untouched despite careful instrumentation. These areas contain tissue remnants and biofilms that only can be

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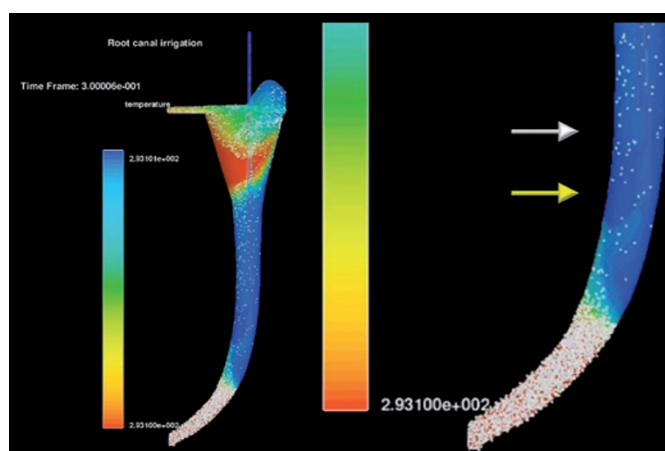


Fig. 1 Particle tracking during irrigation simulated by a computational fluid dynamics model (left), with the high magnification of the root canal (right). The simulation illustrates the weak effect irrigation has on the apical canal. Yellow arrow shows the position of the tip of the irrigation needle, the white arrow shows the place of the side vent

removed by chemical means using irrigation. The apical root canal poses a special challenge to irrigation as the balance between safety and effectiveness is particularly important in this area (Fig. 1).⁶ Negative pressure irrigation was introduced to endodontic treatment several years ago as a safe method to effectively irrigate the most apical canals.^{7,8} Comparative studies on negative pressure and positive pressure irrigation have indicated that the negative pressure method can improve the quality of cleaning of the apical root canal without the risk of extrusion of the solution.^{7,9}

Different means of delivery are used for root canal irrigation, from traditional syringe-needle delivery to various machine-driven systems, including automatic pumps, vibrating tips and sonic or ultrasonic energy.¹⁰⁻¹³ The goal of the various ways to improve irrigation

is to secure optimal spreading of the irrigants throughout the root canal system for more predictable cleaning of the difficult-to-reach areas. Ultrasonic irrigation using ultrasonic tips to deliver the solutions directly into the canal space have shown promising results for cleaning even the most difficult areas such as long and narrow isthmuses between two canals.¹⁴⁻¹⁶

This review is a summary of the present knowledge of effective and safe endodontic irrigation and will include recommendations for optimal irrigation with regard to different solutions, concentration, irrigant sequence and methods of delivery.

GOALS OF IRRIGATION

Irrigation is often regarded as the most important part of endodontic treatment,

in particular for the eradication of root canal microbes. During and following instrumentation, irrigating solutions facilitate the killing and removal of microorganisms, necrotic and inflamed tissue and dentine debris.⁵ Irrigation reduces friction between the instrument and dentine, improves the cutting effectiveness of the files, dissolves tissue, and cools the file and tooth especially during the use of ultrasonic energy. Irrigation may prevent packing of the hard and soft tissue into the apical root canal and extrusion of planktonic and biofilm bacteria out into the periapical tissues.¹⁷ The most important irrigating solutions have tissue-dissolving activity either on organic or inorganic tissue. In addition, several irrigating solutions have antimicrobial activity and actively kill bacteria and yeasts in direct contact with them.¹⁸ However, irrigating solutions show varying degrees of cytotoxicity and sodium hypochlorite may cause severe, immediate and long lasting pain if it is expressed under pressure and then escapes through the apical foramen. In theory, an optimal irrigating solution has the positive characteristics listed in Table 1, but none of the negative or harmful properties mentioned above. Clearly, none of the presently available irrigating solutions can be regarded as optimal, or even close to that. In clinical practice, use of a combination of solutions in a specific sequence is necessary in order to maximally contribute to the success of root canal treatment.⁵

SOLUTIONS USED IN THE IRRIGATION OF THE ROOT CANAL

Sodium hypochlorite (NaOCl) is the most important irrigant in root canal treatment.^{19–21} It is the only presently used solution that can dissolve organic matter in the canal.^{22–24} Therefore the use of hypochlorite is of utmost importance in removing necrotic tissue remnants as well as biofilm. NaOCl ionises in water into sodium (Na^+) and the hypochlorite ions, OCl^- , and establishes an equilibrium with hypochlorous acid (HOCl). At acidic and neutral pH, most of the chlorine exists as HOCl, whereas at pH of nine and above, OCl^- is most abundant.²⁵ Hypochlorous acid has the strongest antibacterial effect while the OCl^- ion is less effective. Hypochloric acid affects directly on the vital functions of the microbial cell, rapidly resulting in cell death.^{26,27}

Hypochlorite is used in concentrations between 0.5–6%.^{28–31} To maximise the effectiveness of hypochlorite irrigation, the solution should be frequently refreshed and kept in motion by agitation or continuous irrigation. The speed of tissue dissolution can be increased with effective agitation and refreshment.^{15,16} While several earlier studies

Table 1 Characteristics of an optimal irrigating solution in root canal treatment

Characteristics
Low cost
Washing action
Reduction of friction
Improving cutting of dentine by the instruments
Temperature control
Dissolution of organic and inorganic matter
Good penetration within the root canal system
Killing of planktonic microbes
Killing of biofilm microbes
Detachment of biofilm
Non-toxic to periapical tissue
Non-allergenic
Does not react with negative consequences with other dental materials
Does not weaken dentin

have reported conflicting results of the comparative effectiveness of hypochlorite at different concentrations, recent studies have confirmed the superiority of high concentration hypochlorite over 1 and 20% solutions.^{32,33} Hypochlorite should be used throughout the instrumentation, as the only solution at this stage, and for one to two minutes after completing the instrumentation. Alternating use of NaOCl and for example, ethylenediaminetetraacetic acid (EDTA) will abolish the antibacterial activity of the NaOCl and should be avoided. According to one recent study, tissue that has been exposed to EDTA is thereafter not effectively dissolved by NaOCl.³⁴ When smear layer removal is completed by EDTA, hypochlorite should not be used again as it causes erosion on dentine after EDTA or citric acid.³⁵ If hypochlorite comes into contact with chlorhexidine, an orange-brown precipitate that contains potentially carcinogenic para-chloroaniline (PCA) is formed.^{36–38} Therefore, the canal should be rinsed, for example, with water or saline, between use of these two solutions.

Sterile water and saline can be used between two irrigating solutions, for example, NaOCl and chlorhexidine, to prevent chemical reactions between them. However, water and saline must not be used as the main irrigants as they have neither tissue-dissolving nor antimicrobial activity.^{39,40} The root canal space will be left with more tissue remnants and bacteria after treatment is completed if NaOCl and EDTA (see below) are not used.

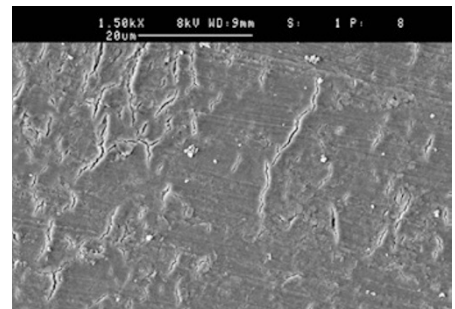


Fig. 2 The smear layer on the wall of the main root canal after instrumentation

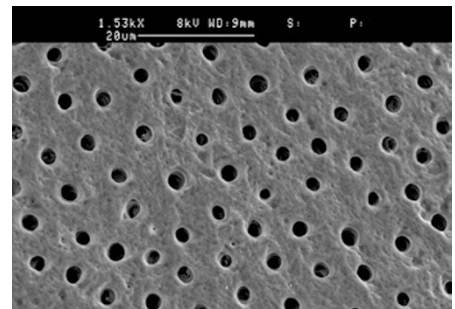


Fig. 3 Instrumented canal wall after removal of the smear layer by NaOCl and a final rinse by EDTA



Fig. 4 The clear zone between the open ended needle tip and the apical blue liquid shows the effect of active irrigation beyond the needle tip during a flow rate of ca. 6 ml/min

EDTA is a chelator, which is used after NaOCl as the final irrigant.^{1–4} EDTA solution is neutral or slightly alkaline; at an acidic pH EDTA precipitates. EDTA is usually used as a 17% or 15% solution, although some studies have suggested that 5% and even 1% EDTA solution is strong enough for smear layer removal. The recommended time for smear layer removal is around two minutes, but thick layers may require longer times of exposure.^{30,41} The smear layer should be removed as it contains microbes

and microbial antigens baked into it during instrumentation of the necrotic, infected root canal (Figs 2 and 3).^{3,42} EDTA only affects the inorganic part of dentine and smear layer (hydroxyapatite) and complete removal of the smear layer can only be achieved when NaOCl has been used before the final rinse with EDTA.^{43,44} EDTA has little or no antimicrobial activity, although some studies have indicated antifungal activity for EDTA.^{45,46} However, EDTA weakens the bacterial cell membrane without killing the cell, but it may work in a synergistic manner with other chemicals, for example, chlorhexidine, which more vigorously attack the bacterial cell wall.⁴⁷ EDTA greatly weakens the effect of NaOCl and should not be used (mixed or alternating) with it. When mixed with chlorhexidine, EDTA forms a white, cloudy precipitate.^{36–38,48}

Citric acid has a long history of use in root canal irrigation. It can be used instead of EDTA as the final rinse to remove the smear layer after use of NaOCl. One to ten percent solutions have been used. Citric acid is somewhat more aggressive than EDTA, and if NaOCl is used after citric acid (not recommended), the root canal wall erosion is more pronounced than in the EDTA–NaOCl sequence.³⁵ Citric acid is used as a component in MTAD and Tetraclean, the combination products for smear layer removal.^{49,50}

Chlorhexidine digluconate (CHX) is used in dentistry for plaque prevention and disinfection because of its good antimicrobial activity.^{51–53} It has also been much used in endodontics as a final irrigant after EDTA. CHX is cytotoxic to human cells but it does not cause pain comparable to NaOCl if accidentally extruded to the periapical area. CHX does not dissolve organic or inorganic matter and therefore it cannot be used as the only irrigating solution. CHX attacks the microbial cell wall or outer membrane resulting in killing of the microbe.²⁵ However, it kills planktonic bacteria much more slowly than NaOCl; against biofilm bacteria its effect is equal to or lower than 1 and 2% NaOCl and much weaker than 5 or 6% NaOCl.^{32,33} CHX binds to hard tissue and remains antimicrobial (substantivity), which has been one reason for its use. However, the potential impact of the continued antimicrobial effect of CHX in the root canal has not been well examined.

Several earlier studies that compared the antibacterial effect of NaOCl and 2% CHX against intracanal infection have shown little or no difference between their antimicrobial effectiveness.^{54–57} However, recent studies using viability staining and more advanced

biofilm models including a dentine biofilm model have shown that 6% NaOCl has a much stronger antibiofilm effect than 2% CHX, which is comparable or weaker than 1 and 2% NaOCl.^{32,33}

Although many bacteria may be killed by CHX, it cannot dissolve the biofilm or other organic debris. Residual organic tissue is likely to weaken the quality of the seal by the permanent root filling, necessitating the use of NaOCl as the main irrigant during instrumentation. On the positive side, CHX as the final rinse after EDTA does not cause erosion of dentine as NaOCl does; therefore 2% CHX may be considered for irrigation after the smear layer is removed.⁵⁸

Much of the research in endodontics on the use of CHX has been done with *Enterococcus faecalis*; it is therefore possible that the studies have given too optimistic a picture of the usefulness of CHX as an antimicrobial agent in endodontics. A recent study suggested that use of CHX as the final rinse may in fact have a negative impact on the healing of apical periodontitis.⁵⁹ More research is needed to identify the optimal irrigation regimen for various types of endodontic treatments.

Combination products for root canal irrigation

In recent years several combination products have been introduced for the irrigation of the root canals. These include sodium hypochlorite mixed with a surfactant (Chlor-Xtra, White King) and EDTA or citric acid products mixed with surfactants and/or antibacterial agents (MTAD, SmearClear, Tetraclean, QMiX).^{5,20,47,49,50,60,61} The role of surfactants in improving the antibacterial or tissue dissolving effect of hypochlorite has been debated in recent years,^{24,62–64} the partly contradictory results may be due to the different types of tissue used for the studies as well as different dilutions of solutions for the experiments.^{24,62} Several studies have shown that the addition of surfactants, which may also have a direct antimicrobial effect, and other antimicrobial agents to EDTA or citric acid adds considerable antimicrobial activity to these solutions.^{32,33} The use of combination products should simplify irrigation and also eliminate the need the use of final NaOCl rinse after EDTA (erosion risk), as the new the smear layer removing 'cocktail' solutions now have a pronounced antimicrobial effect.^{24,32,61}

OLD AND NEW EQUIPMENT FOR IRRIGATION

The classical way of irrigating the root canal is with a syringe and needle. When carefully used, needle irrigation can be

effective and sufficient.^{10,11} Small size 27-gauge or preferably 30-gauge needles should be used to gain access to the apical canal.⁶ Irrigant exchange beyond the needle tip reaches only one to three mm, depending on the needle type and irrigant flow (Fig. 4). Side-vented needles (tip) may offer safer irrigation than open-ended needles in positive pressure irrigation.^{6,65} Agitation of the irrigant and constant refreshment greatly increases the effectiveness of the solutions.²⁴ If the apical canal cannot be easily reached by the irrigation needle, a gutta-percha point in a size corresponding to the dimensions of the apical canal can be used to facilitate irrigant exchange in this region.^{66–68} A recent study²⁴ showed that agitation of the irrigant by active needle irrigation, sonic and ultrasonic activation were equally effective in increasing the speed of tissue dissolution by NaOCl, up to over ten-fold as compared to passive irrigation (no activation or refreshment). This result suggests that movement of the irrigant and refreshment are the key factors in its effectiveness.

New equipment introduced to root canal irrigation includes the EndoActivator⁶⁹, VibriNGe,⁷⁰ and various ultrasonic devices where the irrigant is directed into the canal through the vibrating tip.^{15,16} Several reports have indicated that the various devices may facilitate irrigation, particularly in the difficult-to-reach areas of the canals, such as fins and isthmuses and in large lateral canals.¹⁵ The EndoVac^{71,72} uses negative pressure to achieve safe irrigation of the apical canal. In the EndoVac system the irrigant is applied to the pulp chamber or coronal root canal (teeth with one single root canal) from where it is sucked down the canal and back via the needle. In other words, the direction of the irrigant flow has been reversed, which creates the negative pressure at the apical foramen and thereby prevents the possibility of irrigant extrusion. Some studies have shown improved cleanliness or antimicrobial effect in the most apical canal with the EndoVac as compared to positive pressure irrigation.^{71–73}

CONCLUSION

Instrumentation and irrigation are the most important parts of root canal treatment. Irrigation has several key functions, the most important of which are to dissolve tissue and to have an antimicrobial effect. Apical irrigation poses a special challenge with regard to effectiveness and safety. Small, 30-gauge side-vented needles and/or negative pressure irrigation with NaOCl and EDTA in the apical canal will secure the best results in this important area.

1. Loel D A. Use of acid cleanser in endodontic therapy. *J Am Dent Assoc* 1975; **90**: 148–151.
2. Baumgartner J C, Brown C M, Mader C L, Peters D D, Shulman J D. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. *J Endod* 1984; **10**: 525–531.
3. Baumgartner J C, Mader C L. A scanning electron microscopic evaluation of four root canal irrigation regimens. *J Endod* 1987; **13**: 147–157.
4. Czonstkowsky M, Wilson E G, Holstein F A. The smear layer in endodontics. *Dent Clin North Am* 1990; **34**: 13–25.
5. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. *Dent Clin North Am* 2010; **54**: 291–312.
6. Park E, Shen Y, Khakpour M, Haapasalo M. Apical pressure and extent of irrigant flow beyond the needle tip during positive-pressure irrigation in an *in vitro* root canal model. *J Endod* 2013; **39**: 511–515.
7. Jiang L M, Lak B, Eijsvogels L M, Wesselink P, van der Sluis L W. Comparison of the cleaning efficacy of different final irrigation techniques. *J Endod* 2012; **38**: 838–841.
8. Sarno M U, Sidow S J, Looney S W, Lindsey K W, Niu L N, Tay F R. Canal and isthmus debridement efficacy of the VPro EndoSafe negative-pressure irrigation technique. *J Endod* 2012; **38**: 1631–1634.
9. Howard R K, Kirkpatrick T C, Rutledge R E, Yaccino J M. Comparison of debris removal with three different irrigation techniques. *J Endod* 2011; **37**: 1301–1305.
10. Kahn F H, Rosenberg P A, Gliksberg J. An *in vitro* evaluation of the irrigating characteristics of ultrasonic and subsonic handpieces and irrigating needles and probes. *J Endod* 1995; **21**: 277–280.
11. van der Sluis L W, Gambarini G, Wu M K, Wesselink P R. The influence of volume, type of irrigant and flushing method on removing artificially placed dentine debris from the apical root canal during passive ultrasonic irrigation. *Int Endod J* 2006; **39**: 472–476.
12. Hauser V, Braun A, Frentzen M. Penetration depth of a dye marker into dentine using a novel hydrodynamic system (RinsEndo). *Int Endod J* 2007; **40**: 644–652.
13. Gu L S, Kim J R, Ling J, Choi K K, Pashley D H, Tay F R. Review of contemporary irrigant agitation techniques and devices. *J Endod* 2009; **35**: 791–804.
14. Carr G B, Schwartz R S, Schaudinn C, Gorur A, Costerton J W. Ultrastructural examination of failed molar retreatment with secondary apical periodontitis: an examination of endodontic biofilms in an endodontic retreatment failure. *J Endod* 2009; **35**: 1303–1309.
15. Klyn S L, Kirkpatrick T C, Rutledge R E. *In vitro* comparisons of debris removal of the EndoActivator system, the F file, ultrasonic irrigation, and NaOCl irrigation alone after hand-rotary instrumentation in human mandibular molars. *J Endod* 2010; **36**: 1367–1371.
16. Johnson M, Sidow S J, Looney S W, Lindsey K, Niu L N, Tay F R. Canal and isthmus debridement efficacy using a sonic irrigation technique in a closed-canal system. *J Endod* 2012; **38**: 1265–1268.
17. Park E, Shen Y, Haapasalo M. Irrigation of the apical root canal. *Endodontic Topics* 2012; **27**: 54–73.
18. Basrani B, Haapasalo M. Update on endodontic irrigating solutions. *Endodontic Topics* 2012; **27**: 74–102.
19. Siqueira JF Jr, Rôças I N, Favieri A, Lima K C. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. *J Endod* 2000; **26**: 331–334.
20. Dunavant T R, Regan J D, Glickman G N, Solomon E S, Honeyman A L. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. *J Endod* 2006; **32**: 527–531.
21. Williamson A E, Cardon J W, Drake D R. Antimicrobial susceptibility of monocluster biofilms of a clinical isolate of *Enterococcus faecalis*. *J Endod* 2009; **35**: 95–97.
22. Beltz R E, Torabinejad M, Poursmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. *J Endod* 2003; **29**: 334–337.
23. Cobankara F K, Ozkan H B, Terlemez A. Comparison of organic tissue dissolution capacities of sodium hypochlorite and chlorine dioxide. *J Endod* 2010; **36**: 272–274.
24. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *J Endod* 2010; **36**: 1558–1562.
25. McDonnell G, Russell D. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999; **12**: 147–179.
26. Barrette WC Jr, Hannum D M, Wheeler W D *et al*. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry* 1989; **28**: 9172–9178.
27. McKenna S M, Davies K J. The inhibition of bacterial growth by hypochlorous acid. *Biochem J* 1988; **254**: 685–692.
28. Harrison J W, Hand R E. The effect of dilution and organic matter on the anti-bacterial property of 5.25% sodium hypochlorite. *J Endod* 1981; **7**: 128–132.
29. Gomes B P, Ferraz C C, Vianna M E, Berber V B, Teixeira F B, Souza-Filho F J. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J* 2001; **34**: 424–428.
30. Zehnder M. Root canal irrigants. *J Endod* 2006; **32**: 389–398.
31. Clegg M S, Vertucci F J, Walker C, Belanger M, Britto L R. The effect of exposure to irrigant solutions on apical dentin biofilms *in vitro*. *J Endod* 2006; **32**: 434–437.
32. Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. *J Endod* 2011; **37**: 1380–1385.
33. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. *J Endod* 2012; **38**: 1376–1379.
34. Spangberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surg Oral Med Oral Pathol* 1973; **36**: 856–871.
35. Qian W, Shen Y, Haapasalo M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. *J Endod* 2011; **37**: 1437–1441.
36. Marchesan M A, Pasternak Junior B, Afonso M M, Sousa-Neto M D, Paschoalato C. Chemical analysis of the flocculate formed by the association of sodium hypochlorite and chlorhexidine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103**: 103–105.
37. Basrani B R, Manek S, Sodhi R N, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. *J Endod* 2007; **33**: 966–969.
38. Basrani B R, Manek S, Fillery E. Using diazotization to characterize the effect of heat or sodium hypochlorite on 2.0% chlorhexidine. *J Endod* 2009; **35**: 1296–1299.
39. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol* 1983; **55**: 307–312.
40. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985; **18**: 35–40.
41. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. *Int Endod J* 2003; **36**: 810–830.
42. Yamada R S, Armas A, Goldman M, Lin P S. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: part 3. *J Endod* 1983; **9**: 137–142.
43. Goldman M, Goldman L K B, Cavalieri R, Bogis J, Sun Lin P. The efficacy of several endodontic irrigating solutions: part 2. *J Endod* 1982; **8**: 487–492.
44. Haapasalo M, Qian W, Shen Y. Irrigation: beyond the smear layer. *Endodontic Topics* 2012; **27**: 35–53.
45. Sen B H, Akdeniz B G, Denizci A A. The effect of ethylenediamine-tetraacetic acid on *Candida albicans*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; **90**: 651–655.
46. Ates M, Akdeniz B G, Sen B H. The effect of calcium chelating or binding agents on *Candida albicans*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; **100**: 626–630.
47. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. *Int Endod J* 2012; **45**: 363–371.
48. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. *J Endod* 2005; **31**: 817–820.
49. Torabinejad M, Khademi A A, Babagoli J *et al*. A new solution for the removal of the smear layer. *J Endod* 2003; **29**: 170–175.
50. Torabinejad M, Cho Y, Khademi A A, Bakland L K, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *J Endod* 2003; **29**: 233–239.
51. Shaker L A, Dancer B N, Russell A D *et al*. Emergence and development of chlorhexidine resistance during sporulation of *Bacillus subtilis* 168. *FEMS Microbiol Lett* 1988; **51**: 73–76.
52. Russell A D, Day M J. Antibacterial activity of chlorhexidine. *J Hosp Infect* 1993; **25**: 229–238.
53. Russell A D. Activity of biocides against mycobacteria. *Soc Appl Bacteriol Symp Ser* 1996; **25**: 87S–101S.
54. Vahdaty A, Pitt Ford T R, Wilson R F. Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. *Endod Dent Traumatol* 1993; **9**: 243–248.
55. Jeansonne M J, White R R. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod* 1994; **20**: 276–278.
56. Helling I, Chandler N P. Antimicrobial effect of irrigant combinations within dentinal tubules. *Int Endod J* 1998; **31**: 8–14.
57. Buck R A, Eleazer P D, Staat R H, Scheetz J P. Effectiveness of three endodontic irrigants at various tubular depths in human dentin. *J Endod* 2001; **27**: 206–208.
58. Zamany A, Safavi K, Spangberg L S. The effect of chlorhexidine as an endodontic disinfectant. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; **96**: 578–581.
59. Ng Y L, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. *Int Endod J* 2011; **44**: 583–609.
60. Pappen F G, Shen Y, Qian W, Leonardo M R, Giardino L, Haapasalo M. *In vitro* antibacterial action of Tetraclean, MTAD and five experimental irrigation solutions. *Int Endod J* 2010; **43**: 528–535.
61. Dai L, Khechen K, Khan S *et al*. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. *J Endod* 2011; **37**: 80–84.
62. Clarkson R M, Kidd B, Evans G E, Moule A J. The effect of surfactant on the dissolution of porcine pulpal tissue by sodium hypochlorite solutions. *J Endod* 2012; **38**: 1257–1260.
63. Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L. Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. *Int Endod J* 2012; **45**: 129–135.
64. Rossi-Fedele G, Prichard J W, Steier L, de Figueiredo J A. The effect of surface tension reduction on the clinical performance of sodium hypochlorite in endodontics. *Int Endod J* 2013; **46**: 492–498.
65. Shen Y, Qian W, Chung C, Olsen I, Haapasalo M. Evaluation of the effect of two chlorhexidine preparations on biofilm bacteria *in vitro*: a three-dimensional quantitative analysis. *J Endod* 2009; **35**: 981–985.
66. Wiggins S, Ottino J M. Foundations of chaotic mixing. *Philos Trans A Math Phys Eng Sci* 2004; **362**: 937–970.
67. McGill S, Gulabivala K, Mordan N, Ng Y L. The efficacy of dynamic irrigation using a commercially available system (RinsEndo) determined by removal of a collagen 'bio-molecular film' from an *ex vivo* model. *Int Endod J* 2008; **41**: 602–608.

68. Huang T Y, Gulabivala K, Ng Y L. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. *Int Endod J* 2008; **41**: 60–71.
69. Mancini M, Cerroni L, Iorio L, Armellini E, Conte G, Cianconi L. Smear layer removal and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and Passive Ultrasonic Irrigation): Field emission scanning electron microscopic evaluation in an *in vitro* study. *J Endod* 2013; **39**: 1456–1460.
70. Bolles J A, He J, Svoboda K K, Schneiderman E, Glickman G N. Comparison of Vibriinge, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. *J Endod* 2013; **39**: 708–711.
71. Nielsen B A, Baumgartner C J. Comparison of the EndoVac system to needle irrigation of root canals. *J Endod* 2007; **33**: 611–615.
72. Schoeffel G J. The EndoVac method of endodontic irrigation: part 2—efficacy. *Dent Today* 2008; **27**: 82, 84, 86–87.
73. Malentacca A, Uccioli U, Zangari D, Lajolo C, Fabiani C. Efficacy and safety of various active irrigation devices when used with either positive or negative pressure: an *in vitro* study. *J Endod* 2012; **38**: 1622–1626.