In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*

B. P. F. A. Gomes, C. C. R. Ferraz, M. E. Vianna, V. B. Berber, F. B. Teixeira & F. J. Souza-Filho
Endodontic Unit, School of Dentistry of Piracicaba, State University of Campinas-UNICAMP, Piracicaba – SP, Brazil

Abstract

Gomes BPFA, Ferraz CCR, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *International Endodontic Journal*, **34**, 424–428, 2001.

Aim The aim of this study was to assess, *in vitro*, the effectiveness of several concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%) and two forms of chlorhexidine gluconate (gel and liquid) in three concentrations (0.2%, 1% and 2%) in the elimination of *E. faecalis*.

Methodology A broth dilution test using 24-well cell culture plates was performed and the time taken for the irrigants to kill bacterial cells was recorded. Isolated 24 h colonies of pure cultures of *E. faecalis* grown on 10% sheep blood plus Brain Heart Infusion (BHI) agar plates were suspended in sterile 0.85% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale. One mL of each tested substance was placed on the bottom of wells of 24-well cell culture plates (Corning, NY), including the control group (sterile saline). Six wells were used for each time period and irrigant concentration. Two mL of the bacterial suspension were ultrasonically mixed for 10 s with the irrigants and placed in contact with them for 10, 30, and 45 s; 1, 3, 5, 10, 20, and 30 min; and 1 and 2 h. After each period of time, 1 mL from each well was transferred to tubes containing 2 mL of freshly prepared BHI + neutralizers in order to prevent a residual action of the irrigants. All tubes were incubated at 37°C for 7 days. The tubes considered to have positive growth were those which presented medium turbidity during the incubation period. Data were analysed statistically by the Kruskal–Wallis test, with the level of significance set at $P < 0.05$.

Results All irrigants were effective in killing *E. faecalis*, but at different times. Chlorhexidine in the liquid form at all concentrations tested (0.2%, 1% and 2%) and NaOCl (5.25%) were the most effective irrigants. However, the time required by 0.2% chlorhexidine liquid and 2% chlorhexidine gel to promote negative cultures was only 30 s and 1 min, respectively.

Conclusions Even though all tested irrigants possessed antibacterial activity, the time required to eliminate *E. faecalis* depended on the concentration and type of irrigant used.

Keywords: antimicrobial activity, chlorhexidine, endodontics, sodium hypochlorite.

Received 2 February 2000; accepted 18 September 2000

Introduction

Complete debridement and disinfection of the pulpal space are considered to be essential for predictable long-term success in endodontic treatment. Residual pulpal tissue, bacteria, and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation (Abou-Rass & Piccinino 1982). Therefore, several irrigant solutions have been recommended for use in combination with canal preparation. However, the efficacy of these procedures also depends upon the vulnerability of the involved species. Anaerobic bacteria, especially black-pigmented Gram-negatives, have been linked

Correspondence: Dr Brenda P. F. A. Gomes, Faculdade de Odontologia de Piracicaba – UNICAMP, Endodontia, Avenida Limeira, 901 – Piracicaba -SP, 13414–018 – Brazil (fax: +0055 19 430 5218; e-mail: bpgomes@fop.unicamp.br).

to the signs and symptoms of endodontic disease (Gomes *et al.* 1996a) but facultative bacteria, such as *Enterococcus faecalis*, have also been isolated from pathologically involved root canals, being considered one of the most resistant species in the oral cavity and a possible cause of failure of root canal treatment (Gomes *et al.* 1996b).

An irrigant serves to flush out debris from within the instrumented root canals, dissolve organic tissue remnants, disinfect the root canal space and provide lubrication during instrumentation, without causing irritation to biological tissues (Cheung & Stock 1993, Ingle *et al.* 1994). Sodium hypochlorite (NaOCl) has become the most popular agent for endodontic irrigation, even though its optimum working concentration has not been universally agreed (Cheung & Stock 1993). Chlorhexidine gluconate, a less malodorous and toxic agent, has been suggested as an irrigant based on its antibacterial effects, substantivity and lower cytotoxicity than NaOCl, whilst demonstrating efficient clinical performance (Leonardo *et al.* 1999).

The purpose of this study was to assess *in vitro* the effectiveness of NaOCl and two forms of chlorhexidine gluconate (liquid and gel) at different concentrations in the elimination of *E. faecalis.*

Materials and methods

The irrigants tested in the elimination of *E. faecalis* were several concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%) and two forms of chlorhexidine gluconate (gel and liquid) in three concentrations (0.2%, 1% and 2%). All irrigants were prepared by the same manufacturer (Drogal Farmácia de Manipulação Ltda., Piracicaba, Brazil). NaOCl and chlorhexidine liquid at different concentrations were diluted with sterile water without preservatives. Chlorhexidine gel consisted of gel base (1% natrosol) and chlorhexidine gluconate.

Isolated 24 h colonies of pure cultures of *E. faecalis* (ATCC 29212) grown on 10% sheep blood plus Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) agar plates were suspended in sterile 0.85% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale (1.5×10^8 cfu mL^{-1}).

One mL of each tested substance was placed on the bottom of wells of 24-well cell culture plates (Corning, NY, USA, ref. no. 3524, well Vol. 3.2 mL), including the control group (sterile saline). Six wells were used for each time period and irrigant concentration (i.e. from each well, only one time period and irrigant were tested). Overall, 792 wells were used, comprising 726 for all the test irrigants and 66 for the control group. Two mL of the bacterial suspension were ultrasonically mixed for 10 s with the irrigants and placed in contact with them for 10, 30, and 45 s; 1, 3, 5, 10, 20, and 30 min; and 1 and 2 h. After each period of time, 1 mL from each well was transferred to tubes containing 2 mL of freshly prepared BHI + neutralizers in order to prevent continued action of the irrigants. The neutralizer for NaOCl was 0.6% sodium thiosulphate, whilst 0.5% Tween 80 + 0.07% lecithin was used for chlorhexidine (Siqueira *et al.* 1998b). All tubes were incubated at 37 °C for 7 days. The tubes considered to have positive bacterial growth were those which presented medium turbidity matching the turbidity of a McFarland 4 scale (12×10^8 cfu mL^{-1}) during the incubation period. The purity of the positive cultures was confirmed by Gram staining, by colony morphology on BHI agar + blood and by the use of a biochemical identification kit (API 20 Strep – bioMéuriex SA, Marcy-l'Etoile, France). The results were analysed statistically by the Kruskal–Wallis test, with the level of significance set at $P < 0.05$.

Results

Table 1 shows the contact time required by each tested irrigant to produce 100% of inhibition growth of *E. faecalis*.

All irrigants were effective in killing the bacteria tested, but at different times. Chlorhexidine gluconate liquid (1% and 2%) and NaOCl (5.25%) took significantly less time (>30 s) to eliminate *E. faecalis* than the other irrigants tested. However, the time required by chlorhexidine liquid (0.2%) and chlorhexidine gel (2%) to produce negative cultures was only 30 s and 1 min, respectively.

When used at 0.2% concentration, chlorhexidine gel destroyed bacterial cells after 2 h of contact, as opposed to only 15 min when used at 1.0% concentration.

Table 1 Contact time required by each tested irrigant to produce negative cultures (i.e. 100% inhibition growth) of *E. faecalis*

Irrigants	Contact time
0.2% chlorhexidine gel	2 h
1.0% chlorhexidine gel	15 min
2.0% chlorhexidine gel	1 min
0.2% chlorhexidine liquid	30 s
1.0% chlorhexidine liquid	< 30 s
2.0% chlorhexidine liquid	< 30 s
0.5% sodium hypochlorite	30 min
1.0% sodium hypochlorite	20 min
2.5% sodium hypochlorite	10 min
4.0% sodium hypochlorite	5 min
5.25% sodium hypochlorite	< 30 s

[a]Number of tests performed using the irrigants: 726; number of tests performed using sterile saline (control group): 66.

The antimicrobial activity of NaOCl was related to its concentration, i.e. higher concentrations took less time to inhibit bacterial growth than lower concentrations.

All specimens in the control group yielded positive cultures and *E. faecalis* was always recovered from all positive cultures.

Discussion

Laboratory tests of any kind are only the first steps in a study of the effectiveness of irrigants. Antimicrobial activity of an *in vitro* environment depends upon the pH of the substrates in plates or tubes, sensitivity of the drug, bacterial source (wild strains or collection species), the number of bacteria inoculated, incubation time, and the metabolic activity of the microorganisms. On the other hand, the duration of effectiveness of the drug, temperature, contamination and possible leakage of the agent into the mouth must be considered whilst working *in vivo* (Updegraff & Chaug 1977, Ayhan *et al.* 1999).

Enterococcus faecalis, a facultatively anaerobic Gram-positive coccus, has been implicated in persistent root canal infections (Engström 1964, Cavalleri *et al.* 1989, Gomes *et al.* 1996b, Molander *et al.* 1998) and has been used in several previous studies on the efficacy of endodontic irrigants (Shih *et al.* 1970, Parsons *et al.* 1980, Vahdaty *et al.* 1993, Siqueira *et al.* 1997, Heling & Chandler 1998, Siqueira *et al.* 1998a, Ayhan *et al.* 1999), especially for its high level of resistance to a wide range of antimicrobial agents (Heath *et al.* 1996). In the present study we used ATCC strain because it was also utilized in previous *in vitro* studies investigating the antibacterial effects of endodontic irrigants (Siqueira *et al.* 1997, 1998a, Gomes *et al.* 1999, Ferraz *et al.*, in press).

Sodium hypochlorite solution is, to date, the most commonly employed root canal irrigant. However, no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25%. Kozol *et al.* (1988) evaluated the toxic effects of NaOCl and observed that 0.025% was a safe concentration for clinical use, maintaining the antimicrobial action without harmful effects on the periapical tissues. Many studies (Spångberg *et al.* 1973, Byström & Sundqvist 1983) recommend its use at the concentration of 0.5% in order to obtain acceptable cytotoxic and bactericidal levels. According to a standard undergraduate text book (Harty 1990), 2.5% is the most favoured concentration.

NaOCl provides good tissue solvent action (Grossman & Meiman 1941, Senia *et al.* 1975, Moorer & Wesselink 1982), has a broad spectrum of antimicrobial activity (Spångberg *et al.* 1973, Byström & Sundqvist 1983, Jeansonne & White 1994, Barnard *et al.* 1996, Siqueira *et al.* 1998a, Ayhan *et al.* 1999), acts as a lubricant for instrumentation and can flush loose debris from root canals (Abou-Rass & Piccinino 1982, Baumgartner & Mader 1987, Baumgartner & Cuenin 1992). The major disadvantages of NaOCl are its cytotoxic effect if injected into the periapical tissues (Spångberg *et al.* 1973, Pashley *et al.* 1985, Spångberg *et al.* 1988), its foul smell and taste, its ability to bleach clothes, and its potential for causing corrosion (Neal *et al.* 1983, Busslinger *et al.* 1998). It is also known to produce allergic reactions (Kaufman & Keila 1989).

Exactly how NaOCl destroys microorganisms has never been demonstrated experimentally. The disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution. HClO exerts its germicidal effect by an oxidative action on sulphydryl groups of bacterial enzymes. As essential enzymes are inhibited, important metabolic reactions are disrupted, resulting in the killing of the bacterial cells (Dychdala 1991). However, microorganisms such as *E. faecalis* are resistant to NaOCl, especially at low concentrations (Baumgartner & Cuenin 1992, Heling & Chandler 1998, Ayhan *et al.* 1999). For instance, 0.5% NaOCl took 30 min to destroy bacterial cells, as demonstrated in the present work. On the other hand, the use of NaOCl at high concentrations is undesirable because it is an irritant to periapical tissues (Spångberg *et al.* 1973), even though its antimicrobial action is proportional to its concentration.

The present results showed that 5.25% was the most efficient concentration of NaOCl assessed, killing the bacterial cells in less than 30 s, in agreement with previous studies (Senia *et al.* 1975).

Chlorhexidine gluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At low chlorhexidine concentrations, small molecular weight substances will leak out, especifically potassium and phosphorous, resulting in a bacteriostatic effect. At higher concentrations, chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking (Fardal & Turnbull 1986).

Chlorhexidine gluconate has been used in endodontics as an irrigant solution, but always in a liquid presentation. Chlorhexidine gel was evaluated as an intracanal medication, demonstrating good performance (Siqueira & Uzeda 1997). The natrosol gel (hydroxyethyl cellulose, pH 5.5) used as a base for chlorhexidine gluconate is soluble in water and widely used to thicken shampoos, gels and soaps. The gel formulation may keep the 'active

principle' of chlorhexidine gluconate in contact with the microorganisms longer, inhibiting their growth.

In a previous study (Gomes *et al.* 1999) that tested the antimicrobial activity of several irrigants, including chlorhexidine gluconate (in gel and solution) and different concentrations of NaOCl against selected endodontic microorganisms, by means of the agar diffusion method, chlorhexidine gel was more efficient than the liquid presentation at equivalent concentrations, although no significant difference was detected. In addition, the growth inhibition haloes produced by both forms of 2% chlorhexidine were significantly larger than those created by all concentrations of NaOCl, including 5.25%. All microbial species tested in that study were sensitive to chlorhexidine gluconate, either in gel or in solution, at both tested concentrations. Chlorhexidine demonstrated a broad spectrum of antimicrobial action, in agreement with the results of previous studies (Delany *et al.* 1982, Ringel *et al.* 1982, Jeansonne & White 1994).

The results of the present study confirm those obtained by Ohara *et al.* (1993), although these investigators only used chlorhexidine in a liquid formulation. In both experiments, the antimicrobial activity of chlorhexidine gluconate was superior to that of NaOCl, except for 0.2% chlorhexidine gluconate gel, which took almost 2 h to produce negative cultures. The present investigation tested the antimicrobial activity of chlorhexidine and NaOCl through direct contact with *E. faecalis* suspension and showed that chlorhexidine liquid killed the bacterial cells more rapidly than chlorhexidine gel; this is in contrast to our previous work (Gomes *et al.* 1999). This fact can be explained by the methodologies used; in this study, chlorhexidine liquid mixed very well with the bacterial suspension, immediately exerting its antimicrobial action, whereas the gel formulation, which is more difficult to mix, prevented direct contact between bacterial cells and chlorhexidine, thus requiring a longer time to act against the microorganisms. In the agar diffusion method, the gel formulation kept the active agent in contact with the inoculated media for a longer time, producing the largest inhibition zones. However, during root canal preparation the antimicrobial irrigant used should also act as lubricant, remove the smear layer, be water-soluble, be biocompatible with periapical tissues, and have contact with the microorganisms. A gel formulation has all these advantages (Ferraz *et al.*, in press).

Conclusions

The time required to eliminate *E. faecalis* depended on the concentration and type of irrigant used. The present study confirmed the antimicrobial activity of chlorhexidine and sodium hypochlorite, and also provided new data on the properties of chlorhexidine gel as an endodontic irrigant. Studies using chlorhexidine gel are indicated, especially with respect to its mechanical properties. Further studies involving wild and collection strains, not only of *E. faecalis* but also of other endodontically related bacteria, might be informative. It should be emphasized that, as with most *in vitro* studies, the present findings remain to be confirmed clinically.

Acknowledgements

We thank Dr M. Campbell, University of Manchester, UK, for invaluable statistical help. This work was supported by Brazilian grants from FAPESP (no. 1996/5584–3), CNPq (520277/99–6 & 104238/99–2) and FAEP (1127/97 & 1127/99).

References

Abou-Rass M, Piccinino MV (1982) The effectiveness of four clinical irrigation methods on the removal of root canal debris. *Oral Surgery, Oral Medicine and Oral Pathology* **54**, 323–8.

Ayhan H, Sultan N, Çirak M, Ruhi MZ, Bodur H (1999) Antimicrobial effects of various endodontic irrigants on selected microorganisms. *International Endodontic Journal* **32**, 99–102.

Barnard D, Davies J, Figdor D (1996) Susceptibility of *Actinomyces israelii* to antibiotics, sodium hypochlorite and calcium hydroxide. *International Endodontic Journal* **29**, 320–6.

Baumgartner JC, Cuenin PR (1992) Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *Journal of Endodontics* **18**, 605–12.

Baumgartner JC, Mader CL (1987) A scanning electron microscopic evaluation of four root canal irrigation regimens. *Journal of Endodontics* **4**, 147–57.

Busslinger A, Sener B, Barbakow F (1998) Effects of sodium hypochlorite on nickel–titanium Lightspeed® instruments. *International Endodontic Journal* **31**, 290–4.

Byström A, Sundqvist G (1983) Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surgery, Oral Medicine and Oral Pathology* **55**, 307–12.

Cavalleri G, Cuzzolin L, Urbani G, Benoni G (1989) Root canal microflora: qualitative changes after endodontic instrumentation. *Journal of Chemotherapy* **1**, 101–2.

Cheung GSP, Stock CJR (1993) *In vitro* cleaning ability of root canal irrigants with and without endosonics. *International Endodontic Journal* **26**, 334–43.

Delany GM, Patterson SS, Miller MS, Newton CW (1982) The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. *Oral Surgery, Oral Medicine and Oral Pathology* **53**, 518–23.

Dychdala GR (1991) Chlorine and chlorine compounds. In: Block SS, ed. *Disinfection, Sterilization, and Preservation*, 4th edn. Philadelphia, USA: Lea & Febiger, 133–5.

Engström B (1964) The significance of enterococci in root canal treatment. *Odontologisk Revy* **15**, 87–105.

Fardal O, Turnbull RS (1986) A review of the literature on use of chlorhexidine in dentistry. *Journal of the American Dental Association* **112**, 863–9.

Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Souza-Filho FJ, (in press) *In vitro* assessment of chlorhexidine gel as an endodontic irrigant. *Journal of Endodontics* (in press).

Gomes BPFA, Lilley JD, Drucker DB (1996a) Clinical significance of dental root canal microflora. *Journal of Dentistry* **27**, 47–55.

Gomes BPFA, Lilley JD, Drucker DB (1996b) Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *International Endodontic Journal* **29**, 235–41.

Gomes BPFA, Ferraz CCR, Zaia AA, Souza-Filho FJ (1999) Variations in the susceptibility of selected microorganisms to endodontic irrigants (BES Spring Meeting Abstract). *Journal of Endodontics* **25**, 299.

Grossman L, Meiman B (1941) Solution of pulp tissue by chemical agents. *Journal of the American Dental Association* **28**, 233–5.

Harty FJ (1990) *Endodontics in Clinical Practice*, 3rd edn. London, UK: Wright.

Heath CH, Blackmore TK, Gordon DL (1996) Emerging resistance in *Enterococcus* spp. *Medical Journal of Australia* **164**, 116–20.

Heling I, Chandler NP (1998) Antimicrobial effect of irrigant combinations within dentinal tubules. *International Endodontic Journal* **31**, 8–14.

Ingle JI, Bakland LK, Peters DL, Buchanan S, Mullaney TP (1994) Endodontic cavity preparation. In: Ingle JI, Bakland LK, eds. *Endodontics*. 4th edn. Baltimore, USA: Williams & Wilkins, 92–227.

Jeansonne MJ, White RR (1994) A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *Journal of Endodontics* **20**, 276–8.

Kaufman AY, Keila S (1989) Hypersensitivity to sodium hypochlorite. *Journal of Endodontics* **15**, 224–6.

Kozol RA, Gillies C, Elgebaly AS (1988) Effects of sodium hypochlorite (Dakin's solution) on cells of the wound module. *Archives of Surgery* **123**, 420–3.

Leonardo MR, Tanomaru Filho M, Silva LAB, Nelson Filho P, Bonifácio KC, Ito IY (1999) *In vivo* antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. *Journal of Endodontics* **25**, 167–71.

Molander A, Reit C, Dahlén G, Kvist T (1998) Microbiological status of root-filled teeth with apical periodontitis. *International Endodontic Journal* **31**, 1–7.

Moorer WR, Wesselink PR (1982) Factors promoting the tissue dissolving capability of sodium hypochlorite. *International Endodontic Journal* **15**, 187–96.

Neal RG, Craig RG, Powers JM (1983) Effect of sterilization and irrigants on the cutting abilities of stainless steel files. *Journal of Endodontics* **9**, 93–6.

Ohara PK, Torabinejad M, Kettering JD (1993) Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. *Endodontics and Dental Traumatology* **9**, 95–100.

Parsons GJ, Patterson SS, Miller CH, Katz S, Kafrawy AH, Newton CW (1980) Uptake and release of chlorhexidine by bovine pulp and dentin specimens and their subsequent acquisition of antibacterial properties. *Oral Surgery, Oral Medicine and Oral Pathology* **49**, 455–9.

Pashley EL, Birdsong NL, Bowman K, Pashley DH (1985) Cytotoxic effects of sodium hypochlorite on vital tissue. *Journal of Endodontics* **11**, 525–8.

Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM (1982) *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. *Journal of Endodontics* **8**, 200–4.

Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L (1975) Rapid sterilization of gutta-percha with 5.25% sodium hypochlorite. *Journal of Endodontics* **1**, 136–40.

Shih M, Marshall J, Rosen S (1970) The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. *Oral Surgery, Oral Medicine and Oral Pathology* **29**, 613–9.

Siqueira JF Jr, Batista MMD, Fraga RC, Uzeda M (1998a) Antibacterial effects of endodontic irrigants on black-pigmented Gram-negative anaerobes and facultative bacteria. *Journal of Endodontics* **24**, 414–6.

Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, Uzeda M (1997) Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from root canal, *in vitro*. *International Endodontic Journal* **30**, 279–82.

Siqueira JF Jr, Pereira da silva CHF, Cerqueira MDO, Lopes HP, Uzeda M (1998b) Effectiveness of four chemical solutions in eliminating *Bacillus subtilis* spores on gutta-percha cones. *Endodontic and Dental Traumatology* **14**, 124–6.

Siqueira JF Jr, Uzeda M (1997) Intracanal medicaments: evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. *Journal of Endodontics* **23**, 167–9.

Spångberg L, Engström B, Langeland K (1973) Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surgery, Oral Medicine and Oral Pathology* **36**, 856–70.

Spångberg L, Safavi KE, Kaufman A, Pascon EA (1988) Antimicrobial and toxic effect in vitro of a bisdequalinium acetate solution for endodontic use. Journal of Endodontics 14, 175–8.

Updegraff DM, Chaug RW (1977) Antibacterial activity of dental restorative materials. *Journal of Dental Research* **50**, 382–92.

Vahdaty A, Pitt Ford TR, Wilson RF (1993) Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. *Endodontics and Dental Traumatology* **9**, 243–8.

