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A bio-molecular film *ex-vivo* model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation

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Abstract

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Aims To devise an *ex vivo* model to test the efficacy of irrigation (static/dynamic) in removing a bio-molecular film from root canal walls.

Methodology Forty human teeth with single straight canals were randomly allocated to two groups for static ($n = 20$) or dynamic ($n = 20$) irrigation. The root canals were prepared to different apical sizes (20, 40) and tapers (0.04, 0.08). The teeth were split longitudinally into two, stained collagen was applied to the canal surfaces and the tooth reassembled in a silicone matrix for dynamic or static irrigation. Digital images of the canal surface were taken before and after irrigation with 9, 18, 27 and 36 mL solution. The percentage of canal surface covered with stained collagen was quantified (ipWin4®). The data were analysed using paired *t*-tests and linear regression models.

Results All the five explanatory variables: 'volume of irrigant used', 'mode of irrigation', 'orientation of open port of needle', 'corono-apical level of canal' and 'root canal dimension' had a significant ($P < 0.001$) influence on outcome of irrigation. The corono-apical level of canal was the most dominating factor. After irrigation, the apical third had 19.9% and 33.8% less area covered with the bio-molecular film than the middle and coronal thirds respectively.

Conclusions The stained collagen bio-molecular film could not be removed completely by either static or dynamic irrigation. Factors influencing removal, in rank order of decreasing priority, were: corono-apical level, apical size and taper of canal preparation, and dynamic/static irrigation.

Keywords: bio-molecular film, irrigation, root canal.

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Introduction

Development of a periapical lesion signifies the presence of bacteria in the root canal system (Kakehashi *et al.* 1965, Sundqvist 1976, Möller *et al.* 1981), in particular its apical portion where they can exist in a biofilm or invade dentinal tubules (Nair 1987) and pose a challenge to treatment (Nair *et al.* 2005). Organization of bacteria within biofilms confers a range of phenotypic properties that are not evident in their planktonic counterparts and amongst other characteristics make them more resistant to antimicrobial killing (Costerton *et al.* 1994, 1999, Potera 1999).

Chemo-mechanical debridement and obturation effectively reduce the bacterial load in the root canal system and allow periapical healing in about 80% of cases (Sjögren *et al.* 1990), even though the apical bacterial biofilm survives in 88% (Nair *et al.* 2005). Given that mechanical instruments only plane up to 61% of the canal surface (Mannan *et al.* 2001, Peters *et al.* 2001), the role of canal preparation has undergone a shift from one of fulfilling a prime debriding function, to one regarded more as a radicular access for the irrigant and root filling materials to the complex root canal systems (Gulabivala *et al.* 2005). An impor-

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tant role of root canal irrigation is therefore to remove the residual bacterial biofilm from the inaccessible, un-instrumented surfaces.

The factors affecting the efficacy of irrigation have not been researched systematically, the interpretation of findings often being confounded by different outcome measures, such as removal of debris (Ram 1977), bacterial cells (Sedgley *et al.* 2005) or albumin (Cecic *et al.* 1984), accomplished either in simulated canals in plastic blocks or extracted teeth. More importantly, the interaction between the potential influencing factors has been poorly investigated. It is intuitively obvious that the diameter of canal preparation would affect the penetration of a suitably sized irrigation needle (Chow 1983), in turn affecting the penetration of the irrigant (Senia *et al.* 1971, Ram 1977, Salzgeber & Brilliant 1977, Teplitsky *et al.* 1987) and therefore removal of debris and/or bacteria (Baker *et al.* 1975, Ram 1977, Sedgley *et al.* 2005). The effect of canal preparation taper on the effectiveness of irrigation has only been investigated in the context of ultrasonic agitation and found to be significant (Lee *et al.* 2004b).

Reports on the effect of irrigant volume have been contradictory; larger volumes or repeated irrigation have proved more effective for debris or microorganism removal in some studies (Baker *et al.* 1975, Ram 1977, Moser & Heuer 1982) but not in all (Walters *et al.* 2002). High pressure deposition of irrigant did not increase effectiveness of debris removal and may additionally risk forcing material through the apical foramen (Van de Visse & Brilliant 1975). Agitation of the canal irrigant using hand files or irrigation needles could remove significantly more test albumin medium (Cecic *et al.* 1984) or allow better apical irrigant replacement (Druttman & Stock 1989). This impression is reinforced by unpublished data and practical demonstrations by Pierre Machtou in 2003 (personal communication), which show that dynamic irrigation (push–pull agitation with a well-fitting, tapered gutta-percha point) could improve the penetration and exchange of irrigant apically as compared with static irrigation.

The efficacy of irrigation in removing bacterial biofilms from root canals and the factors affecting such removal have not been properly elucidated. The reasons for such knowledge deficiency relate to the complexity of the problem and the lack of standardization of real bacterial biofilms (Gulabivala *et al.* 2004). The development of a test model using a standard organic layer with the hydrodynamic behaviour of a bacterial biofilm may substantially aid the study of these interactions. The aims of this study were: (i) to devise an *ex vivo* model utilizing a standard bio-molecular film to *simulate* a bacterial biofim and (ii) to use the model to test the influence of several factors (canal diameter and taper, irrigant volume, static or dynamic irrigation) on removal of the bio-molecular film.

Materials and methods

Collection and preparation of extracted tooth samples

Forty extracted human permanent teeth (maxillary, mandibular anterior teeth and mandibular pre-molars) with single canals, straight mature roots and with no caries or resorption, were collected and stored in 4% formal saline. They were randomly assigned to two experimental groups (group A for static irrigation and group B for dynamic irrigation). The 20 teeth in each group were randomly divided into four further equal subgroups (Table 1).

After accessing, the tooth length was determined by placing a size 10 stainless-steel K-flex file (Kerr UK Ltd, Peterborough, UK) in the canal and extending it until visible at the apical foramen. All the teeth were decoronated to give a standardized length of 17.5 mm; the working length was set at 17.0 mm, 0.5 mm short of the apical foramen.

The canals were prepared using SystemGT® instruments (Dentsply Maillefer, Baillaigues, Switzerland) in a 70 : 1 controlled-torque, low-speed rotary handpiece at 300 rpm, to specified dimensions according to subgroup designation (Table 1) following manufacturer's protocol (Dentsply Maillefer). During instrumentation, each tooth was irrigated with 50 mL of 2.5% sodium hypochlorite [(NaOCl) Teepol® bleach, Teepol products, Egham, UK], delivered with a 3 mL Monoject® Luer

Table 1 Allocation of tooth samples

Groups (mode of irrigation)	Subgroups	No. of teeths	Size and taper of canal preparation
Group A (static irrigation)	A1	5	20, 0.04
	A2	5	20, 0.08
	A3	5	40, 0.04
	A4	5	40, 0.08
Group B (Dynamic irrigation)	B1	5	20, 0.04
	B2	5	20, 0.08
	B3	5	40, 0.04
	B4	5	40, 0.08

lock syringe with a 27 gauge needle (Sherwood Medical, St Louis, MO, USA).

Each tooth was partly embedded in silicone putty (President Putty Coltène®, Altstätten, Switzerland) to create a set matrix (Fig. 1) which allowed reassembly of the tooth for irrigation tests after splitting. The tooth was then grooved on the buccal and palatal surfaces along its entire length (Fig. 2) using a diamond disc (Abrasive Technology Inc., Westerville, OH, USA) and placed longitudinally on another silicone matrix (Fig. 3) to allow cushioning when split into two halves using an osteotome and mallet. The two halves of the canal were randomly assigned as side A or B (Fig. 4).

Four layers of organic collagen solution (in 0.6% acetic acid; Type I rat tail collagen, First Link Ltd, Birmingham, UK) mixed with Chinese calligraphic ink (Kai-Ming, Tainan, Taiwan), in a ratio of 5 : 1, were applied to the canal surfaces (Fig. 5) using a small brush and allowed to gel into a three-dimensional matrix by evaporation of the acetic acid solvent at room temperature for 48 h.

Each split half of the root was equally divided into coronal, middle and apical sections and marked with a sharp pencil (Fig. 5). The split tooth was reassembled (Fig. 6) for irrigation tests and disassembled for

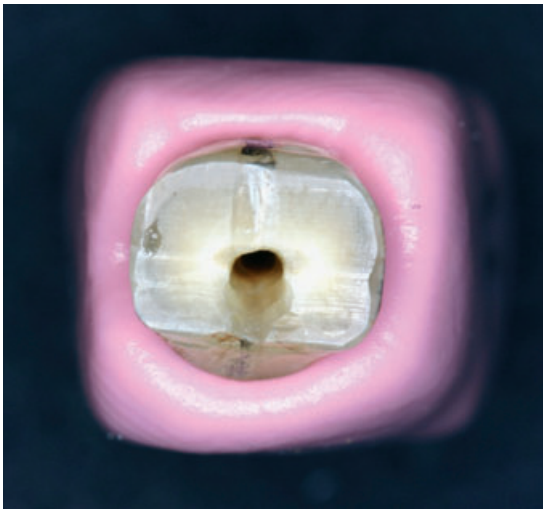


Figure 1 Tooth embedded in silicone putty to create a set matrix which allowed tooth reassembly for irrigation tests.

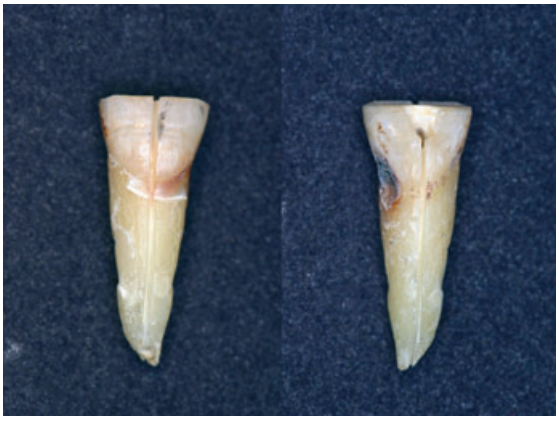


Figure 2 Grooves cut on buccal and palatal surfaces.

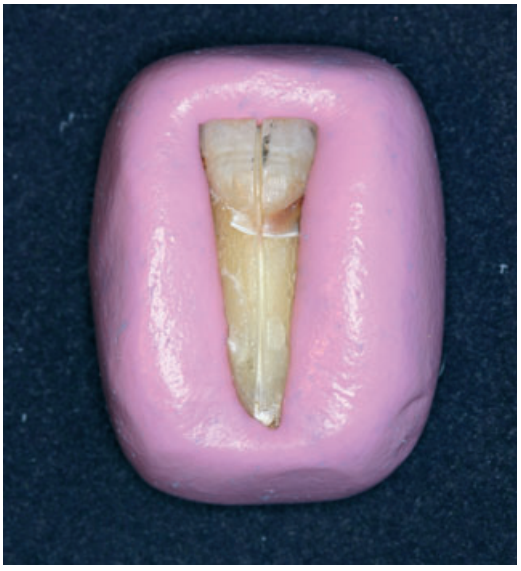


Figure 3 Tooth with longitudinal grooves placed on a silicone matrix for splitting.

Figure 4 Tooth split into two neat halves.

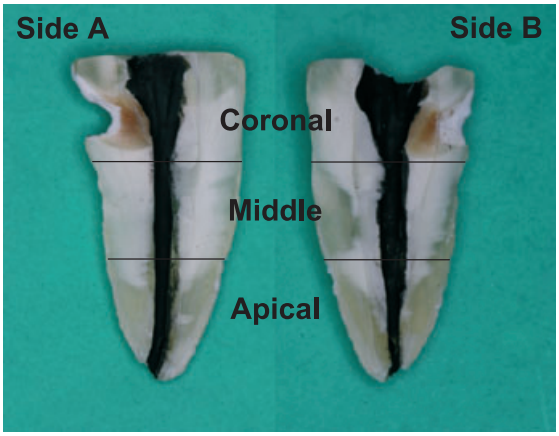


Figure 5 Split tooth divided into coronal, middle and apical sections.

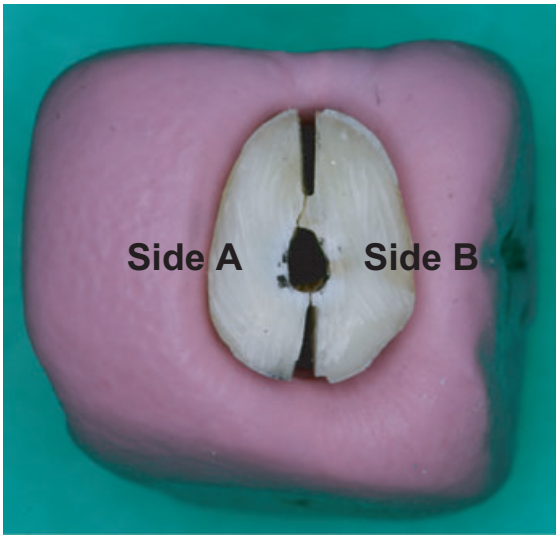


Figure 6 Split tooth reassembled for irrigation tests.

examination. Digital images were taken before and after irrigation with 9, 18, 27 and 36 mL of NaOCl using a digital camera (CoolSNAP-Procf, MediaCybernetics®, Silver Springs, MD, USA).

Irrigation experiments

The reassembled canals in group A (subgroups A1–A4) were irrigated with 2.5% NaOCl, delivered with a Monoject® endodontic 3 mL syringe through a Luer lock 30 gauge Max-I-Prob™ needle (Dentsply Maillefer) at a rate of 6 mL per minute. The irrigating needle was inserted to 4 mm short of the working length and a total of 36 mL of solution was delivered in twelve 3 mL boluses (each 3-mL bolus delivered over 30 s with pauses of sufficient duration to allow syringe recharging and canal surface evaluation after 9, 18, 27 and 36 mL). The open side port of the needle always faced the canal side designated ''A'', in a fixed orientation.

For the canals in group B (subgroups B1–B4, dynamic irrigation), the protocol for irrigation was the same as for group A (static irrigation) with the addition of intracanal push–pull manipulation of a tapered gutta-percha point (SybronEndo, Orange, CA, USA) matching the canal dimensions. One hundred push–pull strokes (each with 5 mm amplitude and reaching the working length) were performed *after* introducing the *first 3 mL* of the irrigant, followed by delivery of the next 6 mL in two 3 mL boli. Therefore, 100 push–pull strokes were used for each 9 mL of irrigation. This pattern was repeated four times until the entire 36 mL was delivered with a total of 400 push–pull strokes.

Image and statistical analyses

The digital images were analysed using ipWin4® (MediaCybernetics®) software to quantify residual canal coverage by the stained collagen. In total, 1200 images (30 images per tooth) were taken and digitized. The means and standard deviations of the percentage of canal surface coverage with *residual* collagen after irrigation were calculated for each corono-apical section (Fig. 5), on each side of the canal. The generalized estimating equation (GEE) approach (STATA 9; STATA Corporation: College Station, TX, USA, 2005) was used to investigate the influence of the potential factors (corono-apical level of canal, canal dimension, volume of irrigant, mode of irrigation and orientation of irrigation needle port) on the efficacy of irrigation using 'percentage of canal coverage with residual collagen' as the independent variable. The 'clustering' effect of the four repeated measurements taken on the same canal surface was accounted for in the GEE linear regression model.

Results

The mean percentages of canal surface coverage with residual collagen after static or dynamic irrigation are summarized in Figs 7–10, respectively. Some obvious trends were evident. The mean percentage of canal coverage with residual collagen increased with coronal level of canal, decrease in apical size and taper of canal

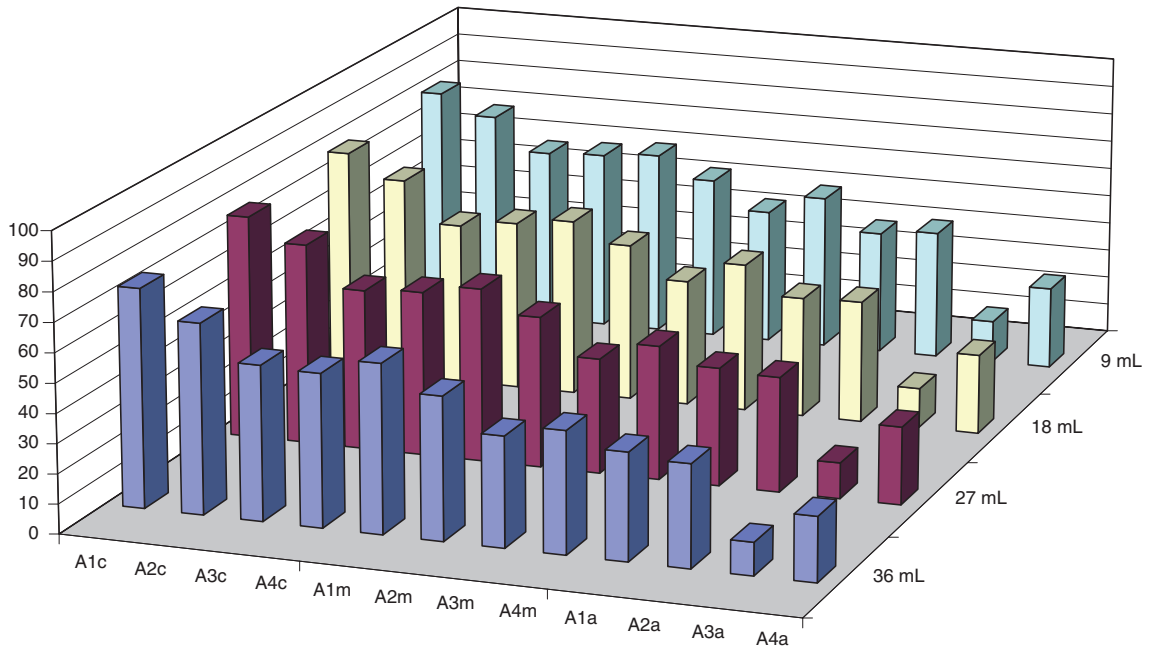


Figure 7 Mean percentage of canal surface coverage with residual collagen on the surface facing side port of needle after static irrigation [A = samples from static irrigation group, 1–4 denote different canal dimensions (1 = size 20/0.04; 2 = size 20/0.08; 3 = size 40/0.04 and 4 = size 40/0.08), c = coronal third of canal, m = middle of third of canal and a = apical third of canal].

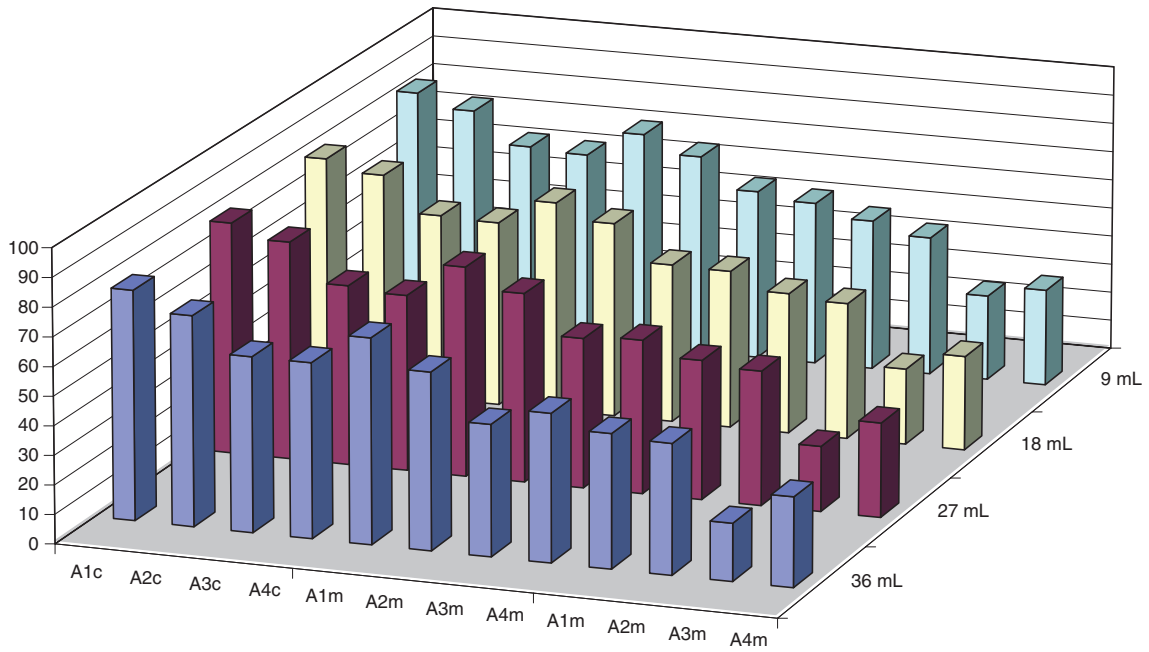


Figure 8 Mean percentage of canal surface coverage with residual collagen on the surface opposite to side port of needle after static irrigation [A = samples from static irrigation group, 1–4 denote different canal dimensions (1 = size 20/0.04; 2 = size 20/0.08; 3 = size 40/0.04 and 4 = size 40/0.08), c = coronal third of canal, m = middle of third of canal and a = apical third of canal].

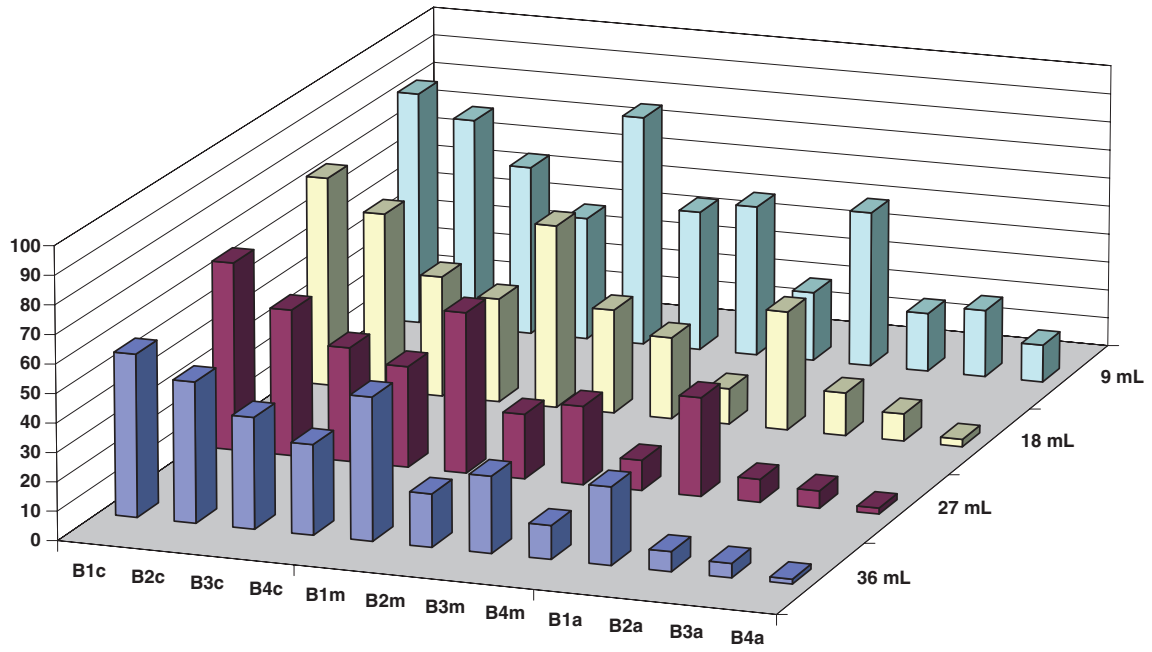


Figure 9 Mean percentage of canal surface coverage with residual collagen on the surface facing side port of needle after dynamic irrigation [B = samples from dynamic irrigation group, 1–4 denote different canal dimensions (1 = size 20/0.04; 2 = size 20/0.08; 3 = size 40/0.04 and 4 = size 40/0.08), c = coronal third of canal, m = middle of third of canal and a = apical third of canal].

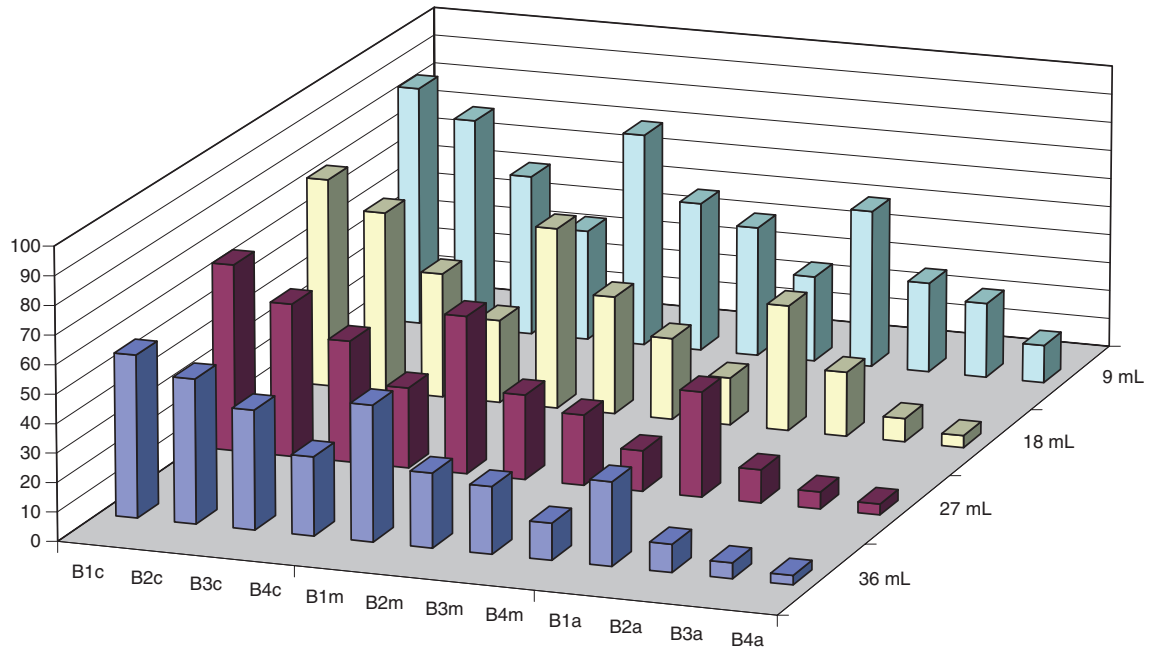


Figure 10 Mean percentage of canal surface coverage with residual collagen on the surface opposite to the side port of needle after dynamic irrigation [B = samples from dynamic irrigation group, 1–4 denote different canal dimensions (1 = size 20/0.04; 2 = size 20/0.08; 3 = size 40/0.04 and 4 = size 40/0.08), c = coronal third of canal, m = middle of third of canal and a = apical third of canal].

Table 2 Linear regression model incorporating five potential explanatory variables simultaneously using 'percentage of surface coverage with residual stained collagen' as the dependent variable

Explanatory variable (reference category)	Coefficient	95% CI for coefficient	*P*-value
Constant	56.75	51.79, 61.72	<0.001
Volume of irrigant (9 mL)			
18 mL	−7.60	−8.46, −6.74	<0.001
27 mL	−11.50	−12.65, −10.35	<0.001
36 mL	−14.53	−15.82, −13.25	<0.001
Mode of irrigation (static)	−17.11	−19.75, −14.47	<0.001
Canal surface (surface facing side port of needle)	4.42	1.78, 7.05	0.001
Corono-apical level of canal (apical third)			
Middle third	19.92	16.45, 23.39	<0.001
Coronal third	33.80	30.69, 36.90	<0.001
Dimension of canal preparation (#20/0.04)			
#20/0.08	−12.64	−16.73, −8.55	<0.001
#40/0.04	−24.32	−27.88, −20.76	<0.001
#40/0.08	−27.45	−31.60, 23.29	<0.001

preparation, and decrease in the volume of irrigant. There was less residual collagen after dynamic irrigation compared with static irrigation. The canal surface facing the open side port of the needle had less residual collagen after irrigation than the opposing surface.

The efficacy of irrigation measured as '*percentage of canal coverage with residual collagen*' was significantly ($P < 0.05$) influenced by all the five explanatory variables. In descending order of effect, the relative ranking was: corono-apical level of the canal, canal dimension, mode of irrigation, volume of irrigant used and orientation of irrigant needle port (Table 2).

Discussion

It is highly likely that in the clinical setting, all the different components of chemo-mechanical root canal debridement, including mechanical instrumentation, the chemical and physical aspects of irrigation and medication contribute to the control of the bacterial biofilm in infected root canal systems. The test model used in the present study was designed to quantify the relative importance of factors affecting the efficacy of removal of a bio-molecular film (simulating a biofilm) by the chemical and physical action of irrigation alone. The test model utilized was developed and refined through a series of pilot studies. The generation of realistic standardized bacterial biofilms in extracted teeth is notoriously difficult (Gulabivala *et al.* 2004) and confounds the testing of the influence of variables affecting irrigation. It is known that certain bacteria can attach to type 1 collagen in dentine (Love & Jenkinson 2002) through expression of surface adhesins and form biofilms (Jagnow & Clegg 2003, Black *et al.* 2004). The use of such bio-molecular substrates to create a standardized organic film, exhibiting the hydrodynamic properties of a bacterial biofilm could act as a suitable surface-adherent test substrate. Four bio-molecular organic materials with an adherent potential were initially screened to act as the test substrate and included: Type I rat tail collagen, Matrix Gel (Sigma®, Poole, UK), Super fibronectin (Sigma®) and Plasma fibronectin (Sigma®). They were applied on flat dentine surfaces and examined under light and scanning electron microscopes to determine consistency of application. The criteria for their selection were that they should be visible, adherent to root dentine and not removed by physical abrasion (gutta-percha point) or fluid turbulence (flushing action of irrigant). Rather, the film should be gradually dissolved and disintegrated from the surface by sodium hypochlorite; its removal synergized by physical abrasion or fluid turbulence. The mechanisms by which bacterial biofilms are degraded in the root canal system have not yet been elucidated and are the subject of ongoing studies. However, it was speculated that bacterial biofilms in root canals would probably succumb in the manner anticipated for the test substrate. It was further speculated that the removal of the bacterial biofilm would be influenced by a number of factors and their interactions, including canal dimensions, properties of irrigant, irrigation regimen and the properties of the biofilm. In the clinical setting, biofilm removal from the instrumented part of the canal may be facilitated by the mechanical contact of the instruments, whilst in the noninstrumented part of the canal, the biofilm is likely to be removed as a function of progressive chemical dissolution and a limited amount of fluid

agitation, as the fluid environment is likely to be viscously dominated.

Matrix Gel was excluded because it could be removed by flushing with water. Type I rat tail collagen, Super fibronectin and Plasma fibronectin met the criteria for adherence but were all transparent and would have to be stained. Type I rat tail collagen was eventually selected because it was the most cost economic and met all other criteria. The selected collagen, which was neither dried nor frozen, retained its native long molecule state, without denaturation in a 0.6% acetic solution. On application, it returns to a nonacidic state and gels into a three-dimensional matrix after evaporation of the acidic solution, a state that is reversible on addition of acetic acid (Type I rat tail collagen).

The effect of smear layer on the adherence of the collagen matrix was tested in a pilot study; 2.5% NaOCl (9 mL) completely removed the collagen layer from flat dentine surfaces in a similar manner whether pre-treated with 17% ethylene diamine tetra-acetic acid (EDTA) to remove the smear layer or not. It was therefore decided not to pre-treat the prepared root dentine surface with EDTA solution prior to the irrigation experiment.

The stain selected to overcome the problem of transparency was chosen by testing on flat dentine surfaces, from six materials: Black Indian ink (Winsor and Newton, London, UK); Methylene Blue (BDH Chemicals Ltd, Poole, UK); blue food dye (Supercook, Sherburn-in-Elmet, Leeds, UK); pen ink (Platinum®, Taipei, Taiwan); poster colour (Pentel Co. Ltd, Saitama, Japan); and Chinese calligraphic ink. The selection criteria for the stain were that it should contrast with dentine, aggregate with the collagen so as not to be decolourized by NaOCl, nor spread to the dentine collagen. In addition, when set, it should not flake or peel off. Chinese calligraphic ink met all the requirements and retained the black colour after exposure to an equal volume of 2.5% NaOCl for 1 day.

The removal characteristics of the stained rat tail collagen layer on canal surfaces were tested using NaOCl and water irrigation under light microscopy. Pilot studies confirmed that water did not remove the layer but that NaOCl did (Figs 11 and 12), leaving a clean surface without evidence of ink spread into dentinal tubules at or beyond the site of initial application (Figs 13 and 14). A comparison of the solubility in NaOCl of the stained and unstained collagen layers on flat dentine surfaces revealed both to behave similarly; the collagen gel (0.5 mL) was dissolved in 0.5 mL of NaOCl (2.5%) solution within 30 s.

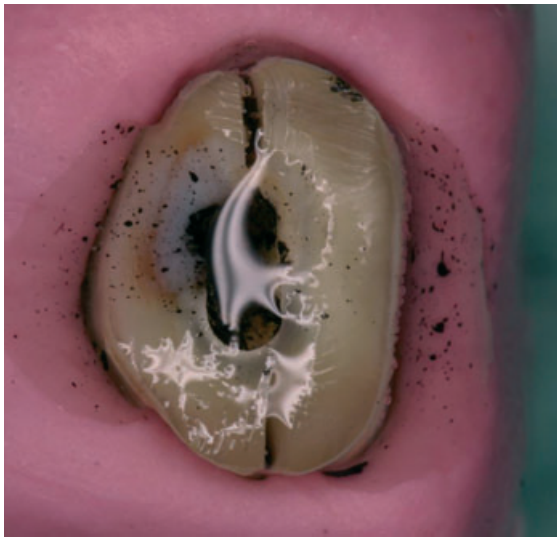


Figure 11 Black particles flushed out during static irrigation.

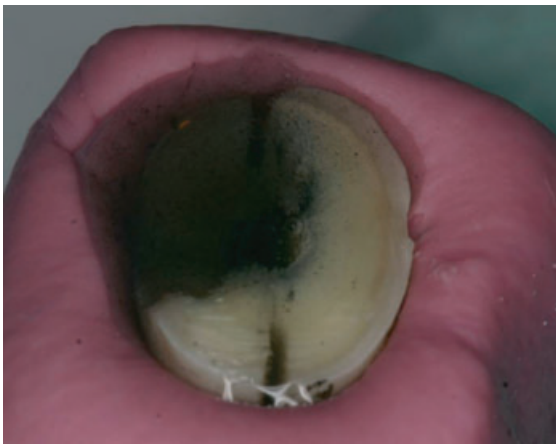


Figure 12 Black particles flushed out during dynamic irrigation.

Four layers of stained collagen gave good contrast and a predictable thickness of coating (5–15 μm) (Fig. 15), a dimension within the range of bacterial biofilm thickness (Distel *et al.* 2002). Teeth with single and straight canals were used because they could be split relatively predictably using the method adopted. It was necessary to use a simple model to establish and test the methodology, accepting the limitations of the inferences that could be drawn. Further ongoing work is evaluating an approach not requiring pre-prepared canals and a split tooth model.

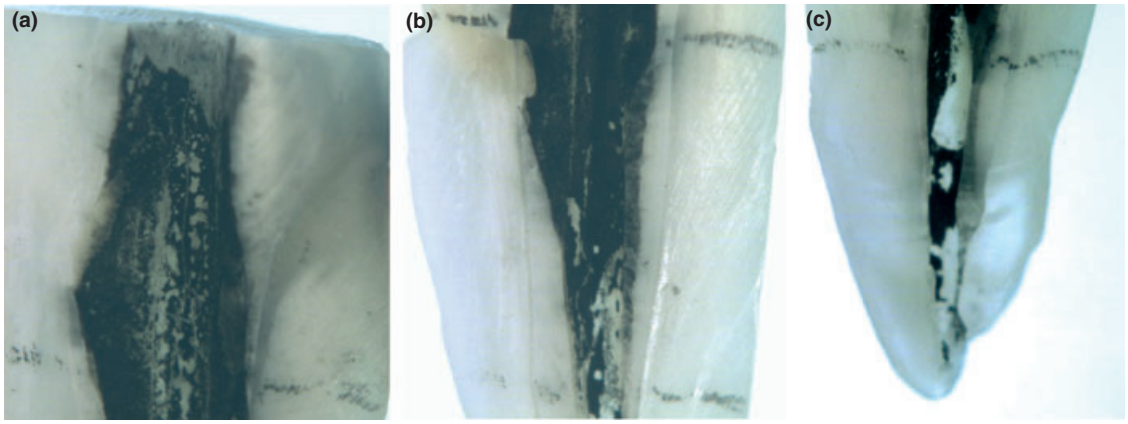
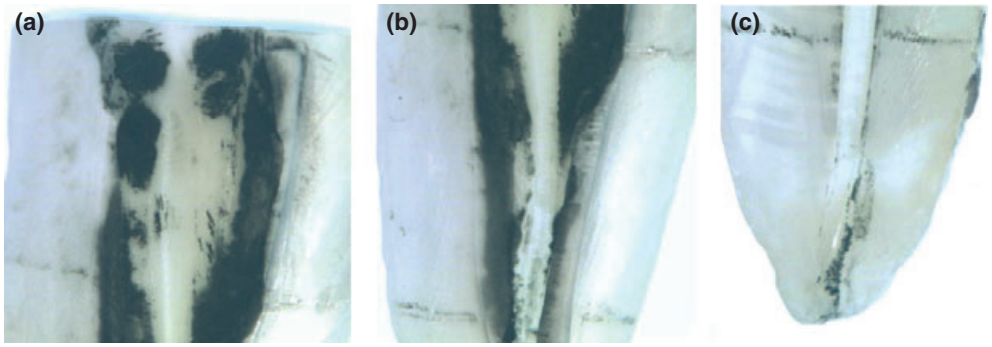


Figure 13 Coronal (a), middle (b) and apical (c) sections of canal surface covered with residual collagen after static irrigation.



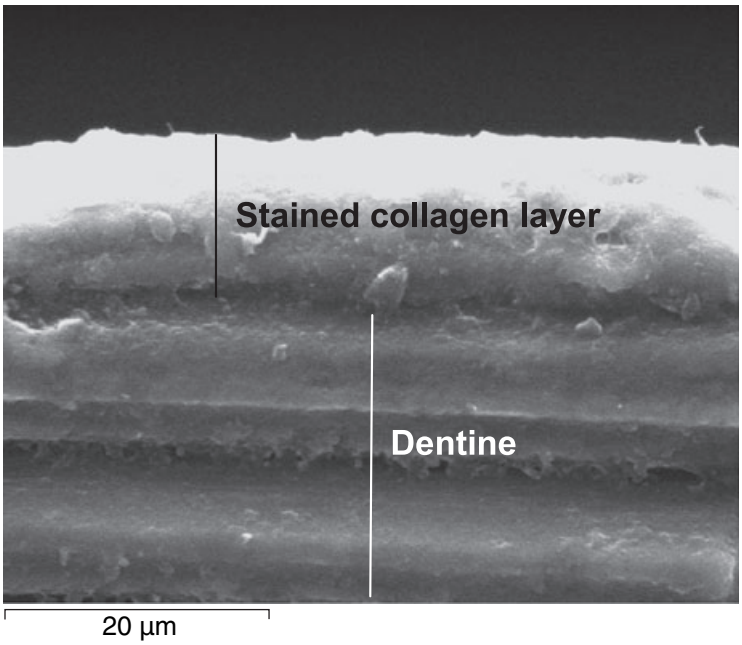


Figure 15 SEM view showing the collagen film on the dentine surface.

It is desirable to control the irrigant delivery by rate and pressure throughout a study (Ram 1977, Chow 1983). Previous studies had used a range of volume rates per unit time expressed through different gauge needles: 16 mL min^{-1} through gauge 23 needle (Chow 1983); 7 mL min^{-1} through gauge 27 needle (Lee *et al.* 2004a) and 12 mL min^{-1} through gauge 28 needle (Sedgley *et al.* 2005). The average rate of irrigant delivery in this study was set at 6 mL min^{-1} through a gauge 30 needle as it seemed clinically optimal and allowed the operator to maintain a constant force during extrusion (Moser & Heuer 1982). This variable was not automated or controlled but by the same token, clinical realism was maintained. The ability to maintain a constant rate would clearly be operator dependent and probably be affected by factors such as strength and length of the fingers, manual dexterity and patience of the operator. The selected needle size was small and allowed deeper penetration without binding and mechanically abrading the coated canal wall (Senia *et al.* 1971, Messing & Ray 1972). To standardize the depth of needle penetration for all the samples, the tip was maintained at 4 mm short of the working length as this was the deepest penetration without the needle tip binding against the canals prepared to apical size 20 with a 0.04 taper.

The imaging method used to quantify residual collagen coating renders three-dimensional irregularities on the root canal surface into two-dimensional surfaces on the images, incurring some loss of accuracy in measurement. However, the random allocation of samples should distribute them evenly to reduce or eliminate bias between groups.

Apart from the verification of the various model elements through pilot studies, the fundamental justification of the validity of the model and this study's thesis is borne out by the results. The clarity of the relationships evident in Figs (7–10) would be impossible to discern without adherence to strict methodological protocol and inherent validity of the model.

This study confirmed the importance of all the tested variables and indicated their relative importance in the test model. Studies have come to contradictory conclusions about the efficacy of apical irrigation. The test model adopted in this study, which effectively removed the direct effect of mechanical preparation from consideration, showed that the effect of irrigation was most marked apically, closest to the position of irrigant deposition (Figs 13 and 14). This confirms the findings of some studies (Senia *et al.* 1971, Sedgley *et al.* 2005) and contradicts others (Druttman & Stock 1989, Walters *et al.* 2002), which found that the removal of debris and exchange of irrigant were less effective apically. The latter did not report standardization of needle penetration.

Consistent with previous studies (Senia *et al.* 1971, Baker *et al.* 1975, Ram 1977, Salzgeber & Brilliant 1977, Teplitsky *et al.* 1987, Wu & Wesselink 1995, Lee *et al.* 2004a), both measures of canal dimension (apical size and taper) were directly associated with increasing removal of residual collagen. It is hypothesized that in this model, a larger apical size probably allowed better apical flushing by the irrigant and that the larger taper allowed better irrigant exchange between apical spent molecules and coronal unreacted molecules of NaOCl.

The particular style of dynamic irrigation adopted in this study (Machtou, personal communication), was significantly ($P < 0.05$) more effective in removal of the stained collagen on canal surface than static irrigation. The principle of this phenomenon is in agreement with previous studies (Cunningham *et al.* 1982a,b, Cecic *et al.* 1984, Goodman *et al.* 1985, Druttman & Stock 1989, Lee *et al.* 2004b) which used alternative methods for creating agitation.

The root canal surface facing the open side port of the needle was significantly ($P < 0.05$) cleaner than the opposing side, reinforcing the notion of the benefit of the physical flushing effect in addition to the chemical effect of the irrigant. This may have significant implications for needle design. The probability of the combined chemical and physical erosive effects is further reinforced by other observations. The data analyses revealed that the volume of irrigant had a significant ($P < 0.05$) influence on the outcome measure (collagen removal), consistent with other studies (Baker *et al.* 1975, Moser & Heuer 1982). Irrigant outflows during the delivery of the first 6 mL of NaOCl often progressively contained some particulate-stained matter, suggesting a gradual degradation of the collagen layer by NaOCl, with dislodgement of small parts of the adherent film because of the physically erosive effect of the irrigant.

Based on the present study and bearing in mind the test model limitations, it is *hypothesized* that the removal of a surface adherent bacterial biofilm from the root canal surface may be facilitated by sufficient canal enlargement, movement of irrigant needle during delivery, a needle design with multiple ports, frequent replacement of irrigant and agitation of the irrigant with a well-fitting gutta-percha point. Although the procedure may seem laborious, it is actually fast and

convenient to perform. Those finding it laborious may adopt automated agitation by endosonics or other commercially available devices. It is intended to test the efficacy of such devices in future studies.

Conclusions

- A bio-molecular film test model has been proposed for evaluation of irrigation variables.
- The stained collagen test substrate on the canal surface could not be completely removed either by static or dynamic irrigation, although the latter was significantly ($P < 0.001$) more effective.
- All the five explanatory variables had significant ($P < 0.001$) influence on the efficacy of irrigation with corono-apical level of the canal, canal dimension and mode of irrigation (static versus dynamic) being the most dominant.

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References

Baker NA, Eleazer PD, Averbach RE, Seltzer S (1975) Scanning electron microscopic study of the efficacy of various irrigating solutions. *Journal of Endodontics* **1**, 127–35.

Black C, Allan I, Ford SK, Wilson M, McNab R (2004) Biofilm-specific surface properties and protein expression in oral *Streptococcus sanguis*. *Archives of Oral Biology* **49**, 295–304.

Cecic PA, Peters DD, Grower MF (1984) The comparative efficiency of final endodontic cleansing procedures in removing a radioactive albumin from root canal systems. *Oral Surgery Oral Medicine Oral Pathology* **58**, 336–42.

Chow TW (1983) Mechanical effectiveness of root canal irrigation. *Journal of Endodontics* **9**, 475–9.

Costerton JW, Lewandowski Z, De Beer D, Caldwell D, Korber D, James G (1994) Biofilms, the customized microniche. *Journal of Bacteriology* **176**, 2137–42.

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**, 1318–22.

Cunningham WT, Martin H, Forrest WR (1982a) Evaluation of root canal debridement by the endosonic ultrasonic synergistic system. *Oral Surgery Oral Medicine Oral Pathology* **53**, 401–4.

Cunningham WT, Martin H, Pelleu GB, Stoops DE (1982b) A comparison of antibacterial effectiveness of endosonic and hand root canal therapy. *Oral Surgery Oral Medicine Oral Pathology* **54**, 238–41.

Distel JW, Hatton JF, Gillespie MJ (2002) Biofilm formation in medicated root canals. *Journal of Endodontics* **28**, 689–93.

Druttman AC, Stock CJ (1989) An *in vitro* comparison of ultrasonic and conventional methods of irrigant replacement. *International Endodontic Journal* **22**, 174–8.

Goodman A, Beck M, Melfi R, Meyers W (1985) An *in vitro* comparison of the efficacy of the step-back technique versus a step-back/ultrasonic technique in human mandibular molars. *Journal Endodontics* **11**, 249–56.

Gulabivala K, Stock CJRS, Lewsey JD, Ghori S, Ng Y-L, Spratt DA (2004) Effectiveness of electrochemically activated water as an irrigant in an infected tooth model. *International Endodontic Journal* **37**, 624–31.

Gulabivala K, Patel B, Evans G, Ng Y-L (2005) Effects of mechanical and chemical procedures on root canal surfaces. *Endodontic Topics* **10**, 103–22.

Jagnow J, Clegg S (2003) *Klebsiella pneumoniae* MrkD-mediated biofilm formation on extracellular matrix- and collagen-coated surfaces. *Microbiology* **149**, 2397–405.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effects of surgical exposures of dental pulps in germ free and conventional laboratory rats. *Journal of Oral Surgery* **20**, 340–9.

Lee SJ, Wu MK, Wesselink PR (2004a) The efficacy of ultrasonic irrigation to remove artificially placed dentine debris from different-sized simulated plastic root canals. *International Endodontic Journal* **37**, 607–12.

Lee SJ, Wu MK, Wesselink PR (2004b) The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. *International Endodontic Journal* **37**, 672–8.

Love RM, Jenkinson HF (2002) Invasion of dentinal tubules by oral bacteria. *Critical Review of Oral Biology and Medicine* **13**, 171–83.

Mannan G, Smallwood ER, Gulabivala K (2001) Effect of access cavity location and design on degree and distribution of instrumented root canal surface in maxillary anterior teeth. *International Endodontic Journal* **34**, 176–83.

Messing JJ, Ray RE (1972) *Operative Dental Surgery*. London: Henry Kimpton.

Möller AJR, Fabricius L, Dahlén G, Ohman AE, Heyden G (1981) Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scandinavian Journal of Dental Research* **89**, 475–84.

Moser JB, Heuer MA (1982) Forces and efficacy in endodontic irrigation systems. *Oral Surgery Oral Medicine Oral Pathology* **53**, 425–8.

Nair RPN (1987) Light and electron microscopic studies of root canal flora and periapical lesions. *Journal of Endodontics* **13**, 29–39.

Nair RPN, Henry S, Cano V, Vera J (2005) Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after ''one-visit'' endodontic treatment. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **99**, 231–52.

Peters OA, Laib A, Göhring TN, Barbakow F (2001) Changes in root canal geometry after preparation assessed by high-resolution computed tomography. *Journal of Endodontics* **27**, 1–6.

Potera C (1999) Forging a link between biofilms and disease. *Science* **283**, 1837–9.

Ram Z (1977) Effectiveness of root canal irrigation. *Oral Surgery Oral Medicine Oral Pathology* **44**, 306–12.

Salzgeber RM, Brilliant JD (1977) An *in vivo* evaluation of the penetration of an irrigating solution in root canals. *Journal of Endodontics* **3**, 394–8.

Sedgley CM, Nagel AC, Hall D, Applegate B (2005) Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging *in vitro*. *International Endodontic Journal* **38**, 97–104.

Senia ES, Marshall FJ, Rosen S (1971) The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. *Oral Surgery Oral Medicine Oral Pathology* **31**, 96–103.

Sjögren U, Hägglund B, Sundqvist G, Wing K (1990) Factors affecting the long-term results of endodontic treatment. *Journal of Endodontics* **16**, 498–504.

Sundqvist G (1976) *Bacteriological Studies of Necrotic Dental Pulps* (dissertation). Umeå, Sweden: Umeå University, 3.

Teplitsky PE, Chenail BL, Mack B, Machnee CH (1987) Endodontic irrigation – a comparison of endosonic and syringe delivery systems. *International Endodontic Journal* **20**, 233–41.

Van de Visse JE, Brilliant D (1975) Effect of irrigation on the production of extruded material at the root apex during instrumentation. *Journal of Endodontics* **17**, 243–6.

Walters MJ, Baumgartner JC, Marshall JG (2002) Efficacy of irrigation with rotary instrumentation. *Journal of Endodontics* **28**, 837–9.

Wu MK, Wesselink PR (1995) Efficacy of three techniques in cleaning the apical portion of curved root canals. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontology* **79**, 492–6.