International Endodontic Journal

doi:10.1111/j.1365-2591.2009.01677.x

Ex vivo assessment of irrigant penetration and renewal during the cleaning and shaping of root canals: a digital subtraction radiographic study

F. Bronnec[1], S. Bouillaguet[2] & P. Machtou[1]

[1]Department of Endodontics and Restorative Dentistry, School of Dentistry, Paris 7-Denis Diderot University, Paris, France; and [2]Department of Cariology and Endodontology, School of Dentistry, University of Geneva, Geneva, Switzerland

Abstract

Bronnec F, Bouillaguet S, Machtou P. *Ex vivo* assessment of irrigant penetration and renewal during the cleaning and shaping of root canals: a digital subtraction radiographic study. *International Endodontic Journal*, **43**, 275–282, 2010.

Aim To assess radiographically irrigant penetration in the root canal system of curved roots during root canal shaping procedures *ex vivo.*

Methodology Thirty extracted mandibular molar teeth with moderate to severe curvature were used. A special aiming device was used to guarantee that each successive radiograph was taken with the same positioning. The mesiolingual canal of each tooth was instrumented using the ProTaper system. For each step of the shaping procedure, two irrigation modalities were repeated in the same order. Active irrigation consisted of a 0.5-mL flush of sodium diatrizoate solution (Hypaque 50%) immediately followed by agitation with a size 08 K-file. Passive irrigation consisted of a 0.5-mL flush of sodium hypochlorite solution delivered with a syringe through a 27-gauge notched tip needle. A digital radiograph was taken after each modality and stored on computer for subsequent digital subtraction and measures of the depths of irrigant penetration. Comparisons were performed within an analysis-of-variance framework in a repeated-measures approach.

Results The penetration of irrigants was significantly greater for each successive step of the shaping procedure when the two modalities were analysed separately ($P < 0.001$). The difference between the two modalities was statistically significant for each step of the shaping procedure ($P < 0.0001$).

Conclusions Shaping root canals improved both penetration and exchange of irrigant inside the root canal system. Complete renewal of the solution was impossible to achieve with a conventional syringe delivery system and a limited volume of solution. Recapitulation with a K-file after flushing improved irrigant penetration.

Keywords: digital radiography, image processing, root canal irrigation, rotary file.

Received 23 February 2009; accepted 25 November 2009

Introduction

The prognosis of root canal treatment is strongly influenced by the presence of periradicular pathosis (Ng *et al.* 2008). As development of this inflammatory lesion is caused by microbial contamination (Kakehashi *et al.* 1965, Möller *et al.* 1981), success depends on the ability to properly disinfect the root canal system (Sjögren *et al.* 1997). Root canal treatment thus relies on both instrumentation and irrigation, and its result is maintained over time by a filling of the root canal system in an attempt to prevent reinfection (Haapasalo *et al.* 2005).

Sodium hypochlorite solution has long been recognized as the gold standard for irrigation, mainly

Correspondence: François Bronnec, Department of Endodontics and Restorative Dentistry, School of Dentistry, Paris 7-Denis Diderot University, 5 rue Garancière 75006 Paris, France (e-mail: bronnec.endo@gmail.com).

because of its solvent action and broad-spectrum antiseptic activity (Zehnder 2006).

Nevertheless, root canal preparation with either manual or rotary instrumentation generates a substantial amount of debris and smear layer (McComb & Smith 1975, Goldman *et al.* 1981, Jeon *et al.* 2003). Smear layer consists in a variably dense aggregate of mixed dentine and pulp particles covering the root canal walls and extending to various depths in the dentinal tubules (Mader *et al.* 1984, Pashley *et al.* 1988).

It has been proposed that smear layer should be removed for optimal results, as it could harbour bacteria and prevent proper disinfection of dentinal tubules (Brännström 1984, Byström & Sundqvist 1985, Ørstavik & Haapasalo 1990). Moreover, studies have shown that smear layer could predispose to leakage of root fillings (White *et al.* 1984, 1987, Kouvas *et al.* 1998). Accordingly, additional irrigation with chelating agents and more potent antibacterial solutions has been recommended to remove the smear layer and to remove bacteria (Yamada *et al.* 1983, Berutti *et al.* 1997, Gambarini 1999). However, this did not produce a smear-free surface in the apical third of the canal (Gambarini & Laszkiewicz 2002) nor the expected bacteria-free state before filling (Coldero *et al.* 2002, McGurkin-Smith *et al.* 2005).

Studies have shown that the apical third of the root canal seemed to represent the limit of effectiveness of irrigating solutions (Senia *et al.* 1971, Salzgeber & Brilliant 1977). These results led to the conclusion that it was necessary to increase apical preparation size (Ram 1977) and to place the tip of the irrigating needle as close as possible to the apical end of the canal (Abou-Rass & Piccinino 1982) to be more effective.

Despite the wealth of studies published on irrigation, the influence of mechanical agitation on irrigant penetration has never been tested specifically, and surprisingly, little effort has been devoted since then to better understand irrigant flow during shaping procedures.

With the advent of rotary nickel–titanium instrumentation techniques and the widespread acceptance of conical-shaped preparations, it is noteworthy that only few data are available on this topic, and no published study to date has investigated the dynamics of irrigation in curved canals.

The objective of this study was to assess the influence of progressive shaping of root canals on irrigant penetration. The hypothesis was that agitation of the solution with a patency file (active irrigation, AI) displaces irrigant deeper into root canals than the level reached with conventional syringe delivery (passive irrigation, PI).

Materials and methods

Collection and preparation of extracted teeth

Thirty freshly extracted human first and second mandibular molar teeth with divergent roots and mature apices were used. The teeth were extracted either for periodontal or carious reasons. None of the teeth had received previous endodontic therapy, and all were vital at the time of surgical removal. Following extraction, the teeth were stored at 4 °C in 0.5% chloramine-T solution to prevent tooth desiccation and bacterial growth.

Conventional access cavities were prepared with appropriate burs in a high-speed handpiece under an operating microscope (Opmi Pico; Carl Zeiss, Oberkochen, Germany). If initial instrumentation to the apical foramen could not be performed with a size 08 K-file (Colorinox; Dentsply Maillefer, Ballaigues, Switzerland), the teeth were excluded. Only teeth with moderately to severely curved canals (Mean = 33.7°; SD = 10.4) according to the Schneider method of canal curvature measurement (Schneider 1971) and root lengths between 9 and 15 mm (Mean = 12.15 mm; SD = 1.38) were included. The occlusal third of each crown was ground flat with a diamond disc to ensure a constant working length (WL) of 19 mm. WL was defined as the distance between the occlusal reference point and the major apical foramen. Two layers of utility wax (Cire à modeler; AJA, Avignon, France) were applied over the root tips to prevent the irrigating solutions from passing through the apical foramina. The orifices of the mesiobuccal and distal canals were filled with a drop of sticky wax (Cire Esvé; Prodont-Holliger, Vence, France) to seal off the canal path of these systems and prevent irrigant penetration during the study.

The teeth were positioned with their occlusal surface at the level of the top of a silicone mould (275 mm × 275 mm × 375 mm), and investment resin (MetaFix-20; Struers, Ballerup, Denmark) was poured into the mould. After the resin had set, the sample was removed from the mould and polished with sandpaper discs of decreasing size before being varnished for optimal transparency of the acrylic resin.

Irrigation experiment

The teeth set into resin, the charge-coupled device (CCD) sensor and the X-ray tube were held together by means of a bench vice to obtain images with identical projection geometry as required for digital subtraction radiography. The device was assembled specifically for the experiment and disassembled only at the end of the experiment. Radiographs of each tooth were taken prior to root canal preparation (Fig. 1).

The mesiolingual canals were prepared using ProTaper instruments (Dentsply Maillefer) in a 16 : 1 controlled-torque, low-speed rotary handpiece at 300 rpm, to apical size 20 with a 0.07 taper, following the manufacturer's instructions. According to the basic sequence, root canal shaping with ProTaper instruments can be broken down into five major steps, namely

1. Scouting the coronal two-thirds with size 10 and 15 manual K-files

2. Shaping the coronal two-thirds with ProTaper rotary files S1 and S2

3. Scouting the apical third with manual K-files from size 10 to 15 to WL

4. Shaping the apical third with ProTaper rotary files S1 and S2 to WL

5. Finishing the apical third with a ProTaper rotary file F1 to WL minus 0.5 mm.

The foramen was kept patent during the experiment, and irrigation was performed with 1 mL of 3% NaOCl (Parcan; Septodont, Saint-Maur-des-Fossés, France) between successive instruments. Upon completion of root canal shaping, the apical diameter of the canals at WL was recorded using a stainless steel K-file as a gauge. If superior to 20, the tooth was excluded and replaced by another.

After each step of the procedure, the canals were irrigated with 0.5 mL of sodium diatrizoate solution (Hypaque Sodium 50%; Amersham Health Inc, Princeton, NJ, USA); a size 08 K-file was inserted in the canals to the WL, stroked repeatedly (three push-pull motions with a 5-mm amplitude), and removed before taking a first radiograph for the AI modality. The canals were then flushed with 0.5 mL of 3% NaOCl before taking a second radiograph for the PI modality (PI).

The canals were irrigated with a Monoject 3-mL syringe through a Luer-Lock 27-gauge notched tip needle (Tyco Kendall, Hampshire, UK) at a flow rate of 12 mL min^{-1}. The needle was inserted as deeply as possible without binding, and irrigating solution was expressed without pressure using an up-and-down motion. Complete removal of Hypaque solution was checked radiographically after each successive step of the shaping procedures before testing AI again.

Direct digital images were obtained using a CCD sensor (Kodal 6100; Trophy Radiology, Croissy-Beaubourg, France) and an X-ray tube operating at 70 kV, 7 mA and 0.08 s (Heliodent MD; Siemens, Bensheim, Germany).

The native images (Trophy large format) were exported to file and converted to TIFF format (Grey

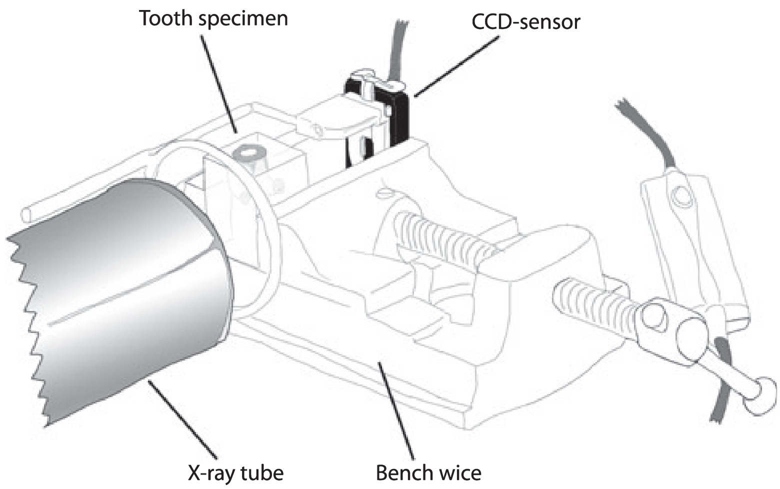

Figure 1 Diagram of the experimental setting illustrating the positioning of the X-ray tube, the embedded tooth sample and the CCD sensor.

Figure 2 Example of preoperative, active and passive irrigation radiographs.

mode, 8-bit intensity resolution and 1372-dpi resolution).

Each of the image pairs were given an alphanumeric code number to keep them anonymous for subsequent analysis and stored in a separate computer file, with each tooth having its own folder (Fig. 2).

Image processing

Radiographs were submitted in pairs to digital subtraction using Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA, USA). For analyses of AI: AI images were subtracted from the preoperative radiograph. For analyses of PI: AI images were subtracted from PI images of the corresponding pair for each step (Fig. 3).

The resulting image of the subtraction process was optimized using the same image-analysis software for brightness and contrast enhancement and grey level adjustment. Tooth and canal outlines were then pasted as layers over the subtraction image, and the level of irrigant penetration was measured using another image-analysis software (ImageJ 1.40g, NIH, Bethesda, MD, USA). This second program allowed direct length measurements using the freehand line selection tool on the resultant image. The result was expressed as a percentage of the length of the root.

Statistical analyses

The assumption of normally distributed data within all experimental groups was checked using the Q-Q plot and the Kolmogorov test-of-fit. Overall comparison was performed within the analysis-of-variance (ANOVA) framework, in a repeated-measures approach. Statistical analyses were processed with SPSS® 16.0 software (SPSS, Chicago, IL, USA). Significance level was set at $P = 0.05$.

Results

The mean percentages of irrigant penetration after passive and AI are presented in Table 1. AI and PI

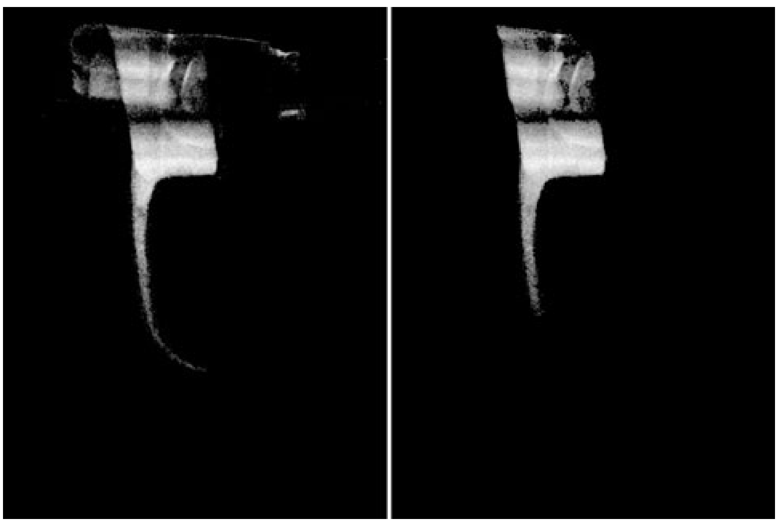

Figure 3 Resulting images of the subtraction process of the previous pictures (Right: passive irrigation; Left: active irrigation).

Table 1 Mean percentage of irrigant penetration during the shaping procedures following active irrigation (AI) and passive irrigation (PI), expressed in per cent of root length

	% AI (mean and SD)	% PI (mean and SD)
LK_1	74.2 ± 18.8	45.4 ± 19.6
$S1–S2_2$	84.4 ± 11.8	60.5 ± 14.2
LK_2	91.9 ± 7.7	67.3 ± 12.1
$S1–S2_2$	94.8 ± 6.9	70.9 ± 13.1
F1	98 ± 3.5	81.4 ± 8.2

revealed a significant difference ($P < 0.0001$) at all of the five steps of the root canal shaping procedure. There was no significant interaction effect between irrigation modality and stage; thus, there was a constant difference between AI and PI. Within-subject effects were significantly different in AI and PI ($P < 0.001$). Complete renewal of the solution was never observed with PI.

Discussion

Several methods have been proposed to assess the effectiveness of root canal preparation in terms of canal wall cleanliness or disinfection results. Most of these studies concluded that the apical third of the roots represent the limit of effectiveness of irrigation, prompting them to recommend enlarging the canals to mechanically eliminate infectious dentine and necrotic pulp remnants (Baugh & Wallace 2005).

However, these studies provide only indirect information on the efficiency of the method of irrigation delivery. There is a general consensus that for a solution to exert its solvent or antiseptic activity, it must get in direct contact with the substrate to be eliminated and maintain its activity for a sufficient amount of time. Therefore, it is fundamental to better understand the dynamics of the irrigating solution and its relationship with canal enlargement.

The only way to gather direct information relies on visualization of solution penetration and renewal of the solution by means of a tracer. Some studies have used coloured dyes to directly visualize solution penetration in acrylic resin artificial canals (Druttman & Stock 1989, Kahn *et al.* 1995), whilst others have used radio-opaque contrast media in natural teeth *in vivo* (Salzgeber & Brilliant 1977) or *in vitro* (Ram 1977, Abou-Rass & Piccinino 1982, Teplitsky *et al.* 1987).

Artificial canals do not represent a valid model for testing the flow of irrigating solution, as the surface characteristics of acrylic resin are not the same as those of dentine. Moreover, images of coloured dyes are difficult to interpret, as the material clears from the canal or is diluted with the flushing solution.

Various radio-opaque dyes have been used in the past to assess irrigant penetration. Sodium diatrizoate and diatrizoate meglumine solutions are radio-opaque contrast media widely used for contrast enhancement in medical settings. Choice of tracer is an important factor because it needs to present physical properties as close as possible with those of the irrigants that are commonly used in clinical practice. Most tracers are unsuitable for the purpose of flushing evaluations as they are six times (MD-60 and Renografin 60) to 16 times (MD-76) more viscous than water or sodium hypochlorite solutions (Abou-Rass & Piccinino 1982, Teplitsky *et al.* 1987). Hypaque sodium 50% has already been chosen for previous research studies on irrigant penetration as its physical properties more closely match sodium hypochlorite solution (Ram 1977, Salzgeber & Brilliant 1977).

An important point to emphasize when analysing the methodology of laboratory studies is that the apical third of the root surface has to be sealed so as to prevent irrigating solution outflow through the foramen, otherwise incorrect conclusions will be drawn.

Salzgeber & Brilliant (1977) investigated irrigant penetration *in vivo* during standardized root canal preparation. Conventional radiographs were obtained after using each successively larger instrument, with Hypaque as sole irrigating solution. It was shown that canals had to be flared coronally and enlarged to apical size 45 for the solution to reach the apex.

Radiography has been used in laboratory studies to investigate the efficacy of irrigation to remove radio-opaque contrast medium from the prepared canals of extracted teeth (Ram 1977). Complete elimination was only possible when enlargement of the apical preparation reached size 60. The conclusions from these studies stated that canals needed to be enlarged apically when a standardized root canal preparation is used. There have been no studies focused on this topic since the introduction of nickel–titanium rotary instrumentation.

Ethical concerns regarding multiple exposures to X-rays are likely to prohibit the realization of radiographic studies *in vivo*. Furthermore, radiographic contrast medium, or its elimination, is not always clearly discernable with conventional radiography because of the surrounding dentine. To overcome the limitations of previous studies, a laboratory model is proposed based on digital subtraction radiography. Digital subtraction radiography was first introduced in dental care in the 1980s. Since then, it has used been used in periodontology (Webber *et al.* 1982), in cariology (Gröndahl *et al.* 1982) and in endodontics (Ørstavik *et al.* 1990, Delano *et al.* 2001) for the early detection of changes in tissue mineral content. It has proven to be a sensitive instrument, but the main drawback of the technique is the lack of reproducibility of the intra-oral radiography, thus limiting its use in clinical experiments (Jeffcoat *et al.* 1987, Carvalho *et al.* 2007). In the present study, the quality of the image standardization was not influenced by sensor positioning or exposure setting, as they remained unchanged during the experiment. The resulting image of the subtraction process was easily interpretable, as even minute traces of solution were visible. The method is simple, effective (sensitive and highly specific) and reliable (showing penetration and nothing else) and allows objective, quantitative measurement.

Solution penetration was greater for each successive step of the shaping procedure following each of the two irrigation modalities tested. The results partly confirm those of previous studies regarding the limited solution penetration with syringe delivery during the shaping procedures, as complete renewal of the solution even at the end of the preparation when the F1 instrument was used as master apical file was not possible. In this study, a limited volume of solution was used via syringe delivery through a 27-gauge notched tip needle. The needle tip was never placed deeper than the junction of the coronal and middle third.

In contrast, AI led to early irrigant penetration in the apical third of root canals well before the completion of the shaping procedure and accounted for almost complete penetration as soon as the coronal two-thirds had been shaped with S1 and S2. This finding clearly reinforces the necessity to intermittently agitate the irrigant with a patency file to improve solution penetration and exchange during shaping procedures.

Schilder was the first to advocate the use of a patency file to clear the apical foramen and put debris into suspension (Schilder 1974). With the advent of rotary instrumentation, early negotiation with hand instruments and apical patency are considered by some as key factors to secure the canal pathway before shaping, suggesting that this helps prevent apical blockage and ledging (Buchanan 2000, Flanders 2002, Ruddle 2005). Surprisingly, no study has ever mentioned the use of a patency file after flushing the canal between each instrument use. The main reason for not using it is the fear of extrusion of debris or solution through the foramen (Van de Visse & Brilliant 1975); patency filing has been confused with over-instrumentation. Contrary to common belief endodontic instruments do not push the solution unless a file is tight in a parallel-shaped canal; the solution actually flows into the canal when the file is moved outward.

'Passive' and 'active' are the appropriate terms to distinguish the two modalities of irrigation as the needle tip never remains static during pressureless ejection of the solution, whereas AI relies only on mechanical agitation with an appropriate instrument.

The study proves the validity of the experimental setting to record the penetration and renewal of irrigating solution. As continuous replenishment of the irrigant is essential for maintaining its solvent (Moorer & Wesselink 1982) or antibacterial activity (Harrison & Hand 1981), there is a need to identify potential variables that may affect solution penetration inside the canal at the end of the shaping procedures during final irrigation.

Conclusions

Within the limitations imposed by the model, the flow of irrigating solution was improved by progressive shaping of the root canal. Mechanical agitation of the irrigant with a small-sized file at WL after each instrument further improved irrigant penetration. Complete renewal of the solution in the apical third was impossible to achieve with PI at the end of the preparation.

Acknowledgements

The authors are deeply grateful to Dr Bernard Goldfarb (CEREMADE, Paris-Dauphine University, Paris, France) for his major contribution to statistical analyses.

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.

Baugh D, Wallace J (2005) The role of apical instrumentation in root canal treatment: a review of the literature. *Journal of Endodontics* **31**, 333–40.

Berutti E, Marini R, Angeretti A (1997) Penetration ability of different irrigants into dentinal tubules. *Journal of Endodontics* **23**, 725–7.

Brännström M (1984) Smear layer: pathological and treatment considerations. *Operative Dentistry* **3**(Suppl.), 35–42.

Buchanan LS (2000) Negotiating root canals to their termini. *Dentistry Today* **19**, 60–71.

Byström A, Sundqvist G (1985) The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *International Endodontic Journal* **18**, 35–40.

Carvalho FB, Gonçalves M, Tanomaru-Filho M (2007) Evaluation of chronic periapical lesions by digital subtraction radiography by using Adobe Photoshop CS: a technical report. *Journal of Endodontics* **33**, 493–7.

Coldero LG, McHugh S, MacKenzie D, Saunders WP (2002) Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. *International Endodontic Journal* **35**, 437–46.

Delano EO, Ludlow JB, Ørstavik D, Tyndall D, Trope M (2001) Comparison between PAI and quantitative digital radiographic assessment of apical healing after endodontic treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **92**, 108–15.

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

Flanders DH (2002) Endodontic patency. How to get it. How to keep it. Why it is so important. *New York State Dental Journal* **68**, 30–2.

Gambarini G (1999) Shaping and cleaning the root canal system: a scanning electron microscopic evaluation of a new instrumentation and irrigation technique. *Journal of Endodontics* **25**, 800–3.

Gambarini G, Laszkiewicz J (2002) A scanning electron microscopic study of debris and smear layer remaining following use of GT rotary instruments. *International Endodontic Journal* **35**, 422–7.

Goldman LB, Goldman M, Kronman JH, Lin PS (1981) The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. *Oral Surgery, Oral Medicine, and Oral Pathology* **52**, 197–204.

Gröndahl HG, Gröndahl K, Okano T, Webber RL (1982) Statistical contrast enhancement of subtraction images for radiographic caries diagnosis. *Oral Surgery, Oral Medicine, and Oral Pathology* **53**, 219–23.

Haapasalo M, Endal U, Zandi H, Coil JM (2005) Eradication of endodontic infection by instrumentation and irrigation solutions. *Endodontic Topics* **10**, 77–102.

Harrison JW, Hand RE (1981) The effect of dilution and organic matter on the anti-bacterial property of 5.25% sodium hypochlorite. *Journal of Endodontics* **7**, 128–32.

Jeffcoat MK, Reddy MS, Webber RL, Williams RC, Ruttimann UE (1987) Extraoral control of geometry for digital subtraction radiography. *Journal of Periodontal Research* **22**, 396–402.

Jeon IS, Spangberg LSW, Yoon TC, Kazemi RB, Kum KY (2003) Smear layer production by 3 rotary reamers with different cutting blade designs in straight root canals: a scanning electron microscopic study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **96**, 601–7.

Kahn FH, Rosenberg PA, Gliksberg J (1995) An in vitro evaluation of the irrigating characteristics of ultrasonic and subsonic handpieces and irrigating needles and probes. *Journal of Endodontics* **21**, 277–80.

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

Kouvas V, Liolios E, Vassiliadis L, Parissis-Messimeris S, Boutsioukis A (1998) Influence of smear layer on depth of penetration of three endodontic sealers: an SEM study. *Endodontics and Dental Traumatology* **14**, 191–5.

Mader CL, Baumgartner JC, Peters DD (1984) Scanning electron microscopic investigation of the smeared layer on root canal walls. *Journal of Endodontics* **10**, 477–83.

McComb D, Smith DC (1975) A preliminary scanning electron microscopic study of root canals after endodontic procedures. *Journal of Endodontics* **1**, 238–42.

McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A (2005) Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and Ca(OH)2. *Journal of Endodontics* **31**, 359–63.

Möller AJ, 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.

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

Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K (2008) Outcome of primary root canal treatment: systematic review of the literature – Part 2. Influence of clinical factors. *International Endodontic Journal* **41**, 6–31.

Ørstavik D, Haapasalo M (1990) Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endodontics and Dental Traumatology* **6**, 142–9.

Ørstavik D, Farrants G, Wahl T, Kerekes K (1990) Image analysis of endodontic radiographs: digital subtraction and quantitative densitometry. *Endodontics and Dental Traumatology* **6**, 6–11.

Pashley DH, Tao L, Boyd L, King GE, Horner JA (1988) Scanning electron microscopy of the substructure of smear layers in human dentine. *Archives of Oral Biology* **33**, 265–70.

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

Ruddle CJ (2005) The Protaper technique. *Endodontic Topics* **10**, 187–90.

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.

Schilder H (1974) Cleaning and shaping the root canal. *Dental Clinics of North America* **18**, 269–96.

Schneider SW (1971) A comparison of canal preparations in straight and curved canals. *Oral Surgery, Oral Medicine, and Oral Pathology* **32**, 271–5.

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

Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *International Endodontic Journal* **30**, 297–306.

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.

Webber RL, Ruttimann UE, Grondahl HG (1982) X-ray image subtraction as a basis for assessment of periodontal changes. *Journal of Periodontal Research* **17**, 509–11.

White RR, Goldman M, Lin PS (1984) The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. *Journal of Endodontics* **10**, 558–62.

White RR, Goldman M, Lin PS (1987) The influence of the smeared layer upon dentinal tubule penetration by endodontic filling materials. Part II. *Journal of Endodontics* **13**, 369–74.

Yamada RS, Armas A, Goldman M, Lin PS (1983) A scanning electron microscopic comparison of a high-volume final flush with several irrigating solutions: Part 3. *Journal of Endodontics* **9**, 137–42.

Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389–98.