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Cleanliness of dentinal tubules following gutta-percha removal with and without solvents: a scanning electron microscopic study

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Abstract

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Aim To determine the influence of solvents on gutta-percha and sealer remaining on root canal walls and in dentinal tubules.

Methodology The root canals of 70 teeth were prepared chemomechanically to apical size 40. In group 1 (n = 10; control group), the canals remained unfilled. In groups 2–4 (n = 20 each), the canals were filled using lateral compaction with gutta-percha and sealer. Removal of root fillings was undertaken after 2 weeks using Gates Glidden burs and hand files without solvent (group 2), with eucalyptol (60 μL; group 3) and with chloroform (60 μL; group 4) to size 50. After further irrigation using sodium hypochlorite and ethylenediaminetetraacetic acid, the roots were split, photographed and scanning electron microscopy (SEM) was performed. The number of filled dentinal tubules (SEM) and the surface covered by root filling remnants (photographs) were evaluated for the coronal, middle and apical third of each root half. Statistical analysis was performed via mixed model for clustered data followed by Tukey's test.

Results After pooling the results of all thirds of the canal, open tubules were more prevalent in the control group, followed by the nonsolvent group, the eucalyptol group and the chloroform group ($P < 0.05$ between all groups). Less surface was covered by root filling remnants in the nonsolvent group than in the eucalyptol group and the chloroform group ($P < 0.05$); again, fewer remnants were found in the control group than in all other groups ($P < 0.05$).

Conclusions Solvents led to more gutta-percha and sealer remnants on root canal walls and inside dentinal tubules.

Keywords: chloroform, dentinal tubules, endodontic retreatment, eucalyptol, gutta-percha removal, scanning electron microscopy.

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Introduction

Because of the increasing demand to preserve teeth, including cases with post-treatment disease following root canal treatment, there is a growing interest in conventional retreatment. The procedure requires the removal of the existing root filling, further instrumentation, disinfection and refilling (Stabholz & Friedman 1988). The successful removal of gutta-percha and sealer is an important step; however, it has not been proved that the complete removal of root filling materials will ensure success of root canal retreatment and that remaining material will cause the retreatment to fail. Nevertheless, removing the maximum amount of filling material from inadequately prepared and/or filled root canal systems appears to be essential in order

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to uncover remaining necrotic tissue or bacteria that may be responsible for the persistent disease and enable thorough chemomechanical reinstrumentation and redisinfection of the root canal system (Bergenholtz *et al.* 1979).

The removal of root filling material can be achieved with endodontic hand files, engine-driven rotary instruments, heat-carrying or ultrasonic devices (Wilcox 1989, Friedman *et al.* 1990, Zakariasen *et al.* 1990, Schirrmeister *et al.* 2006a). Furthermore, solvents can be used to soften and dissolve gutta-percha in the root canal to facilitate its penetration and removal. Whether solvents are helpful during gutta-percha removal or not, is inconclusive. One study showed less gutta-percha remnants after retreatment of curved canals using ProFile instruments in combination with chloroform than without a solvent (Ferreira *et al.* 2001), whereas no difference was found in another study using ProFile instruments with and without chloroform (Sae-Lim *et al.* 2000). Both studies failed to reveal a significant reduction in working time when using chloroform. In another study, the use of eucalyptol was also unable to decrease working time, or to increase canal wall cleanliness (Hülsmann & Bluhm 2004) .

After gutta-percha removal, open dentinal tubules would be necessary in order to eliminate bacteria using irrigants. Bacterial invasion of dentinal tubules has been demonstrated (Love & Jenkinson 2002). To date, there is no literature regarding the cleanliness of dentinal tubules after retreatment with and without a solvent using scanning electron microscopy (SEM). Therefore, the aim of this study was to determine whether root filling material remained in dentinal tubules, visualized by SEM, and to evaluate macroscopic root filling remnants on the root canal surface after gutta-percha removal with chloroform, eucalyptol and without using a solvent.

Materials and methods

Specimen preparation

Soft tissue and calculus of 70 extracted human maxillary incisor and canine teeth were removed from root surfaces and the teeth stored in an aqueous solution containing 0.001% thymol for no longer than 6 months. Radiographic evaluation revealed patent canals and canal curvature angles of 0–10° (Schneider 1971). Access cavity preparations were achieved using high-speed burs and water spray. A size 10 K-type file was inserted into the canal until it was visible at the apical foramen. The working length was established 1 mm short of this length. The incisal edge was adjusted, so that the working length of each tooth was 18 mm. The root surfaces were grooved horizontally at a distance of 2, 6 and 10 mm from the anatomical apex, in order to define the position for the SEM images.

Canal preparation

Root canals were prepared using FlexMaster instruments sizes 30, 25 and 20 with a 0.06 taper (VDW, Munich, Germany) in a crown-down technique according to the manufacturer's recommendation. FlexMaster instrument size 30, 0.04 taper reached the working length. Apical enlargement was completed with FlexMaster instruments sizes 35 and 40 with a 0.02 taper. Irrigation was performed using 3% sodium hypochlorite (NaOCl) during instrumentation. Finally, the root canals were rinsed for 1 min using 17% ethylenediaminetetraacetic acid (EDTA, 5 mL), followed by 3% NaOCl (10 mL). A 28-gauge irrigation needle (VMK Endoneedle, Vedevar, Dilbeek, Belgium), inserted 1–2 mm short of the working length was used for irrigation. All root canals were dried with paper points.

All samples were randomly divided into four groups (group 1: $n = 10$; groups 2–4: $n = 20$). The roots in group 1 (control group) remained unfilled.

Canal filling

The root canal of each tooth in groups 2–4 was filled using lateral compaction of gutta-percha. A master gutta-percha cone size 40 was selected and tug-back was checked. AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) was mixed according to the manufacturer's instructions. The master cone was coated with sealer and positioned into the canal. Thereafter, accessory gutta-percha cones sizes 20 were laterally compacted using nickel-titanium finger spreaders size 20, until they could not be introduced more than 5 mm into the canal. The extent of the root filling was limited to 14 mm from the apex, so that the volume of gutta-percha was nearly equal for all roots. The roots were radiographed in buccolingual and mesiodistal directions in order to confirm the adequacy of the root filling. The access cavities were filled temporarily (Coltosol, Coltène/Whaledent, Altstätten, Switzerland). All teeth were stored in a humidor at 37 °C and 100%

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humidity for 2 weeks to allow the complete setting of the sealer.

Retreatment technique

In groups 2–4, 6 mm of root filling material was removed from the cervical part of the canal using Gates Glidden burs sizes 2, 3 and 4. In the middle and apical part of the canal, Hedström files sizes 25–50 were used in a circumferential quarter-turn push–pull filing motion in order to remove gutta-percha and sealer from the canal. No solvent was used for gutta-percha removal in group 2. Eucalyptol served as a solvent in group 3, chloroform in group 4; 15 μL of solvent was applied four times in each canal (overall: 60-μL solvent per canal). During reinstrumentation, 3% NaOCl was used as an irrigant. After gutta-percha removal, the canals were irrigated for 1 min with 17% EDTA (5 mL), followed by 3% NaOCl (10 mL) using the aforementioned irrigation needle 1–2 mm short of the working length. Finally, all canals were dried with paper points.

Evaluation

The teeth were grooved with a diamond saw, split longitudinally, and both root halves were photographed (EOS 20D, with lens EF 100 mm 1:2.8 Macro USM, and MacroRing Lite MR-14EX; Canon, Tokyo, Japan). The total canal wall area and the area covered by root filling remnants were measured in mm^2 for the coronal, middle and apical thirds of the specimens at 20 × magnification using image analyzer software (Adobe Photoshop CS4, Adobe Systems Inc., San Jose, CA, USA).

For SEM analysis, the specimens were dehydrated at 37 °C for 7 days and sputtered with gold (SCD 050 Sputter Coater, Bal-Tec, Balzers, Liechtenstein). The coronal, middle and apical thirds of all root halves were examined using a SEM (LEO 435VP; LEO Electron Microscopy, Cambridge, UK) at 10–15 kV and at a standard magnification of 2000 ×. One image was made at the position of each groove prepared in the root surface. For statistical analysis, the total number of dentinal tubules and the number of dentinal tubules either completely or partially filled with material were recorded.

The evaluation of the coded photographs and SEM images was performed by an operator who was unaware of the method of retreatment. The digital images were displayed at 20 × magnification for the photographs and 2000 × magnification for the SEM images and simultaneously examined on a 15-inch thin film transistor computer monitor operating at 1024 × 768 × 16 bit in a darkened room to minimize glare.

Statistical analysis

A mixed model (analysis of clustered data) was used. Model assumptions were graphically checked by residuals and other regression diagnostics (including Cook's distance). Normality of error terms can be assumed. In the main analysis, all observations were included without distinguishing between the coronal, middle and apical third. In the second analysis (subanalysis), the thirds of the root canal were considered separately. The group effect was calculated and the *P*-values for the pairwise comparison were adjusted by the method of Tukey–Kramer. All calculations were completed using Proc Mixed with the repeated statement from the statistical software SAS 9.1.2 (SAS Institute Inc., Cary, NC, USA). Significance was established at 5% ($P < 0.05$).

Results

The results of the SEM analysis for dentinal tubules and the photographic analysis for surface remnants are summarized in Table 1.

Dentinal tubules

Figure 1 shows representative SEM images from all groups. Because of tubular sclerosis and/or artefacts only 367 of 420 SEM images could be evaluated with regard to open dentinal tubules (group 1: 53 of 60, group 2: 103 of 120, group 3: 101 of 120 and group 4: 110 of 120).

The SEM analysis showed the highest rate of open tubules was in group 1, followed by groups 2–4 ($P < 0.05$ between all groups). More open tubules were found in the apical third than in the middle third ($P < 0.05$).

Surface remnants

Photographs revealed less remnants in group 2 than in groups 3 and 4 ($P < 0.05$), and less remnants in group 1 than in all other groups ($P < 0.05$, Table 1). No significant differences were found between coronal, middle and apical thirds ($P < 0.05$).

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Table 1 Estimated least square means (mean), standard errors (SE) of the ratios evaluated in SEM (number of obturated dentinal tubules/total number of dentinal tubules) and photographic (area covered by root filling material remnants in mm^2/total area of the root canal third in mm^2) analysis and numbers of evaluated images (*n*)

	SEM analysis				Photographic analysis			
	Mean	SE	*n*	Tukey	Mean	SE	*n*	Tukey
All thirds								
Control group	0.00	0.04	53	A	0	0.02	60	A
Without solvent	0.37	0.03	103	B	0.08	0.02	120	B
Eucalyptol	0.56	0.03	101	C	0.16	0.02	120	C
Chloroform	0.78	0.03	110	D	0.20	0.02	120	C
Coronal third								
Control group	0	0.07	19	A	0	0.04	20	A
Without solvent	0.44	0.05	39	B	0.07	0.03	40	A
Eucalyptol	0.53	0.05	38	B	0.11	0.03	40	A, B
Chloroform	0.75	0.05	39	C	0.24	0.03	40	B
Middle third								
Control group	0	0.06	20	A	0	0.04	20	A
Without solvent	0.51	0.05	34	B	0.10	0.03	40	A
Eucalyptol	0.58	0.05	37	B	0.21	0.03	40	B
Chloroform	0.83	0.05	39	C	0.21	0.03	40	B
Apical third								
Control group	0	0.08	14	A	0	0.04	20	A
Without solvent	0.14	0.05	30	A	0.08	0.03	40	A, B
Eucalyptol	0.59	0.06	26	B	0.15	0.03	40	B
Chloroform	0.75	0.05	32	B	0.14	0.03	40	B
All groups								
Coronal third	0.43	0.03	135	A, B	0.10	0.01	140	A
Middle third	0.48	0.03	130	A	0.13	0.01	140	A
Apical third	0.37	0.03	102	B	0.09	0.01	140	A

Groups with the same characters did not show significant differences ($P < 0.05$).

Discussion

In earlier studies on gutta-percha removal, gutta-percha remnants were assessed radiographically (Ferreira *et al.* 2001), or roots were split longitudinally and residual gutta-percha and sealer were measured using evaluation scales (Hülsmann & Stotz 1997, Sae-Lim *et al.* 2000, Baratto *et al.* 2002). In recent studies, the roots were cleared to allow measurement of the area of residual gutta-percha or sealer (Schirrmeister et al. 2006b–e). Only one study using SEM has been reported (Ezzie *et al.* 2006). However, only a few representative SEM images were taken, without evaluating the dentinal tubules.

The removal of the root filling material from dentinal tubules seems to be essential in order to uncover bacteria that might be responsible for post-treatment disease and to eliminate them using irrigant solutions. Furthermore, root filling material remnants might reduce adaptation and adhesion of sealers and cements used for posts. In the present study, chloroform and eucalyptol were used as they are two commonly used gutta-percha solvents (Hunter *et al.* 1991).

The evaluation of canal filling remnants using SEM has been questioned, as studies did not report the magnifications used (Goldberg *et al.* 1988, Haikel & Allemann 1988) or used different magnifications during the investigation (Prati *et al.* 1994). Goldberg *et al.* (1988) also did not evaluate the results of different parts of the root separately. Furthermore, the area the image is taken from is chosen by the operator and thus open to bias (Hülsmann *et al.* 2005). Therefore, in this study, a magnification of 2000 × was set for all images and the results were evaluated both with and without respect to the part of the root the images were made. Operator bias was limited by grooves in the root surface 2, 6 and 10 mm from the anatomical apex specifying the area for investigation.

At most six repeated measures were taken from each tooth [two tooth halves and three tooth parts (coronal, middle and apical third)]. A mixed model has been used to take this within-teeth dependency into account. Because of tubular sclerosis and/or artefacts, open dentinal tubules could not be evaluated in all SEM images. Tubular sclerosis is a physiological phenomenon that starts in the third decade of life in the apical

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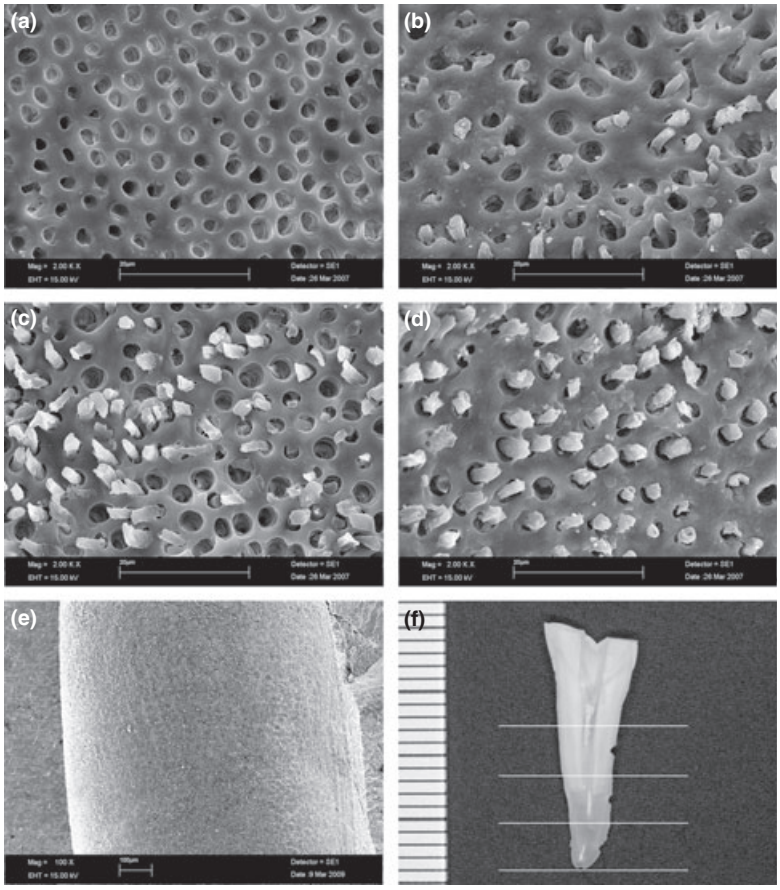

Figure 1 Representative SEM images at a magnification of 2000 × for each group (1: control group, 2: nonsolvent group, 3: eucalyptol group and 4: chloroform group); 5: overview SEM image of the root canal at a magnification of 100 × ; 6: representative photograph of a specimen.

part of the root canal and advances coronally with age (Paqué *et al.* 2006). This could explain the lowest number of evaluated samples in the apical third, compared with the middle and coronal thirds. The teeth used in this study were taken from a tooth bank. Therefore, the age of the specimens could not be controlled, although age would have had an influence on patent tubules.

The SEM analysis showed significantly more open tubules in the apical third of the root canal compared with the middle third. The photographic analysis revealed no significant differences between the root canal thirds. The result of the SEM analysis is in contrast to studies that found more remnants in the apical third of the root canal (Wilcox *et al.* 1987, Ferreira *et al.* 2001, Gergi & Sabbagh 2007). This might be due to different study designs regarding root canal curvature and preparation technique. In the present study, straight canals were reprepared using files two sizes larger than the ones used in the initial preparation. Furthermore, these studies did not use SEM to visualize dentinal tubule debris. SEM analysis generally revealed a higher ratio of dentinal tubule debris than the photographic analysis of surface remnants. This leads to the conclusion that the macroscopic evaluation of surface remnants does not reveal the extent of dentinal tubule debris on a microscopic level.

None of the techniques tested was able to completely remove the root filling material. This is in accordance with previous studies (Ferreira *et al.* 2001, de Oliveira *et al.* 2006, Ezzie *et al.* 2006, Schirrmeister *et al.* 2006b, d,e). It seems that more remnants were found in irregularities of the root canal wall and in dentinal tubules with increasing dissolution of the root filling material. This might be explained by the fact that softened root filling material may easily be compacted into these irregularities and into dentinal tubules from where they can no longer be removed. This effect was observed more often in the solvent groups than in the nonsolvent group, and more often in the chloroform group than in the

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eucalyptol group (with regard to SEM images). The difference between the chloroform group and the eucalyptol group might be explained by the higher solubility of gutta-percha in chloroform compared with eucalyptol (Wourms *et al.* 1990, Pecora & Andreana 1993).

Remaining root filling material inside dentinal tubules rarely blocked the entire cross-section of the dentinal tubule. This might be due to the dissolution of the root filling material by the solvent, but was also observed in the nonsolvent group. Another explanation might be that the gutta-percha/sealer 'tags' were pulled out of the tubules to a minor degree during instrumentation. The motion of the cutting edges of the instruments might explain why the gutta-percha/sealer 'tags' did not block the entire cross-section of the tubule and were not pulled out axially.

Conclusion

Solvents led to more gutta-percha and sealer remnants on root canal walls and inside dentinal tubules. Therefore, solvents should not be standard practice during endodontic retreatment. They should only be applied if the working length cannot be reached without a solvent.

Further studies should evaluate the effect of ultrasonic irrigation on the cleanliness of dentinal tubules during endodontic retreatment. Moreover, the influence of remnants inside dentinal tubules on diffusion or penetration of the tubules by different irrigation solutions should be assessed.

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References

Baratto FilhoF, Ferreira EL, Fariniuk LF (2002) Efficiency of the 0.04 taper ProFile during the re-treatment of gutta-percha-filled root canals. *International Endodontic Journal* **35**, 651–4.

Bergenholtz G, Lekholm U, Milthon R, Hedén G, Ödesjö B, Engström B (1979) Retreatment of endodontic fillings. *Scandinavian Journal of Dental Research* **87**, 217–24.

Ezzie E, Fleury A, Solomon E, Spears R, He J (2006) Efficacy of retreatment techniques for a resin-based root canal obturation material. *Journal of Endodontics* **32**, 341–4.

Ferreira JJ, Rhodes JS, Ford TR (2001) The efficacy of gutta-percha removal using ProFiles. *International Endodontic Journal* **34**, 267–74.

Friedman S, Stabholz A, Tamse A (1990) Endodontic retreatment-case selection and technique. 3. Retreatment techniques. *Journal of Endodontics* **16**, 543–9.

Gergi R, Sabbagh C (2007) Effectiveness of two nickel-titanium rotary instruments and a hand file for removing gutta-percha in severely curved root canals during retreatment: an ex vivo study. *International Endodontic Journal* **40**, 532–7.

Goldberg F, Soares I, Massone EJ, Soares IM (1988) Comparative debridement study between hand and sonic instrumentation of the root canal. *Endodontics and Dental Traumatology* **4**, 229–34.

Haikel Y, Allemann C (1988) Effectiveness of four methods for preparing root canals: a scanning electron microscopic evaluation. *Journal of Endodontics* **14**, 340–5.

Hülsmann M, Bluhm V (2004) Efficacy, cleaning ability and safety of different rotary NiTi instruments in root canal retreatment. *International Endodontic Journal* **37**, 468–76.

Hülsmann M, Stotz S (1997) Efficacy, cleaning ability and safety of different devices for gutta-percha removal in root canal retreatment. *International Endodontic Journal* **30**, 227–33.

Hülsmann M, Peters OA, Dummer PM (2005) Mechanical preparations of root canals: shaping goals, techniques and means. *Endodontic Topics* **10**, 30–76.

Hunter KR, Doblecki W, Pelleu GB Jr (1991) Halothane and eucalyptol as alternatives to chloroform for softening gutta-percha. *Journal of Endodontics* **17**, 310–1.

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

de Oliveira DP, Barbizam JV, Trope M, Teixeira FB (2006) Comparison between gutta-percha and Resilon removal using two different techniques in endodontic retreatment. *Journal of Endodontics* **32**, 362–4.

Paqué F, Luder HU, Sener B, Zehnder M (2006) Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. *International Endodontic Journal* **39**, 18–25.

Pecora G, Andreana S (1993) Use of dental operating microscope in endodontic surgery. *Oral Surgery, Oral Medicine, and Oral Pathology* **75**, 751–8.

Prati C, Selighini M, Ferrieri P, Mongiorgi R (1994) Scanning electron microscopic evaluation of different endodontic procedures on dentin morphology of human teeth. *Journal of Endodontics* **20**, 174–9.

Sae-Lim V, Rajamanickam I, Lim BK, Lee HL (2000) Effectiveness of ProFile.04 taper rotary instruments in endodontic retreatment. *Journal of Endodontics* **26**, 100–4.

Schirrmeister JF, Strohl C, Altenburger MJ, Wrbas KT, Hellwig E (2006a) Shaping ability and safety of five different rotary nickel-titanium instruments compared with stainless steel hand instrumentation in simulated curved root canals. *Oral*

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Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics **101**, 807–13.

Schirrmeister JF, Hermanns P, Meyer KM, Goetz F, Hellwig E (2006b) Detectability of residual Epiphany and gutta-percha after root canal retreatment using a dental operating microscope and radiographs-an ex vivo study. *International Endodontic Journal* **39**, 558–65.

Schirrmeister JF, Meyer KM, Hermanns P, Altenburger MJ, Wrbas KT (2006c) Effectiveness of hand and rotary instrumentation for removing a new synthetic polymer-based root canal obturation material (Epiphany) during retreatment. *International Endodontic Journal* **39**, 150–6.

Schirrmeister JF, Wrbas KT, Meyer KM, Altenburger MJ, Hellwig E (2006d) Efficacy of different rotary instruments for gutta-percha removal in root canal retreatment. *Journal of Endodontics* **32**, 469–72.

Schirrmeister JF, Wrbas KT, Schneider FH, Altenburger MJ, Hellwig E (2006e) Effectiveness of a hand file and three nickel-titanium rotary instruments for removing gutta-percha in curved root canals during retreatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **101**, 542–7.

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

Stabholz A, Friedman S (1988) Endodontic retreatment-case selection and technique. Part 2: treatment planning for retreatment. *Journal of Endodontics* **14**, 607–14.

Wilcox LR (1989) Endodontic retreatment: ultrasonics and chloroform as the final step in reinstrumentation. *Journal of Endodontics* **15**, 125–8.

Wilcox LR, Krell KV, Madison S, Rittman B (1987) Endodontic retreatment: evaluation of gutta-percha and sealer removal and canal reinstrumentation. *Journal of Endodontics* **13**, 453–7.

Wourms DJ, Campbell AD, Hicks ML, Pelleu GB Jr (1990) Alternative solvents to chloroform for gutta-percha removal. *Journal of Endodontics* **16**, 224–6.

Zakariasen KL, Brayton SM, Collinson DM (1990) Efficient and effective root canal retreatment without chloroform. *Journal of the Canadian Dental Association* **56**, 509–12.

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