Influence of tooth age and root section on root dentine dye penetration

A. Thaler, J. Ebert, A. Petschelt & M. Pelka

Dental Clinic 1, Operative Dentistry and Periodontology, University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

Thaler A, Ebert J, Petschelt A, Pelka M. Influence of tooth age and root section on root dentine dye penetration. *International Endodontic Journal*, **41**, 1115–1122, 2008.

Aim To investigate dye permeability of root dentine according to patients' age, root section and dye penetration time.

Methodology A total of 96 extracted human single-rooted teeth, assigned to four age groups (<30, 30–45, 45–60 and >60 years) were separated at the cemento–enamel junction and root canals were enlarged. The root surfaces were coated with cyanocrylate to prevent external dye penetration and centrifuged in distilled water to eliminate air. For dye penetration the root canals were filled with methylene blue 5%. After 1, 30 and 60 days eight roots per age group were cross-sectioned in 1 mm slices. Dye penetrated areas and the complete dentine areas were digitized and measured. Differences between groups were judged with ANOVA and LSD, $P < 0.05$ or $P < 0.01$.

Results The root section, the patients' age and the penetration time influenced significantly the penetrated areas ($P < 0.05$). After 1 and 30 days significant differences could be found only in the apical root sections between all age groups ($P < 0.05$). Dye penetration areas systematically decreased with increasing age and also from coronal to apical ($P < 0.01$).

Conclusions Age influenced dye penetration significantly. Dye penetration also depended on the location (coronal, middle and apical) within the root canal. These findings indicate that there may be a correlation between the tooth age and permeability of root dentine, which may influence the distribution and effectiveness of drugs used for root canal disinfection.

Keywords: disinfection, dye penetration, permeability, root canal, root dentine, sclerosis.

Received 1 April 2008; accepted 31 August 2008

Introduction

One important aim of endodontic treatment is prevention or treatment of the infection within the root canal system (European Society of Endodontology 2006). In the course of a bacterial infection or reinfection of the root canal system, bacteria grow from the root canal into the dentinal tubules, infecting dentine, cement and the surrounding bone (Armitage *et al.* 1983, Pissiotis & Spångberg 1994, Barthel *et al.* 1999, Haapasalo *et al.* 2003). To remove the infection, the main canals are instrumented, resulting in a considerable reduction of bacterial counts within the root canal, but also in clogging of the dentinal tubules by smear layer (Marshall *et al.* 1960, Fogel & Pashley 1990, Tao *et al.* 1991, Deardorf *et al.* 1994, Sen *et al.* 1995, Paqué *et al.* 2006). This can be partly prevented using chelating agents during instrumentation (Hülsmann *et al.* 2002, 2003) or after instrumentation by irrigation with chelating agents or acids (Torabinejad *et al.* 2002, 2003, Hülsmann *et al.* 2003). Besides removal of the smear layer irrigation of the root canal system with disinfecting solutions as well as the application of medicaments into the root canal have

Correspondence: PD Dr Matthias Pelka, Dental Clinic 1, Operative Dentistry and Periodontology, University of Erlangen, Nuremberg, Glückstrasse 11, D-91054 Erlangen, Germany (Tel.: +49 9131 8536310; fax: +49 9131 8533603; e-mail: pelka@dent.uni-erlangen.de).

a potential influence in reduction of the bacterial load inside the root dentine (Fogel *et al.* 1988, Ørstavik & Haapasalo 1990, Gutierréz *et al.* 1991, Deardorf *et al.* 1994, Hanks *et al.* 1994, Haapasalo *et al.* 2000, Gomes *et al.* 2003, Sirén *et al.* 2004). The presence or absence of a smear layer also has an important influence on the permeability of dentine (Sen *et al.* 1995).

The permeability of dentine has been evaluated extensively within a lot of studies (Outhwaite *et al.* 1976, Pashley & Livingston 1978, Pashley *et al.* 1978, 1987, Garberoglio & Bassa 1982, Fogel *et al.* 1988, Maroli *et al.* 1992, Mjör *et al.* 2001, Özok *et al.* 2002). This permeability is dependent on the configuration of the intertubular dentine (Pashley *et al.* 1978, Montgomery 1984, Oliver & Abbott 1991, Pissiotis & Spångberg 1994, Peters *et al.* 2000, Gladys *et al.* 2001, Paqué *et al.* 2006), that is the product of the number and diameter of the dentinal tubules, dentine thickness, temperature and the distance from the pulp chamber. In the case of dye penetration it is also dependent on solubility, concentration and particle size of the dye. Other factors are capillary forces, osmotic gradients and concentration gradients (Pashley *et al.* 1978, Vasiliadis *et al.* 1983, Montgomery 1984, Oliver & Abbott 1991, Tagami *et al.* 1992, Barthel *et al.* 1999, Haapasalo *et al.* 2000, Gladys *et al.* 2001, Hülsmann *et al.* 2003, Nair *et al.* 2005, Paqué *et al.* 2006).

Dentinal sclerosis is an important factor, because sclerosis reduces the volume that is available for penetration of bacteria and their metabolic products, as well as for penetration of irrigant solutions and intracanal drugs. This may play an important role in the outcome of endodontic treatment (Ørstavik & Haapasalo 1990). Nevertheless, knowledge about the spatial and time-dependant development of dentinal sclerosis is still lacking, because the age of the patients donating teeth for experimental studies is rarely known.

Thus, the purpose of this *ex vivo* study was to assess the relationship between root dentine permeability, patients' age, the root canal section and the penetration time to clarify the influence of dentine age on the penetration of dye solution into the dentine. The following hypotheses were tested:

1. Passive dye penetration in root canals does not depend on the tooth age.

2. Dye penetration is equal in all root canal sections.

3. Penetration time does not influence dye penetration.

Materials and methods

Test teeth

One hundred and seventy-eight single-rooted teeth with mature apices scheduled for extraction were collected. All met the following inclusion criteria: single-rooted teeth, no large carious lesions, restorations or previous endodontic treatment, mature apices. The age of the patients was recorded. Teeth were divided into four age groups: <30 years, 30–45 years, 45–60 years and >60 years. All teeth were stored in 0.2% of sodium azide (NaN_3) at 4 °C in separate containers according to the different age groups. The teeth were stored for a maximum of 4 weeks.

Instrumentation and irrigation procedure

The root surfaces of all teeth were cleaned with scalers. Subsequently the crowns were sectioned 2 mm coronal to the cemento–enamel junction. The root length was determined by inserting a size 08 K-File (Maillefer, Dentsply, Ballaigues, Switzerland) into the root canal until the tip was just visible at the apical foramen. Working length was calculated as this distance minus one millimetre. Root canals were prepared to at minimum size of 35, taper 0.04 with ProFile instruments (Maillefer) and to three instrument sizes greater than the first binding file at working length. After the use of each instrument the root canals were alternately irrigated with 1 mL of 3% sodium hypochlorite and 1 mL of 40% citric acid.

To complete the chemo-mechanical preparation and to remove the smear layer, a final irrigation with 2 mL of 3% sodium hypochlorite and 2 mL of 40% citric acid in five repeats followed (Pérez-Heredia *et al.* 2006). All root canals were rinsed with 2 mL of 70% ethanol to remove remnants of citric acid. To prepare the roots for the dye penetration test, they were rinsed with water and dried by compressed air. The root surfaces were coated with five layers of cyanoacrylate (Renfert, Hilzingen, Germany) to prevent external dye penetration. The specimens were then centrifuged in distilled water (30 min at 4 000 *G*) to remove air within the dentinal tubules, which may inhibit the diffusion process.

After inital preparation, 96 of the 170 teeth were available for the study. That is, 24 teeth for each age group. The discarded teeth were sclerotic, had obliterated root canals or had two root canals or became cracked during centrifugation. The four age groups

were divided into three subgroups in terms of penetration time (1 day, 30 days and 60 days; $n = 8$ per subgroup) and stored in 0.2% sodium acid at 4 °C.

Dye penetration and cross-sectioning

Four boxes, one for each age group, were prepared. Each box contained an antibacterial impregnated foam rubber (Incidur® 5 mL; Ecolab, Wien, Austria) and 100 mL distilled water). This impregnation avoided the colonization of the foam rubber by bacteria and mould spores during the incubation time. Eight randomized chosen roots from each age group were fixed in the corresponding box and the root canals were filled with filtrated 5% methylene blue solution using a syringe and a 0.3×23 mm gauge needle (Transcoject, Neumünster, Germany). The closed boxes were stored in an incubator at 37 °C during the penetration test. With exception of the 1-day penetration group, the experimental setup was examined every day and the dye solution as well as the antibacterial solution was replenished.

At the end of the dye penetration procedure, the roots and root canals were thoroughly rinsed with water, gently dried, glued face down onto the bottom of a casting mould and embedded in resin (Modralit-3K; Dreve-Dentamid, Unna, Germany). The hardened resin blocks were serially sectioned in 1 mm steps with a water-cooled inner-diameter saw (The Roditi Int. Corp., Hamburg, Germany). The first slice in the coronal root section and the last apical slice were discarded. Twelve to 20 sections per root were available for analysis.

For each root, the number of utilizable cross-sections was divided into three. The number of sections assigned to the coronal, middle and apical third in every root was approximately equal. The overall number of slices in the three root sections was similar.

Data analysis and photo documentation

All cross-sections were serially numbered, according to age group, root section and dye penetration time with the aid of evaluation lists, which determined exactly each single root cross-section. The total dentine area of each slice was digitized using a stereomicroscope (Wild M3Z Type S®; Leica Heerbrugg AG, Heerbrugg, Switzerland) equipped with a video camera, a frame grabber card and digitizing software (TIFMES 1.7; Dental Clinic, Erlangen, Germany). For the coronal and middle root sections a 6.5-fold magnification was used, and for the apical sections 10-fold magnification was used. A

millimetre scale placed next to each slice served as a calibration standard. The dye penetrated areas, the total dentine areas and the root canal areas were measured at each level with the same software. The digitized pictures were evaluated as follows. First the complete dye penetrated dentine area was gradually identified. In the same manner the area of the root canal was interactively marked. Third the complete dentine area was marked. Thus, in each sample three polygons were marked. The software computed the three areas using the pixel resolution of the microscopic pictures (calculated with the calibration standard).

In addition, cross-section series of two teeth for each subgroup and some special findings were digitally photographed using a Nikon D100® digital camera (Nikon GmbH, Düsseldorf, Germany) and a stereomicroscope (Zeiss Stemi SV6®; Carl Zeiss Jena GmbH, Jena, Germany).

The percentage of the dye penetrated area related to the complete dentine area in each section was measured in the following manner: three values were measured per cross-section, first the whole cross-sectional area including the root canal (a), then the whole stained area including the root canal (b), and then the area of the root canal (c). Dye penetration was calculated as a percentage of the whole cross-sectional area excluding the root canal {stained area in per cent = $[(b-c)/(a-c)] \times 100$}.

Statistical analysis was carried out with SPSS 15.0® (SPSS Inc., Chicago, IL, USA) using an analysis of variance (three-way ANOVA, one-way ANOVA and LSD). The level of significance was set to $P = 0.01$ and $P = 0.05$. The threefold null hypothesis was that neither patients' age, nor root section, nor penetration time had an influence on dye penetration into the dentine tubules.

Results

Table 1 summarizes the mean results from all age groups, localization and penetration groups with the accompanying statistical differences. Three-way ANOVA revealed significant differences for all three factors (age, penetration time and root section) tested ($P < 0.001$). Thus, the relative dye penetration decreased from coronal to apical, decreased with the age of the patient and increased with the dye penetration time – with some exceptions. Furthermore all possible combinations of two of the three factors showed significant influences ($P < 0.01$), but the combination of all three factors together did not show a significant influence ($P = 0.7$).

Table 1 Summary of the results. The table shows the mean relative penetration areas with standard deviations (SD) in per cent of the complete dentin area

Age		<30		30–45		45–60		>60	
Root section	Time (days)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Coronal	1	35.29[a]	11.35	34.00[a]	10.69	31.85[a]	12.12	30.05[a]	15.66
	30	53.71[a]	11.81	52.70[a]	11.51	49.55[a b]	14.95	47.13[a b]	20.10
	60	75.91[c]	13.11	63.12[c]	17.55	52.18[a b]	18.77	49.14[a b]	26.60
Middle	1	23.67[a]	11.66	22.15[a]	14.52	18.71[a b]	6.95	15.63[a b]	9.78
	30	38.99[a]	11.26	31.98[a]	11.96	23.82[a b]	13.31	18.13[a b]	13.10
	60	74.32[c]	13.59	57.59[c]	19.64	40.81	16.69	28.60	18.73
Apical	1	13.80[a b]	12.01	10.60[a b]	11.81	6.19[a b]	6.56	2.83[a b]	2.17
	30	20.82[a b]	12.87	17.80[a b]	10.94	10.11[b]	5.93	5.04[b]	7.48
	60	46.74	27.02	30.92	20.96	15.26[b]	12.65	6.20[b]	6.88

[a]marks not significant differences between age groups.
[b]marks not significant differences between different penetration times.
[c]marks not significant differences between the root sections.
Not marked results differed significantly ($P < 0.01$, ANOVA, LSD).

In the age groups <30 years and 30–45 years the dye penetrated areas increased with increasing penetration time. In the apical subgroups no significant difference could be found between the 1 day and 30 days results (ANOVA, LSD, $P > 0.05$). After 60 days no differences between the coronal and middle root sections could be found. The coronal parts of the roots of teeth older than 45 years remained penetrable, but resulting in significant lower dye penetration in comparison with teeth from patients younger than 45 years (ANOVA, LSD, $P < 0.01$). For the middle root sections, penetrability decreased substantially with age, resulting in <40% dye penetration for teeth of patients over 45 years and 60 days penetration time (Fig. 1). In teeth of young patients (<30 years) the dentinal tubules in the apical regions allowed dye penetration of up to 50% of the dentine area. This value clearly decreased with age resulting in <7% penetrability for the apical sections of teeth in the >60 years group (ANOVA, LSD, $P < 0.01$).
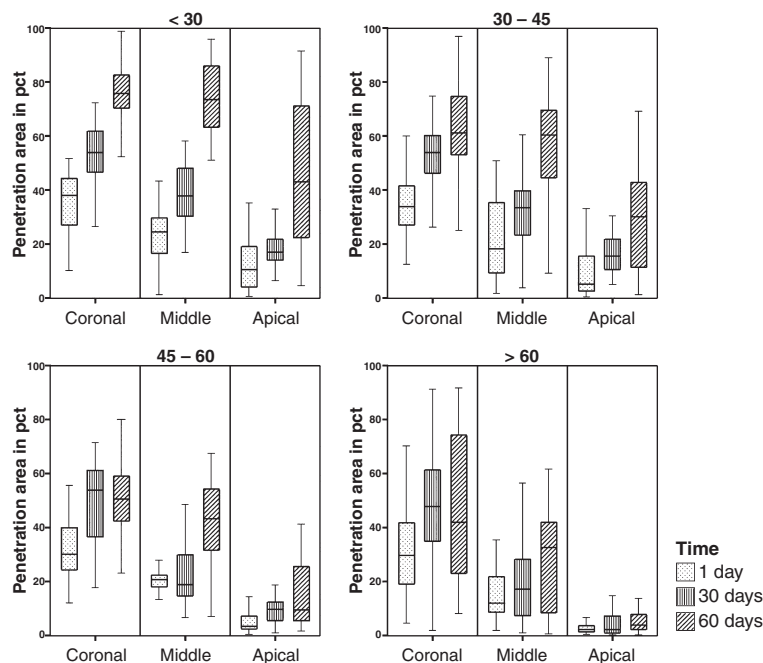
Figure 1 Box plots depicting the relative penetrated dentin areas in percentage. Horizontal bars, medians; boxes, interquartile areas; error bars, 10th and 90th percentile. Clearly visible are the differences in penetration areas between teeth younger than 45 and older. The relative apical penetration area was smallest in the >60 years group. Significances read out from Table 1.

Noticeable in the age group of 45–60 years was the minor difference in the coronal third between 30 and 60 days of incubation (ANOVA, LSD, $P > 0.05$). Overall, the dye penetration in the age groups <30 years and 30–45 years depended more strongly on the incubation time within the different root sections than in the age groups of 45–60 years and >60 years (Table 1).

Additional findings

When reviewing the photographs, it was evident that dye penetration into the dentinal tubules was not equally distributed. Generally, deeper penetration was found in the bucco-lingual direction compared with the mesio-distal direction. In several cases a wide variation was found within the same slice of one root (Fig. 2). So called 'transparent teeth' were found mainly in the apical root sections of older (>45 years) and old (>60 years) specimens. This 'transparency' is a sign of complete dentinal sclerosis.

Discussion

Significant differences were obvious for all three factors evaluated. Thus, all three parts of the null hypothesis were rejected. Furthermore, the results were dependent on combinations of two of the three factors. The decrease of dentine permeability with age did not follow a uniform pattern. It did not have a linear relationship with age or tooth section. Thus additional penetration time within the dye penetration tests did not cause a linear increase of penetrated dentine area.

The experimental design could be a model for simulating the diffusion of medicaments or bacterial toxins into the dentinal tubules. This simulation could also be achieved by radioactively marked molecules (Nalbandian *et al.* 1960), but such substances would have been difficult to handle and the detection of the diffusion pattern would require microradiography that has a very low resolution (Takagi *et al.* 1985) and could not detect the complex diffusion pattern shown in Fig 2.

Dye leakage can detect small voids between restorations or root canal fillings and cavity walls (Going 1972). This method was used to assess dye diffusion into dentinal tubules. Within leakage studies, a broad range of dyes is used including methylene blue or india ink (Spångberg *et al.* 1989, Prati 1994, Ahlberg *et al.* 1995, Youngson *et al.* 1998). Methylene blue has small molecules that allows deeper penetration than other dyes (Ahlberg *et al.* 1995) or radioactive substances (Matloff *et al.* 1982). Furthermore, the molecular size of methylene blue is in the same order as bacterial toxins (Spångberg *et al.* 1989). Due to these properties mentioned above, methylene blue dye was selected as a suitable marker for penetration of dentinal tubules by diffusion.

Entrapped air inside the root canal system may inhibit the penetration of dyes (Oliver & Abbott 1991, Wu *et al.* 1994). Thus, dye penetration tests under negative or high pressure are recommended (Spångberg *et al.* 1989, Wu *et al.* 1994). In the present study a passive dye penetration was applied, but the teeth were centrifuged within distilled water prior to dye diffusion testing to exclude the possible influence of
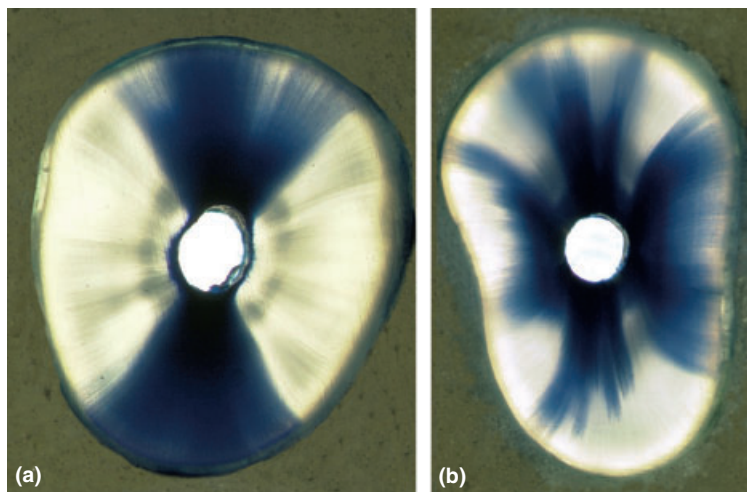
Figure 2 The light microscope pictures show two samples of the middle root section within the 45–60 years group. Dye penetration is not regular distributed around the root canal. (a) The maximum penetration depths were found vestibular and oral. Sclerosis primary occurs in the mesial and distal parts of the root dentine. (b) Frequently we found a lot of irregularities. Only the registration of the complete dye penetrated dentine area produced comparable results.

entrapped air. The high pressure during centrifugation should have ensured that entrapped air within the dentine tubules was replaced by water allowing unhindered passive dye diffusion.

Smear layer can prevent dye solution from diffusion into the dentinal tubules. With the rinsing protocol adapted the smear layer was removed (Pérez-Heredia *et al.* 2006). The selection of teeth with straight roots and round or oval root cross-sections enabled instrumentation of most of the root canal walls with only a few non treated areas remaining. If there were unprepared areas the irrigation with sodium hypochlorite and citric acid would open all dentinal tubules. When a smear layer could not be removed by the irrigation procedure, it was coloured by the dye solution and could be detected easily. Only a few dentinal slices, especially in the apical region had shown persisting smear layer, and these were excluded from further dye penetration evaluation.

Dye leakage tests mostly exhibit broad standard deviations (Schuurs *et al.* 1993, Wu & Wesselink 1993). The variance in dentine tubule content of the apical and middle regions of human teeth as found within the present study may contribute to some of the great variance, which is normally found in apical leakage studies. Until now, the age of patients donating teeth for *ex-vivo* studies is rarely recorded. In future studies the effect of age on apical leakage should be evaluated.

The results demonstrated that relative dentinal dye penetration was strongly dependent on the location of the root canal section and decreased form coronal to apical. If the dye penetrated into the dentine areas had not been related to the complete dentine area, the absolute values for dye penetration decreased even more strongly from coronal to apical. This decrease of dye penetration is simple to explain: The number of dentinal tubules decreases from coronal near the pulp from 40 000 mm^{-2} to 14 400 mm^{-2} in the apical region (Mjör *et al.* 2001).

Within the present study, teeth of older patients (>60 years) had a dentine penetration below 7% of the entire dentinal volume in the apical third because of advanced dentine sclerosis. This sclerosis may be one reason for the better clinical outcome of root canal treatments of older patients as found by Ørstavik *et al.* (2004). Ørstavik *et al.* (2004) assumed that the reduction of canal ramifications with age limited the volume available for infection (Ørstavik *et al.* 2004). Because of the reduction of the tubule diameter through sclerosis and according to the Poiseuille-Hagen equation, such small changes in the functional diameter of dentinal tubules can greatly modify the dentine permeability and reduce the space for residual bacteria. In consequence, the apical root dentine of older patients is most likely impermeable, both to disinfectants and bacteria (Mjör *et al.* 2001). Bacterial colonies in teeth affected by apical periodontitis are often found in and around the apical portals of the root canal system, where they have access to tissue fluid (Nair *et al.* 2005). Thus, new apical inflammation after endodontic treatment by penetration of residual bacteria through root dentine is dramatically reduced by naturally occurring sclerosis of the dentine.

Conclusions

Passive dye penetration of dentine tubules is strongly dependent on the age of the patient, particularly for the middle and apical parts of the roots. Thus, the volume available for infection as well as for the action of irrigants and intracanal medicaments may also be strongly influenced by the age of the patients. This should be taken into account when performing apical leakage studies as well as endodontic treatment procedures. On the basis of these findings and within the limits of the study we can propose that endodontic treatment concepts in terms of root canal enlargement, preparation length and placement of intracanal drugs or disinfectants should consider the age dependency of root dentine permeability.

References

Ahlberg KM, Assavanop P, Tay WM (1995) A comparison of the apical dye penetration patterns shown by methylene blue and india ink in root-filled teeth. *International Endodontic Journal* **28**, 30–4.

Armitage GC, Ryder MI, Wilcox SE (1983) Cemental changes in teeth with heavily infected root canals. *Journal of Endodontics* **9**, 127–30.

Barthel CR, Moshonov J, Shuping G, Ørstavik D (1999) Bacterial leakage versus dye leakage in obturated root canals. *International Endodontic Journal* **32**, 370–5.

Deardorf KA, Swartz ML, Newton CW, Brown CE Jr (1994) Effect of root canal treatments on dentin permeability. *Journal of Endodontics* **20**, 1–5.

European Society of Endodontology (2006) Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. *International Endodontic Journal* **39**, 921–30.

Fogel HM, Pashley DH (1990) Dentin permeability: effects of endodontic procedures on root slabs. *Journal of Endodontics* **16**, 442–5.

Fogel HM, Marshall FJ, Pashley DH (1988) Effects of distance from the pulp and thickness on the hydraulic conductance of human radicular dentin. *Journal of Dental Research* **67**, 1381–5.

Garberoglio R, Bassa S (1982) The transparent tooth. *Mondo odontostomatologico* **24**, 3–6.

Gladys S, Van MB, Lambrechts P, Vanherle G (2001) Microleakage of adhesive restorative materials. *American Journal of Dentistry* **14**, 170–6.

Going RE (1972) Microleakage around dental restorations: a summarizing review. *Journal of the American Dental Association* **84**, 1349–57.

Gomes BP, Souza SF, Ferraz CC *et al.* (2003) Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *International Endodontic Journal* **36**, 267–75.

Gutierréz JH, Donoso E, Villena F, Jofré A (1991) Diffusion of medicaments within root canal dentin. A scanning electron microscopic study. *Oral Surgery, Oral Medicine, and Oral Pathology* **72**, 351–8.

Haapasalo HK, Sirén EK, Waltimo TM, Ørstavik D, Haapasalo MP (2000) Inactivation of local root canal medicaments by dentine: an in vitro study. *International Endodontic Journal* **33**, 126–31.

Haapasalo M, Udnaes T, Endal U (2003) Persistent, recurrent, and acquired infection of the root canal system post-treatment. *Endodontic Topics* **6**, 29–56.

Hanks CT, Wataha JC, Parsell RR, Strawn SE, Fat JC (1994) Permeability of biological and synthetic molecules through dentine. *Journal of Oral Rehabilitation* **21**, 475–87.

Hülsmann M, Heckendorff M, Schäfers F (2002) Comparative in-vitro evaluation of three chelator pastes. *International Endodontic Journal* **35**, 668–79.

Hülsmann M, Heckendorff M, Lennon A (2003) Chelating agents in root canal treatment: mode of action and indications for their use. *International Endodontic Journal* **36**, 810–30.

Maroli S, Khera SC, Krell KV (1992) Regional variation in permeability of young dentin. *Operative Dentistry* **17**, 93–100.

Marshall FJ, Massler M, Dute HL (1960) Effects of endodontic treatments on permeability of root dentine. *Oral Surgery, Oral Medicine, and Oral Pathology* **13**, 208–23.

Matloff IR, Jensen JR, Singer L, Tabibi A (1982) A comparison of methods used in root canal sealability studies. *Oral Surgery, Oral Medicine, and Oral Pathology* **53**, 203–8.

Mjör IA, Smith MR, Ferrari M, Mannocci F (2001) The structure of dentine in the apical region of human teeth. *International Endodontic Journal* **34**, 346–53.

Montgomery S (1984) External cervical resorption after bleaching a pulpless tooth. *Oral Surgery, Oral Medicine, and Oral Pathology* **57**, 203–6.

Nair PN, 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" endodon-tic treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **99**, 231–52.

Nalbandian J, Gonzáles F, Sognnaes RF (1960) Sclerotic age changes in root dentin of human teeth as observed by optical, electron, and x-ray microscopy. *Journal of Dental Research* **39**, 598–607.

Oliver CM, Abbott PV (1991) Entrapped air and its effects on dye penetration of voids. *Endodontics & dental traumatology* **7**, 135–8.

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

Ørstavik D, Qvist V, Stoltze K (2004) A multivariate analysis of the outcome of endodontic treatment. *European Journal of Oral Sciences* **112**, 224–30.

Outhwaite WC, Livingston MJ, Pashley DH (1976) Effects of changes in surface area, thickness, temperature and post-extraction time on human dentine permeability. *Archives of Oral Biology* **21**, 599–603.

Özok AR, Wu MK, Wesselink PR (2002) Comparison of the in vitro permeability of human dentine according to the dentinal region and the composition of the simulated dentinal fluid. *Journal of Dentistry* **30**, 107–11.

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.

Pashley DH, Livingston MJ (1978) Effect of molecular size on permeability coefficients in human dentine. *Archives of Oral Biology* **23**, 391–5.

Pashley DH, Livingston MJ, Reeder OW, Horner J (1978) Effects of the degree of tubule occlusion on the permeability of human dentine in vitro. *Archives of Oral Biology* **23**, 1127–33.

Pashley DH, Andringa HJ, Derkson GD, Derkson ME, Kalat-hoor SR (1987) Regional variability in the permeability of human dentine. *Archives of Oral Biology* **32**, 519–23.

Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP (2006) The effectiveness of different acid irrigating solutions in root canal cleaning after hand and rotary instrumenta-tion. *Journal of Endodontics* **32**, 993–7.

Peters OA, Laib A, Ruegsegger P, Barbakow F (2000) Three-dimensional analysis of root canal geometry by high-resolution computed tomography. *Journal of Dental Research* **79**, 1405–9.

Pissiotis E, Spångberg LS (1994) Dentin permeability to bacte-rial proteins in vitro. *Journal of Endodontics* **20**, 118–22.

Prati C (1994) What is the clinical relevance of in vitro dentine permeability tests? *Journal of Dentistry* **22**, 83–8.

Schuurs AH, Wu MK, Wesselink PR, Duivenvoorden HJ (1993) Endodontic leakage studies reconsidered. Part II. Statistical aspects. *International Endodontic Journal* **26**, 44–52.

Sen BH, Wesselink PR, Türkün M (1995) The smear layer: a phenomenon in root canal therapy. *International Endodontic Journal* **28**, 141–8.

Sirén EK, Haapasalo MP, Waltimo TM, Ørstavik D (2004) In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on *Enterococcus faecalis*. *European Journal of Oral Sciences* **112**, 326–31.

Spångberg LS, Acierno TG, Yongbum CB (1989) Influence of entrapped air on the accuracy of leakage studies using dye penetration methods. *Journal of Endodontics* **15**, 548–51.

Tagami J, Hosoda H, Burrow MF, Nakajima M (1992) Effect of aging and caries on dentin permeability. *Proceedings of the Finnish Dental Society* **88**(Suppl. 1), 149–54.

Takagi S, Chow LC, Brown WE, Dobbyn RC, Kuriyama M (1985) Application of an x-ray image magnifier to the microradiography of dental specimens. *Journal of Dental Research* **64**, 866–9.

Tao L, Anderson RW, Pashley DH (1991) Effect of endodontic procedures on root dentin permeability. *Journal of Endodontics* **17**, 583–8.

Torabinejad M, Handysides R, Khademi AA, Bakland LK (2002) Clinical implications of the smear layer in endodontics: a review. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **94**, 658–66.

Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S (2003) The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *Journal of Endodontics* **29**, 233–9.

Vasiliadis L, Darling AI, Levers BG (1983) The histology of sclerotic human root dentine. *Archives of Oral Biology* **28**, 693–700.

Wu MK, Wesselink PR (1993) Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. *International Endodontic Journal* **26**, 37–43.

Wu MK, De Gee AJ, Wesselink PR (1994) Fluid transport and dye penetration along root canal fillings. *International Endodontic Journal* **27**, 233–8.

Youngson CC, Jones JC, Manogue M, Smith IS (1998) In vitro dentinal penetration by tracers used in microleakage studies. *International Endodontic Journal* **31**, 90–9.