Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals

F. Paqué[1], H. U. Luder[2], B. Sener[1] & M. Zehnder[1]

[1]Division of Endodontology, Department of Preventive Dentistry, Periodontology, and Cariology; and [2]Section for Orofacial Structures and Development, Institute for Oral Biology, University of Zürich Center for Dental Medicine, Zürich, Switzerland

Abstract

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Aim To evaluate the effect of different root canal irrigating regimes on dentine penetration of Patent Blue dye.

Methodology Eighty extracted single-rooted human mandibular premolar teeth with narrow root canals were prepared using ProFile instruments. After each instrument, canals were irrigated with 1% sodium hypochlorite. Subsequently, teeth were randomly assigned to receive a 10 mL rinse of aqueous 17% (w/v) ethylenediaminetetraacetic acid or tap water for 2 or 10 min, followed by a final rinse with a 2% Patent Blue dye solution for 2 or 10 min (eight groups, $n = 10$ teeth per group). Teeth were then horizontally sectioned 3, 6 and 9 mm from the apex. Sections were digitally photographed and dye penetration was calculated as percentage of total dentine area using NIH Image J. Values were compared using one-way ANOVA and Bonferroni correction with the alpha-type error set at <0.05. Representative tooth sections from all groups were further analysed using scanning electron microscopy.

Results No significant impact of irrigating protocols on dye penetration was found. Dye penetration was significantly ($P < 0.001$) greater in the coronal than middle, and in middle than in apical root thirds. When observed microscopically, irrigant penetration was independent of the presence of a smear layer, but was rather a function of tubular sclerosis.

Conclusions Tubular sclerosis, a physiological phenomenon that starts in the third decade of life in the apical root region and advances coronally with age, was the main factor influencing penetrability of root dentine.

Keywords: dentine, dye penetration, ethylenediaminetetraacetic acid, root canal irrigation, sclerosis.

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Introduction

The importance of irrigating root canal systems during and after canal preparation has been known for some time (Grossman 1943). Grossman (1943) distinguished three phases of root canal treatment: mechanical preparation, chemical preparation and disinfection. Methods relying solely on chemical preparation and disinfection of root canal systems thus far have proved to be clinically unsatisfactory (Attin *et al.* 2002). Consequently, root canals are still shaped mechanically to be accessible to disinfectants. Shaping of the root canal wall produces a so-called smear layer, which consists of inorganic dentine components held together by organic debris derived from the pulp–dentine complex (Dautel-Morazin *et al.* 1994). Removal of this smear layer after root canal preparation using a sodium hypochlorite (NaOCl) solution for the organic phase and a calcium-chelating solution for the inorganic phase has been recommended (Baumgartner & Mader

Correspondence: Dr Matthias Zehnder, Department of Preventive Dentistry, Periodontology, and Cariology, University of Zürich Center for Dental Medicine, Plattenstrasse 11, CH 8032 Zürich, Switzerland (Tel.: +41 44 634 3438; fax: +41 44 634 4308; e-mail: matthias.zehnder@zzmk.unizh.ch).

1987). Currently used chelating preparations inhibit the action of NaOCl (Zehnder *et al.* 2005). A sequence of NaOCl (throughout instrumentation)-chelating solution-final irrigant is thus recommended to remove the smear and possibly allow optimal efficacy of the final irrigant.

The root dentine of teeth with apical periodontitis can be infected (Armitage *et al.* 1983). It has been surmised that the smear layer may protect microorganisms in dentinal tubules from the antiseptic action of irrigating solutions and local root canal medicaments (Ørstavik *et al.* 1990). It would appear that dentine antisepsis depends on the diffusion of active antimicrobial moieties into the root canal wall. The role of a smear layer regarding this diffusion, however, remains unclear. The hydraulic conductance of root dentine was reduced by 50% in the presence of the smear layer (Fogel & Pashley 1990). However, removing a smear layer using ethylenediaminetetraacetic acid (EDTA)- and NaOCl-irrigating solutions had only a minimal effect on the diffusion of hydroxide ions from root canals dressed with calcium hydroxide (Foster *et al.* 1993). A similar NaOCl/EDTA-irrigating regimen even decreased the diffusion of $^{3}H_2O$ through human roots when compared with specimens with the smear layer (Galvan *et al.* 1994). Conflicting results have been reported regarding the penetration of isotopes through root dentine after EDTA and/or NaOCl treatment (Marshall *et al.* 1960, Hampson & Atkinson 1964). Furthermore, no information is currently available on the effect that the duration of EDTA application exerts on dentine penetrability. On the contrary, physiological changes in root dentine, such as tubular sclerosis, may have an impact on its permeability that is independent of the presence/absence of the smear layer (Wach *et al.* 1955). A correlation between the influence of a smear layer and underlying structures on dentine permeability, however, has never been made.

It was thus the goal of this *ex vivo* study to assess the impact of a 2-min and a 10-min flush with 10 mL of a 17% EDTA solution on the penetration into root dentine of Patent Blue from a final aqueous irrigant applied in instrumented human mandibular premolars. Dye penetration per total area of dentine was recorded on digital photographs of coronal, middle and apical root cross sections. To assess the impact of underlying structures on dentine permeability, selected root dentine specimens were sectioned or fractured and further analysed using scanning electron microscopy (SEM).

Materials and methods

Dye solution

The dye solution used in this study was an aqueous 2% (w/v) Patent Blue V sodium salt ($C_{27}H_{31}N_2NaO_7S_2$; Fluka, Buchs, Switzerland) solution. Chemicals from local root canal medicaments and irrigants penetrate into root dentine by molecular diffusion via dentinal tubules, which are filled with liquid (Spreter von Kreudenstein & Stüben 1955). Thus, the ability of active irrigant moieties to penetrate dentine is influenced by the irrigant's osmolarity. Furthermore, it has been stated that the surface tension of irrigating solutions may have an effect on their permeation through narrow root canals (Abou-Rass & Patonai 1982). Osmolarity of the Patent Blue solution and common endodontic irrigating solutions, i.e. 1% and 5% NaOCl and 0.2% and 2% chlorhexidine digluconate used in this study, was measured using an osmometer according to the manufacturer's guidelines (One-Ten; Fiske, Needham Heights, MA, USA). Surface tension of these solutions was assessed at 25 °C using the Wilhelmy plate method (Tensiometer K100; Krüss, Hamburg, Germany).

Experimental teeth and instrumentation procedures

Eighty single-rooted mandibular premolars of similar size and root shape, which had been stored in a 0.1% thymol solution at 5 °C, were selected from a collection of extracted teeth. The age of the patients from whom these teeth were extracted was not known. Radiographs taken from the mesio-distal aspect were taken of the experimental teeth to verify that they contained only one root canal. Teeth were cleaned externally from periodontal tissues with scalers, and then immersed in a 1% NaOCl solution for 15 min in an ultrasonic bath. Subsequently, root surfaces were washed in deionized water, dried using compressed air and covered with nail varnish. After gaining access to the root canal system with a diamond-coated bur, canals were instrumented using ProFile (Dentsply Maillefer, Ballaigues, Switzerland) instruments size 40.06–30.06 in a crown-down manner. A size 10 K-File (Dentsply Maillefer) was inserted into the root canal until the tip was just visible beyond the apex. Working length was determined by subtracting 1 mm from this length and root canals were prepared to ProFile size 45.04 at full working length. After each instrument, apical patency was maintained by using a

size 10 K-file, and root canals were irrigated with 1 mL of a 1% NaOCl solution using a 30-gauge irrigating needle 1 mm short of working length (Hawe Neos, Bioggio, Switzerland). After instrumentation all root canals were rinsed with 10 mL of water to remove remnants of NaOCl. Teeth were then randomly assigned to eight groups to receive a rinse with either 17% (w/v) EDTA or water, and subsequently a final irrigation using the 2% Patent Blue solution. In detail, the final irrigation in each experimental group was as follows:

Group I: Water (10 mL in 2 min), Patent Blue solution (10 mL in 2 min)
Group II: Water (10 mL in 2 min), Patent Blue solution (10 mL in 10 min)
Group III: Water (10 mL in 10 min), Patent Blue solution (10 mL in 2 min)
Group IV: Water (10 mL in 10 min), Patent Blue solution (10 mL in 10 min)
Group V: EDTA (10 mL in 2 min), Patent Blue solution (10 mL in 2 min)
Group VI: EDTA (10 mL in 2 min), Patent Blue solution (10 mL in 10 min)
Group VII: EDTA (10 mL in 10 min), Patent Blue solution (10 mL in 2 min)
Group VIII: EDTA (10 mL in 10 min), Patent Blue solution (10 mL in 10 min)

In view of the chemical actions and interactions of NaOCl and EDTA (Grawehr *et al.* 2003), root canals were mechanically prepared in this investigation using solely NaOCl as an irrigant, so as to remove pulpal remnants, predentine and the organic smear layer components (Baumgartner & Mader 1987). Subsequently, as recommended clinically, canals were irrigated using 17% EDTA followed by a final irrigant (Yamada *et al.* 1983). Before and after irrigation with Patent Blue, root canals were rinsed with 10 mL of water to remove remnants of EDTA and the dye solution respectively. Root canals were then dried with paper points (Roeko, Langenau, Germany) inserted to working length.

Root sectioning and evaluation of dye penetration

After drying of the root canals, the teeth were immediately attached to stubs using polymethylmethacrylate (Heraeus Kulzer GmbH, Hanau, Germany). The roots were sectioned perpendicular to the long axis at distances of 3, 6 and 9 mm from the apex using a kerosene-cooled diamond-coated disc (Wirtz-Buehler GmbH, Düsseldorf, Germany). Kerosene was used instead of water to avoid washing out of the penetrated dye solution (Haenni *et al.* 2003). Cross sections were photographed using a Tessovar photographic unit (Zeiss, Oberkochen, Germany). A millimetre scale was placed next to specimens for calibration. For morphometric measurements, slides were scanned (Super Coolscan 400 ED; Nikon, Tokyo, Japan) and imported into a MacIntosh G4 computer (Apple, Cupertino, CA, USA). Penetrated and total dentine areas were determined at each level using Image J software (NIH, Bethesda, MD, USA). Dye penetration was then calculated as a percentage of the whole cross sectional area stained with Patent Blue.

Microscopic evaluations

Specimens with typical penetration patterns in each group were further examined microscopically. First, root sections assessed in the penetration analysis were dehydrated using an ascending ethanol series, infiltrated and finally embedded in ascending concentrations of an isobornyl methacrylate resin (Technovit 7200 VLC; EXAKT, Norderstedt, Germany). Resin blocks polymerized with white and blue light were mounted on stubs and ground using 1200 and then 2400 grit silicon paper so as to expose the root cross sections. Thereafter, specimens were polished using diamond pastes of particle sizes down to 0.5 μm. The exposed surfaces were photographed using the Tessovar unit described above, and subsequently carbon coated in an electron-beam evaporator (BAL-TEC MED 020; Balzers, Liechtenstein).

A second series of root slices was fractured along the root canal axis using a hammer and a scalpel. One-half of these longitudinal sections, each, was glued on a stub with the fractured surface facing upwards, photographed and carbon coated as described above.

Embedded and fractured specimens were examined in a SEM (Tescan VEGA TS 5316 XM; Brno, Czech Republic) equipped with an annular mono-crystal scintillation type (YAG) backscatter detector in backscatter mode at an accelerating voltage of 20 kV and 20–23 mm working distance. Digital images were taken at magnifications of 1000 and 3000× and acquired at a resolution of 2048 × 1536 pixels.

Data analysis

Percentage values of dye penetration with the various irrigation protocols in apical, middle and coronal root thirds, and overall percentage of dyed areas irrespective

of the irrigating protocol in the three evaluated root areas were compared using one-way analysis of variance (ANOVA) followed by Bonferroni correction for multiple comparisons (StatView; SAS Institute Inc., Cary, NC, USA). The level of significance was set at <0.05.

Results

Osmolarity and surface tension of the 2% Patent Blue dye solution were comparable with corresponding values of a 2% chlorhexidine digluconate solution (Table 1).

No significant differences in dye penetration were noted between the irrigating protocols under investigation (Fig. 1). Regardless of these protocols, coronal root sections showed significantly ($P < 0.001$) larger stained areas than middle or apical root slices, and apical root areas were significantly ($P < 0.001$) less penetrated by the dye than mid-root or coronal specimens (Fig. 2).

With the majority of root cross sections that showed dye penetration, the stained area had a barbell shape extending in a bucco-lingual direction (Fig. 3). Dye penetration was independent of whether the canal wall had been circumferentially instrumented or whether uninstrumented canal extensions were present (Fig. 3a,b).

Further examination of specimens in a SEM consistently revealed that the penetration of the dye was prominent in areas with open dentinal tubules (Fig. 4b) and absent in regions with tubular sclerosis (Fig. 4c,d). Evaluating longitudinally fractured root specimens, a complete absence or only negligible amounts of the smear layer in all specimens irrigated with EDTA (groups V–VIII) was evident, whilst marked amounts of smear were found in roots irrigated with water (Fig. 5a,b). However, dye penetration was observed in dentine areas with patent tubules, irrespective of the presence or absence of a smear layer.

Table 1 Parameters of the dye solution under investigation in comparison with commonly used endodontic irrigating solutions

Solution (percentages: w/v of water)	Osmolarity (mOsm kg^{-1})	Surface tension (mN m^{-1})
Distilled water	–	73
Patent Blue (2%)	65	48
Sodium hypochlorite (1%)	850	73
Sodium hypochlorite (5%)	>2000[a]	76
Chlorhexidine digluconate (0.2%)	6	71
Chlorhexidine digluconate (2%)	58	53

[a]The osmolarity of a 5% hypochlorite solution was beyond the measuring range of 1–2000 mOsm kg^{-1} of the method described in this study.

Discussion

The current study revealed that chemical removal of the smear layer after mechanical root canal preparation using a 17% EDTA solution did not affect dentine penetration of a final irrigant. Tubular sclerosis, on the other hand, impaired dye penetration.

Penetration of a blue dye into dentinal tubules is a simple yet reliable method to assess the diffusion of molecules through the dentine. It must be conceded, however, that the diffusion through individual tubules could not be monitored using the current assay. Therefore, the reported results should be considered an estimate of overall dentine rather than individual tubule permeability. Nevertheless, as consistently observed on backscattered electron micrographs of root sections, the blue staining of the dentine matched the area with open tubules (Fig. 4). In theory, the orientation of tubules within the observed cross sections might further have obscured the findings. This, however, is also unlikely, as the tubules run perpendicular to the root axis (Garberoglio & Brännström 1976), i.e. parallel to the plane of the cut surface. Dentine is a somewhat transparent tissue, and stained blue areas observed and measured on photographs thus not only represented the cut surface but also subsurface layers. Hence, the orientation of dentinal tubules within the section was unlikely to influence the stain and therefore the measurements.

The smear layer does not appear to be a diffusion barrier for small molecules such as the dye used in the current investigation. However, it should be kept in mind that certain irrigants such as EDTA and citric acid have an affinity with the dentine, and theoretically, their capacity to penetrate into the dentine may be affected by the dentine debris that forms the smear layer. Penetration of molecules with dentine affinity was not investigated in the present study, and results can therefore not be extrapolated. Furthermore, it may not be deduced from the current results that the smear layer produced by root canal instrumentation should not be removed, as this deposit can be penetrated by bacteria (Akpata & Blechman 1982) and may offer protection to biofilms adhering to root canal walls (Sen *et al.* 1999). It should also be kept in mind that hypochlorite was used throughout instrumentation as an irrigant in all specimens, and the organic smear

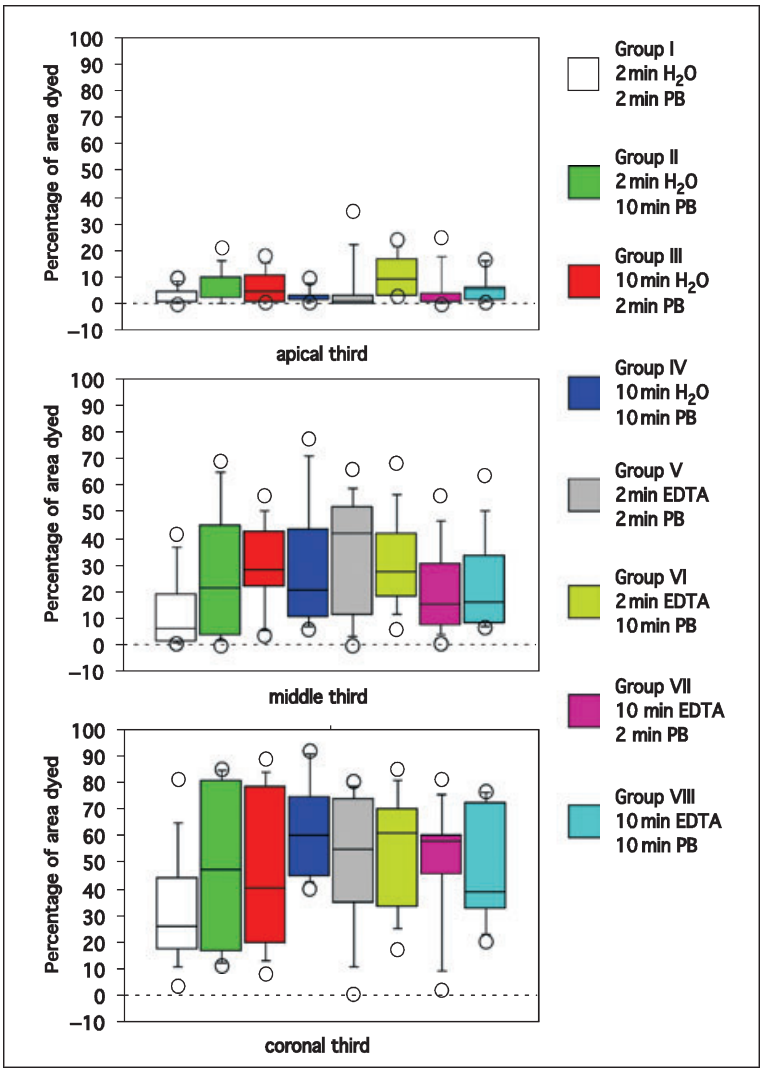

Figure 1 Box plots depicting the percentage of stained root dentine in coronal, middle and apical cross section of roots treated with various irrigation regimens (groups I–VIII). Horizontal bars: medians; boxes: inter-quartile areas; error bars: 10th and 90th percentile; dots: extreme values. No differences were detected between groups at any root level (ANOVA/Bonferroni).

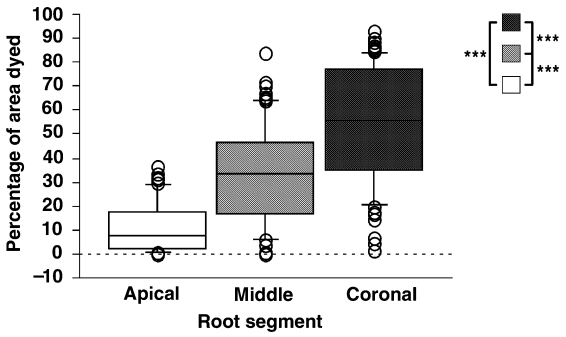

Figure 2 Box plots indicating the dyed areas irrespective of irrigating protocol. Horizontal bars: medians; boxes: inter-quartile areas; error bars: 10th and 90th percentile; dots: extreme values. Differences between all groups were significant at the 0.001 level (ANOVA/Bonferroni).

layer components were thus dissolved (Baumgartner & Mader 1987, Dautel-Morazin *et al.* 1994). Mechanical root canal treatment without the aid of a hypochlorite irrigant could have led to a more densely adhering smear layer and penetration of smear material into dentinal tubules (Aktener *et al.* 1989), a phenomenon not observed in the current investigation.

Tubular sclerosis or intratubular calcification is the physiological deposition of increasing amounts of peritubular dentine that begins in the third decade of life in the apical root region and advances coronally with age. It has been used for age determination in forensic dentistry (Vasiliadis *et al.* 1983). Knowledge of the impact of intratubular calcification on dentine permeability is not new, but the phenomenon has never gained the attention it probably deserves in

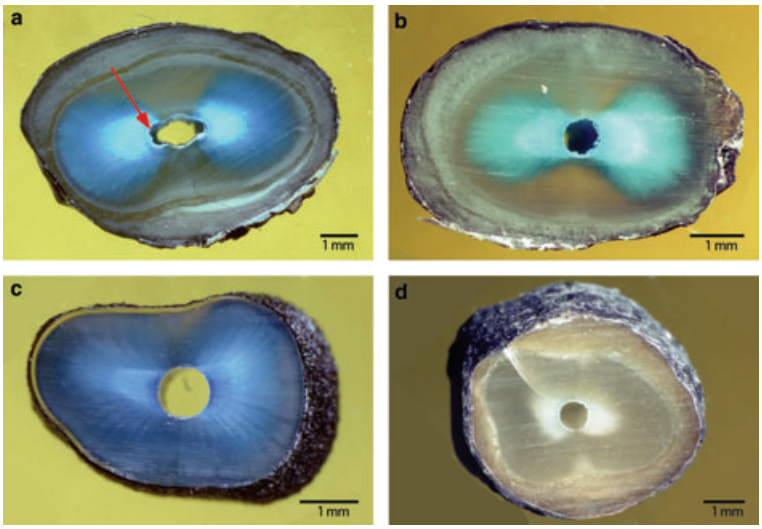

Figure 3 Frequently observed patterns of dye penetration into root dentine (a, b). Note the barbell shape of the blue area in panels a and b. Dye penetration was independent of whether the canal wall had been circumferentially instrumented (b) or whether uninstrumented canal extensions were present (a, arrow). (c) Specimen with total dentine penetration. (d) Specimen with minimal penetration.

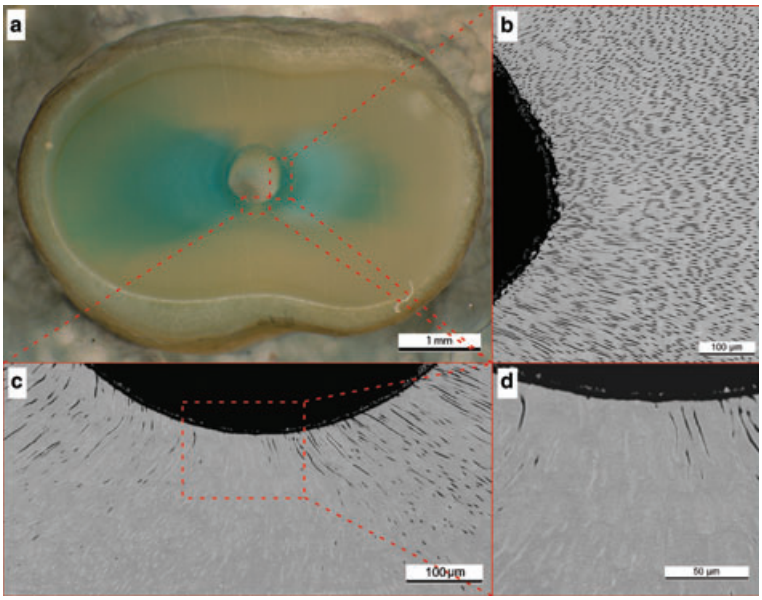

Figure 4 A light microscopic overview of the cross section of a specimen embedded in methacrylate resin showing the typical barbell shape of dye penetration (a). (b–d) Backscattered electron micrograph of areas with (b) and without (c, d) penetration of Patent Blue. Note the patent dentinal tubules in (b) and marked tubular sclerosis in (c) and (d). Original magnifications: (a) 16×; (b), (c) 1000×; (d) 3000×.

endodontics. Using extracted human teeth filled with methylene blue, Fish (1932) was one of the first to report that dentine was a permeable tissue. However, he merely made passing reference to dentine areas that were impermeable. The impact of 'transparent root dentine' on root penetration of ^{35}S-labelled penicillin was only realized 20 years later (Wach *et al.* 1955). In agreement with the latter study, a lower permeability of apical compared with coronal and middle root areas has been found in the current investigation. In sections transverse to the long axis of the root, a 'butterfly' shape of the unstained and sclerotic dentine was evident as seems typical for tubular sclerosis of root dentine (Vasiliadis *et al.* 1983), i.e. mesial and distal root aspects showed more prominent intratubular calcification than bucco-lingual counterparts (Fig. 3). In teeth with apical periodontitis, bacteria invading dentine were only found in areas with open tubules (Shovelton 1964). In single-rooted teeth, open tubules with bacterial invasion were observed more frequently in the bucco-lingual than in the mesio-distal direction of cross-sectioned roots (Shovelton 1964).

The age of the patients from whom the teeth used in this study had been extracted was not known.

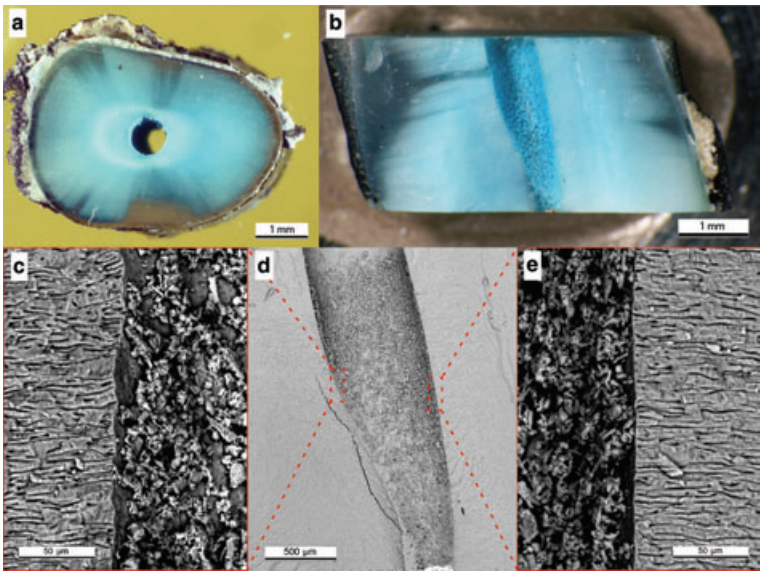

Figure 5 Instrumented root segment of one of the specimens irrigated for 2 min with water followed by 2 min irrigation using the Patent Blue solution (group I). (a, b) Light microscopic overviews of cross section (a) and fractured surface (b) of the specimen. (c–e) Backscattered electron micrographs of root canal and adjacent dentine (d) as well as of two selected areas (c, e) of the canal wall. Note the massive amounts of inorganic smear on root canal walls (a–e) and the patent dentinal tubules (c, e).

Nevertheless, as mandibular premolars with relatively narrow root canals were specifically selected, and as most of the specimens in the department's collection of extracted teeth come from the patients suffering from advanced periodontal disease; it must be assumed that most patients were over the age of 30. Hence, they compared well with the majority of patients receiving root canal treatment (Lazarski *et al.* 2001). As periodontal disease does not seem to affect root dentine transparency (Vasiliadis *et al.* 1983), the degree of tubular sclerosis observed in this study would appear to be representative. Consequently, the apical root dentine of the average endodontic case is most likely impermeable, both to disinfectants and bacteria (Mjör *et al.* 2001). Bacterial clusters in teeth affected by apical periodontitis are most frequently found around the apical portals of exit of the root canal system, where they have access to tissue fluid (Nair 2004). When considering this, the clinical relevance of laboratory models utilizing infected human or bovine dentinal tubules would appear somewhat questionable.

Conclusions

Rather than a smear layer produced by root canal instrumentation, tubular sclerosis, a physiological phenomenon that starts in the third decade of life in the apical root region and advances coronally with age, was the main factor influencing permeability of root dentine.

Acknowledgements

The current study was presented in a short lecture at the 2005 AAE meeting in Dallas, TX, USA, and an abstract was thus published in *Journal of Endodontics* 31 (3), p. 228, OR 50 (2005).

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