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ORIGINAL ARTICLE

Assessment of different dyes used in leakage studies

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Abstract The goal of this in vitro study was to identify the most suitable dye for endodontic dye leakage studies, which could be a further step towards standardisation. The root canals of 70 extracted, single-rooted human adult teeth were enlarged to apical size 50 using hand instruments. The teeth were divided into seven groups (n=10 each), and all root canals were completely filled by injection with one of the following dyes: methylene blue 0.5% and 5%, blue ink, black ink, eosin 5%, basic fuchsine 0.5% and drawing ink. Transverse root sections from the coronal, middle and apical part of the roots were examined, and the percentage of the dentine penetrated by dye was evaluated by software-supported light microscopy. In addition, the range of particle size of drawing ink particles was evaluated. There were conspicuous differences in the relative dye penetration into the root dentine and the penetration behaviour in the different root sections (two-way ANOVA, both p<0.0001). One dye (drawing ink) penetrated less into the root dentine compared with all the others (p<0.0001). The particle size of this agent (0.1–2 μm) corresponds best with the size range of a representative selection of 21 species of pathogenic endodontic bacteria. Compared to the other dyes tested, drawing ink appears to be superior for use in endodontic dye leakage studies. The penetration behaviour into the root dentine of all the other dyes tested might be one factor that limits the applicability of these dyes in dye leakage studies.

Key words Apical leakage · Dye penetration · Microleakage · Dentinal penetration · Dyes

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Introduction

Coronal or apical leakage, as a result of inadequate root canal filling or permeable coronal restoration, is an important factor in successful endodontic treatment because of the risk of re-infection [1–4]. The "Washington Study" showed that 58.66% of unsuccessful endodontic treatments were associated with imperfect root canal treatment [5]. Similar results were also ascertained by other authors [6].

This demonstrates the importance of the sealing ability of root canal fillings and the necessity to evaluate such parameters pre-clinically before new root canal filling materials or techniques are used on patients. One of the most commonly used methods for evaluating apical leakage is dye penetration because it can be performed very easily [7, 8].

If the large number of dye penetration studies is analysed closely, a wide variance in the procedures clearly emerges [9–11]. The logical conclusion of this lack of standardisation is that most results of dye leakage studies can neither be compared with each other [10, 12] nor with other

methods for evaluating apical leakage [13–15]. Strong doubts have been cast on the usefulness of dye penetration studies since the publication of the study by Oliver and Abbott [16] that questioned the correlation between the clinical success of root canal fillings and apical dye penetration. These doubts were further strengthened by the publication of the study by Susini et al. [17], which examined the correlation between dye penetration and the presence of apical radiolucencies.

Probably in order to discourage the execution and publication of further leakage studies with widely differing methodology, the Editorial Board of the Journal of Endodontics decided to publish a statement [18]. At the same time, however, the editors of the Journal of Endodontics encourage investigations, which examine the methods of sealability studies themselves as an aid to improving their validity.

Using clinical data and radiographs Oliver and Abbott [19] had classified the endodontic therapy of 116 teeth, which were scheduled for extraction as successful or unsuccessful. After extraction, they applied methylene blue dye solution and established apical dye penetration in 99.5% of the experimental teeth.

Several studies have shown that most root fillings are permeable to small molecules within a short time after placement [20–23]. In spite of this, a high percentage of root canal treatments still succeeds clinically and radiographically [24–27].

The methylene blue solution used by Oliver and Abbott [16] consists of dye molecules, which are 10^3-fold smaller than bacteria [16]. Thus, it appears very possible that the extremely small size of the molecules of the dye solution used in this study could have been the decisive factor, which led to excessive dye penetration in 99.5% of all the test specimens.

It has already been observed that penetration behaviour depends very much on the type of dye used [28, 29]. Moreover, there is a consensus that the size or molecular weight of the dye particles have a crucial influence on penetration behaviour [10, 12, 29–31], and further testing of these parameters was recommended [30, 31]. Kersten and Moorer [30] suggested that the dye particles should correspond to the size of the various species of bacteria. They pointed out that leakage of low molecular weight substances alone is unlikely to play a decisive role in periapical disease, but that leakage of high-molecular toxic antigenic substances or bacteria into the periapical tissue is probably an important factor. Kersten and Moorer [30] therefore used coloured latex particles with a defined diameter of 0.945 µm. However, these latex particles represented only a portion of the size range of bacteria found in infected root canals [32, 33], and smaller species of bacteria in particular were not represented by these particles.

Is there a dye solution which is more suitable for dye penetration studies than the most frequently used methylene blue? What properties should this dye have? On the one hand, the size range of the dye particles should correlate with the size range of the bacteria in an infected root canal [30]; on the other hand, it should not penetrate too deeply into the root dentin because this may result in misinterpretation when evaluating dye leakage [34, 35].

The first intention of this study was to examine the penetration behaviour of various dyes, as well as to set their molecule (particle) size in relation to the size of a representative assortment of bacteria, which could be identified in infected root canals [32, 33]. Hence, it was the aim of this study to identify a particularly suitable dye for dye penetration studies, whose dye particles correspond best with the size range of bacteria in infected root canals, in order to avoid false positive results and undesirable penetration in dentinal tubules as far as possible.

Materials and methods

Selection of teeth

All specimens were prepared by one operator. Seventy single-rooted maxillary incisors and canines were inspected to exclude the presence of cracks, fissures, previous root fillings or caries and repairs extending to the root or the neck of the tooth. All test specimens were cleaned with a scalpel (Feather Safety Razor, Japan) to remove residual periodontal tissue and calculus from the root surface and stored, until use, in distilled water with a few thymol crystals to suppress bacterial growth [36].

Preparation of root canals

All teeth were radiographed before the root canals were prepared in order to obtain the necessary data for making the subsequent saw cuts in the dentin. The canals were accessed, and working length was established by inserting a size 10 K-type file (VDW, Munich, Germany) until it emerged at the apical foramen and then by subtracting 1 mm. Passing the instrument through the foramen also established apical patency. The canals were then enlarged with reamers and Hedstroem files (VDW) while being intermittently irrigated with 2.5% NaOCl and finally flushed with 20 ml of EDTA 17% (SmearClear™, SybronEndo, Orange, CA, USA) to remove the smear layer [37]. The canals of all 70 teeth were enlarged to size 50 at working length. The middle and coronal thirds of the canals were shaped in a step-back manner with files sizes

55 to 80. Apical patency was confirmed by passing a size 15 K-File (VDW).

Choice of dyes

Dyes were chosen, which have been used frequently in internationally published dye penetration studies [9, 11]. In contrast to leakage studies, no gutta-percha root canal fillings and sealer were applied to these prepared root canals, but the canals were rather completely filled by injection with one of the following dyes:

1. Drawing ink (A 17, no. 201665)
   Pelikan, Hanover, Germany
2. Methylene blue 0.5%
   Merck, Darmstadt, Germany
3. Methylene blue 5%
   Merck
4. Black ink
   Pelikan
5. Blue ink
   Pelikan
6. Eosin 5%
   Merck
7. Basic fuchsin 0.5%
   Merck

Dye penetration

The root canals of 10 teeth at a time were completely filled with one of the above dyes, which were applied with disposable cannulae and syringes. The specimens were positioned vertically [20] in a vacuum device (Heraeus-Kulzer, Hanau, Germany), and a vacuum of 620 mm of mercury [10] was applied for 5 min. Passive dye penetration followed at 37°C and 100% humidity for 7 days. All root canals were dried (removal of surplus dye) using paper points before the saw cuts were made (VDW).

Slice preparation

Three 1-mm-thick transverse dentine sections were taken from different regions of the roots of the trial teeth with a saw microtome (Leitz, Bensheim, Germany; Fig. 1A). The regions for the saw cuts were defined as follows:

1. Dentine section from the coronal part of the root, 2 mm apical from the root canal entrance (Fig. 1A, section a).
2. Dentine section from the apical part of the root, 2 mm coronal of the apical constriction (Fig. 1A, section c).
3. Dentine section from the middle part of the root (Fig. 1A, section b), exactly in the middle of the root between saw cuts a and b.

Evaluation of dye penetration

All dentine cuts were photographed digitally using a stereomicroscope (Carl Zeiss, Oberkochen, Germany) at ×10 magnification and a camera attached to it (Nikon Coolpix 5000, Nikon Corporation, Tokyo, Japan; Fig. 1B).

The digital pictures were imported into a custom-made programme in order to evaluate the extent of the relative dye penetration, This software was developed (by A.D.) at the Institute of Medical Biometry and Informatics in Heidelberg with the application development system Microsoft Visual C++.Net (Microsoft, Redmond, WA, USA), using the Halcon software library image processing operators (MVTec, Munich, Germany). Based on a digitised image, the software measured the surface content of the entire dentine cut and subtracted the area of the dyed dentine. Hence, it was possible to calculate the percentage of dye penetration for every transversal dentine cut in relation to the thickness of the root dentin.

Determination of the size of dye particles and molecules

The molecular size of the majority of the dyes used in our experiments (methylene blue, eosin, basic fuchsine, as well as blue and black ink) was estimated on the basis of the molecular structure formula (e.g. Fig. 1C) [16]. Drawing ink is not a real dye, but a dispersion of soot particles and shellac. In order to evaluate the size of the drawing ink particles, this dispersion was diluted in particle-free distilled water (1/100), and images of 1,395 particles were visualised with transmitted-light microscopy (microscope DMIRE2, objective PLAPO 100x, Leica Microsystems, Wetzlar, Germany). The size distribution of drawing ink particles was calculated using Openlab software (Version 4.0.2, Improvision, Coventry, UK).

Determination of the size range of endodontically relevant bacteria

A representative selection of pathogenic endodontic bacteria species, commonly associated with different forms of periradicular diseases, was used as described by [32, 33], and the reference ranges for the diameters of these bacteria were calculated according to [38].

Statistical analysis

Statistical analysis of dye penetration

The data were analysed with SAS (version 9.1, SAS, Cary, NC, USA) and SPSS, version 15.0 (SPSS, Chicago, IL, USA).

Descriptive statistics comprising mean, median and standard deviation were calculated, as well as the mini-

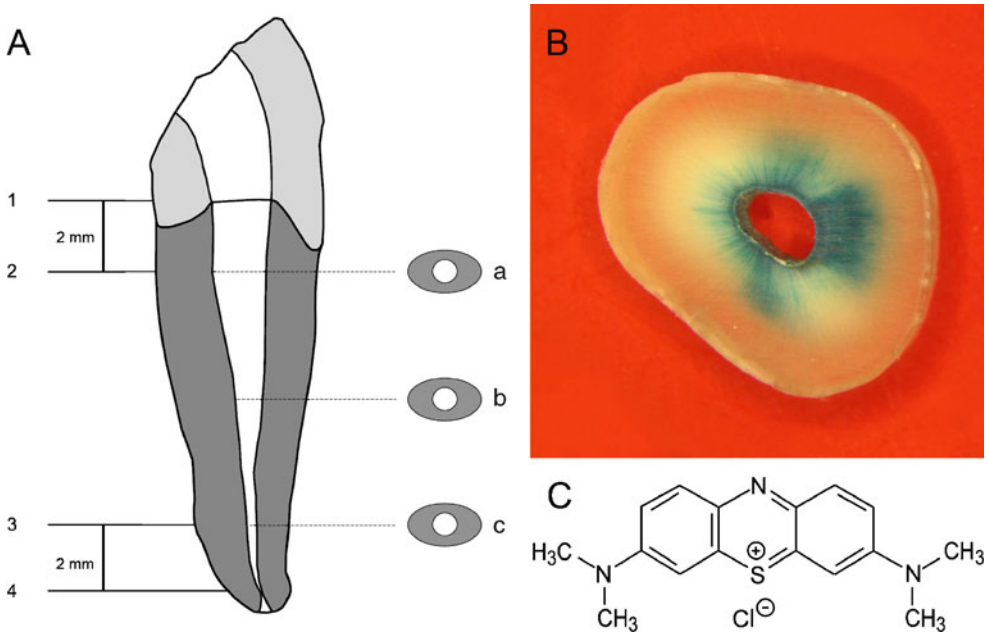

Fig. 1 **A** Diagram of the region from which the dentine cuts were taken, *a* coronal dentine cut, *b* dentine cut from the middle root section, *c* dentine cut from the apical section. *1* root canal entry, *2* extraction point for coronal dentine cut, *3* extraction point for apical dentine cut, *4* apical constriction. **B** Dentine cut from the middle root section, prepared for digital measuring after penetration with methylene blue 0.5% (original magnification ×10). **C** Chemical structure of methylene blue

mum, maximum, absolute and relative frequencies. In addition, box plots are presented showing the percentage of dye penetration in relation to the total surface of the dentine cut. Two-way analysis of variance was carried out for the factors "dye" and "root dentine section" and their interaction in order to evaluate the influence of these factors on relative dye penetration.

In order to assess the differences of the relative dye penetration into the different root regions (coronal, middle and apical thirds), group comparisons were made using one-sample *t* tests. The comparison of the relative dye penetration of drawing ink and all other tested dyes was made using two sample *t* tests for each dentine cut region.

Based on the results of a pilot study, the sample size for the two-way analysis of variance was calculated for the factor dye using the following parameters: a standard deviation of 25.5, type I error of 0.05, type II error of maximum 0.20. This scenario results in a sample size of nine teeth per dye. Because of possible dependencies between the three root sections in any one tooth, a sample size of 10 teeth was considered sufficient to identify differences in the dyes. In view of the explorative character of these analyses, no adjustment was made for multiple testing, and statistical significance was accepted at $p<0.05$.

Statistical analysis of drawing ink particle evaluation compared with the diameter of bacteria

The diameters of 1,395 ink particles were determined. A non-parametric 95% reference interval was constructed from the 2.5% and the 97.5% quantiles of the empirical distribution of the untransformed diameter.

The size range of 21 important pathogenic root canal bacteria (see above) was graphically illustrated, showing the minimum, maximum and mean of the reference range for the diameters of these bacteria (Fig. 3).

Results

Results of dye penetration

Two-way analysis of variance showed that the factor dye had a conspicuous influence on the extent of dye penetration ($p<0.0001$). The relative dye penetration into all three root areas (see Fig. 1A and B) as a percentage of the total dentine in the root sections are shown in Fig. 2. Median, mean, standard deviation and minimum and maximum of the relative dye penetration (in percent) are presented in Table 1. Differences ($p<0.0001$) were identified in all root sections when comparing the relative dye penetration of drawing ink with that of the other dyes tested (see Fig. 2).

Differences in the relative dye penetration (in percent) of all the dyes were found between the coronal and apical root sections ($p<0.0001$) and the middle and apical root sections ($p<0.0001$). The data showed no conspicuous differences with regard to the relative dye penetration (in percent) in the coronal and middle root sections ($p=0.252$).

Size of the ink particles and ink molecules

The molecular size of most of the dyes used in our experiments (methylene blue, eosin, basic fuchsin, as well

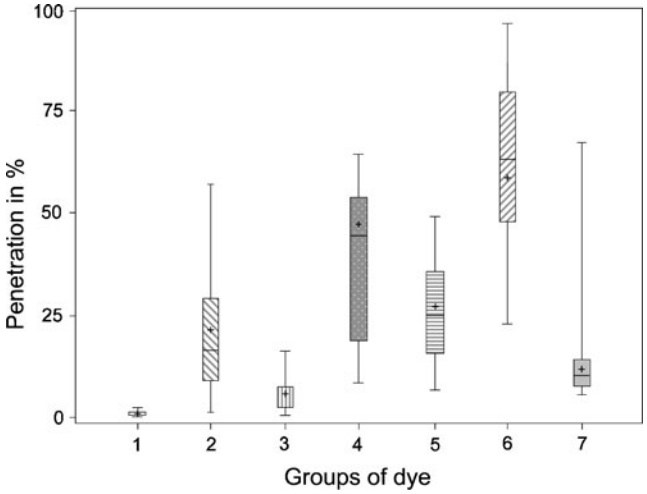

Fig. 2 Box plot diagram of the distribution of the relative dye penetration in percentage of the total area of dentine cut in all root canal sections a, b and c (see also Fig. 1) with medians, means (*plus signs*) and 25% quartiles. 1=drawing ink, 2=black ink, 3=blue ink, 4=methylene blue 5%, 5=methylene blue 0.5%, 6=eosin 5%, 7=basic fuchsine 0.5%

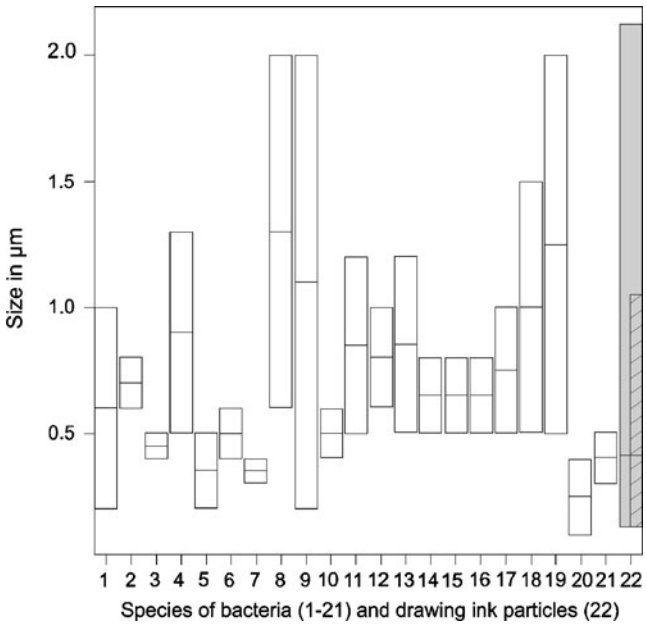

Fig. 3 Diagram of the reference areas, including median of a representative selection of 21 different pathogenic endodontic bacteria species [32, 33], compared with the size range of drawing ink particles after measuring 1,395 ink particles using a computer-supported microscope. *Empty bars* reference ranges for the diameter of pathogenic endodontic bacteria. *Shaded bars* minimum and maximum diameters of all ink particles. *Hatched bars* 95% reference range of the ink particles. *1 Actinomyces*, *2 Bacteroides forsytus*, *3 Bacteroides gracilis*, *4 Bifidobacterium*, *5 Camphylobacter*, *6 Capnocytophaga*, *7 Eikenella*, *8 Enterococcus*, *9 Eubacterium*, *10 Fusobacterium*, *11 Lactobacillus*, *12 Neisseria*, *13 Peptostreptococcus*, *14 Porphyromonas*, *15 Prevotella*, *16 Propionibacterium*, *17 Pseudomonas*, *18 Staphylococcus*, *19 Streptococcus*, *20 Treponema*, *21 Veilonella*, *22* drawing ink particles

as blue and black ink) is about 1 to 2 nm [16]. The size (diameter) of the drawing ink soot particles ranges from 0.13 to 2 µm.

Size of ink particles compared with the size of endodontic pathogens

Figure 3 shows the measurement of the ink particles with the software-assisted light optical microscope and the 95% reference range in comparison with the size range of bacteria from the infected root canal.

Discussion

Confounding factors should be standardised as far as possible in all laboratory research models for assessing sealability in endodontics pre-clinically [39]. Standardisation has often been called for to improve the validity of dye penetration studies [9–11, 40–42].

With regard to the methodology, there is evidence that dye penetration should be performed under reduced pressure [20, 43–45] and that the vertical positioning of the teeth during application of the dye is a relevant issue [20]. These studies, aimed at standardising the execution of dye penetration studies, need no further confirmation. Hence, in the present study, dye penetration in the test specimens was performed under vacuum conditions (620 mm of mercury) and with the teeth positioned vertically.

It is also known that the number and diameter of dentinal tubules per square millimetre are affected by the age of the teeth and the distance from the pulp as well as the presence and type of coronal restoration [46–48]. For this reason, teeth with restorations or caries extending to the root or the neck of the tooth (class V) were excluded from the present study.

In the endeavour to ensure a standardised approach, the procedure for cleaning and shaping and the size of enlargement was identical in all the test teeth. Teeth which were too large or too small for this standardised approach were excluded. In order to remove the smear layer and thus ensure unhindered dye penetration, the root canals were rinsed with 17% EDTA solution when root canal preparation was nearly completed. Likewise, with the aim of standardising the removal of the sample dentine sections, identically sized dentine cuts were taken from comparable root sections (the coronal, middle and apical thirds) of each tooth (Fig. 1A). This minimised the influence of different dentine tubule diameters on the relative dye penetration, subject to the distance from the pulp [46, 48]. A pilot study served to determine the necessary sample size. In the course of this pilot study, it was also observed that the extent of

Table 1 Medians, means, standard deviations and range (minima and maxima) of the relative dye penetration (in percent) of the dyes into the root dentine of 10 teeth (three root sections per tooth)

Dye	Samples (teeth)	Median	Mean	Minimum	Maximum	SD
Drawing ink	10	0.0	0.2	0.0	1.3	0.4
Methylene blue 0.5%	10	25.5	26.3	7.3	49.7	11.8
Methylene blue 5%	10	44.9	39.6	9.5	65.9	17.7
Blue ink	10	5.2	5.4	0.4	15.9	3.6
Black ink	10	16.8	22.1	1.5	57.5	14.9
Basic fuchsine 0.5%	10	10.7	13.1	5.9	68.0	11.0
Eosin 5%	10	63.8	62.0	23.6	97.3	20.9

circular dye penetration in a dentine cut was often irregular (see also Fig. 1B). In order to permit a comparison of relative dye penetration in the dentine sections of the different dyes, a special software programme for measuring irregular areas was developed by one of the authors of this study (A.D.) during the pilot study (see above "Evaluation of dye penetration").

All small molecule-sized tracers in the dyes tested in this study (methylene blue, eosin, as well as the blue and black inks and the basic fuchsine) penetrated massively into the root canal dentine (Figs. 1B and 2). This reduces the specificity of a dye penetration test, i.e. areas of potential leakage appear larger than they really are [34] or they are not clearly identifiable [35].

With diameters between 0.13 and 2 μm, the size range of bacteria in the infected root canal (0.2–2 μm) correlates with the size range of the drawing ink particles (Fig. 3), but not with the molecular size (1–2 nm) of all other dyes tested (methylene blue, eosin, basic fuchsine as well as blue and black ink). The most important difference between drawing ink and the other dyes tested is that the soot particles in the drawing ink are larger by a factor of 10^3 than the pigment molecules in all the other dyes tested in our study. This enormous difference in size may be the reason for the different penetration behaviour in root dentine (Fig. 1B and C).

A greater degree of dye penetration into the surrounding dentine in the coronal parts of the roots compared to the apical parts, as was observed in this study, may be attributed to the fact that the dentinal tubules in the vicinity of the coronal pulp cavity have larger diameters than those in the apical area of the root canal [46, 48].

Youngson et al. [36] also examined the particle sizes of a drawing ink. Although that publication refers to Indian ink, the range of particle sizes reported (from 0.5 up to 600 μm) indicates that this was rather a drawing ink dispersion.

The range in the above study differs from that in the present study (0.1–2 μm), which may be due to different manufacturing methods: Youngson et al. [36] used a Winsor and Newton ink, whereas the one used in this study was drawing ink (A 17, no. 201665, Pelikan), a dispersion of soot particles and shellac.

However, as the size of the particles in the different makes of drawing ink varies, it would be helpful if the range of particles sizes in a specific ink were stated before it is used for dye leakage studies.

The results from studies where very small molecule dyes were used may be the reason why no correlation between apical dye penetration and clinical success of root fillings [19] or dye penetration and the presence of apical radiolucencies [17] could be established to date.

The Oliver and Abbott study [19], which is frequently cited as an argument against the validity of dye leakage studies, reported a significant difference between the successful and unsuccessful groups, with the mean percentage of linear dye penetration greater in the unsuccessful specimens [19]. Susini et al. [17] also reported a significant correlation between the quality of the root canal filling and the dye penetration. Some authors reported that the results of dye penetration studies can, indeed, correlate with other methods of leakage assessment [41, 49]. Hence, dye penetration studies should not be rejected per se or simply because the results have not been demonstrated to be related to the radiographic outcome of treatment [39].

However, the use of different low molecular weight dyes is not the only potential source of discrepancies in leakage test results. In some other studies in which "dye penetration" is judged critically [13, 14], no vacuum was used, although several studies mentioned above have emphasised the need to remove all entrapped air from root-filled teeth prior to performing apical dye penetration. The validity of dye leakage studies using drawing ink and the clinical relevance of this research method might be reconsidered and examined in further studies, taking into account all findings to date on standardisation of this research method.

Conclusions

1. The size range of drawing ink particles correlates with the size range of a representative selection of species of pathogenic endodontic bacteria in the infected root canal.

2. In contrast to drawing ink, all low molecular weight dyes tested in this study penetrate deeply into the root dentin. This may result in misinterpretation when using these dyes in dye leakage studies.
3. Drawing ink appears to be superior to the other dyes tested for use in endodontic dye leakage studies.

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References

1. Dugas NN, Lawrence HP, Teplitsky PE, Pharoah MJ, Friedman S (2003) Periapical health and treatment quality assessment of root-filled teeth in two Canadian populations. Int Endod J 36:181–192
2. Kirkevang LL, Orstavik D, Horsted-Bindslev P, Wenzel A (2000) Periapical status and quality of root fillings and coronal restorations in a Danish population. Int Endod J 33:509–515
3. Sidaravicius B, Aleksejuniene J, Eriksen HM (1999) Endodontic treatment and prevalence of apical periodontitis in an adult population of Vilnius, Lithuania. Endod. dent. traumatol 15:210–215
4. Tronstad L, Asbjornsen K, Doving L, Pedersen I, Eriksen HM (2000) Influence of coronal restorations on the periapical health of endodontically treated teeth. Endod. dent. traumatol 16:218–221
5. Ingle JI, Glick DN (1965) Modern endodontic therapy. Endodontics, 1st edn. Lea & Febinger, Philadelphia, pp 54–76
6. Petersson K, Petersson A, Olsson B, Hakansson J, Wennberg A (1986) Technical quality of root fillings in an adult Swedish population. Endod. dent. traumatol 2:99–102
7. Bohsali K, Pertot WJ, Hosseini B, Camps J (1998) Sealing ability of super EBA and Dyract as root-end fillings: a study in vitro. Int Endod J 31:338–342
8. Pommel L, Camps J (2001) In vitro apical leakage of system B compared with other filling techniques. J. endod. 27:449–451
9. Raskin A, D'Hoore W, Gonthier S, Degrange M, Dejou J (2001) Reliability of in vitro microleakage tests: a literature review. J. Adhes. Dent. 3:295–308
10. Tamse A, Katz A, Kablan F (1998) Comparison of apical leakage shown by four different dyes with two evaluating methods. Int Endod J 31:333–337
11. Wu MK, Wesselink PR (1993) Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. Int Endod J 26:37–43
12. 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. Int Endod J 28:30–34
13. Barthel CR, Moshonov J, Shuping G, Orstavik D (1999) Bacterial leakage versus dye leakage in obturated root canals. Int Endod J 32:370–375
14. Gilbert SD, Witherspoon DE, Berry CW (2001) Coronal leakage following three obturation techniques. Int Endod J 34:293–299
15. Pommel L, Jacquot B, Camps J (2001) Lack of correlation among three methods for evaluation of apical leakage. J. Endod 27:347–350
16. Staab H (1970) Einführung in die theoretische organische Chemie. Weinheim/Weinstraße, Verlag Chemie
17. Susini G, Pommel L, About I, Camps J (2006) Lack of correlation between ex vivo apical dye penetration and presence of apical radiolucencies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102:e19–23
18. Editorial JOE (2007) Wanted: a base of evidence. J. Endod 33:1401–1402
19. Oliver CM, Abbott PV (2001) Correlation between clinical success and apical dye penetration. Int Endod J 34:637–644
20. Goldman M, Simmonds S, Rush R (1989) The usefulness of dye-penetration studies reexamined. Oral Surg. Oral med. Oral pathol 67:327–332
21. Johnson WT, Zakariasen KL (1983) Spectrophotometric analysis of microleakage in the fine curved canals found in the mesial roots of mandibular molars. Oral Surg. Oral med. Oral pathol 56:305–309
22. Kennedy WA, Walker WA 3 rd, Gough RW (1986) Smear layer removal effects on apical leakage. J. Endod. 12:21–27
23. Pitt Ford TR (1983) Relation between seal of root fillings and tissue response. Oral Surg. Oral Med. Oral Pathol 55:291–294
24. Gesi A, Hakeberg M, Warfvinge J, Bergenholtz G (2006) Incidence of periapical lesions and clinical symptoms after pulpectomy—a clinical and radiographic evaluation of 1- versus 2-session treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101:379–388
25. Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S (2006) Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment. J. Endod. 32:299–306
26. Orstavik D (1996) Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. Int Endod J 29:150–155
27. Sjogren U, Hagglund B, Sundqvist G, Wing K (1990) Factors affecting the long-term results of endodontic treatment. J. Endod. 16:498–504
28. Tanomaru Filho M, Figueiredo FA, Tanomaru JM (2005) Effect of different dye solutions on the evaluation of the sealing ability of Mineral Trioxide Aggregate. Pesqui Odontol Bras 19:119–122
29. Vogt BF, Xavier CB, Demarco FF, Padilha MS (2006) Dentin penetrability evaluation of three different dyes in root-end cavities filled with mineral trioxide aggregate (MTA). Braz Oral Res 20:132–136
30. Kersten HW, Moorer WR (1989) Particles and molecules in endodontic leakage. Int Endod J 22:118–124
31. Michailesco P, Boudeville P (2003) Calibrated latex microspheres percolation: a possible route to model endodontic bacterial leakage. J. Endod. 29:456–462
32. Baumgartner JC (2004) Microbiologic aspects of endodontic infections. J. Calif. Dent. Assoc. 32:459–468
33. Siqueira JF Jr (2002) Endodontic infections: concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 94:281–293
34. Gale MS, Darvell BW (1999) Dentine permeability and tracer tests. J. Dent. 27:1–11
35. Scott AC, Vire DE, Swanson R (1992) An evaluation of the Thermafil endodontic obturation technique. J. Endod. 18:340–343
36. Youngson CC, Jones JC, Manogue M, Smith IS (1998) In vitro dentinal penetration by tracers used in microleakage studies. Int Endod J 31:90–99
37. Hülsmann M, Heckendorff M, Lennon A (2003) Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 36:810–830

38. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams S, Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore
39. Wu MK (2008) Letter to the editor. Int Endod J 41:721–722
40. Al-Ghamdi A, Wennberg A (1994) Testing of sealing ability of endodontic filling materials. Endod Dent Traumatol 10:249–255
41. Chong BS, Pitt Ford TR, Watson TF, Wilson RF (1995) Sealing ability of potential retrograde root filling materials. Endod Dent Traumatol 11:264–269
42. Verissimo DM, Vale MS (2006) Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. J. Oral Sci. 48:93–98
43. Oliver CM, Abbott PV (1991) Entrapped air and its effects on dye penetration of voids. Endod Dent Traumatol 7:135–138
44. Spangberg LS, Acierno TG, Yongbum Cha B (1989) Influence of entrapped air on the accuracy of leakage studies using dye penetration methods. J. Endod 15:548–551
45. Wimonchit S, Timpawat S, Vongsavan N (2002) A comparison of techniques for assessment of coronal dye leakage. J. Endod 28:1–4
46. Garberoglio R, Brännström M (1976) Scanning electron microscopic investigation of human dentinal tubules. Arch Oral Biol 21:355–362
47. Mjör IA, Sveen OB, Heyeraas KJ (2001) Pulp-dentin biology in restorative dentistry. Part 1: Normal structure and physiology. Quintessence Int 32:427–446
48. Tronstad L (2008) The Endodontium. In: Tronstad L (ed) Clinical endodontics: a textbook, 3rd edn. Thieme, Stuttgart, pp 1–30
49. Delivanis PD, Chapman KA (1982) Comparison and reliability of techniques for measuring leakage and marginal penetration. Oral Surg. Oral Med. Oral Pathol. 53:410–416