Journal of Oral Science, Vol. 48, No. 3, 93-98, 2006

Review

Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review

Denusa Moreira Veríssimo and Mônica Sampaio do Vale

Department of Dental Clinic, Dentistry School,
Federal University of Ceará, Ceará, Brazil

(Received 2 March and accepted 20 July 2006)

Abstract: Apical leakage continues to be a topic of great interest, because in spite of the advances in Endodontics, clinical failures still occur. Most failures are probably attributed to the proliferation of bacteria that remain viable after chemical-mechanical preparation and cause periapical tissue irritation. Coronal leakage has aroused researchers' interest over the last few years, since canals may be re-contaminated after loss of coronal sealing or fracture of the remaining tooth. In this review, the various methodologies used for assessing root canal sealing capacity are critically analyzed, as they are not standardized, which makes it difficult to compare the results obtained when different methodologies are used, even though the same substrate is being assessed. (J. Oral Sci. 48, 93-98, 2006)

Keywords: dye dilution technique; dyes; filtration.

Introduction

Microorganisms present inside root canals may remain active in the dentinal tubules even after vigorous chemical-mechanical preparation. Thus, perfect apical sealing is desirable to prevent the remaining bacteria and their endotoxins from reaching the root apex (1,2). Apical leakage is considered to be a common cause for endodontic therapy failure, and is influenced by many variables such as different filling techniques, the physical and chemical properties of sealers and the presence or absence of a smear layer (1,3). In coronal leakage, the canal may be recontaminated in various ways such as contact between the oral bacterial flora and root canal tubule inlets. However, it most frequently occurs as a result of loss of temporary filling, or inadequate endodontic filling or crown sealing (4,5).

According to Timpawat et al. (6), endodontic sealers are used to eliminate the interface between the gutta-percha and the dentinal walls. Leakage may, however, occur at the interfaces between the sealer and dentin, sealer and gutta-percha and in spaces within the sealer itself. Thus, the quality of the filling depends largely on the sealing capacity offered by sealers (7,8).

Methodologies *in vitro* are used to estimate sealing quality, generally by measuring microleakage that allows the tracer agent to penetrate the filled canal. Commonly used tracers are dyes, radioisotopes, bacteria and their products, such as endotoxins. Bacteria used as tracers most closely approximate what happens clinically in terms of leakage (9-11). Other methodologies, such as fluid filtration and dye extraction methods have also been used, their main advantage being high reproducibility (1,12-14).

The aim of this review was to critically analyze the various methodologies reported in the literature for assessing root canal sealing capacity. Apparently, they are not standardized, which makes it difficult to compare the results obtained when different methodologies are used, even though the same substrate is being assessed.

Literature Review

Although there are many leakage studies, there is no consensus about the endodontic sealer and core material sealing capacities. One of the reasons is that investigations do not use a standardized methodology and this frequently

Correspondence to Dr. Denusa Moreira Veríssimo, Av. José Leon 2740, Casa 18 Cidade dos Funcionários, Fortaleza, Ceará, Cep: 60822-670, Brazil
Tel.: +55-85-32713141
Fax: +55-85-32713141
E-mail: denusa.verissimo@terra.com.br

leads to contradictions (15,16).

Methodology that uses dyes

The methodology using tooth immersion in various types of dyes (eosin, methylene blue, black India ink, Procion brilliant blue, and others), reported for the first time by Grossman in 1939, is perhaps most widely used, mainly because it is easy to perform (16-20). The phenomenon of capillarity is of utmost importance in this passive method used mainly for assessing apical leakage, as the tooth apex is submerged in the dye that penetrates through any space between the canal walls and filling material (19). Next, the teeth are sectioned longitudinally, transversely, or cleared and the linear penetration of the dye is recorded (2,21,22).

The longitudinal sectioning method enables examination of the exposed filling material and any dye penetration into the material and at the interface of the dentinal wall on one side (21). Ahlberg et al. (21) suggested a variation of this technique; whereby the roots are worn down to visualize the leakage through a thin layer of dentin, thus reducing the risk of dye dissolution during sectioning. They also affirmed that this technique provides more reliable information about the real leakage pattern than transverse sections or clearing. The disadvantages of longitudinal dentinal sectioning seem to be the random choice of the cut axis and the very low probability of the sections being made through the deepest dye penetration point, with consequent underestimation of leakage and recording of unreliable data (19). Schafer and Olthoff (23) stated that although greater linear dye penetration does not furnish data about area, it provides sufficient data about apical leakage.

According to Martin et al. (2) and Ahlberg et al. (21), transverse root sectioning results in loss of part of the dentinal tissue and dye due to the technique itself (saw thickness), and only allows one to determine whether or not there is penetration in each section.

The clearing technique recommended by Okumura in 1927, in which the teeth become transparent after a process of demineralization, dehydration and immersion in methyl salicylate, provides a three-dimensional view of the internal anatomy of root canals without the loss of dental substance, making it easier to view the leakage area. It is simple, fast, performed with substances low in toxins and does not require complex equipment (3,23-27). Martin et al. (2) also affirmed that the technique makes it easier to observe the lateral and accessory canals, and clearly reflects the relation between the sealing material and the apical foramen. However, Malvar et al. (27) stated that some of the samples submitted to clearing may present deficient demineralization, which would compromise the final transparency of the specimen. Furthermore, the demineralization times differs, as greater the weight of the dentinal part, the greater the mineral content present and the longer it would take to complete the process. According to Tagger et al. (25), the endpoint of this step could be easily assessed by inserting a thin needle in an unimportant area of the crown or by radiography. Another potential problem is that incomplete dehydration will leave opaque areas in the teeth, but this can be corrected by additional dehydration in 100% ethyl alcohol (24). Ahlberg et al. (21) pointed out that immersion in acids such as nitric acid and alcohol for a long period may cause dye dissolution in this technique. Martin et al. (2) showed that the clearing technique was more precise than the transverse section for detecting apical leakage, as it allows the leakage to be visualized in tenths of millimeters, while transverse sectioning only determines whether or not leakage has occurred in each section. The clearing system could not, however, be used to measure the volume of tracer ingress (28).

With regard to dyes, particle molecule size, pH and chemical reactivity are expected to affect the degree of penetration (21). A large number of studies used methylene blue as dye (17,19,29) because it is inexpensive, easy to manipulate, has a high degree of staining and a molecular weight even lower than that of bacterial toxins (17). It has been suggested that methylene blue presents the same leakage as butyric acid (30), a microbial metabolic product that has greater penetration than Indian ink. This dye presents a few disadvantages such as dissolution during the demineralization and clearing process, in addition to being difficult to observe its maximum penetration point in some cases (23). On the other hand, Barthel et al. (9) suggested that the molecular size of the dye may not be a relevant parameter in leakage tests.

Indian ink particles with diameter smaller than or equal to 3 μm, are also widely used, as it is unlikely that bacterial invasion would occur in spaces inside the canal where this dye is unable to penetrate (23). However, it has been reported that the weight and size of Indian ink molecules are smaller than those of the bacterial molecules found in the root canal. Therefore, this substance may also not faithfully represent the molecules of fluids coming from periradicular tissues, giving false-positive results during analysis of the leakage (3).

One of the major considerations with respect to dye penetration studies is that air entrapped in voids along the root canal filling may hinder fluid movement. It has been recommended that dye penetration should be performed under reduced pressure, incorrectly referred to as vacuum (12). However, it is much more difficult to remove the

trapped air by applying reduced pressure to small empty spaces, such as those of 2 μm in diameter which, in principle, are permeable to bacteria (12). Kontakiotis et al. (29) investigated the influence of hydration on voids along root fillings through a fluid transport model and dye penetration, in which transport air was applied to remove water from gaps in one group, and found that methylene blue penetrates more easily in dry gaps than in water-filled gaps. Methylene blue passes along air-filled gaps by capillary action, whereas in water-filled gaps, it passes by diffusion. Wimonchit et al. (31) comparing different coronal dye leakage test techniques, observed that the vacuum method resulted in significantly more dye penetration than fluid filtration and passive dye penetration. The result of this study emphasized the importance of the use of reduced air pressure in dye penetration. Spångberg et al. (32) found that passive dye penetration resulted in incomplete void filling, regardless of void size, whereas vacuum dye delivery resulted in complete void filling. Katz et al. (33) found no significant differences between a horizontally positioned experimental group under reduced pressure and groups in passive immersion, but when the apices were in an upright position, the mean leakage was significantly higher under reduced pressure. Thus, the authors showed that tooth positioning had a significant effect on linear dye penetration under reduced pressure and emphasized the need to standardize factors that may influence penetration when assessing the methodology of leakage studies.

It was noted that teeth with apical leakage generally show leakage at all of their surfaces. Very few cases present dye penetration at only one surface, and when this is the case, there would be very little of it (34).

Fluid filtration or transportation methodology

The fluid filtration method, in which the sealing capacity is measured by means of air bubble movement inside a capillary tube, was developed by Pashley's group in 1987 and modified by Wu et al. in 1993 for use in root canals. It consists of a filled canal that has its coronal portion connected to a tube filled with water under atmospheric pressure, and its apex to a 20 μl glass capillary tube 170 mm long and of uniform caliber filled with water. Finally, a pressure of 0.1 atm is applied through the coronal part, which forces the water through the empty spaces along the root canal (12). The results are generally expressed in μl/min (13).

The above-mentioned method presents many advantages in comparison with dye penetration methods, as the samples are not destroyed, therefore it allows both the apical and coronal sealing to be assessed after a long period. Furthermore, the results are recorded automatically, thus providing quantitative measurements and avoiding operator errors; the results are precise, as small volumes can be recorded (1,8,18); and it would be more sensitive than dye penetration in detecting empty spaces along the canal (8,12,18). System sensitivity can be adjusted by altering the pressure used or the diameter of the micropipette (8). However, according to Pommel, Camps (13) the materials and methods used in this technique are not standardized, as the pressure used may range from 10 to 20 psi, and the measuring time from 1 min to 3 h. This would alter the results obtained, since lower filtration values have been found associated with longer recording time, and the values recorded were higher when high pressure was used in comparison with low pressure. According to the authors, 20 psi pressure would appear to be far too high because it corresponds to 1406 cm H_2O pressure. Therefore, to be as close as possible to physiological pressures, 15 cm by H_2O would appear to be sufficient when highly sensitive equipment is used. The pressure should be included in the results and should be expressed as μl/min cm H_2O instead of μl/min.

Thus, various parameters that could change the test results such as diameter of the capillary that contains the bubble, bubble length, measuring time and the pressure applied (13), must be mentioned in the materials and methods section.

According to Miletić et al. (35), it is very important to assess leakage not only after filling, but also after some time, because this assessment is required to ensure that sealers are clinically effective.

Orucoglu et al. (36) developed a new computerized fluid filtration meter based on light refraction at the starting and ending positions of air bubble movement inside micropipette. It has some advantages over the conventional ones with the computer control and digital air pressure arrangement. Additionally, the movement of air bubbles can be observed by laser diodes which are computer controlled rather than visual findings.

Younson et al. (28) did not find any statistically significant correlation between the fluid filtration measurements and dye penetration measured by a scoring system, but fluid filtration was more sensitive.

Dye Extraction Method

In the dye extraction or dissolution method, the teeth are dissolved in acids that release all the dye from the interface and the optical density of the solution is measured by a spectrophotometer. It is fast and can be carried out with equipment available at most universities (19).

According to Camps, Pashley (19), there was no

correlation between dye penetration and the fluid filtration and dye extraction techniques which determine microleakage. The fluid filtration technique gave similar results to those of dye extraction, because both take into consideration the porosity of the interface between the filling material and the root. Both techniques are based on quantitative measurements of liquids passing through these interfaces. The dye extraction method presents an advantage over the fluid filtration method, because the filtration values tend to diminish over time, as the water penetrates all the irregularities until a plateau is reached.

Bacteria and toxin infiltration method

According to Timpawat et al. (6), the use of bacteria to assess leakage (mainly coronal) is considered to be of greater clinical and biological relevance than the dye penetration method. Many different strains of bacteria have been used to assess marginal leakage and this has lead to contradictory results, because the methods depend on the type of bacteria used. Moreover, if the sealer has antimicrobial activity, it is unfeasible to employ the bacteria method (23,37). The systems generally comprise two chambers and enable the apical and coronal extremities of each specimen to be completely separated. The turbidity of the broth in the apical chamber is the first indication of contamination by microorganisms (4,38).

If the pulp chamber becomes contaminated, it may serve as a reservoir of microorganisms and toxins. This could cause a problem in either of two ways. First, the apical seal may be affected adversely and cause the root canal treatment to fail. Second, movement of microorganisms and toxins through accessory canals in the floor of the pulp chamber may result in periodontal furcation involvement (39).

These bacterial studies have been qualitative rather than quantitative. If only one bacterium passes through the obturated root canal, it may multiply in the enriched broth and cause turbidity (38,39).

It may be observed that in their studies, Barthel et al. (9) used Staphylococcus epidermidis; Timpawat et al. (6) Enterococcus faecalis; Carratù et al. (4) used P. mirabilis and S. epidermides; Miletic et al. (10) assessed the penetration of Candida albicans and the bacteria S. mutans, S. mitis, Prevotella melaninogenica, and Lactobacillus acidophilus; Michailesco et al. (11) tested Actinomyces odontotylicus, Lactobacillus acidophilus, and Pseudomonas fluorescens; Chailertvanitkul et al. (39,40) studied anaerobic streptococci and Fusobacterium nucleatum; and Maltezos et al. (37) Streptococcus salivarius. Enterococcus faecalis is frequently used, because it is part of the normal mouth flora and is frequently found in infections with other optional aerobic and anaerobic bacteria; it is also one of the microorganisms most frequently isolated inside root canals (6). The fungus Candida albicans has the same capacity as bacteria to penetrate filled canals (10). Polymicrobial infections involving black-pigmented Gram-negative anaerobes are likely to have a greater pathogenic potential than infections with a single species (40).

According to Barthel et al. (9), bacteria or bacterial product penetration may start or reactivate the inflammatory process, and saliva leakage may stimulate the growth of the bacteria that persist inside the root canals. The size of the test agent molecule must be representative of the bacteria and/or components of the bacterial cell wall and/or nutrient fluids.

The differences in behavior between bacteria and endotoxins must be related to their chemical activities. Endotoxins are lipopolysaccharides of the external membrane of Gram-negative bacteria and consist of a lipid portion, Lipid A, and a polysaccharide portion, which is the external part of the membrane. The possibility of the bacteria exerting enzymatic action on the gutta-percha, sealer and dentin and creating a passage through the seal, has not yet been demonstrated (4). It has been reported that endotoxin preceded bacterial penetration of the canal system (41).

Xu et al. (42) introduced a new method for analysis of endodontic microleakage based on the filtration rate of glucose along the root canal filling. The amount of leakage was quantified with spectrophotometry. Glucose was selected as the tracer because of its small molecular size (MW = 180 Da) and as it is a nutrient for bacteria. So, if glucose could enter the canal from the oral cavity, bacteria that survive root canal preparation and obturation could multiply and lead to periapical inflammation.

Michailesco et al. (11) using latex microspheres, showed that they can be used to simulate bacterial leakage and that smaller particles penetrate more deeply than bacteria and the larger ones, less than they do.

The ability of viable microorganisms to change their shape and size and to move actively and multiply may play an important role inside the root canal, as they cannot be represented by any aqueous dye solution (9).

Conclusions

From a review of the literature, it is noted that various methodologies are available for assessing leakage, some are shown to be simple, such as dye penetration, and others more complex, such as bacterial leakage. It was found that there is a real lack of technique standardization, as even when one and the same methodology is used, small variations may be included, which are able to interfere in

the final outcome of the research.

References

1. Pommel L, Jacquot B, Camps J (2001) Lack of correlation among three methods for evaluation of apical leakage. J Endod 27, 347-350
2. Lucena-Martín C, Ferrer-Luque CM, González-Rodríguez MP, Robles-Gijón V, Navajas-Rodríguez de Mondelo JM (2002) A comparative study of apical leakage of Endomethasone, Top Seal, and Roeko Seal sealer cements. J Endod 28, 423-426
3. Valois CRA, de Castro AJR (2002) Comparison of the apical sealing ability of four root canal sealers. J Bras Endod 3, 317-322 (in Portuguese)
4. Carratù P, Arnato M, Riccitiello F, Rengo S (2002) Evaluation of leakage of bacteria and endotoxins in teeth treated endodontically by two different techniques. J Endod 28, 272-275
5. Saunders WP, Saunders EM (1994) Coronal leakage as a cause of failure in root-canal therapy: a review. Endod Dent Traumatol 10, 105-108
6. Timpawat S, Amornchat C, Trisuwan WR (2001) Bacterial coronal leakage after obturation with three root canal sealers. J Endod 27, 36-39
7. Oliver CM, Abbott PV (1998) An in vitro study of apical and coronal microleakage of laterally condensed guttapercha with Ketac-Endo and AH-26. Aust Dent J 43, 262-268
8. Çobankara FK, Adanir N, Belli S, Pashley DH (2002) A quantitative evaluation of apical leakage of four root-canal sealers. Int Endod J 35, 979-984
9. Barthel CR, Moshonov J, Shuping G, Ørstavik D (1999) Bacterial leakage versus dye leakage in obturated root canals. Int Endod J 32, 370-375
10. Miletić I, Prpić-Mehičič G, Maršan T, Tambić-Andrašević A, Pleško S, Karlović Z, Anić I (2002) Bacterial and fungal microleakage of AH26 and AH Plus root canal sealers. Int Endod J 35, 428-432
11. Michailesco P, Boudeville P (2003) Calibrated latex microspheres percolation: a possible route to model endodontic bacterial leakage. J Endod 29, 456-462
12. Wu MK, De Gee AJ, Wesselink PR (1994) Fluid transport and dye penetration along root canal fillings. Int Endod J 27, 233-238
13. Pommel L, Camps J (2001) Effects of pressure and measurement time on the fluid filtration method in endodontics. J Endod 27, 256-258
14. Pommel L, About I, Pashley D, Camps J (2003) Apical leakage of four endodontic sealers. J Endod 29, 208-210
15. Kopper PMP, Figueiredo JAP, Della Bona A, Vanni JR, Bier CA, Bopp S (2003) Comparative *in vivo* analysis of the sealing ability of three endodontic sealers in post-prepared root canals. Int Endod J 36, 857-863
16. 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
17. Brandão CG, de Moraes IG, Bramante CM (2001) Apical sealing ability of ionomeric endodontic sealers. Rev FOB 9, 29-34 (in Portuguese)
18. Miletić I, Anić I, Pezelj-Ribarić S, Jukić S (1999) Leakage of five root canal sealers. Int Endod J 32, 415-418
19. Camps J, Pashley D (2003) Reliability of the dye penetration studies. J Endod 29, 592-594
20. Dandakis C, Kaliva M, Lambrianidis T, Kosti E (2005) An *in vitro* comparison of the sealing ability of three endodontic sealers used in canals with iatrogenic enlargement of the apical constriction. J Endod 31, 190-193
21. Ahlberg KMF, 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
22. Zmener O, Pameijer CH, Macri E (2005) Evaluation of apical seal in root canals prepared with a new rotary system and obturated with a methacrylate based endodontic sealer: an in vitro study. J Endod 31, 392-395
23. Schāfer E, Olthoff G (2002) Effect of three different sealers on the sealing ability of both thermafil obturators and cold laterally compacted Gutta-Percha. J Endod 28, 638-642
24. Robertson D, Leeb IJ, McKee M, Brewer E (1980) A clearing technique for the study of root canal systems. J Endod 6, 421-424
25. Tagger M, Tamse A, Katz A, Tagger E (1983) An improved method of three-dimensional study of apical leakage. Quintessence Int 14, 981-998
26. Santa Cecília M, de Castro AJR, de Moraes IG (1997) The use of cleared teeth in Endodontics. Rev ABO Nac 4, 27-30 (in Portuguese)
27. Malvar MFG, Gomes MR, Pereira MRS (2002) Root canal anatomy study of lower incisors by clearing technique. J Bras Endod 3, 202-207 (in Portuguese)
28. Youngson CC, Jones JCG, Fox K, Smith IS, Wood DJ, Gale M (1999) A fluid filtration and clearing technique to assess microleakage associated with three dentine bonding systems. J Dent 27, 223-233
29. Kontakiotis EG, Georgopoulou MK, Morfis AS

(2001) Dye penetration in dry and water-filled gaps along root fillings. Int Endod J 34, 133-136
30. Kersten HW, Moorer WR (1989) Particles and molecules in endodontic leakage. Int Endod J 22, 118-124
31. Wimonchit S, Timpawat S, Vongsavan N (2002) A comparison of techniques for assessment of coronal dye leakage. J Endod 28, 1-4
32. Spångberg LSW, 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
33. Katz A, Rosenwasser R, Tamse A (1998) Root positioning and leakage to dye in extracted teeth using reduced pressure. Int Endod J 31, 63-66
34. Antonopoulos KG, Attin T, Hellwig E (1998) Evaluation of the apical seal of the root canal fillings with different methods. J Endod 24, 655-658
35. Miletić I, Ribarić SP, Karlović Z, Jukić S, Bošnjak A, Anić I (2002) Apical leakage of five root canal sealers after one year of storage. J Endod 28, 431-432
36. Orucoglu H, Sengun A, Yilmaz N (2005) Apical leakage of resin based root canal sealers with a new computerized fluid filtration meter. J Endod 31, 886-890
37. Maltezos C, Glickman G, Ezzo P, He J (2006) Comparison of the sealing of Resilon, Pro Root MTA, and Super-EBA as root-end filling materials: a bacterial leakage study. J Endod 32, 324-327
38. Britto LR, Grimaudo NJ, Vertucci FJ (2003) Coronal microleakage assessed by polymicrobial markers. J Contemp Dent Pract 4, 1-10
39. Chailertvanitkul P, Saunders WP, Saunders EM, MacKenzie D (1997) An evaluation of microbial coronal leakage in the restored pulp chamber of root-canal treated multirooted teeth. Int Endod J 30, 318-322
40. Chailertvanitkul P, Saunders WP, Saunders EM, MacKenzie D (1998) Polymicrobial coronal leakage of super EBA root-end fillings following two methods of root-end preparation. Int Endod J 31, 348-352
41. Williamson AE, Dawson DV, Drake DR, Walton RE, Rivera EM (2005) Effect of root canal filling/sealer systems on apical endotoxin penetration: a coronal leakage evaluation. J Endod 31, 599-604
42. Xu Q, Fan MW, Fan B, Cheung GSP, Hu HL (2005) A new quantitative method using glucose for analysis of endodontic leakage. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 99, 107-111