Comparability of results from two leakage models

Erick Miranda Souza, MSc,[a] Min-Kai Wu, MD, MSD, PhD,[b] Hagay Shemesh, DMD,[c] Idomeo Bonetti-Filho, PhD,[d] and Paul R. Wesselink, DDS, PhD,[e] São Paulo, Brazil and Amsterdam, The Netherlands
SÃO PAULO STATE UNIVERSITY AND ACADEMIC CENTRE FOR DENTISTRY AMSTERDAM

Objective. The goal of this study was to check whether leakage results of the same specimens measured by 2 different leakage models are similar.
Study design. Canine root canals were prepared and filled with cold gutta-percha cones and 1 of 4 sealers (20 canals for each sealer). The 80 specimens were first connected to a fluid transport model where air-bubble movement was measured. The same specimens were later connected to a glucose penetration model where the concentration of glucose was measured. In both models, a headspace pressure of 30 kPa was used to accelerate leakage.
Results. In both models, 4 sealers ranked the same regarding the leakage they allowed, and a significant correlation between the results of the 2 models was confined (Spearman test coefficient = 0.65; $P = .000001$).
Conclusion. Under the conditions of this study, leakage results of 80 specimens recorded in the fluid transport model and glucose penetration model were similar. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:309-13)**

To achieve periapical healing, root fillings should prevent coronal reinfection and entomb remaining bacteria.[1] In a study by Felippe et al.,[2] roots of dogs' teeth and periapical tissues were examined histologically 5 months after different endodontic treatments. Prepared but unfilled canals were associated with severe chronic periapical inflammatory reaction and severe bone and root resorption.

Defective root fillings, which provide pathways for bacteria and toxins to the periapex, are not always identified with 2-dimensional radiographs. A recent treatment outcome study reported reduced success rates when the root filling contained radiographically detectable voids (poor root filling density or dark lines along the filling).[3] Therefore, root fillings should present as few voids as possible, and, once present, voids should be as narrow as possible.

Erick Miranda Souza was supported by MEC/CAPES (Foundation for the Coordination of Higher Education and Graduate Training) with an exchange Scholarship. Study conducted at Academic Centre for Dentistry Amsterdam, The Netherlands.
[a]PhD student, Department of Restorative Dentistry, Araraquara Dental School, São Paulo State University.
[b]Senior Scientist, Department of Cariology, Endodontology, and Pedodontology, Academic Centre for Dentistry Amsterdam.
[c]Lecturer, Department of Cariology, Endodontology, and Pedodontology, Academic Centre for Dentistry Amsterdam.
[d]Assistant Professor, Department of Restorative Dentistry, Araraquara Dental School, São Paulo State University.
[e]Professor and Chairman, Department of Cariology, Endodontology, and Pedodontology, Academic Centre for Dentistry Amsterdam.
Received for publication Jan 22, 2008; returned for revision Feb 17, 2008; accepted for publication Feb 19, 2008.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2008.02.025

Different leakage models, including fluid transport[4] and glucose penetration,[5] have been used in vitro to determine the presence of voids along the root filling. Because new materials are continuously developing, so is the need for assessing their sealing ability. Results of different in vitro leakage tests are used to rank various materials. However, it has rarely been studied whether the results of the same specimens recorded in different leakage models are similar.

The purpose of the present study was to investigate the comparability of leakage results of the same specimens recorded in the fluid transport model and glucose penetration model.

MATERALS AND METHODS

One hundred recently extracted maxillary and mandibular canines were selected, and proximal radiographs were taken to confirm the presence of a single canal. The coronal parts were removed, leaving roots 15 mm in length.

Instrumentation

Canals were prepared with K-files #15 to #50 (Dentsply Maillefer, Ballaigues, Switzerland) to 1 mm short of the apical foramen. This enlargement was chosen following the recommendations of Tronstad.[6] A step-back flaring technique was performed at 2-mm increments with Gates-Glidden burs #2 to #6. File #50 was used to smooth the irregularities left by this flaring regimen. Canals were rinsed between each instrument with 2 mL 2% NaOCl solution. One minute of passive ultrasonic irrigation was performed using a #15 Endosonore file (Dentsply Maillefer).[7] Because the apical

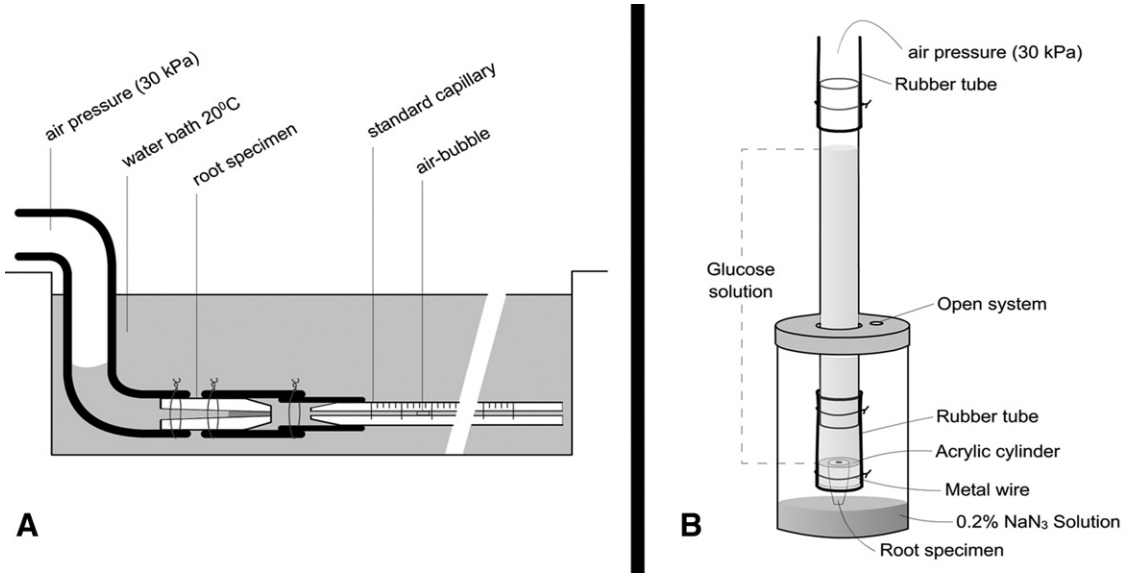

Fig. 1. Schematic illustration of 2 models. A, Fluid transport. B, Glucose penetration.

diameter of canines could be larger than 0.2 mm,[8] a K-file #30 was used to verify patency and assure that the apical foramen was not smaller than #30. Canals were rinsed with NaOCl and dried using paper points.

Roots were randomly divided into 4 experimental groups (20 each) according to the sealers tested—AH26 (Dentsply Detrey, Konstanz, Germany), AH Plus (Dentsply Detrey), RSA (Roeko Dental Products, Langenau, Germany), an experimental castor oil polymer (Polifil; Poliquil Araraquara Polímeros Químicos, Araraquara, Brazil)—and 2 control groups (10 each).

Obturation

Polifil is commercially available and consists of a paste (polyester), liquid (biphenyl methane isocyanine), and zinc oxide. The manufacturer's instructions were followed and 2.5 g of supplied zinc oxide was mixed with 1.0 g of paste and inserted into a plastic syringe. This was then mixed with the liquid in a 3:1 ratio on a glass plate. The other 3 sealers were also prepared following manufacturer's recommendations.

Sealers were introduced into the canal twice, 5 s each, using a bidirectional spiral #25 (EDS, Hackensack, NJ). A gutta-percha cone #50 (Henry Schein, Mexico City, Mexico), coated with sealer, was placed into the canal followed by 2 accessory cones #25 placed to a depth where resistance was met. No spreader was used.

Eleven millimeters of the coronal gutta-percha was removed immediately after obturation with a heated plugger, leaving the apical 4 mm to be subjected to the leakage tests.

In the positive control group, lateral compaction of gutta-percha was performed without sealer. Negative controls were filled with 3 gutta-percha cones and AH26, and the external root surface was completely covered with cyanoacrylate.[9]

To facilitate the leakage setting up, the coronal 8 mm of each specimen was embedded in acrylic resin to form a cylinder around the root. Specimens were then stored for 1 month at 37°C and 100% humidity for sealers' setting.

Fluid transport

Roots were mounted on a fluid transport model previously described[4] and shown in Fig. 1, *A*. A 30-kPa (360 cm H_2O) headspace pressure was applied, and after 3 h, 6 h, and 24 h, the air bubble movement (in μL) in the capillary tube (Fig. 1, *A*) was recorded.

Glucose penetration

Twenty-four hours after finishing fluid transport readings, samples were mounted on a glucose penetration model (Fig. 1, *B*), where glucose solution was placed in the coronal reservoir. A headspace pressure of 30 kPa was created by connecting the open orifice of the pipette to a pressure source (Fig. 1, *B*). After 24 h, a sample of 100 μL was taken from the apical reservoir and the glucose concentration was measured.

The samples were analyzed using a Glucose kit (Megazyme, Wicklow, Ireland) in a spectrophotometer (Spectra Max 384 Plus; Molecular Devices, Sunnyvale, CA) at a wavelength of 340 nm.[5] Glucose concentrations were presented in mg/mL.

Table I. Leakage of four sealers: fluid transport and glucose penetration

	Fluid transport (μL)			*Glucose concentration (mg/mL)*
Sealer	*3 h*	*6 h*	*24 h*	*24 h*
AH26 (n = 20)				
Median (range)	0 (0-1)	0 (0-2)	0 (0-4)	0.01 (0-1.2)
Mean (SD)	0.2 (0.41)	0.25 (0.55)	0.85 (1.73)	0.10 (0.30)
RSA (n = 20)				
Median (range)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-0.05)
Mean (SD)	0 (0)	0 (0)	0.05 (0.22)	0.01 (0.01)
AH Plus (n = 20)				
Median (range)	0 (0-1)	0 (0-2)	1 (0-4)	0.02 (0-1.3)
Mean (SD)	0.2 (0.41)	0.35 (0.67)	1.20 (1.20)	0.23 (0.43)
Polifil (n = 20)				
Median (range)	0 (0-0)	0 (0-0)	0 (0-2)	0 (0-0.1)
Mean (SD)	0 (0)	0 (0)	0.25 (0.55)	0.02 (0.03)

Kruskal-Wallis and Mann-Whitney tests checked differences among the 4 sealer groups and Spearman test verified the correlation between the results of two models (SPSS, version 12.0.1; Chicago, IL). Chi-squared test detected differences in the number of leaking samples demonstrated by the fluid transport model at different time intervals (Sigma Stat, version 3.1; San Jose, CA).

RESULTS

In the fluid transport test, no movement of the air bubble was detected in the negative controls. All positive controls showed bubble movement that exceeded the pipette length within 3 h. In the glucose penetration test, no glucose penetration was detected in the negative controls. In all positive controls, glucose penetrated completely to the apical reservoir within 24 h.

Tables I and II show the results of the experimental groups. After 24 h, in both models, RSA showed the best sealing, and AH Plus leaked the most ($P < .01$); no significant difference between RSA and Polifil was observed ($P = 0.15$, fluid transport; $P = 0.89$, glucose penetration).

When the fluid transport model was used, significantly more leaking samples were detected after 24 h than after 3 h or 6 h (Table II; $P < 0.01$).

A positive correlation was observed between the results of both models (Fig. 2; coefficient = 0.65; $P = 0.000001$).

Table II. Number of leaking samples detected by the fluid transport model at different time intervals

	Number of leaking samples		
Sealer	*3 h*	*6 h*	*24 h*
AH26 (n = 20)	4	4	8
RSA (n = 20)	0	0	1
AH Plus (n = 20)	4	5	14
Polifil (n = 20)	0	0	3
Total (n = 80)	8	9	26

DISCUSSION

In the original glucose model,[5] the headspace pressure was 15 cm of glucose solution. In the present study a higher headspace pressure of 30 kPa (360 cm H_2O), was applied to accelerate glucose penetration and create conditions comparable to those for the fluid transport. Thus, water or glucose solution that moved to the apical chamber under the same headspace pressure was quantified. In fluid transport, air-bubble movement was measured to indicate the volume of water penetration; in glucose penetration, the concentration of glucose in the apical chamber was measured to indicate the volume of glucose solution movement.

Fluid transport and glucose penetration occur only through voids that are completely open, while cul-de-sac type voids prevent fluid transport or glucose penetration.[10,11] It has been shown that neither water nor glucose solution penetrate through root dentin.[12] Thus, evidence of fluid transport or glucose penetration demonstrates the existence of at least 1 continuous void along the root filling. A higher value for fluid transport or glucose penetration indicates that the total volume of the voids is bigger.[10,11]

In this study, 24 h after fluid transport the specimens were connected to the glucose model, where the concentration of glucose was measured. When the same specimens are tested by 2 models, similarity of the results could be considered to be a measure of the models' reliability. However, one may argue that running the specimens through the fluid transport system under 30 kPa pressure had changed the property of the filling and affected the results of glucose penetration. In a study by Wu et al., the amount of fluid transport along

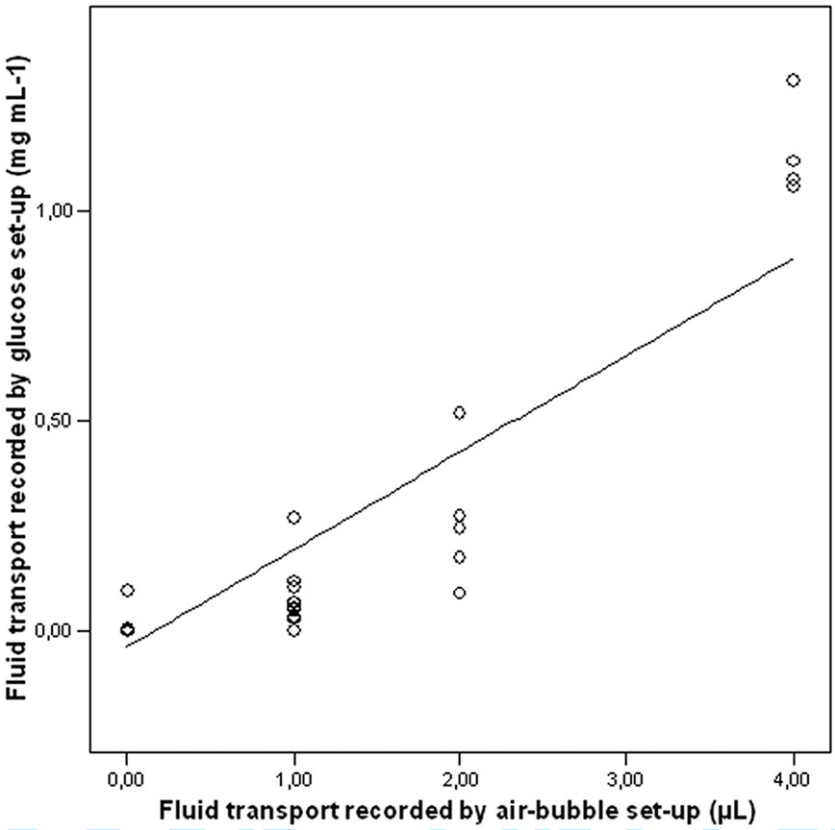

Fig. 2. Correlation between the 24 h results of 80 specimens recorded in 2 models (coefficient = 0.65; P = .000001).

the same root fillings was examined.[13] Fluid transport under headspace pressure of 60 kPa was measured every 2 h. All specimens filled by lateral compaction, vertical compaction, and single cone showed consistent values, indicating that the 60 kPa headspace pressure did not result in detectable damage to the root fillings.

In earlier studies where fluid transport was measured, the headspace pressure was applied for a certain period of time from a few minutes to 24 h.[4,14,15] To investigate whether the time of fluid transport plays a role in detection of leaking samples, fluid transport was recorded also after 3 h and 6 h. Significantly more leaking samples were detected after 24 h than after 3 h and 6 h (Table II). Presumably, samples presented with narrow voids required longer pressure time to display detectable fluid accumulation in the apical reservoir. This is supported by the observation that only 1 to 2 µL of fluid transport was recorded after 24 h (Table I).

The purpose of this study was to examine whether the same root fillings displayed a similar amount of leakage in 2 different leakage models, not to test specific root filling techniques. Only 3 gutta-percha cones were used to fill each canal to simplify the procedure. Spreader was not used because of the difficulty to standardize the spreader load and the number and size of spreader tracks.[16,17] Furthermore, using no spreader may prevent root fracture.

Similar techniques, where no compaction forces are used, have been included in several recent studies and in some of them showed a comparable sealing ability to compaction techniques.[18-21] Therefore, the results of the present study should provide useful information about the 4 sealers used whenever these noncompaction techniques are accepted.

AH Plus is considered to be a new generation of AH26, having a faster setting time.[22] The accelerated setting time may cause shrinkage stress, leading to debonding of sealer from the root canal wall.[23,24] In addition, silicon oils present in the sealer have been claimed to affect its sealing properties.[24,25] In accordance with other studies, RSA sealer displayed effective sealing,[21,26,27] which may be due to its slight expansion during setting[28,29] and close adaptation to dentinal walls.[30] Polifil is a polyurethane (polyester)[31] mixed with zinc oxide. According to our results, Polifil may become a promising endodontic sealer. Similar tissue response has been observed to Polifil and to calcium hydroxide sealer.[32]

Under the conditions in this study, results of the same specimens recorded in the fluid transport model and glucose penetration model were similar.

REFERENCES

1. Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. J Endod 1990;16:566-9.
2. Felippe WT, Felippe MCS, Rocha MJC. The effect of mineral trioxide aggregate on the apexification and periapical healing of teeth with incomplete root formation. Int Endod J 2006;39:2-9.
3. Ørstavik D, Qvist V, Stoltze K. A multivariate analysis of the outcome of endodontic treatment. Eur J Oral Sci 2004;112:224-30.
4. Wu MK, De Gee AJ, Wesselink PR, Moorer WR. Fluid transport and bacterial penetration along root canal fillings. Int Endod J 1993;26:203-8.
5. Xu Q, Fan MW, Fan B, Cheung GS, Hu HL. A new quantitative method using glucose for analysis of endodontic leakage. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:107-11.
6. Tronstad L. Clinical endodontics. New York: Thieme; 1991. p. 190, 196.
7. van der Sluis LW, Shemesh H, Wu MK, Wesselink PR. An evaluation of the influence of passive ultrasonic irrigation on the seal of root canal fillings. Int Endod J 2007;40:356-61.
8. Wu MK, R'oris A, Barkis D, Wesselink PR. Prevalence and extent of long oval canals in the apical third. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:739-43.
9. Campos TN, Inoue CH, Yamamoto E, Araki AT, Adachi LK, Rodriguez JE. Evaluation of the apical seal after intraradicular retainer removal with ultrasound or carbide bur. Braz Oral Res 2007;21:253-8.
10. Pashley DH. Dentine permeability: theory and practice. In: Spånberg LSW, editor. Experimental endodontics. Boca Raton, FL: CRC Press; 1990. p. 19-49.
11. Van den Berg HR, Ten Seldam CA, Van der Gulik PS. Compressible laminar flow in a capillary. J Fluid Mech 1993;246:1-20.
12. Shemesh H, van den Bos M, Wu M-K, Wesselink PR. Glucose penetration and fluid transport through coronal root structure and filled root canals. Int Endod J 2007;40:866-72.
13. Wu MK, van der Sluis LWM, Ardila CN, Wesselink PR. Fluid movement along the coronal two-thirds of root fillings placed by three different gutta-percha techniques. Int Endod J 2003;36:533-40.
14. Cobankara FK, Adanir N, Belli S, Pashley DH. A quantitative evaluation of apical leakage of four root-canal sealers. Int Endod J 2002;35:979-84.
15. Pommel L, Camps J. Effects of pressure and measurement time on the fluid filtration method in endodontics. J Endod 2001;27:256-8.
16. Budd CS, Weller RN, Kulild JC. A comparison of thermoplasticized injectable gutta-percha obturation techniques. J Endod 1991;17:260-4.
17. Jarrett IS, Marx D, Covey D, Karmazin M, Lavin M, Gound T. Percentage of canals filled in apical cross sections: an in vitro study of seven obturation techniques. Int Endod J 2004;37:392-8.
18. Dalat DM, Spångberg LS. Comparison of apical leakage in root canals obturated with various gutta-percha techniques using a dye vacuum tracing method. J Endod 1994;20:315-9.
19. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. The evaluation of apical leakage for three endodontic fill systems. Gen Dent 1998;46:618-23.
20. Pommel L, Camps J. In vitro apical leakage of System B compared with other filling techniques. J Endod 2001;27:449-51.
21. Wu MK, van der Sluis LW, Wesselink PR. A 1-year follow-up study on leakage of single-cone fillings with RoekoRSA sealer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:662-7.
22. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. An in vitro study of the cytotoxicity of the two root canal sealers. J Endod 2000;26:228-9.
23. Zmener O, Spielberg C, Lamberghini F, Rucci M. Sealing properties of a new epoxy resin-based root-canal sealer. Int Endod J 1997;30:332-4.
24. Miletic I, Anic I, Pezelj-Ribaric S, Jukic S. Leakage of five root canal sealers. Int Endod J 1999;32:415-8.
25. De Moor RJ, De Boever JG. The sealing ability of an epoxy resin root canal sealer used with five gutta-percha obturation techniques. Endod Dent Traumatol 2000;16:291-7.
26. Cobankara FK, Adanir N, Belli S, Pashley DH. A quantitative evaluation of apical leakage of four root-canal sealers. Int Endod J 2002;35:979-84.
27. Roggendorf MJ, Ebert J, Petschelt A, Frankenberger R. Influence of moisture on the apical seal of root canal fillings with five different types of sealer. J Endod 2007;33:31-3.
28. Kazemi RB, Safavi KE, Spångberg LSW. Dimensional changes of endodontic sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1993;76:766-71.
29. Ørstavik D, Nordahl I, Tibballs JE. Dimensional change following setting of root canal sealer materials. Dent Mater 2001;17:512-9.
30. Gençoglu N, Türkmen C, Ahiskali R. A new silicon-based root canal sealer (Roekoseal-Automix). J Oral Rehabil 2003;30:753-7.
31. Pereira-Júnior OCM, Rahal SC, Iamaguti P, Felisbino SL, Pavan PT, Vulcano LC. Comparison between polyurethanes containing castor oil (soft segment) and cancellous bone autograft in the treatment of segmental bone defect induced in rabbits. J Biomater Appl 2007;21:283-97.
32. Perassi, FT. Tecidual response of EndoRez sealer and a castor oil-based sealer compared to Endofill and Sealapex: a morphologic study (PhD thesis). Araraquara, Brazil: São Paulo State University; 2004.

Reprint requests:
Dr. Min-Kai Wu
Department of Cariology, Endodontology, and Pedodontology
Academic Centre for Dentistry Amsterdam (ACTA)
Louwesweg 1
1066 EA Amsterdam
The Netherlands
m.wu@acta.nl