

Leakage along apical root fillings with and without smear layer using two different leakage models: a two-month longitudinal *ex vivo* study

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Abstract

Shemesh H, Wu M-K, Wesselink PR. Leakage along apical root fillings with and without smear layer using two different leakage models: a two-month longitudinal *ex vivo* study. *International Endodontic Journal*.

Aim To compare two different experimental models when measuring leakage along root fillings with or without smear layer.

Methodology One hundred and twenty single-rooted teeth were prepared to size 50 and allocated to two groups: fluid transport model ($n = 60$) and glucose penetration model ($n = 60$). The roots in each group were divided into three subgroups of 20 teeth each. Smear layer was left in place in group 1 but removed in groups 2 and 3. In groups 1 and 2 canals were filled with laterally compacted gutta-percha cones and AH 26. Group 3 was laterally compacted with Resilon cones and Epiphany sealer. The coronal portion of the filling was removed to assure only 4 mm of filling remained in the canal. Leakage of glucose was evaluated by measuring its concentration once a week for a total period of 56 days using a glucose penetration model. Fluid transport was evaluated by measuring the movement of an air-bubble using a fluid transport model, 1 and 8 weeks after canal filling. Differences

between the groups in glucose concentrations and fluid transport were statistically analysed with the Kruskal–Wallis and the Mann–Whitney tests. The level of significance was set at $\alpha = 0.05$.

Results Glucose penetration was significantly different between the three groups after the first 8 days ($P < 0.05$). Resilon leaked the most throughout the experiment period. No significant difference ($P > 0.05$) existed between the two gutta-percha groups at all time intervals (Mann–Whitney test). In the fluid transportation model, no statistically significant differences were observed between all three experimental groups ($P > 0.05$) at either 1 or 8 weeks after filling (Kruskal–Wallis test).

Conclusions Under the conditions of this study, the glucose penetration model was more sensitive in detecting leakage along root fillings. Removing the smear layer before filling did not improve the sealing of the apical 4 mm of filling. Resilon allowed more glucose penetration but the same amount of fluid transport as the gutta-percha root fillings.

Keywords: gutta-percha, leakage, Resilon, root canal filling, smear layer.

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Introduction

The purpose of a root filling is to prevent bacterial growth and penetration of fluid and antigenic agents

between the canal and periapical tissues (Sundqvist *et al.* 1998).

A variety of laboratory-based experimental models are used to detect and measure leakage along root fillings. Dye leakage, fluid transport and bacterial penetration are currently the methods used most often. Recently Xu *et al.* (2005) discussed a new model that measures the leakage of glucose molecules. The model consists of a tube containing concentrated glucose

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solution that is connected to the coronal aspect of the tooth, whilst the apical region is dipped in water. Glucose that accumulates in the apical chamber is measured with a spectrophotometer following an enzymatic reaction. Glucose has a low molecule weight of 180 Da, and may be used as an indication for toxins that might penetrate the canal (Xu *et al.* 2005).

Leakage studies consistently show bacterial penetration through root fillings. Torabinejad *et al.* (1990) reported that 50% of filled single-rooted teeth were contaminated along the whole length of the canal after 19 and 42 days of exposure depending on the infecting microorganism. Khayat *et al.* (1993) reported that all root canals filled with laterally or vertically condensed gutta-percha were contaminated in less than 30 days after exposure to human saliva.

One of the methods previously described for improving the seal and for minimizing leakage is the removal of the smear layer before filling (Clark-Holke *et al.* 2003). This has been claimed to improve sealer penetration inside the dentinal tubules, achieving a potentially greater adherence to the canal wall (Kokkas *et al.* 2004). Indeed, some studies that investigated the removal of the smear layer concluded that a better seal was achieved when the smear layer was removed (Kennedy *et al.* 1986, Cergneux *et al.* 1987, Taylor *et al.* 1997, Clark-Holke *et al.* 2003, Çobankara *et al.* 2004). Other studies have suggested that removing the smear layer increases dentine permeability and might impair the sealing ability, and even allow bacteria to grow inside the dentinal tubules (Pashley *et al.* 1981, Drake *et al.* 1994, Galvan *et al.* 1994, Love 1996). Two review articles on the clinical implications of the smear layer in endodontics (Şen *et al.* 1995, Torabinejad *et al.* 2002) confirmed the uncertainty and debate relating to the removal of smear layer before filling. More recently Gulabivala *et al.* (2005) discussed the effects of mechanical and chemical procedures including the removal of the smear layer on the seal and stated that the mechanisms leading to successful root canal treatment remained to be determined.

Current filling materials and techniques fail to provide a leak-free seal (Wu & Wesselink 1993, Wu *et al.* 1993). Gutta-percha is the most popular filling material and has been used for this purpose for many years. Systems like warm injection and carrier-coated root fillings have been developed but have been shown to leak to a certain extent (Mannocci *et al.* 1999, Abarca *et al.* 2001, Wu *et al.* 2003, Chu *et al.* 2005).

Recently, a new thermoplastic synthetic polymer-based root filling material was introduced (Resilon;

Pentron Clinical Technologies, Wallingford, CT, USA). This material resembles gutta-percha in appearance, has similar handling properties and is available both in cone format and in pellets for warm injection. The corresponding sealer (Pentron Clinical Technologies) is a dual curable dental resin composite. This so-called 'Epiphany' system (Resilon and sealer combined with self-etching of the canal wall) is claimed to form a 'monoblock' which adheres to the dentine walls, prevents leakage and increases resistance to fracture (Shipper *et al.* 2004, Teixeira *et al.* 2004).

The purpose of this study was to compare two different experimental models in measuring leakage along apical root fillings with and without the smear layer.

Materials and methods

Selection and preparation of teeth

One hundred and sixty recently extracted single-rooted human teeth were selected and stored in 0.2% sodium azide, NaN₃ (E. Merck, Darmstadt, Germany) at +4 °C until use. Mandibular incisors were excluded because of their morphological diversity (Kaffe *et al.* 1985). Premolars were used only when a radiograph indicated a single canal. Teeth with open apices or large carious lesions were excluded.

The coronal portions of all teeth were removed so that each root specimen was 15 mm long. A diamond bur (FG 173 Horico, Berlin, Germany) was used to gain straight-line entry to the root canal. A size 20 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal to verify patency (Kuttler 1955). All samples were examined under a microscope (Zeiss Stemi SV6, Jena, Germany) to exclude cracks. The coronal 4 mm of the root specimens were then embedded in acryl (Vertex; Dentimex BV, Zeist, the Netherlands) to form an acrylic cylinder around the root and enable intimate contact between the rubber tube used to connect the specimen during the leakage phase of the study and the root specimen. All procedures and treatments were performed by one individual.

Instrumentation and obturation of root canals

The working length was determined by subtracting 1 mm from the total length of the root. The apical portion of the canal was instrumented to a size 50 master file using the balanced force technique (Roane & Sabala 1985) with K-Flexofiles (Dentsply Maillefer). A

step-back flaring technique was then performed at 1 mm increments with Gates Glidden burs number 2–6 (Dentsply Maillefer) making the taper 0.2 mm mm^{-1} (Wu *et al.* 2002). The purpose of this preparation regimen was to create a uniform size of canal and to overcome the variation in natural morphology. Each canal was irrigated with freshly prepared 2% NaOCl with a 27-gauge needle after every instrument and ensuring patency by extrusion of the solution beyond the apical foramen. A minimum of 10 mL NaOCl solution was used for each root. The prepared roots were randomly divided into three experimental groups of 40 roots, and two control groups of 20 roots each.

Group 1

After preparation was completed, canals were rinsed with an additional 5 mL 2% NaOCl solution and then with 5 mL deionized water. Each canal was dried using paper point size 50.

A size 50 gutta-percha master cone coated with AH 26 sealer (Dentsply Maillefer) was inserted into the canal. Light pumping motions were used to fill the canal with sealer and bring the cone to full working length. Lateral compaction was achieved using a size C finger spreader (Dentsply Maillefer) and size 25 accessory gutta-percha cones that initially reached to within 1 mm of the working length. The tip of each accessory cone was lightly coated with sealer, placed and compacted laterally. The process was repeated until cones could not be inserted more than 10 mm into the canal. An estimation of the total amount of sealer used was achieved by using a $0.5 \text{ cm} \times 0.5 \text{ cm}$ square of mixed sealer for each tooth.

The coronal gutta-percha was removed with a hot plugger (0.5 mm diameter, Dentsply Maillefer) and vertically packed, leaving the apical 4 mm of root filling subjected to the leakage test (Fan *et al.* 1999).

Group 2

After completion of preparation canals were rinsed with 5 mL 17% EDTA for 3 min to remove the smear layer (Hülsmann *et al.* 2003) and then rinsed with 5 mL deionized water. The filling was completed in the same way as group 1.

Group 3

All canals were rinsed with 5 mL 17% EDTA for 3 min and then with 5 mL deionized water. After drying, a self-etching primer (Epiphany primer; Pentron Clinical Technologies) was placed into the canal with a 26-gauge needle. Two drops of primer were used for each

root. Three paper points size 50 were used to remove excess primer after 1 min from each root. Roots were then filled with lateral compaction of Resilon cones and Epiphany sealer (Pentron Clinical Technologies) in the same way as in group 1. The filling was removed from the coronal portion of the canal in the same manner as group 1, leaving 4 mm of the apical filling intact.

Positive control group

Canals were filled using lateral compaction of gutta-percha cones without any sealer. No warm vertical forces were used and the whole length of the filling remained.

Negative control group

All roots were sealed with laterally compacted gutta-percha and AH 26 for the whole length of the canal and completely covered with nail varnish.

After filling all specimens were maintained for 1 week at 37°C and 100% humidity to allow the materials to set. Specimens in each group were then divided equally between the two different models, glucose penetration and fluid transport.

Glucose penetration model – preparation and measurements

The difference between the current version of the glucose penetration model and the original model introduced by Xu *et al.* (2005) lies mainly in the environment in which the equipment was stored: in order to overcome evaporation of fluids, specimens were placed in a closed jar with 100% humidity. From a pilot study it was concluded that this method would eliminate the effect of fluid evaporation on glucose concentration measurements.

The resin block around the coronal part of each root was connected to a rubber tube with stainless steel wires, which was in itself connected to a 16 cm long pipette (Pyrex, Acton, MA, USA). The assembly was then placed in a sterile glass bottle with a screw cap and sealed with sticky wax. A uniform hole was drilled in the screw cap with a diamond bur (No.173 Horico, Berlin, Germany) to assure an open system at all times (Fig. 1). Two millilitres of 0.2% NaN_3 solution were inserted into the glass bottle, such that the root samples were immersed in the solution. NaN_3 was used to inhibit the growth of microorganisms that might influence the glucose readings. The tracer used in the present study was 1 mol L^{-1} glucose solution (pH 7.0). Glucose has a low molecular weight and is hydrophilic

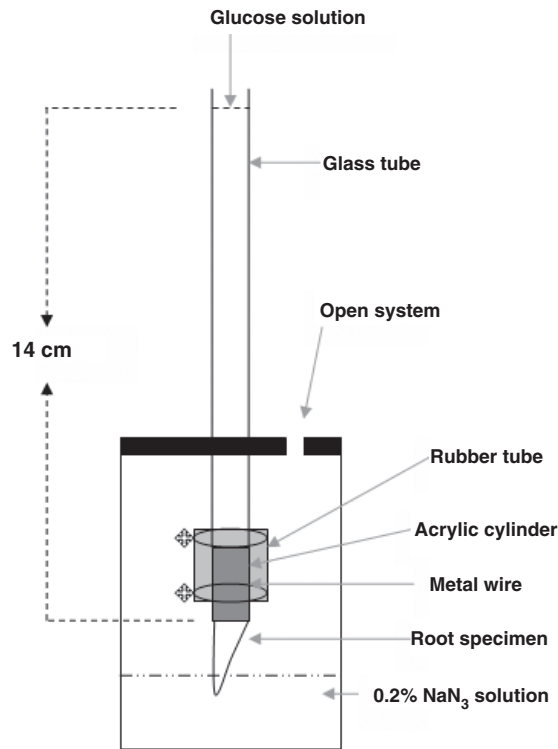


Figure 1 Glucose penetration model.

and chemically stable. About 4.5 mL of the glucose solution, containing 0.2% NaN_3 , was injected into the pipette until the top of the solution was 14 cm higher than the top of gutta-percha in the canal, which created a hydrostatic pressure of 1.5 kPa or 15 cm H_2O (Xu *et al.* 2005). All specimens were then returned to the incubator at 37 °C for the duration of the observation period. A total of 25 μL of solution was drawn from the glass bottle using a micropipette at 8, 13, 20,

33, 40, 48 and 56 days. The same amount of fresh 0.2% NaN_3 was added to the glass bottle reservoir to maintain a constant volume of 2 mL. The sample was then analysed with a Glucose kit (Megazyme, Wicklow, Ireland) in a spectrophotometer (Molecular Devices, Spectra max 384 plus) at a wavelength of 340 nm. Concentrations of glucose in the lower chamber were presented in mmol L^{-1} at each time interval following filling. The lowest glucose level for which the current procedure is believed to be accurate is 0.003 mmol L^{-1} which derives from an absorbance difference of 0.02 (D-Glucose-HK assay procedure; Megazyme, 2004). Below this level, the absorbance readings become relatively small, and results are subject to greater error from technique variables. Concentrations smaller than this were thus ignored. Similarly, once leakage exceeded 21 mmol L^{-1} samples were no longer observed as the glucose concentration in the lower chamber suggested substantial leakage had occurred.

Fluid transport model – preparation and measurements

Roots were mounted in the fluid transport device (Fig. 2) previously described by Wu *et al.* (1993). The pipettes used were 22 mm long 1 mL glass pipettes (Witeg, Wertheim, Germany). All connections were tightly closed by twisting pieces of stainless steel wire in a water bath at 20 °C. Fluid transport along the root filling was measured under a headspace pressure of 30 kPa (0.3 atm) and after 3 h the volume of fluid transport was recorded. The results were expressed as $\mu\text{L min}^{-1}$. After measurements teeth were carefully disconnected from the assembly, placed in 0.2% NaN_3 solution and returned to the incubator for a period of 8 weeks. The medium was changed with a fresh NaN_3

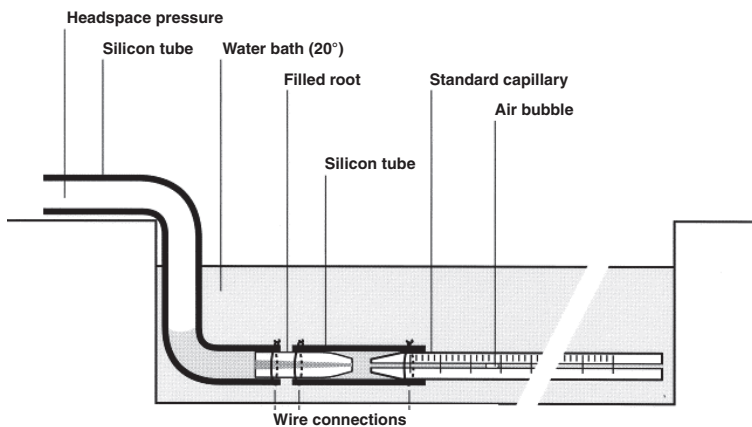


Figure 2 Fluid transport model.

solution every week. After 8 weeks the roots were mounted again and checked for fluid transport in the same way.

Statistical analysis

The differences between the groups with regard to glucose concentrations and fluid transport were statistically analysed with the Kruskal–Wallis and the Mann–Whitney tests (version 12.0.1, SPSS, Chicago, IL, USA). The level of significance was set at $\alpha = 0.05$.

Results

The results for the glucose model are shown in Table 1 and Figs 3 and 4. The positive control group had substantial leakage of glucose from the first day which increased over time. After 2 weeks all samples had maximum leakage (21 mmol L⁻¹). In the negative control group no glucose was detected in the apical reservoirs throughout the experiment. Glucose concentrations in the experimental groups revealed that after the first 8 days the difference between the three groups was significant (Kruskal–Wallis test, $P < 0.05$).

Resilon laterally compacted had the most leakage at all time intervals. However, no significant difference existed between the two gutta-percha groups (Mann–Whitney test, $P > 0.05$) at all time intervals. The statistical significance of the differences between all three groups is summarized in Table 2.

The results of the fluid transport model are shown in Table 3. The positive control group had bubble movement that exceeded the pipette length after 3 h and was impossible to measure. The negative control group had no movement of the bubble. No significant difference

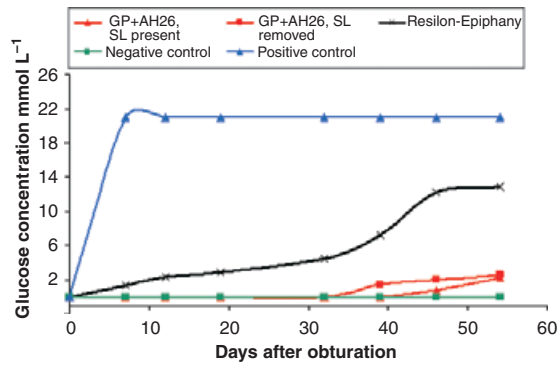


Figure 3 Median glucose penetration in mmol L⁻¹ after 2 months.

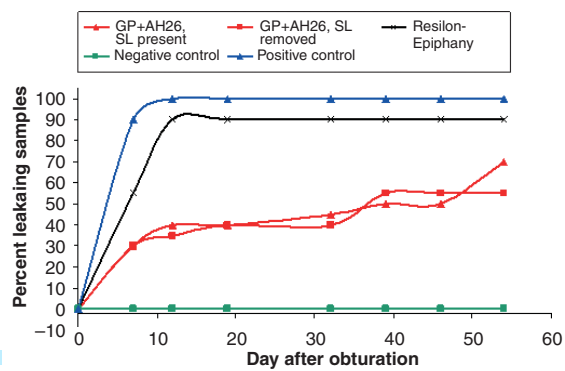


Figure 4 Percentage of leaking samples in the glucose penetration model – after 2 months.

($P > 0.05$) existed between the three experimental groups at both time intervals, 1 and 8 weeks, after filling (Kruskal–Wallis test).

Table 1 Mean and median of glucose leakage in mmol L⁻¹ at different times after obturation

Group	Day							
	8	13	20	33	40	48	56	
GP AH 26 (smear layer present)								
Mean (SD)	3.1 (6.0)	4.1 (7.3)	4.6 (7.9)	5.1 (7.9)	5.5 (8.0)	6.0 (8.4)	7.3 (8.7)	
Median (range)	0 (0–21)	0 (0–21)	0 (0–21)	0 (0–21)	0.2 (0–21)	0.8 (0–21)	2.2 (0–21)	
Percentage leaking	30	40	40	45	50	50	70	
GP AH 26 (smear layer removed)								
Mean (SD)	3.2 (6.6)	3.6 (6.8)	4.5 (7.3)	4.8 (7.5)	6.3 (8.7)	6.8 (9.0)	7.0 (9.0)	
Median (range)	0 (0–21)	0 (0–21)	0 (0–21)	0 (0–21)	1.5 (0–21)	2.0 (0–21)	2.5 (0–21)	
Percentage leaking	30	35	40	40	55	55	55	
Resilon–Epiphany (smear layer removed)								
Mean (SD)	3.5 (5.6)	6.2 (7.0)	6.6 (7.0)	8.0 (7.2)	9.6 (7.4)	12.0 (8.0)	12.8 (7.9)	
Median (range)	1.4 (0–13.5)	2.4 (0–21)	2.9 (0–21)	4.4 (0–21)	7.2 (0–21)	12.3 (0–21)	12.9 (0–21)	
Percentage leaking	55	90	90	90	90	90	90	

Time after filling (days)	Kruskal–Wallis test	Mann–Whitney test		
	<i>P</i> (groups 1–3)	<i>P</i> (groups 1 and 2)	<i>P</i> (groups 1 and 3)	<i>P</i> (groups 2 and 3)
8	0.415	–	–	–
13	0.012	0.799	0.015	0.013
20	0.026	0.989	0.020	0.033
33	0.034	0.799	0.038	0.026
40	0.031	0.799	0.023	0.030
48	0.020	0.820	0.013	0.026
56	0.035	0.738	0.035	0.024

Table 2 *P* values – statistical significance of the difference in glucose concentrations between the groups at specific time intervals

Table 3 Average fluid transportation and percentage of leaking samples 1 and 8 weeks after obturation

Group	Average fluid transport in μL (percentage leaking samples)	
	1 week	8 weeks
GP/AH26 (smear layer present; $n = 20$)	0.5 (20)	0.2 (10)
GP/AH26 (smear layer removed; $n = 20$)	0.1 (15)	0.05 (5)
Resilon/Epiphany (smear layer removed; $n = 20$)	0.0 (0)	0.0 (0)

Discussion

Several test methods have been described to evaluate the sealing quality of filled root canals. In the present study, two different models were used: the fluid transport model (Wu *et al.* 1993) and the glucose penetration model (Xu *et al.* 2005). The latter can be seen as a further development of the fluid transportation concept: both measure passage of fluid along root filled teeth after subjecting them to constant pressure. However, the glucose model allows measurements of diffusion of the marker molecules as well. The glucose test might be more sensitive than the measurement of air-bubble movement, not only because the detected threshold measurement by eye is higher than that of the spectrophotometer, but also because the convective fluid transport was combined with glucose molecule diffusion.

Time difference is an important factor when comparing the results from the two different models. In the glucose penetration model the tooth is continuously subjected to the pressure of the glucose solution in the coronal chamber for a period of 2 months. The fluid penetration model detects leakage after subjecting the filling to pressure for 3 h. This enormous time difference might result in detection of smaller voids in the

filling, making the glucose test more sensitive. Furthermore, summated glucose leakage during 2 months was measured whereas fluid transportation was measured for 3 h and observed at two different time intervals, 1 and 8 weeks after filling.

Evaporation of fluids during the 56 days experiment duration could alter the glucose concentrations both in the apical and the coronal chambers. Evaporation will inevitably occur as these two compartments have to have an opening to release pressure build-up and cannot be closed hermetically in order to allow leakage to occur. Xu *et al.* (2005) refers only to evaporation from the apical chamber, compensating it with water according to a representative sample. The method used here, storing the models in a closed humid jar, addresses the evaporation factor from both chambers and proved to be effective in initial pilot studies.

The effect of the removal of smear layer before obturation has been the subject of extensive debate. According to the current findings, the smear layer did not affect the seal with gutta-percha and AH 26 in the apical 4 mm, when checked with the fluid transport or the glucose penetration models. These results are in agreement with those of Saunders & Saunders (1994a) who found no significant difference in dye leakage after 4 months between root fillings when the smear layer was removed or present. Saunders & Saunders (1994b) assessed dye leakage of Thermafil fillings and laterally condensed gutta-percha with glass-ionomer sealer no significant difference was observed after 4 months between any of the groups. Madison & Krell (1984) and Evans & Simon (1986) also found no difference in leakage when the smear layer was removed or not. Although dye-leakage results have debatable relevancy (Wu & Wesslink 1993) they were the most frequently used to assess the influence of smear layer. In contrast to these findings, Clark-Holke *et al.* (2003) checked a mixed culture of bacteria penetrating through root fillings. A total of 30 teeth were used, amongst which

10 served as controls. During the 2-month observation period, leakage was not observed in the group where smear layer was removed, whilst 60% of the specimen having a smear layer leaked. In spite of the small number of specimen and the authors' own claim that leakage could occur after the 2-month observation period, it was suggested that removing the smear layer decreased bacterial leakage. Similarly, Kennedy *et al.* (1986) and Cergneux *et al.* (1987) observed that smear layer removal reduced dye leakage. These conflicting reports might be attributed to difference in the type of sealer and filling technique, the method of producing and removing the smear layer and different laboratory procedures to check the leakage (Şen *et al.* 1995). More recently, Paqué *et al.* (2006) found no effect of the smear layer on dye penetration through root dentine, and suggested that tubular sclerosis rather than the smear layer that influences penetrability.

Resilon is a new root filling material that consists of a composite that may be bonded to the wall of the canal. Results from the current experiment indicate that the apical 4 mm of Resilon–Epiphany root canal fillings allowed more glucose penetration than gutta-percha.

Shipper *et al.* (2004) detected more rapid bacterial leakage in gutta-percha and AH 26 fillings when compared with Resilon–Epiphany during a period of 31 days when the whole length of the root canal was filled with laterally or vertically compacted material. In the current experiment only the 4 apical mm of filling were checked. The dentinal tubules configuration which is less dense in the apical part than the coronal part (Fogel *et al.* 1988) might lead to compromised bonding apically. Tay *et al.* (2005a) observed in a transmission electron micrograph gaps of about 2 μm between the root dentine and the Resilon primer. These imperfections in the bonding might be too small to be detected by bacterial penetration models, as the average length of bacteria varies from 0.2 to more than 10 μm , the width from 0.2 to 1.5 μm (Hobot 2002).

The influence of the geometric variables involved in the use of adhesive sealers was previously discussed by Feilzer *et al.* (1993) and more recently by Tay *et al.* (2005a). The latter study simulated different scenarios and appraised the C-factors that arise from thin resin films. In a Class I cavity, the bonded surface area is five times more than the unbonded surface area (C-factor is 5). As the unbonded surface area becomes smaller, as in a root canal, the C-factor becomes much higher, there is insufficient stress relief by flow and a high probability that one or more bonded areas will debond. The probability of imperfect dentine bonding in a root

canal during polymerization might exceed the bond strength, and a high volumetric shrinkage of the sealer might occur when it polymerizes. In cross-sections of filled roots, gaps were observed between the dentine and the Epiphany layer (Tay *et al.* 2005a). These imperfections in the bonding to the walls of the canal might be too small to be detected by bacterial penetration models.

The dimensional stability of Resilon should also be addressed. Preliminary unpublished studies have shown that Resilon cones discharged a coloured substance to the surrounding medium it, that may affect the measurements of optical density. As this colour (pink) is not absorbed at the same wavelength that is assessed by the glucose kit, the results were not compromised. However, every new material that is about to be checked with this method, should be assessed for its colour properties when it is immersed in fluid for an extended period. Gutta-percha and AH 26 on the other hand, did not show any colour discharge when soaked in water.

Tay *et al.* (2005b,c) discussed the susceptibility of Resilon to degradation in two different studies: in the first, 15 mm diameter Resilon and gutta-percha discs were immersed in sodium etoxide for 20 and 60 min. The treated discs were then examined with a scanning electronic microscope and dispersive X-ray analysis. The surface of the Resilon discs was hydrolysed after 20 min exposing the filler, whilst gutta-percha discs were unaffected. The second experiment examined 15 mm diameter discs of Resilon, gutta-percha and polycaprolactone that were incubated with phosphate-buffered saline, Lipase PS or cholesterol esterase. Resilon and polycaprolactone discs had significant weight loss and surface thinning when compared with the gutta-percha discs. The influence of this phenomenon on glucose penetration may be greater in the current setting than in that of Shipper *et al.* (2004) because of the longer observation period. These results challenge the claims of the manufacturer ('Epiphany Newsletter', July 2005, Pentron Clinical Technologies) that the colour discharge from Resilon cones is only food grade dye 'leaching out into the tooth'. However, it may provide an explanation for the increased leakage in the Resilon group.

Conclusions

- The glucose penetration model is a sensitive method to detect leakage along root fillings.

- Under the conditions of this study, no statistically significant difference in glucose penetration or fluid transportation was observed along the 4 mm apical root filling with gutta-percha and AH 26 whether or not the smear layer was removed prior to filling.
- Canals filled with Resilon had more glucose penetration than gutta-percha and AH 26 during a period of 56 days, whilst no statistically significant difference was observed between the Resilon and gutta-percha filled teeth in the fluid transportation model either at 1 or 8 weeks.

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