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Glucose penetration and fluid transport through coronal root structure and filled root canals

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Abstract

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Aim To measure glucose penetration and fluid transport through coronal root structure and compare it with leakage along the coronal region of root fillings.

Methodology A total of 50 single-rooted teeth were selected and divided into three groups. Ten roots were sectioned longitudinally and the apical portion was removed leaving a total length of 9 mm. These 20 half-roots served as group 1: root structure ($n = 20$). The canals of the remaining 40 roots were prepared to size 50 and filled with vertically compacted injectable filling material and sealer. Group 2: Resilon + Epiphany ($n = 20$) and group 3: gutta-percha + AH26 ($n = 20$). The apical portion of the root was removed. Glucose penetration through the coronal root structure and coronal root fillings was checked over a period of 4 weeks and fluid transport was measured after completion of the glucose penetration test. Differences between the groups were statistically analysed with the Kruskal–Wallis test and the Mann–Whitney test.

Results The three groups presented significantly different glucose penetration ($P < 0.05$). The two groups of filled canals showed significant glucose leakage whilst the root structure group did not show any leakage. In the fluid transport model, the root structure group also did not show any leakage. No significant difference in leakage existed between the two vertically compacted filling materials, Resilon with Epiphany sealer and gutta-percha with AH26 in both models ($P > 0.05$).

Conclusion Under the conditions of this study, in both models used, no leakage was observed through root structure. Filled canals were associated with penetration of glucose regardless of the material used.

Keywords: coronal microleakage, fluid transport model, glucose penetration model, root structure.

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Introduction

A variety of laboratory-based experimental models are used to detect and measure leakage along root fillings. Whilst dye leakage, fluid transport and bacterial penetration were the most frequently used, other methods such as radio-labelled isotopes (Haikel *et al.* 1999), electromechanical tests (von Fraunhofer *et al.* 2000) and glucose penetration (Xu *et al.* 2005) have also been described. These models check penetration of different tracers through the root canal, assuming it travels along the canal and reaches the apical region.

The clinical relevancy of these studies has been debated. Pitt Ford (1983) showed no correlation between dye penetration through human teeth and periapical tissue response to four different filling materials in dogs. Oliver & Abbott (2001) reported dye penetration through root-filled teeth that had been clinically successful. Karagenç *et al.* (2006) demonstrated a poor correlation between different leakage model results and concluded that their clinical relevancy was questionable. Pommel *et al.* (2001) claimed that filtration, diffusion or electrical phenomena governed the outcome of leakage studies in the corresponding models and thus raised doubt on the relevancy of these findings. The size of the tracer might

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also influence the results (Barthel *et al.* 1999) as well as the potential of the tracer to react or affect the filling material itself. Methylene blue, for example, frequently used in dye leakage studies, might react with different filling materials and calcium hydroxide, making the results from some of these tests unreliable (Wu *et al.* 1998). A different problem with leakage tests might be that different leakage tracers could penetrate through root dentine and not through the canal. This claim is supported by the findings that dentinal tubules are permeable to bacteria (Love *et al.* 1996, Perez *et al.* 1996), adhesive agents (Pashley *et al.* 1993), cements (Çalt & Serper 1999, Weis *et al.* 2004), and fluids (Ozok *et al.* 2002). Furthermore, dentinal tubules are oriented perpendicular to the root canal walls (Ferrari *et al.* 2000) and the contact surface area may affect the seal provided by the filling material. As the smear layer is usually removed prior to obturation (Torabinejad *et al.* 2002), the tubules might be open and allow the tracer used in leakage studies to penetrate through them. Previously performed leakage studies did not address this issue and used a negative control group of similar filled roots to those checked in the experimental groups, usually coated with nail varnish or sticky wax (Khayat *et al.* 1993, Taylor *et al.* 1997, Abarca *et al.* 2001). These control groups prevent the tracer from penetrating the canal, but also the dentine itself. If, however, small molecules such as glucose or water can leak through root dentine, it would make results from such leakage studies questionable.

The purpose of this experiment is to compare leakage through root structure with leakage through the coronal part of root-filled canals using both the glucose penetration model and the fluid transport model.

Materials and methods

Selection and preparation of teeth

Seventy freshly extracted single-rooted teeth were selected and stored in water. Teeth with open apices or large carious lesions were excluded. The roots were randomly divided into two control groups and three experimental groups. The coronal portions of all teeth were removed so that each root specimen was 16 mm long.

Group 1 ($n = 20$)

Ten roots were cut vertically with a diamond-coated bur (H-327, Horico, Berlin, Germany), so 20 half-roots were formed (Fig. 1). The canal area was flattened with a diamond disc and the apical area was removed, leaving a total of 9 mm root structure. A 7-mm thick acrylic cylinder was prepared around the root, which left 1 mm root protruding from each side of the acrylic cylinder. The contact between the acrylic and the root was covered with methacrylate glue (Permacol, Ede, The Netherlands).

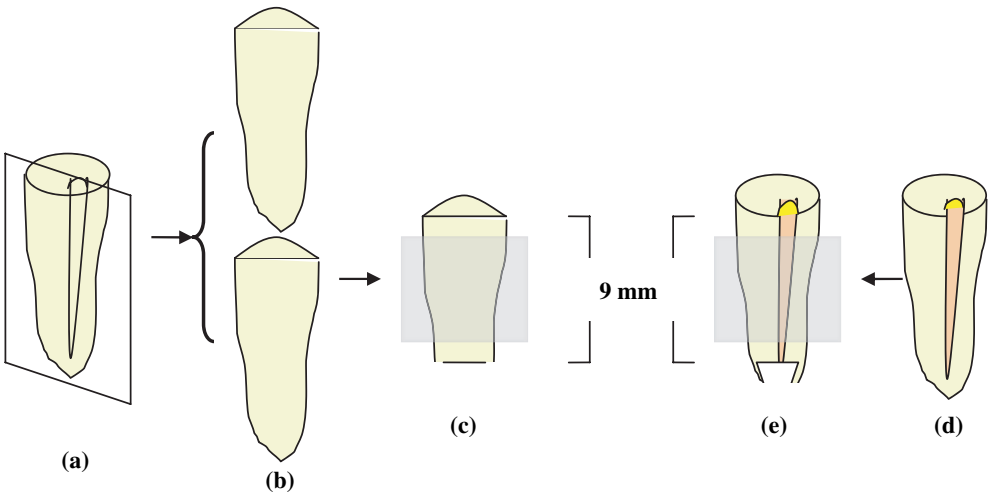

Figure 1 Preparation of the experimental groups. Root vertically sectioned (a) to result in two halves where the root canal was polished away (b); apical part removed and embedded in acrylic resin block to form group 1 specimens (c); root canal prepared and filled (d); apical part removed, prepared and embedded in acrylic resin block to from group 2, 3 specimens (e). Both the length of the root canal filling and the halved roots measured 9 mm.

Preparation of root canals

All samples were examined under a microscope (Zeiss Stemi SV6, Jena, Germany) to exclude cracks. A diamond bur (FG 173 Horico, Berlin, Germany) was used to gain straight-line access to the root canal. An ISO size 20 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal to verify patency. The coronal 7 mm of the root specimens was embedded in acrylic (Vertex, Dentimex BV, Zeist, The Netherlands) to form a cylinder around the root and enable intimate contact between the root and the rubber tube used to connect the specimen during the leakage phase of the study.

Instrumentation and filling of root canals

All teeth were treated by the same operator. The working length was determined by subtracting 1 mm from the total length of the root (16 mm). The apical portion of the canal was instrumented to an ISO size 50 master file using the balanced force technique (Roane *et al.* 1985) with K-Flexofiles. Step-back flaring was performed at 1 mm increments with Gates Glidden burs numbers 2–6 (Dentsply Maillefer) making the taper 0.2 mm mm^{-1} (Wu *et al.* 2002). The purpose of this preparation was to create a uniform size of canal and to overcome variation in natural morphology. Each canal was irrigated with freshly prepared 2% sodium hypochlorite solution (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. After preparation, canals were ultrasonically irrigated for 20 s and rinsed with 2 mL of 2% NaOCl. This procedure was repeated thrice (van der Sluis *et al.* 2006). All canals were then rinsed with 5 mL 17% ethylenediamine tetraacetic acid (EDTA) for 3 min (Scelza *et al.* 2003) and with 5 mL de-ionized water. Each canal was dried with an ISO size 50 paper point and filled according to the relevant group.

Group 2 ($n = 20$)

Canals were filled with gutta-percha and AH26 (Dentsply Maillefer) using the continuous-wave technique with a System B device and Obtura II system (Obtura Corporation, Fenton, MO, USA). An ISO size 50 gutta-percha cone coated with AH26 sealer was inserted into the canal. Light pumping motions were used to fill the canal with sealer and bring the cone to full working length.

The coronal part of the gutta-percha was removed with a heated System B plugger reaching the apical 5 mm and compacted with a pre-fitted hand plugger. The coronal section was compacted vertically with gutta-percha using the Obtura II system leaving the coronal 2 mm of the canal empty.

Group 3 ($n = 20$)

Canals were filled with the Resilon–Epiphany system (Pentron Clinical Technologies, Wallingford, CT, USA). A self-etching primer was placed into the canal with a 26-gauge needle. Two drops of primer were used for each root. Three paper points ISO size 50 were used to remove the excess primer after 1 min from each root. Roots were filled with Resilon cones and Epiphany sealer with the continuous-wave technique using the System B device and compacted vertically with the Obtura II system. An ISO size 50 Resilon cone coated with Epiphany sealer was inserted into the canal. Light pumping motions were used to fill the canal with sealer and bring the cone to full working length. The coronal part of the filling was removed with a heated System B spreader extending to the apical 5 mm and compacted with a pre-fitted hand plugger. The coronal part was filled with Resilon using the Obtura system, leaving the coronal 2 mm of the canal empty.

Controls

The Positive control group ($n = 10$) consisted of canals that were filled using lateral compaction of gutta-percha cones without sealer. No warm vertical forces were used and the whole length of the filling remained intact. The negative control group ($n = 10$) consisted of roots that were sealed with laterally compacted gutta-percha and AH26 for the whole length of the canal and completely covered with nail varnish.

Storage and apical preparation

The filled roots were stored for 1 week at 37 °C and 100% humidity to allow the materials to set. The apical 2 mm of the roots were cut with a diamond bur (FG 109 Horico, Berlin, Germany) and 3 mm of the apical root fillings were removed mimicking apical surgery preparation, leaving a total of 9 mm root canal filling (Fig. 1).

Model assembly and measurements: glucose penetration model

All samples were mounted on a glucose leakage model. This model was first used by Xu *et al.* (2005) and was further developed and described in detail in other publications (Shemesh *et al.* 2006, van der Sluis *et al.* 2007). The acrylic resin block around the root was connected to a rubber tube with stainless steel wires, which were connected to a 16-cm long pipette (Pyrex, Acton, MA, USA). The assembly was 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. Sterile water (2 mL) was injected into the glass bottle, such that the root samples were immersed in the solution. The tracer used in the present study was 1 mol L^{-1} glucose solution. Glucose has a low molecular weight and is hydrophilic and chemically stable. About 4.5 mL of the glucose solution was injected into the pipette until the top of the solution was 14 cm higher than the top of the root canal filling, which created an hydrostatic pressure of 1.5 kPa or 15 cm H_2O (Xu *et al.* 2005). All specimens were returned to the incubator at 37 °C for the duration of the observation period. A total of 100 μL of the solution was drawn from the glass bottle using a micropipette every week for 4 weeks. The same amount of fresh de-ionized water was added to the glass bottle reservoir to maintain a constant volume of 2 mL. All assemblies were stored in a closed plastic container at 100% humidity to prevent evaporation.

The sample taken every week was 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 mg L^{-1} at each time interval. The lowest glucose level for which the current procedure is believed to be accurate is 0.6 mg L^{-1} which derives from an absorbance difference of 0.02 (D-Glucose HK assay procedure, Megazyme International Limited, 2004). Below this level, the absorbance readings become relatively small, and results are subjected to greater error from technique variables. Concentrations smaller than this were thus ignored. Similarly, once the optical density reading exceeded 1.2 (corresponds to a glucose concentration of 79.6 mg L^{-1}), samples were no longer observed as the glucose concentration in the lower chamber suggested substantial leakage had occurred.

Fluid transport model

After completion of the glucose penetration, test roots were mounted on a fluid transport model (Wu *et al.* 1993). Fluid transport was measured under a head-space pressure of 30 kPa (0.3 atm), and after 3 h the movement of an air bubble was recorded and expressed in microlitre.

Statistical analysis

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

Results

The negative control group showed no leakage in either model throughout the experiment period. The positive control group leaked immediately. The results obtained from the experimental groups are summarized in Table 1.

The difference in glucose leakage between the three experimental groups was highly significant ($P < 0.0001$). Group 1 (root structure) had no leakage, whilst the two other groups (gutta-percha, Resilon) did leak (Fig. 2). Although more roots in the Resilon group showed glucose penetration, there was no statistical significance between them ($P = 0.174$). In the fluid transport model, no samples leaked at the end of 4 weeks in the Resilon–Epiphany group, and one sample leaked (4 μL) in the gutta-percha group. No

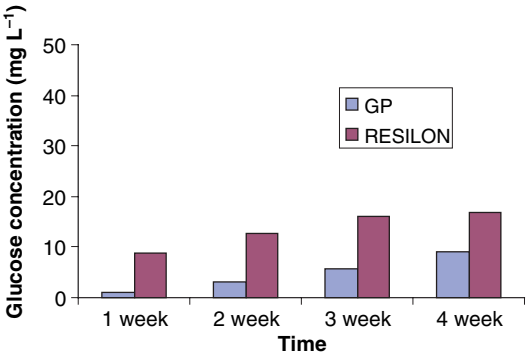

Figure 2 Mean glucose penetration in mg L^{-1} after 1–4 weeks.

Table 1 Mean glucose leakage in mg L^{-1}

	1 week	2 week	3 week	4 week
GP and AH26				
Mean	0.96	3.14	5.58	9.05
SD	1.23	4.61	12.22	20.73
Median	0.50	1.62	1.13	1.23
Range	0–4.97	0.20–19.30	0–41.46	0–79.60
% Leaking samples	0	15	20	20
Resilon–Epiphany				
Mean	8.75	12.69	16.04	16.85
SD	20.45	24.78	27.76	27.67
Median	0.13	2.88	4.97	5.67
Range	0–79.60	0–79.60	0–79.60	0–79.60
% Leaking samples	15	40	50	50
Root structure				
Mean	0.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0
% Leaking samples	0	0	0	0

statistical significance was observed between groups in the fluid transport model ($P = 0.368$).

Discussion

At the end of 4 weeks, both tracers penetrated all 10 roots of the positive control group and none of the negative control group. Root structure showed no penetration of any of the tracers. Overall, 14 of the 40 filled roots allowed glucose to pass through the canal and reach the apical portion.

Most studies in this field found fluid movement or tracer penetration through root-filled teeth regardless of the filling material or filling method used (Abarca *et al.* 2001, Wu *et al.* 2003). The current finding that root dentine did not allow fluid movement or glucose penetration is important when assessing the relevancy of such studies. It proves that the tracer passes through the canal and the measurements reflect the ability of the canal filling to prevent tracer penetration through it. Nevertheless, we cannot exclude the possibility that parts of the root dentine might be permeable to the tracer: only if the whole length of the checked root structure (9 mm long) could allow the tracer to pass through it, would we detect leakage at the apical side. The possibility that certain parts of the root dentine might be permeable is of little concern to the present study, however, as it does not affect the detection of tracer at the other side of the root.

Advances in bonding techniques have led to the development of composite resin root filling materials such as Resilon. Early studies with Resilon have presented a decrease in the amount of bacterial penetration through root canals treated using Resilon when compared with gutta-percha (Shipper *et al.* 2004) and an increase in the fracture resistance of root-filled teeth (Teixeira *et al.* 2004). However, Most of the more recent studies comparing Resilon–Epiphany root fillings to more conventional gutta-percha techniques concluded that there is no apparent advantage of using Resilon–Epiphany over gutta-percha with different sealers (Biggs *et al.* 2006, Onay *et al.* 2006, Pitout *et al.* 2006, Sagsen *et al.* 2006, Baumgartner *et al.* 2007). Consistent with these reports, the current experiment indicates no statistically significant difference between the two different materials: Resilon with Epiphany sealer or gutta-percha with AH26 in terms of fluid movement and glucose penetration.

The apical portion of root fillings with Resilon or gutta-percha allow glucose penetration when checked with the glucose model (Shemesh *et al.* 2006). However, the coronal portion of the root canal is wider, more accessible, and it is assumed that the filling of this part of the canal is more efficient than the apical portion. In the case of Resilon, it could be hypothesized that the bond between the filling material and the conditioned canal walls may be stronger in the coronal portion. However, this has not been substantiated. For example, Wu *et al.* (2003) assessed the coronal two-thirds of gutta-percha and RoekoSeal root fillings with the fluid transport model. Comparing their results to a previously published study (Wu *et al.* 2002) on the same materials and at similar conditions, the coronal portion allowed more fluid movement than the apical portion. In the current experiment, leakage along the coronal portion of the canal was assessed: Resilon allowed more glucose penetration than gutta-percha, but this difference was not statistically significant because of the small group size ($n = 20$). However, comparing previous reports on the sealing ability of the apical 4 mm of canal filling (Shemesh *et al.* 2006) to the current findings, Resilon performed better in the coronal part of the canal. This difference could be explained by methodological differences between the two experiments, and possibly by the different location of the filling along the canal. In the present study, the length of the root filling was significantly longer (9 mm as compared with 4 mm in the previous experiment) and the longer the root filling, the less leakage observed (Mattison *et al.* 1984).

There were differences between results in the two experimental models used. The glucose test may be more sensitive than the measurement of the fluid transport observations: measurements of fluid transportation are carried out by observing an air bubble

and its movement along a capillary whilst the glucose concentration is determined by a highly sensitive enzymatic reaction measured by the spectrophotometer. Furthermore, the glucose concentration is measured continuously for a period of 4 weeks, whilst specimens in the fluid transport model are subjected to pressure for only 3 h.

Concerns have been raised over the possibility that different filling materials might react with the tracer used. A pilot study was conducted where all filling materials involved in the current experiment were immersed in a glucose solution for up to a month and glucose concentrations were checked periodically. Resilon, Epiphany, gutta-percha and AH26 did not influence the concentration of glucose over time.

Conclusion

Under the conditions of this study, in both models used, samples of coronal root structure did not show any leakage. Root canals filled with vertically compacted Resilon and Epiphany or gutta-percha and AH26 allowed penetration of glucose with no statistical difference between them. However, there was a trend for Resilon–Epiphany-filled roots to allow more penetration of glucose.

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