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# Bacterial penetration along different root canal filling materials in the presence or absence of smear layer

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## Abstract

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**Aim** To study the effect of the smear layer on the penetration of bacteria along different root canal filling materials and to examine the dentine/sealer and sealer/core material interfaces for the presence of bacteria.

**Methodology** A total of 110 human root segments were instrumented to size 80 under irrigation with 1% sodium hypochlorite. Half of the roots were irrigated with a 5-mL rinse of 17% EDTA. Roots with and without smear layer were filled with gutta-percha (GP) and AH Plus sealer (AH), GP and Apexit sealer (AP), or RealSeal cones and sealer (RS). Following storage in humid conditions at 37 °C for 7 days, the specimens were mounted into a bacterial leakage test model for 135 days. Survival analyses were performed to calculate the median time of leakage and log-rank test was used for pairwise comparisons of groups. The level of significance was set at $P = 0.05$. Selected specimens were longitudinally sectioned and inspected by scanning electron microscopy for the presence of bacteria at the interfaces.

**Results** In the presence of the smear layer, RS and AP leaked significantly more slowly than in its absence. In the absence of the smear layer, AH leaked significantly more slowly than RS. SEM results indicated a differential pattern of bacterial penetration among the sealers.

**Conclusions** Removal of the smear layer did not impair bacterial penetration along root canal fillings. A comparison of the sealers revealed no difference except that AH performed better than RS in the absence of the smear layer.

**Keywords:** bacterial leakage, root canal sealers, scanning electron microscopy, smear layer.

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## Introduction

One of the requirements for a successful root filling is the achievement and maintenance of a tight seal, chemical and/or mechanical, along the root canal system (Johnson & Gutmann 2005). A tight seal should prevent the ingress of bacteria and their by-products to the periradicular tissues or entomb the remaining microorganisms (Sundqvist & Figdor 1998) and hence prevent or heal apical periodontitis.

Standard methods for filling the root canal system make use of a core material, which usually is gutta-percha (GP), in combination with a root canal sealer. Recent advances in dentine bonding have led to the development of a root filling system that consists of a thermoplastic synthetic resin core and a dual curable dental resin composite sealer (Teixeira *et al.* 2004). This product is used in combination with a self-etching primer, with the intention of creating a root filling that acts as a solid monoblock.

A smear layer is formed on the surface of dentinal walls when the root canals are instrumented (McComb

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& Smith 1975) And its significance in endodontics has been the subject of extensive debate since it was first described. Retaining the smear layer on the root canal walls has been considered to be beneficial as it may discourage bacterial penetration and colonization of the dentinal tubules (Michelich *et al.* 1980, Drake *et al.* 1994). It has also been argued that the removal of the smear layer increases dentine permeability with possible diffusion of noxious substances from the root canal to the external root surface (Galvan *et al.* 1994). More recently, it has been found that the removal of the smear layer may impair adhesion of some sealers to dentine (Lalh *et al.* 1999, Saleh *et al.* 2002). Other factors would indicate removal of the smear layer prior to root filling. Not only can the smear layer act as a reservoir or substrate for microorganisms (Pashley 1984), but its presence can also inhibit or significantly delay the penetration of antimicrobial agents such as intracanal irrigants and medications into the dentinal tubules (Lester & Boyde 1977, Byström & Sundqvist 1985, Foster *et al.* 1993). The smear layer may also interfere with adhesion and penetration of sealers into dentinal tubules (Kouvas *et al.* 1998).

It is now generally advocated that the smear layer should be removed prior to the insertion of the root filling (Johnson & Gutmann 2005). This is assumed to facilitate adaptation of the filling material to the dentine wall and to improve adhesion and resistance to bacterial penetration (Hülsmann *et al.* 2003). However, the results on smear and adhesion are conflicting, and it is unclear whether possible beneficial effects of smear layer removal is a general phenomenon or is dependent on the materials and techniques used (Lalh *et al.* 1999, Saleh *et al.* 2002). It is therefore of interest to examine whether the adhesive properties, as influenced by the smear layer, will affect bacterial penetration along different root fillings. Leakage through a filled root canal may occur at the interfaces between sealer and dentine or sealer and GP, or through voids within the sealer (Wu *et al.* 1994).

The aim of the present work was to test whether the removal of the smear layer aids in preventing bacterial leakage along root fillings with different sealers, and to examine the dentine/sealer and sealer/core material interfaces in leaking specimens for the presence of bacteria.

## Materials and methods

### Materials tested

The materials used, their manufacturers and batch numbers are listed in Table 1.

### Preparation and filling of root canals

A total of 110 single-rooted human teeth were stored in 0.01% NaOCl after extraction. The crowns were removed and root segments with a length of 10 mm from the cemento-enamel junction were prepared by cutting off the root tips, using a rotating diamond saw (Accutom; Struers, Copenhagen, Denmark) under water cooling. The roots were inspected for the presence of cracks with an operating microscope at ×8 magnification. Stainless steel K-files (Dentsply Maillefer, Ballaigues, Switzerland) were used to prepare each root canal to size 80 under irrigation with 10 mL 1% NaOCl. After instrumentation, half of the roots were irrigated with a 5-mL rinse of 17% EDTA for 5 min each. The use of NaOCl and EDTA effectively removes the smear layer, as demonstrated by SEM (Saleh *et al.* 2004). The roots were rinsed thoroughly with distilled water and sterilized by autoclaving for 20 min at 121 ± 2 °C. The root canals were then blotted dry with sterile paper points (Dentsply Maillefer). Roots with and without smear layer were then assigned to six experimental groups ($n = 15$) and four control groups ($n = 5$) as shown in Table 2.

The sealers tested were mixed and applied according to the manufacturer's instructions. For groups 3 and 4, a self-etching primer (RealSeal; SybronEndo, Glendora, CA, USA) was placed into the canal with a micro-brush and excess primer was blotted with paper points. A GP or RS cone size 70 coated with the freshly mixed sealer was introduced into the prepared canal with tweezers until fully seated. Excess core material, coronal and

**Table 1** Materials tested

| Sealer | Code | Manufacturer | Batch number |
|---|---|---|---|
| Gutta-percha | GP | Dentsply Maillefer, Ballaigues, Switzerland | 031002 |
| AH Plus | AH | Dentsply De Trey GmbH, Konstanz, Germany | 9810000713 |
| RealSeal sealer and cones | RS | SybronEndo, Glendora, CA, USA | 04J7 |
| | | SybronEndo, Glendora, CA, USA | 114305 |
| Apexit | AP | Vivadent, Schaan, Liechtenstein | 912697 |

**Table 2** Experimental design and results of leakage

| Group | Material | Code | *n* | *P* | m |
|---|---|---|---|---|---|
| 1 | Gutta-percha + AH Plus | AH-s | 15 | 11/15 | 31 |
| 2 | Gutta-percha + AH Plus + EDTA | AH-ns | 15 | 14/15 | 29[c] |
| 3 | RealSeal core & sealer | RS-s | 15 | 13/15 | 44[a] |
| 4 | RealSeal core & sealer + EDTA | RS-ns | 15 | 15/15 | 11[a,c] |
| 5 | Gutta-percha + Apexit | AP-s | 15 | 8/15 | 105[b] |
| 6 | Gutta-percha + Apexit + EDTA | AP-ns | 14[d] | 14/14 | 25[b] |
| 7 | Positive control | PC-s | 5 | 5/5 | 0 |
| 8 | Positive control + EDTA | PC-ns | 5 | 5/5 | 0 |
| 9 | Negative control | NC-s | 5 | 0/5 | 135+ |
| 10 | Negative control + EDTA | NC-ns | 5 | 0/5 | 135+ |

n, number of specimens; p, proportions of leaking specimens; m, median time of leakage in days.
[a]$P < 0.05$ for RS-s vs RS-ns, [b]$P < 0.05$ for AP-s vs AP-ns, [c]$P < 0.05$ for AH-ns vs RS-ns (log-rank test). No other pairwise comparison showed a *P*-value below 0.05.
[d]One specimen was excluded before mounting because of a macroscopic fracture.

apical, was cut off with a scalpel. Aseptic techniques were maintained throughout the procedure. The specimens were kept in sealed tubes, under humid conditions, and placed in an incubator for 7 days at 37 ± 1 °C to allow the sealers to set.

### Bacterial leakage test

Bacterial leakage was tested using the two-chamber method described by Torabinejad *et al.* (1990) (Fig. 1). The tip portions of 15-mL polyethylene tubes were cut off to accommodate the coronal ends of the root-filled specimens. The specimens were attached with sticky wax to the tubes that served as bacterial reservoir (=upper chambers), leaving the apical 1–2 mm uncovered. The negative controls were completely covered with sticky wax before being attached to the tubes. The mounts were then tightly sealed with sticky wax to 20-mL sterile glass vials (=bottom chambers) containing 8-mL of sterile trypticase soy broth (TSB; Oxoid Ltd, Basingstoke, UK) with 2 mg $mL^{-1}$ streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The apices extruding from the polyethylene tubes were hanging vertically 1–2 mm into the broth. A 3-mL of an overnight culture of a streptomycin-resistant strain of *Enterococcus faecalis* (strain A197A, adapted to and maintained on TSB with 2 mg $mL^{-1}$ streptomycin) was added to each top chamber. The mounts were kept at 37 ± 1 °C throughout the experiment (135 days). The bacteria and medium in the upper chamber were replaced with freshly grown cultures twice weekly to maintain viability and numbers of bacteria. To determine the viability of the bacteria throughout the week, samples of the old inoculums of randomly chosen tubes were incubated and specified. The bottom chambers of all mounts were checked daily for turbidity as evidence for bacterial penetration along the root filling. On observation of turbidity in the lower chamber, the seal was broken, and the


**Figure 1** Two-chamber bacterial leakage test model. a: clear medium in bottom chamber indicating no leakage; b: leaking specimen with bacterial growth in lower chamber.

nature and purity of the organism growing there were confirmed by cultural morphology and streptomycin resistance. The day of leakage was recorded for each leaking sample and the number of leaking samples was recorded per group.

Statistical analysis was performed using the Kaplan Meier test for survival analysis, which includes calculation of the median time of leakage, and pairwise comparisons of groups with the log-rank (Mantel Cox) test (Table 2). A *P*-value of $<0.05$ was considered to be significant.

### Scanning electron microscope (SEM) examination

On completion of the leakage study, three specimens from each experimental group and one from the positive control group were randomly chosen. The specimens were fixed in buffered formalin solution. Longitudinal grooves were cut in the roots and they were split into two halves so that the dentine-filling interface could be obtained. The specimens were mounted onto a SEM specimen stub and coated with a gold/palladium film with a Bio-Rad Sc 5000 (Fisons Instruments, Uckfield, UK) sputter coater. Specimens were viewed with a Philips XL 30 ESEM scanning electron microscope (Eindoven, The Netherlands) at 15-kV accelerating voltage. The specimens were inspected systematically, from the coronal to the apical area of the root, for the presence of bacteria at the dentine/sealer interface or sealer/core material interface.

## Results

The results of the bacterial penetration test are summarized in Table 2 and Fig. 2. All sealers leaked more slowly in the presence of the smear layer than in its absence. The difference was not statistically significant for AH ($P = 0.636$), whereas for RS ($P = 0.011$) and AP ($P = 0.002$) the difference was significant. In the presence of the smear layer, bacterial penetration occurred more slowly for AP than the other materials, but the difference was not significant. In the absence of the smear layer, AH leaked significantly more slowly than RS, while the other intergroup comparisons did not show significant differences.

### Scanning electron microscopy

In AH-s specimens, bacteria were mainly observed on the GP surface (Fig. 3a) and among the AH sealer particles adhering to GP (Fig. 3b). In the absence of a smear layer (AH-ns specimens), bacteria were observed on the sealer remaining on the dentine surface (Fig. 3c). Sealer tags were seen inside the dentinal tubules with bacterial colonization of the dentine surface (Fig. 3d).

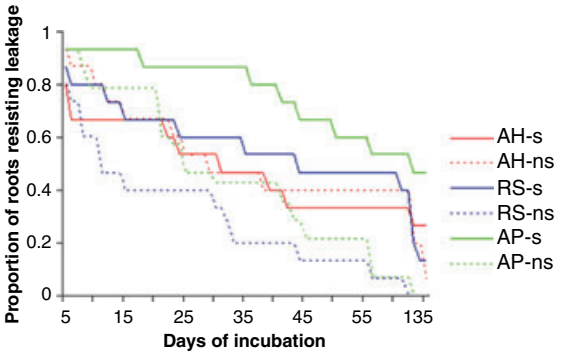


**Figure 2** Kaplan Meier plot of leakage showing the proportion of roots resisting leakage in each experimental group over time.

Examination of the sealer/dentine interface for RS-s specimens revealed cohesive failure within RS sealer with bacterial colonization of the RS remaining on the dentine surface (Fig. 4a). Bacteria colonizing the smear layer and the dentinal tubules could also be observed (Fig. 4b). In RS-ns specimens, bacteria were observed among the sealer particles and on the dentine surface at the sealer/dentine interface (Fig. 4c,d).

In both AP-s specimens and AP-ns specimens, bacteria were observed at both interfaces: the GP/sealer interface (Fig. 5a,c) and the sealer/dentine interface (Fig. 5b,d).

## Discussion

Despite ongoing research and recent developments in endodontic materials, complete sealing of the root canal system with currently accepted materials and obturation techniques is not a predictable procedure. Numerous *in vitro* studies have reported that bacteria and bacterial elements leak along root fillings when exposed to the oral environment (coronal leakage), which is a major cause of failure of root fillings (Madison *et al.* 1987, Torabinejad *et al.* 1990, Trope *et al.* 1995). However, in a recent clinical investigation, Ricucci & Bergenholtz (2003) reported that well-prepared and filled root canals resist bacterial penetration despite exposure to bacterial plaques and caries for a prolonged period.

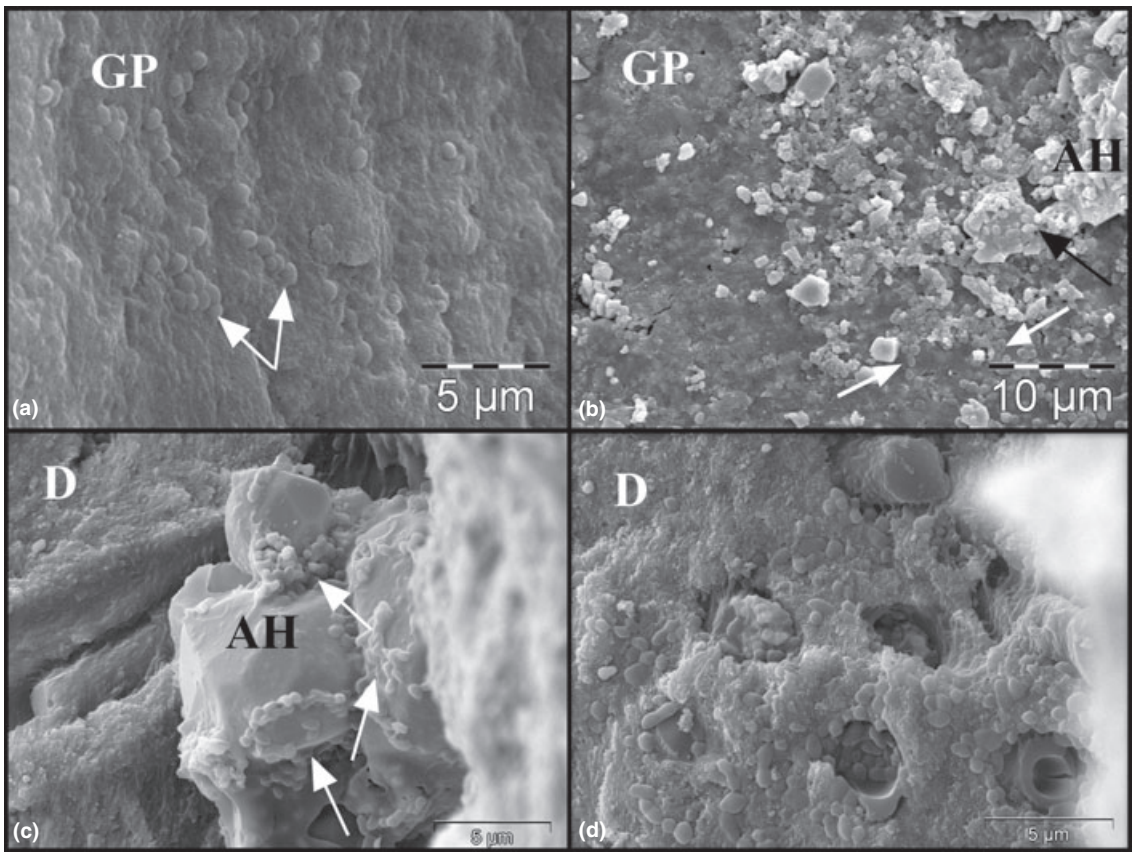


**Figure 3** Scanning electron micrographs of AH specimen. (a, b) Specimen with smear. At the gutta-percha/sealer interface, bacteria are observed on the GP surface [white arrows in (a,b) and among the AH sealer particles adhering to GP (black arrow in (b)]. (c, d) Specimen without smear. At the sealer/dentine interface, bacteria are observed on the sealer (AH) remaining on the dentine surface (D) [arrows in (c)]. Sealer tags are seen inside the dentinal tubules with bacterial colonization of the dentine surface (d).

Bacterial leakage tests (Goldman *et al.* 1980) are frequently used for evaluation of the sealing ability of endodontic sealers. The model described by Torabinejad *et al.* (1990) was used in the present study. *E. faecalis* was chosen as the test bacteria, as they are part of normal flora in humans and are frequently isolated in failed endodontically treated teeth together with other facultative anaerobes (Molander *et al.* 1998). For standardization of the procedure, the root apex (1–3 mm), where apical deltas and anatomical variations are common, was removed. In addition, a single cone of the core material was used in combination with a sealer to produce a sealer layer of near uniform thickness in all specimens. This would consequently provide similar conditions for the bacterial penetration test and facilitate the examination of the interface with SEM. The sealers AH and AP were selected as they possessed the strongest and weakest bond strengths to dentine, respectively, when previously tested in the presence and absence of the smear layer (Saleh *et al.* 2002). They both showed increased adhesion when the smear layer was present.

There has been no disagreement in the endodontic literature that the smear layer itself may be infected and may protect the bacteria already present in the dentinal tubules (Pashley 1984). Previous studies also agreed that the presence of the smear layer can inhibit or significantly delay the penetration of antimicrobial agents such as intracanal irrigants and medications into the dentinal tubules (Lester & Boyde 1977, Byström & Sundqvist 1985, Foster *et al.* 1993). Only recently, Paqué *et al.* (2006) showed that the smear layer does not appear to be a diffusion barrier. They observed microscopically that irrigant penetration was

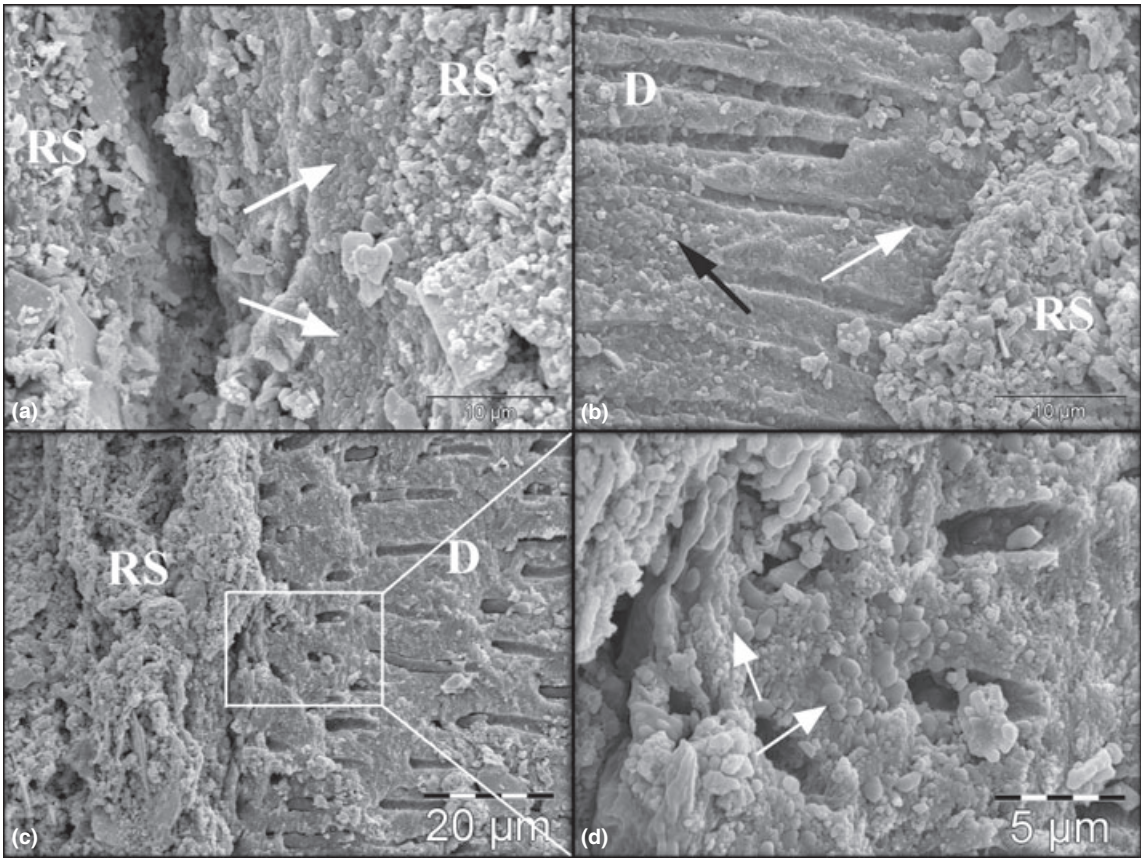


**Figure 4** Scanning electron micrographs of RS specimen. (a, b) Specimen with smear. At the sealer/dentine interface, (a) there is cohesive failure within RealSeal sealer (RS) with bacterial colonization of RS sealer remaining on the dentine surface (arrows). (b) RS remnants on the dentine surface (D) are seen with bacterial colonization of the smear layer (white arrow) and the dentinal tubules (black arrow). (c, d) Specimen without smear, at the sealer/dentine interface (c, low magnification). At higher magnification (d), bacteria are observed among the sealer particles (RS) and on the dentine surface (D) (arrows).

not influenced by the presence of a smear layer, but was rather a function of tubular sclerosis. Another argument for removal of the smear layer has been that it may act as a physical barrier interfering with adhesion and penetration of sealers into dentinal tubules, which may affect the sealing efficacy of root filling (White *et al.* 1984, Kouvas *et al.* 1998). This statement is not in accordance with the results of a previous study (Saleh *et al.* 2002) on the effect of the smear layer on adhesion, which showed that the removal of the smear layer with EDTA significantly reduced, rather than increased, the adhesion of AH ($P < 0.01$) and AP ($P < 0.05$). Similarly, the bond strength for RS tended to decrease when dentine was pre-treated with EDTA (I.M. Salch *et al.*, unpublished data). The results of the present leakage study also showed that AH and AP sealers leaked more quickly in the absence of the smear layer. The difference was not statistically significant for AH, while for AP ($P = 0.002$) the difference was significant.

As with AH and AP sealers, the resin-bonded filling system RealSeal leaked significantly ($P = 0.011$) more when the smear layer was removed. The RealSeal system is assumed to be chemically identical with the Epiphany/Resilon system (Pentron, Wallingford, CT, USA). Resilon is claimed to be a resin core root canal filling that contains dimethacrylate, bioactive glass and radiopaque fillers (Teixeira *et al.* 2004). It bonds with a dentine-bonding system to a resin sealer that itself bonds to the canal wall. The core material performs like GP and has similar handling properties. For retreatment purposes, it may be softened with heat or dissolved with solvents like chloroform.

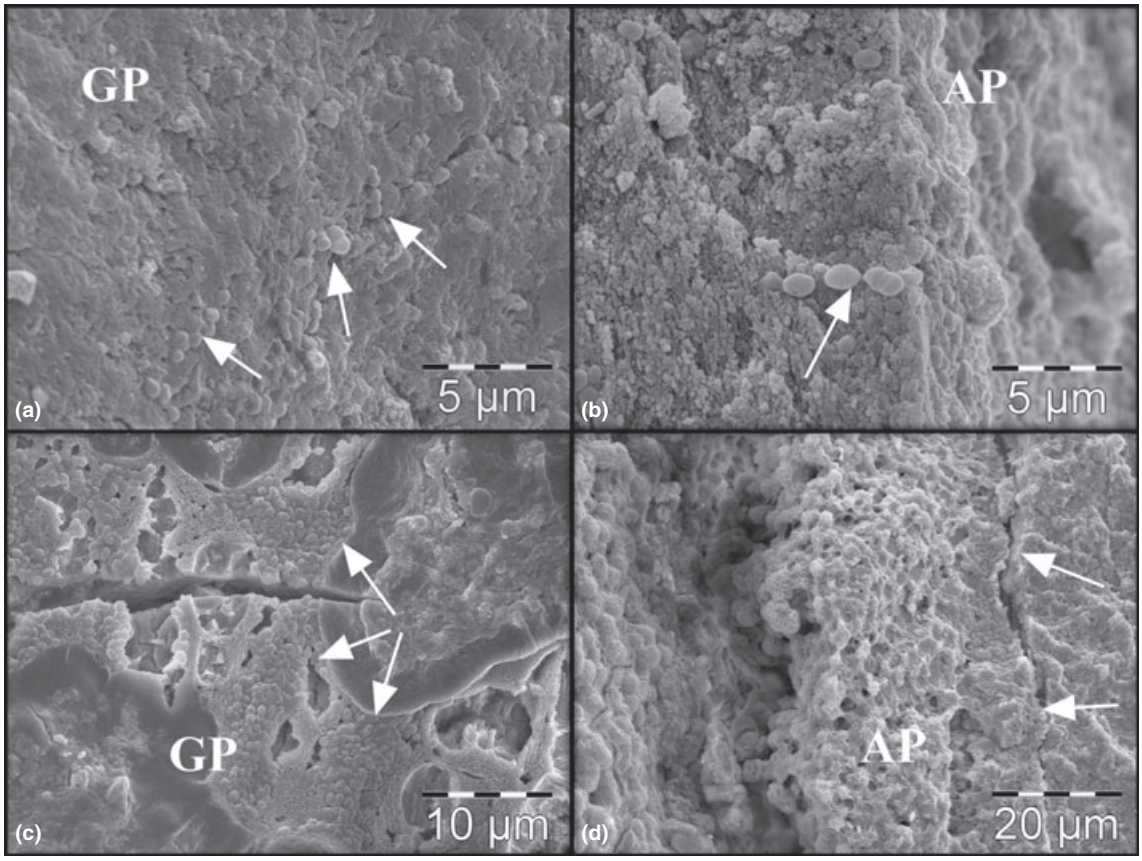


**Figure 5** Scanning electron micrographs of AP specimen. (a, b) Specimen with smear. At the gutta-percha/sealer interface (a) and at the sealer/dentine interface (b), bacteria are observed on the AP surface [arrows in (a)], and on the sealer (AP) remaining on the dentine surface [arrow in (b)]. (c, d) Specimen without smear. At the gutta-percha/sealer interface (c) and at the sealer/dentine interface (d), Bacteria are observed on the sealer remaining on the GP surface [arrows in (c)], and along the interface on the sealer (AP) remaining on the dentine surface [arrows in (d)].

The ratio of the bonded to the unbonded surface area is called the configuration factor or C-factor (de la Macorra & Gomez-Fernandez 1996). It has been suggested that the very high C-factor in root canals is a major obstacle for producing gap-free adhesive fillings (Tay *et al.* 2005). During polymerization, a large unbonded surface of the setting material can move and flow, thereby relieving shrinkage stresses. However, as the unbonded surface area becomes small relative to the bonded area, as in a long narrow root canal, there may be insufficient stress relief by flow and a high probability that one or more bonded areas will pull off or debond. This may be one reason why significant leakage was observed along bonded fillings with RS in this study. The fact that no bacteria could be observed on the RS core may indicate that the core and associated sealer may be impenetrable to bacteria.

The results of the present study are contrary to earlier findings which have indicated that removal of the smear layer reduces leakage of bacteria through the root canal system (Clark-Holke *et al.* 2003, Çobankara *et al.* 2004, Khayat & Jahanbin 2005). However, they are in agreement with those of a recent study (Shemesh *et al.* 2006) that has indicated that the removal of the smear layer did not improve the sealing ability of AH 26 sealer. Direct comparisons among the studies are difficult, as they have employed different methods for the assessment of leakage.

The difference in median times for leakage were in some pairwise comparisons quite large without reaching statistical significance. This probably reflected a substantial variation within some groups, particularly the AP-s specimens (Table 2). The three sealers tested leaked similarly (no statistical significance) in the

presence of the smear layer. Although AP had the weakest bond strength and AH had the highest bond strength in a previous study (Saleh *et al.* 2002), AP performed better in the leakage study, with seven specimens still not leaking at 135 days (median = 105 days). This indicates that increased adhesion does not necessarily equate with improved resistance to bacterial ingress. Other factors, such as antibacterial properties and physical hindrance, may operate.

In the absence of the smear layer, AH leaked significantly more slowly than RS, which is in contrast to the findings of Shipper *et al.* (2004). But Pitout *et al.* (2006) have reported that the bacterial micro-leakage of a root canal sealed with Resilon and Epiphany sealer was similar to that of root canal sealed using GP and Roth root canal cement, and Shemesh *et al.* (2006) found that Resilon and Epiphany leaked significantly more quickly than GP AH fillings in a glucose penetration model.

The results of the present study support the view that retaining the smear layer on the root canal walls may be beneficial in preventing bacterial penetration and colonization (Michelich *et al.* 1980, Drake *et al.* 1994). The smear layer may also act as filler for the sealer, thus reducing the contraction stresses that lead to the pulling out of the sealer tags from the dentinal tubules. This would provide a more intimate contact at the sealer dentine interface. The results also lend credence to speculations (Torabinejad *et al.* 2002, Saleh *et al.* 2002) that it may be advantageous to recreate the smear layer under aseptic conditions prior to root canal filling.

## Conclusions

Removal of the smear layer did not impair bacterial penetration along root canal fillings. In fact, bacterial penetration along root canals filled with GP and AP or with RS system occurred more slowly in the presence of the smear layer. No difference in leakage among the three sealers could be established when a smear layer was present. In its absence, RS fillings leaked more quickly than AH fillings.

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