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A laboratory assessment of coronal bacterial leakage in root canals filled with new and conventional sealers

A. U. Eldeniz[1,2] & D. Ørstavik[3]

[1]Nordic Institute of Dental Materials, Haslum, Norway; [2]School of Dentistry, Selcuk University, Konya, Turkey; and [3]Institute of Clinical Dentistry, University of Oslo, Oslo, Norway

Abstract

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Aim To evaluate the resistance to *ex vivo* bacterial leakage over a 40-day period of root canal fillings with five new root canal sealers: RC Sealer, Epiphany, EndoREZ, GuttaFlow and Acroseal, compared with Apexit, AH Plus and RoekoSeal.

Methodology One hundred and forty-four single rooted human teeth were divided randomly into eight test ($n = 15$) and two control groups ($n = 12$). The root canals were filled using a single cone technique with gutta-percha except in the Epiphany and EndoREZ groups. These were filled with Resilon and resin-coated gutta-percha, respectively. The gutta-percha surface of the GuttaFlow group was coated with an experimental primer prior to filling. Positive controls were filled with gutta-percha without sealer and tested with bacteria, whereas negative controls were sealed with wax to test the seal between the chambers. Filled roots were incorporated in a split chamber model system using *Streptococcus mutans* as a microbial marker. Leakage was assessed for turbidity of the broth in the lower chamber every day for 40 days. Survival analysis was performed using the Kaplan–Meier product limit method and event times were compared using the Log-rank test ($\alpha = 0.05$).

Results Epiphany, GuttaFlow with test primer and Apexit prevented leakage significantly better than AH Plus, RC Sealer, RoekoSeal, EndoREZ and Acroseal ($P < 0.05$). None of the specimens in the AH Plus, RC Sealer, RoekoSeal and EndoREZ groups resisted bacterial penetration for 40 days.

Conclusion The new sealers, Epiphany and GuttaFlow with primer, along with Apexit, showed better resistance to bacterial penetration than the other new or traditional sealers tested.

Keywords: bacterial leakage, methacrylate, new sealers, polycaprolactone, silicone.

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Introduction

The aim of a root filling is to create a bacteria-tight seal, thus minimizing the risks of infection or reinfection of the root canal system (Siqueira *et al.* 1999) and preventing periradicular pathosis. No available material and/or technique produce a complete seal of the entire root canal system. Therefore, root canal filling materials should be developed that possess an improved capacity to prevent bacterial ingress in the long term.

Recently, biodegradable aliphatic polyester incorporated with polycaprolactone (Jia & Alpert 2003) has been introduced as a root canal filling material which performs like gutta-percha. It contains dimethacrylate resins (Jia & Alpert 2003), and it can couple to a variety of dentine adhesives and resin-type sealers, including Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA), RealSeal (SybronEndo, Orange, CA, USA) and Next (Heraeus-Kulzer, Armonk, NY,

Correspondence: Ayce Unverdi Eldeniz, Faculty of Dentistry, Department of Endodontics, Selcuk University, Konya, Turkey (Tel.: 90 332 223 12 57; fax: 90 332 241 00 62; e-mail: ayce71@hotmail.com; aunverdi@selcuk.edu.tr).

USA). Another resin-based root canal sealing material is EndoREZ (Ultradent, South Jordan, Utah, USA), which is a hydrophilic, urethane-dimethacrylate-based resin sealer. It is recommended for use with the same company's resin-coated gutta-percha points for bonding between sealer and point.

The methyl methacrylate/tributylborane (MMA/TBB) resin-based RC Sealer (Test sealer-Sun Medical, Moriyama, Shiga, Japan) is modified from resin cements C & B Metabond (Parkell, Farmingdale, NY, USA) and Super Bond C & B (Sun Medical, Moriyama, Shiga, Japan) by Imai & Komabayashi (2003). The major problems with the original formulations, including a short working time, low radiopacity and difficulty in the removal of the resin from the root canal have been solved to some extent by substituting the polymer component with poly (methyl-methacrylate) (PMMA) (Imai & Komabayashi 2003). This material also contains partially oxidized tri-n-butyl borane as a catalyst and 4-methacryloxyethyl trimellitate anhydride/methyl methacrylate (4-META/MMA).

Many endodontic sealers containing calcium hydroxide are available. These sealers present leakage values comparable to other types of sealers commonly used in endodontics (Chailertvanitkul *et al.* 1996, 1997, Haikel *et al.* 1999, Xu *et al.* 2005). The advantages of the presence of calcium hydroxide in the composition of this type of sealers have been shown by many investigators (Sonat *et al.* 1990, Eldeniz *et al.* 2007a). Acroseal is a new epoxy-based sealer with calcium hydroxide.

Promising clinical and laboratory data have been reported for the silicone-based sealer RoekoSeal (Wu *et al.* 2002, Huumonen *et al.* 2003, Al-Awadhi *et al.* 2004). Another silicone-based material, GuttaFlow, has been developed; this sealer also contains nanosilver and gutta-percha particles. The manufacturer claims a better seal and good adaptability because of the increased flowability of GuttaFlow and because of the slight expansion of this material on setting (ElAyouti *et al.* 2005).

A wide variety of test methods have been used to assess the seal of endodontic materials (Wu & Wesselink 1993), including: methylene blue (Dummer *et al.* 1993, Roggendorf *et al.* 2007), india ink (Baumgardner *et al.* 1995), fluid filtration (Wu *et al.* 1995, Brackett *et al.* 2006, Stratton *et al.* 2006), radioisotopes (Rhome *et al.* 1981), electrochemical circuits (Jacobson & von Fraunhofer 1976), saliva (Torabinejad *et al.* 1995), lipopolysaccharide (Bouillaguet *et al.* 2004), endotoxin (Trope *et al.* 1995, Alves *et al.* 1998, Carratù *et al.* 2002) and bacteria (Torabinejad *et al.* 1990, Timpawat *et al.* 2001, Carratù *et al.* 2002). Diffusion of dyes or other media into an obturated canal space when a tooth is suspended or submerged is still a problematic issue (Trope *et al.* 1995). Thus, due to inadequacies associated with these types of testing methods and as a result of the nonexistence of a universally accepted model, bacterial leakage studies might be meaningful and clinically relevant.

Therefore, the purpose of this *ex vivo* study was to assess the penetration of *S. mutans* through coronally unsealed, filled root canals and the effectiveness of five new sealers (Epiphany, EndoREZ, RC Sealer, Acroseal and GuttaFlow) to resist bacterial leakage compared with conventional sealers (AH Plus, Apexit and RoekoSeal).

Materials and methods

A total of 144 single-rooted human teeth with fully developed apices were used. Data about age, gender or reason for extraction were not available. The teeth were stored in 1% NaOCl solution until use. Bone, calculus or soft tissues on the roots were removed with scalpel blades, with care not to damage the root surface. Before the experiment, the teeth were rinsed thoroughly under running tap water for 20 h.

The crowns of all specimens were removed with a diamond saw (Accutom, Struers, Copenhagen, Denmark) with water coolant and the coronal surfaces of the roots were sectioned perpendicular to the long axis of the root. Three milimetres of the root apices were similarly removed. In an attempt to standardize the length of canal involved in each experimental group, the length of all roots was measured from the coronal surface to the cut apex, and root specimens ranging 11–16 mm were distributed equally to the groups. Apical patency was ensured throughout instrumentation. Each root canal was coronally enlarged with Largo Peeso Reamers (Dentsply Maillefer, Ballaigues, Switzerland) to ISO size 090 or 110. Due to the presence of equally distributed oval shaped root canals in experimental groups (20% oval-shaped canals), further preparation with ISO size 110 Largo Peeso Reamer was completed in order to obtain a round root canal shape. Hand K-files (to ISO size 090 or 110) were also used to finish the enlargement and achieve better adaptation of core materials to the root segments except the EndoREZ group (see below). A total of 10 mL of distilled water, applied with a syringe and a 26 G needle (Terumo Europe N.V., Leuven, Belgium) was

used for irrigation. Organic and inorganic debris including the smear layer were removed by treatment in an ultrasonic bath (Finn Sonic m03/m, Lahti, Finland) in 5% NaOCl followed by 17% EDTA, for 3 min each, and the specimens were rinsed with distilled water for 3 min to remove remnants of these solutions and autoclaved in vials containing distilled water for 20 min at 121 °C. The root canals were dried with sterile paper points (Roeko, Colténe/Whaledent, Langenau, Germany) before filling.

The experimental groups had 15 and the control groups had 12 root segments, respectively.

Group 1: The roots were filled with gutta-percha and AH Plus sealer (De Trey/Dentsply, Konstanz, Germany) using a single cone technique. AH Plus sealer was applied to the canal with a lentulo spiral filler (Dentsply, Maillefer) and an approriate size gutta-percha cone was coated with AH Plus sealer and placed into the root canal with tweezer until fully seated.

Group 2: A single cone of Resilon with Epiphany sealer (Pentron, Wallingford, CT, USA) was used. Epiphany primer was applied to the root canals for 20 s, the excess was removed with sterile paper points, and the root canals were filled as in Group 1 using Epiphany sealer and an appropriate size of Resilon point (ISO 090 or 110).

Group 3: EndoREZ sealer (Ultradent, South Jordan, Utah, USA) and the manufacturer's special type of resin-coated gutta-percha, 0.06 tapered with a size of 35 EndoREZ points (0.06 Taper Assorted, Lot no. 4226C-A, Ultradent Products Inc.,Köln-Porz, Germany) were used to fill this group. Because of the lack of ISO 090 and 110 sizes of this special type gutta-percha, Profile 0.06 instruments (Profile, Dentsply Maillefer, Ballaigues, Switzerland) were used in the enlargement of these root canals to ISO size 30 with a crown-down technique to achieve adequate adaptation of coated tapered gutta percha to the dentine walls. The teeth were otherwise treated as in Group 1.

Group 4: RC sealer (Sun Medical, Moriyama, Shiga, Japan) in combination with conventional gutta-percha points was used. The Green activator from the sealer kit was applied to the root canal walls with paper points for 10 s, and the root canals were rinsed with sterile distilled water and dried. Five drops of monomer were dispensed into the chilled dispensing dish and one drop of catalyst S was added to the dispensed monomer to activate the liquid. Then one small cup of the polymer powder from the RC Sealer kit was added to the activated liquid and stirred lightly for 5–10 s to form a slurry, which was applied to the root canals as in Group 1.

Group 5: These roots were filled with gutta-percha and Apexit sealer (Ivoclar-Vivadent, Liechtenstein) using a single cone technique similar to Group 1.

Group 6: These roots were filled with gutta-percha and Acroseal sealer (Septodont, Saint-Maur-des-Fossés, Cedex, France) using a single cone technique similar to Group 1.

Group 7: These roots were filled with gutta-percha and RoekoSeal sealer (Coltène/Whaledent, Langenau, Germany) using a single cone technique similar to Group 1.

Group 8: The root segments in this group were filled with GuttaFlow sealer (Coltène/Whaledent, Langenau, Germany), applying a test primer (Guttapercha Primer H, Lot S17848-104, Coltène/Whaledent, Langenau, Germany) provided by the manufacturer for better adhesion of GuttaFlow to gutta-percha. GuttaFlow sealer was mixed according to the manufacturer's instruction and applied to the canal with a lentulo spiral filler. After applying primer to the gutta-percha surface, the gutta-percha cone was placed into the root canal.

Group 9: The teeth in this positive control group were filled with a single cone of gutta-percha but without any sealer.

Group 10: The surfaces of the roots as well as the canal orifices coronally in this negative control group were completely covered by sticky wax. The canals were filled with a single cone of gutta-percha without any sealer.

During all procedures throughout the experiment the teeth were kept moist by holding the roots in gauze moistened with sterile distilled water. Excess core material and sealer, coronally and apically, was carefully removed with sterile scalpels, and the root surfaces were wiped with gauze and ethanol to remove excess sealer. All groups were stored in an incubator and allowed to set for 14 days at 37 °C and 100% humidity.

A modification of the microbial leakage model consisting of an upper chamber and a lower chamber as described by Torabinejad *et al.* (1990) was used. The upper chamber consisted of a Corning 15-mL polycarbonate centrifuge tube (Corning Inc., Corning, NY, USA) with a small hole prepared at the bottom to receive the root-end. The tooth was inserted into the tube and gently pushed through the opening until approximately one-half of it protruded through the tube. The space between the tube and the tooth was then sealed with sticky wax (Sticky Wax, Kerr Corporation, Orange, CA, USA). Approximately 4 mm of root remained in the upper chamber.

Testing with *S. mutans*

Streptococcus mutans, strain ATCC 10449, was adapted to and maintained on trypticase soy broth (TSB; Oxoid Ltd, Basingstoke, UK) with 2 mg mL^{-1} streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and used as a test organism. The tip of the centrifuge tube with the tooth attached was introduced into and sealed to the neck of a flat-bottomed, 20-mL, transparent scintillation vial. The tip of the root was mounted to reach approximately 2 mm into a reservoir of 10 mL sterile TSB with 2 mg mL^{-1} streptomycin in the lower chamber. To the upper chamber 2 mL of an overnight culture of resistant *S. mutans* in TSB with 2 mg mL^{-1} streptomycin was added (Fig. 1a). Every second day 1.9 mL of broth was removed from the upper chamber and replaced with fresh broth. The centrifuge tube cap was replaced to prevent evaporation and contamination. The mount was stored in an aerobic incubator at 37(±1) °C and any changes in opacity of the broth in the apical chamber checked daily for 40 days. Bacteria penetrating along the root filling were detected by turbidity observed in the lower chamber (Fig. 1b). The time taken for this to occur was recorded as an indicator of complete root canal contamination. When this occurred, the seal was broken, and the nature and purity of the organism growing in the lower chamber confirmed by Gram stain, cultural morphology and streptomycin resistance.

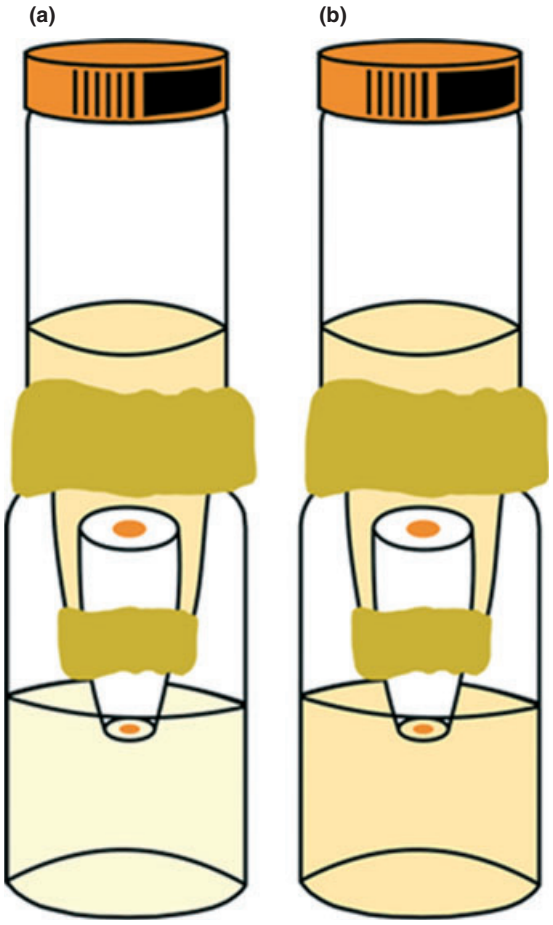

Figure 1 (a) Apparatus set-up demonstrating fresh broth in lower chamber. (b) Evident turbidity of broth in lower chamber after *Streptococcus mutans* penetration through the specimen.

Data analysis

Using the nonparametric Kaplan–Meier analysis, survival curves were constructed illustrating leaking specimens over time, and the median time of leakage in days was estimated for all groups. Specimens that did not leak over the 40 experimental days were computed with an event time of 40 days as censored variables. Bacterial leakage was statistically compared amongst the groups using the log-rank (Mantel Cox) test, with the alpha type error set at 0.05.

Results

All positive control teeth exhibited bacterial leakage rapidly and consistently within 24–48 h, whereas the lower chamber of negative control teeth remained uncontaminated throughout the experiment. All samples which were taken from the bottom chambers after the occurence of turbidity showed the presence of *S. mutans* only. The number of leaking samples and the mean day of leakage per group are presented in Table 1.

The resistance of all the tested sealers to the penetration of the bacteria was better when compared with Group 9 in which no sealer was used ($P < 0.05$). The percentage of specimens without leakage was highest for the Epiphany group (73.33%). Many specimens in the Apexit (66.67%) and GuttaFlow (60%) groups also resisted bacterial penetration up to 40 days. There was no significant difference between the Epiphany, GuttaFlow and Apexit groups ($P > 0.05$). None of the specimens from the AH Plus, RC Sealer, RoekoSeal and EndoREZ groups resisted bacterial penetration for 40 days. Statistically no difference was found between these groups and Acroseal group ($P > 0.05$). Kaplan–Meier survival

Table 1 Number of leaking specimens and the median time of leakage in days

Test Sealers	n	P	p	m	D
AH Plus	15	15/15	100	4	a
Epiphany	15	4/15	26.7	40	b
EndoREZ	15	15/15	100	5	a
RC Sealer	15	15/15	100	2	a
Apexit	15	5/15	33.3	40	b
Acroseal	15	11/15	73.3	10	a
RoekoSeal	15	15/15	100	5	a
GuttaFlow	15	6/15	40	40	b
Positive Control	12	12/12	100	0	
Negative Control	12	0/12	0	40+	

n, number of specimens
P, proportions of leaking specimens
p, percentage of leaking specimens
m, median time of leakage in days
D, Log-rank test ($P < 0.05$): experimental groups with different letter are significantly different from each other.

probabilities for all the test groups are presented in Fig. 2.

Discussion

This study confirmed the findings of other studies that used different techniques for assessment of coronal leakage and demonstrated that leakage occurs after the loss of coronal seal in filled root canals to different extents (Barthel *et al.* 1999, Milétic *et al.* 2002, Monticelli *et al.* 2007). Removal of the smear layer may improve the resistance of filled canals to bacterial challenge from a coronal direction (Behrend *et al.* 1996). This could be because of the relatively weak bond of the smear layer to the underlying dentine, approximately 5 MPa (Taylor *et al.* 1997), which may be insufficient to withstand the shrinkage associated with the curing of resins, and the smear layer may be pulled away from the dentine and provide an avenue for microleakage (Shipper & Trope 2004). The combination of 17% EDTA and 5.25% NaOCl is a generally preferred and effective method in removing the smear layer from the canal walls and dentinal tubules (Oksan *et al.* 1993). NaOCl is a strong oxidizing agent and may cause problems when used as the last irrigant. It leaves behind an oxygen-rich layer on the dentine surface, which results in reduced bond strengths (Lai *et al.* 2001, Erdemir *et al.* 2004) by inhibiting the polymerization of resins (Rueggeberg & Margeson 1990), and increased microleakage (Yiu *et al.* 2002, Stratton *et al.* 2006). Therefore, it has been proposed to use NaOCl first, followed by EDTA for removal of the smear layer after the instrumentation, and then distilled water as a final rinse in order to minimize the compromising effect of NaOCl on primer/resin-sealer polymerization (Lai *et al.* 2001), and to achieve better adhesion of the sealers by permitting penetration of sealers into dentine tubules (Behrend *et al.* 1996, Eldeniz *et al.* 2005), a procedure which was followed in the present study.

Although the validity of laboratory leakage tests has been criticized (Wu *et al.* 1993, Al-Ghamdi & Wennberg 1994), the use of bacteria as markers in an *ex vivo* model was introduced to overcome some of the limitations of dye leakage studies (Torabinejad *et al.*

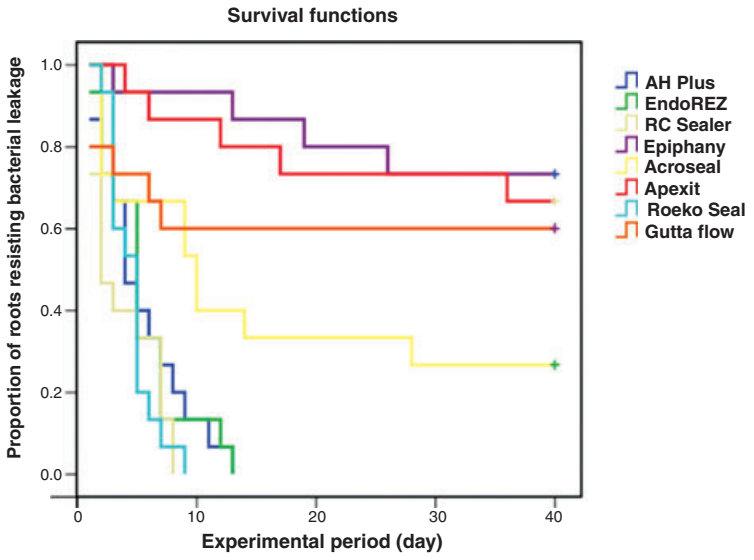

Figure 2 Kaplan–Meier survival curves for all sealer groups.

1990, Michailesco *et al.* 1996, Barrieshi *et al.* 1997, Malone & Donnelly 1997). The model used in this study was patterned after that designed by Torabinejad *et al.* (1990) and has been modified and used by several other researchers for coronal leakage studies (Shipper & Trope 2004, Shipper *et al.* 2004). Streptomycin-resistant bacteria and a medium containing streptomycin sulphate were used to eliminate false-positive results. The apical 3 mm of the roots were removed to eliminate the variations induced by the apical pulp ramifications (Vertucci 1984).

The strain of *S. mutans* used as a bacterial marker in this study is a nonmotile coccus that moves by Brownian movement (Wu *et al.* 1993). It is a facultative anaerobic organism with a size of 0.5–1.2 μm (Behrend *et al.* 1996). It was used because facultative bacteria are predominant in infections of previously treated canals (Molander *et al.* 1998); *Streptoccocus* species are often found in endodontic infections (Sundqvist 1994); they penetrate easily along root canal fillings (Milétic *et al.* 2002), and *S. mutans* is convenient and practical to use for the purpose. The number of microorganisms that caused turbidity in the lower chamber was not measured as the purpose was only to test if *S. mutans* was capable of penetrating through the root filled speciman.

Kaplan–Meier survival analyses were chosen for statistical analyses. They allow visualization of the event-time patterns of all materials under investigation. Event-times were compared using the log rank test. This approach facilitates differentiation between early and late failures during the 40 days of observation period (Zehnder *et al.* 2007).

Most sealers have both antibacterial and cytotoxic effects, and these properties may limit the ingress of bacteria. The materials' physical properties, such as adhesion, adaptability and degradation, may also be important for their resistance to bacterial penetration (Timpawat *et al.* 2001).

This study found that bacterial leakage occurred in a small proportion of specimens in root canals filled with the polycaprolactone-based Resilon and Epiphany sealer after applying dentine primer. This result is in agreement with the results of Shipper *et al.* (2004) and could be attributable to many factors. Pre-treatment of dentine before filling with a self-etching Epiphany primer may prevent shrinkage of the resin sealer away from the dentine wall and the bonding of Resilon to resin type sealers (Tay *et al.* 2005a) may create a 'monoblock' composed of Resilon filling material, resin sealant, bonding agent and dentine (Teixeira *et al.* 2004). As cavity-configuration factors (C-factors: the ratio of the bonded to the unbonded surface area) are extremely high in root canals (Tay *et al.* 2005b), sometimes this primer-containing Epiphany filling technique may not always suffice to form monoblocks within the root canals (Skidmore *et al.* 2006). Epiphany sealer is made to auto-polymerize in 45 min at room temperature in order to improve the chance for the relief of shrinkage stress via resin flow (Tay *et al.* 2005b). As the manufacturer's instructions about light-curing of dual cure Epiphany sealer might cancel out the benefits derived from a sealer that is designed for very slow auto-curing dynamics and in order to not add one more variable amongst the test groups, photo polymerization was not applied to this group. The antibacterial activity of the Epiphany sealer may also contribute to resistance to the bacterial penetration (Eldeniz & Ørstavik 2007c). Some of the Epiphany specimens showed leakage in this group (26.67%) within 40 days, which is more than found previously (Shipper *et al.* 2004). This could be attributable to a higher film thickness and greater polymerization shrinkage as a result of single cone filling technique used in this study: it has previously been demonstrated that when the thickness of the adhesive is increased, the volumetric shrinkage is increased, which results in an increase in shrinkage stress (Tay *et al.* 2005b). Moreover, it has also been shown that Resilon core exhibits extensive surface thinning and weight loss after incubation with bacterial and salivary enzymes (Tay *et al.* 2005c). Biodegradation of Resilon may thus contribute to the leakage of bacteria at the sealer-Resilon interface.

As a result of increasing interest in the use of methacrylate resin-based sealers in endodontics (Eldeniz *et al.* 2005, Sevimay & Kalayci 2005, Tay *et al.* 2005d) in order to obtain chemical union between the polyisoprene component of gutta-percha and methacylate resins, another strategy was introduced by coating conventional gutta-percha cones with resins (Haschke 2004). A special resin is created first and grafted to gutta-percha producing a resin coat that is bondable to a methacylate-based resin sealer (Grubbs *et al.* 2000, Haschke 2004). The resin-coated gutta-percha cone is recommended for use as a single master cone with the EndoREZ sealer. It has been demonstrated that although long resin tags are formed with the thin hybrid layer of dentine, gaps may form along the sealer-dentine interface (Sevimay & Kalayci 2005) as a result of sealer tags being pulled out of the tubules during polymerization shrinkage (Pashley *et al.* 1995,

Bergmans *et al.* 2005) and leakage could not be prevented in canals filled with this technique (Tay *et al.* 2005d). In the present study, even if the inner surface area of the root canals were less when compared with the other experimental groups due to the increased taper of the instruments used during the preparation of these root segments, all the specimens in this group showed bacterial leakage within 13 days. This could be due to polymerization shrinkage of the methacrylate-based sealer (Schwartz & Fransman 2005) following the increased sealer thickness as a result of using a single cone technique (De-Deus *et al.* 2006); the high C-factors of the root canals (Tay *et al.* 2005b); the incomplete removal of the smear layer in isolated areas of the root canal; and also debonding of resin sealer from resin coated gutta-percha due to the lack of an oxygen inhibition layer which is necessary for optimal coupling of methacrylate-based resins (Ruyter 1981). After the application of the coating, this inhibition layer is removed to avoid sticking of the gutta-percha cones during storage (Haschke 2004). This may have resulted in weak bonds between the resin-coated gutta-percha and the resin sealer (Tay *et al.* 2005d). Another reason for the rapid bacterial leakage in this sealer group could be that no or very little antibacterial activity has been demonstrated for EndoREZ (Sipert *et al.* 2005, Eldeniz *et al.* 2006).

Although the RC Sealer has satisfactory physical properties (film thickness, flow, radiopacity) according to the ISO standards 6876–1984 and 2001, it demonstrates higher film thickness values than the other sealers in the present study (Eldeniz & Ørstavik 2005). Previous studies also demonstrated that it is slightly toxic to human gingival fibroblasts (Eldeniz *et al.* 2007b) and it shows similar apical leakage values as AH Plus and Rocanal 2 (Cobankara *et al.* 2006). In the present study, all the specimens filled with this sealer leaked within eight days. Again, this material also has very limited antibacterial activity (Erdemir *et al.* 2003).

The epoxy-resin-based AH Plus sealer was chosen as a reference (Brackett *et al.* 2006). All the specimens filled with AH Plus sealer leaked within 13 days. This could be because of shrinkage of this epoxy type sealer during setting (Ørstavik *et al.* 2001), and/or as a result of diminished antibacterial activity of this sealer some time after setting (Pizzo *et al.* 2006) with no inhibitory effect on *S. mutans* (Kaplan *et al.* 1999). The results are in aggreement with the findings of Yücel *et al.* (2006) and Timpawat *et al.* (2001), who showed that early bacterial penetration and increased penetration after 14 days for AH Plus sealer respectively, but they are in contrast of the findings of other researchers (De-Deus *et al.* 2006) who demonstrated that a greater film thickness did not negatively influence the sealing property and good performance of AH Plus sealer to polymicrobial leakage. The relatively poor bonding between gutta-percha and the sealer (Sevimay & Kalayci 2005) may also contribute to bacterial leakage in the present study.

The high pH of calcium hydroxide-containing sealers is known to inhibit growth of bacteria. Whilst calcium hydroxide released by Apexit may be partly neutralized by other compounds in the formula and thus limit its antibacterial effect (Kaplan *et al.* 1999, Timpawat *et al.* 2001), this sealer demonstrated relatively high resistance to bacterial penetration in the present study. This could be attributable to the highly hydrophobic, zinc stearate content of this sealer that might prevent water ingress and solubility (Eldeniz *et al.* 2007a), and it could be because of toxic substances in the Apexit effective against *S. mutans* (Eldeniz *et al.* 2007b). The recently introduced calcium hydroxide-based Acroseal sealer contains diglycidyl ether of bisphenol A and methenamine, which are known epoxy compounds and also found in the formula of AH 26 and Sealer 26. Most of the specimens in this group leaked within 28 days (73.33%). This could be because of polymerization contraction of the sealer as a consequence of epoxy component, and to the presence of voids in this type of sealer (Mutal & Gani 2005); voids might occur as a result of formaldehyde release during setting and through ionization of calcium hydroxide (Eldeniz *et al.* 2007a).

All RoekoSeal filled specimens showed leakage within 9 days. These results are in contrast with the favourable leakage results reported by previous researchers (Bouillaguet *et al.* 2004, Roggendorf *et al.* 2007). This could be attributable to different leakage evaluation methods and/or tracers used. The present results confirmed the findings reported by Economides *et al.* (2005) that RoekoSeal in combination with gutta percha did not yield better sealing ability than other sealers tested. Whitworth & Baco (2005) suggested that the use of RoekoSeal sealer only as backfills provided leak-proof coronal seals. This may be because the sealer-gutta percha interface is eliminated (Brackett *et al.* 2006). The GuttaFlow sealer, when used together with its test primer, showed comparable leakage values to the Resilon-Epiphany and Apexit sealer groups. The primer may be important for this promising result; also, the moderate expansion of the GuttaFlow sealer (ElAyouti *et al.* 2005, Monticelli *et al.* 2007) may be beneficial.

Conclusion

Within the limitations of this laboratory study Epiphany, GuttaFlow with test primer, and Apexit sealers resisted bacterial penetration for a longer period of time than EndoREZ, RC Sealer, AH Plus, Acroseal and RoekoSeal.

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