

An *in vitro* comparison of adhesive systems to seal pulp chamber walls

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Abstract

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Aim To compare *in vitro* the sealing properties of five different dentine adhesive materials (Prime&Bond NT (PBNT); Prompt L-Pop (PLP); Clearfil SE Bond (CSEB); Scotchbond Multi Purpose Plus (SMPP); EBS-Multi (EBSM)) inside the pulp chamber.

Methodology Seventy-five recently extracted human molar teeth were used. The roof of the pulp chambers and roots were removed under water cooling. Pulp tissue was removed, and the canal orifices were sealed. The pulp chambers were then treated with 5% sodium hypochlorite (NaOCl) for 1 min. The teeth were randomly divided into five groups of 15 teeth each. Adhesive systems were applied to the pulp chamber walls according to the manufacturers' instructions. The samples were connected to Plexiglass plates, and a fluid filtration method was used for quantitative evaluation of leakage. Measurements of fluid movement were made at 2-min intervals for 8 min. The quality of seal of each specimen was measured immediately, after 24 h, 1 week and 1 month. The data were statistically analysed by repeated-measurements multivariate ANOVA, Friedman

test, Wilcoxon signed rank test, Kruskal–Wallis of one-way ANOVA and Mann–Whitney *U*-tests. The pulp chamber wall with and without NaOCl and resin–dentine interfaces of specimens were observed under a scanning electron microscope (SEM).

Results The leakage values of the materials were significantly different at different measurement periods. In all groups, leakage values decreased with time ($P < 0.05$). PBNT and PLP had the least leakage during immediate measurements ($P < 0.05$). After 1 month, leakage of all adhesive systems was not significantly different ($P < 0.05$). SEM observation of pulp chamber walls demonstrated that the irregular dentine surface without smear layer was present in the nontreated group. However, NaOCl application removed the collagen fibrils leaving the dentine surface smooth. At resin–dentine interfaces of specimens, no hybridization zone was observed.

Conclusions None of the materials had created a perfect seal to the pulp chamber walls. PBNT and PLP had better sealing over the short term, but over the long term, there were no differences between the materials.

Keywords: coronal leakage, coronal sealing, fluid filtration method.

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Introduction

When the filled root canal system is exposed to the oral environment, penetration of microorganisms from a coronal direction potentially contributes to failure of the root canal treatment (Swanson & Madison 1987, Madison

& Wilcox 1987). Therefore, the coronal seal is likely to be a factor in the long-term success of root canal treatment (Madison & Wilcox 1987, Swanson & Madison 1987, Saunders & Saunders 1990, Hommez *et al.* 2002). Coronal leakage is particularly important in multirooted teeth, where accessory canals may be present in the furcation area (Vertucci & Anthony 1986). These canals may allow inflammatory changes to occur in the periodontal tissues because of a direct spread of microorganisms from the pulp chamber (Gutmann 1978). Consequently, the primary purpose of sealing access cavities is to prevent contamination of the root canal

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system by fluids, organic material or bacteria from the oral environment.

Swartz *et al.* (1983) found that the failure rate of root filled teeth in cases without an adequate restoration was almost twice as high as that in cases that were restored properly. Several materials have been used within the pulp chamber in an attempt to provide a second line of defence against the leakage of bacteria, when the material used to restore the access preparation fails (Leonard *et al.* 1996, Belli *et al.* 2001).

The structure of the pulp chamber wall is complicated, including predentine, physiological secondary dentine and tertiary dentine. (Berkovitz *et al.* 1992, Bath-Balogh & Fehrenbach 1997). The dentine contains many tubules ($8000\text{--}58\,000\text{ mm}^{-2}$), and the diameter and permeability of the tubules can vary in different parts of the dentine (Mjör & Nordahl 1996). In the absence of cavity preparation, smear layer will not have been created. The tooth structure remaining after endodontic therapy may exhibit various altered physical characteristics (Chow 1983, Nikaido *et al.* 1999, Saleh & Ettman 1999). This is because the application of sodium hypochlorite (NaOCl) provides gross debridement, lubrication, destructions of microbes, dissolution of tissues, removal of the collagen layer and dehydration of the dentine (Grossman & Melman 1941, Gutmann 1992, Vivacqua-Gomes *et al.* 2002). Consequently, the ability to create a high-quality seal against the pulp chamber wall with adhesive is complicated arguably more than that with other dental surfaces.

Derkson *et al.* (1986) described an *in vitro* system to measure the efficacy of sealing the dentine–pulp complex by quantification of dentinal permeability before and after obturation with different materials. This permeability is expressed by measuring the amount of fluid that comes through the area studied per unit time. This method has been used in numerous studies to determine the sealing efficacy of many materials (Derkson *et al.* 1986, Pashley *et al.* 1993, Del Nero & Macorra 1999, Youngson *et al.* 1999, Bouillaquet *et al.* 2000). A common observation in such studies was that the filtration through dentine slowed but did not stop with any of the materials studied. That is, most materials do not perfectly seal immediately, although the seal improves with time in some cases. This experimental system has been adapted for endodontics (Wu & Wesselink 1993). Several investigations have assessed the sealing of the pulp chamber floor with a different restorative materials. However, none of the materials studied was able to reduce leakage completely (Belli *et al.* 2001, Galvan *et al.* 2002, Wells *et al.* 2002).

The purpose of this *in vitro* study was to compare the sealing properties of five different dentine adhesive materials placed in the pulp chambers of human molar teeth. A fluid filtration method was used for quantitative evaluation of leakage in a nondestructive longitudinal study.

Materials and methods

Seventy-five sound molar teeth with fully developed apices were used within 6 months following extraction. The roofs of the pulp chambers were removed using an isomet saw (Buehler Ltd., Lake Bluff, IL, USA), and the roots were removed 1–2 mm below the bifurcation. Pulp tissue was removed carefully with a spoon excavator and endodontic instruments. Canal orifices were widened with Gates–Glidden drills (numbers 2–3, Mani Inc., Tochigiken, Japan). The pulp chambers were treated with 5% NaOCl for 1 min and then rinsed with water for 1 min. Canal orifices were obturated with a gutta-percha master cone (size 35–40, DiaDent, Choongchong Buk Do, Korea) without sealer. The teeth were randomly divided into five groups of 15 teeth each. Adhesive systems and resin composites belonging to the same manufacturers were then applied to the pulp chamber floor and walls according to the manufacturers' directions (Table 1). All groups had an approximately 2-mm-thick layer of restorative material placed onto the pulp chamber floor (Beckham *et al.* 1993, Zaia *et al.* 2002). A probe was used to measure the distance between the pulp chamber floor and the occlusal surface; 2 mm was then subtracted from this measurement, and the restorative material was placed onto the pulp chamber floor to that depth. The probe was used to check the thickness of the materials. The cut surfaces of the pulp chambers were then cemented on to $2\text{ cm} \times 2\text{ cm} \times 0.7\text{ cm}$ pieces of Plexiglass with a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA; Fig. 1).

The pieces of Plexiglass had 18-gauge stainless steel tubes placed through their centres, ending flush with the upper surfaces. The access openings of the tooth segments were then positioned over the tubes to permit a direct communication between the pulp chamber and the micropipette/microsyringe system as shown in Fig. 2. Unsealed gutta-percha cones were removed, and the pulp chambers were filled with water through the 18-gauge needle, taking care to remove all air bubbles that could be seen through the transparent Plexiglass (Belli *et al.* 2001). Empty root canals beneath the sealing materials were also filled with water to maintain hydration of the dentine. During the study, the specimens were

Table 1 Materials, components and bonding procedures used in this study

Materials	Components	Bonding procedures
PBNT	Acid: 36% phosphoric acid Bond: PENTA, UDMA, resin R5-62-1T-resin, D-resin, nanofillers photoinitiators, stabilizers, cetylamine hydrofluoride, acetone	Acid-etching 15 s, rinse 15 s and air dry gently, apply bond 20 s, light cure 10 s, apply TPH kompozit resin (B1) in 2 mm thickness, light cure 40 s
PLP	Di-HEMA-phosphate, water, complex fluoride	Apply 15 s, air dry gently, apply Hytac compomer (B2) in 2 mm thickness, light cure 40 s
SMPP	Acid: 35% phosphoric acid Primer: polyalkenoic acid copolymer, HEMA, water Bond: Bis-GMA, HEMA, photoinitiator	Acid-etching 15 s, rinse and air dry gently, apply primer, air dry 5 s, apply bond, light cure 10 s, apply Filtek Z-250 (B2) in 2 mm thickness, light cure 40 s
CSEB	Primer: MDP, HEMA, Hydrophilic dimethacrylate, <i>N</i> -diethanol <i>p</i> -toluidine, water Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, <i>N</i> -diethanol <i>p</i> -toluidine, silanated colloidal silica	Apply primer 20 s, air dry gently, apply bond, light cure 10 s, apply Clearfil AP-X (B2) in 2 mm thickness, light cure 40 s
EBSM	Acid: 32% phosphoric acid Primer: HEMA, HEMA-salt, water Bond: MAC-10	Acid-etching; apply 20 s, 15 s rinse and air dry gently, apply primer, air dry, apply adhesive resin, light cure 20 s, apply Pertac II (B2) in 2 mm thickness, light cure 40 s

PENTA, dipentaerythritol penta acrylate monophosphate; UDMA, urethane dimethacrylate; HEMA, 2-hydroxyethyl Methacrylate; Bis-GMA, bisphenyl-glycidyl-methacrylate; MDP, 10-methacryloxydecyl-dihydrogen phosphate; and MAC-10, 11-methacryloxy-11-undecadecarboxylic acid.

stored in distilled water at 37 °C for 24 h. After immediate measurements, all the specimens were subjected to 250 thermal cycles of 5 and 55 °C with dwell times of 15 s in each water bath before each measurement.

A fluid filtration method previously described by Belli *et al.* (2001) was used for the quantitative evaluation of leakage. The sealing qualities of the five testing materials

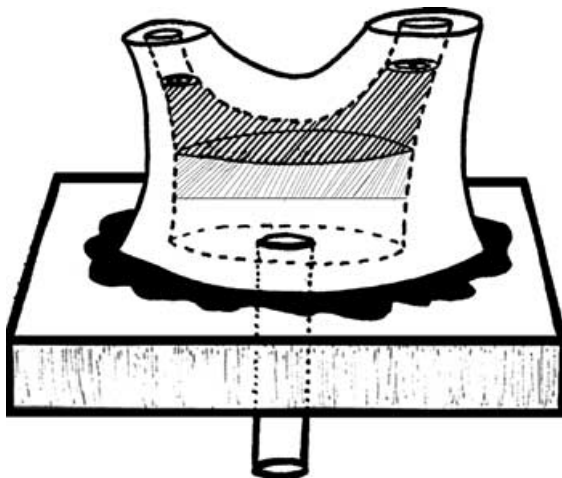


Figure 1 Schematic illustration of the tooth segment created by removal of the upper half of the tooth and removal of the distal half of the roots. After application of the sealing material (cross-hatching), the sealed specimen was inverted onto a piece of Plexiglass and bonded with cyanoacrylate adhesive for fluid filtration measurements.

were quantitated by following the progress of a tiny air bubble traveling within a 25- μ L micropipette (Microcaps, Fisher Scientific, PA, USA). All tubing, pipette and syringe were filled with distilled water under a pressure of 23.4 kPa or 239 cmH₂O (Fig. 2).

Fluid exited from the pressurized reservoir through tubing containing a micropipette connecting to the tooth segment. The movement of a tiny air bubble, controlled by the microsyringe, was proportional to the leakage. Measurements of fluid movement were made at 2-min intervals for 8 min. The quality of the seal of the each specimen was measured immediately, after 1 day, 1 week and 1 month. Fluid flow rate through the 18-gauge needle in the Plexiglass in unsealed specimens was measured by weighing the amount of water that could flow through the needle in 1 min (17.03 g min⁻¹ at 194 H₂O or 80.75 μ L min⁻¹ cmH₂O⁻¹); this value served as both a positive control and 100% leakage, to which the sealed values could be expressed (as a percentage).

For scanning electron microscope (SEM) evaluation, seven extracted human sound, unrestored and noncarious third maxillary molar teeth were used. One tooth was prepared as a control without any treatment. The others were prepared as follows: one tooth, 5% NaOCl for 1 min was applied to the pulp chamber wall and left unrestored; five teeth, 5% NaOCl for 1 min was applied to the pulp chambers and restored according to the manufacturers' directions (Table 1), with adhesive systems and resin composites used in this study. Specimens were cut perpendicular to the long axis of the teeth, using a

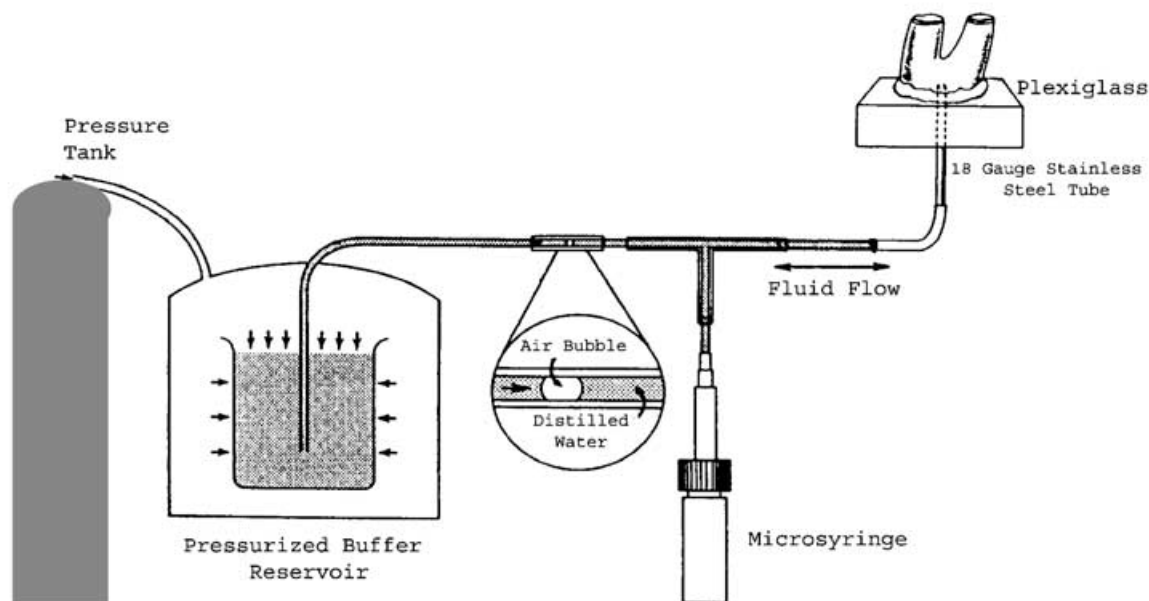


Figure 2 Schematic of the apparatus used to measure fluid flow around the sealed floor of the pulp chamber as a hydraulic conductance. Distilled water was exited from the pressurized reservoir through tubing containing a micropipette to the tooth segment. The movement of the air bubble was measured and controlled by the microsyringe.

low-speed diamond saw under water-cooling. The cut surfaces of specimens were then subjected to 10% phosphoric acid for 10 s and 5% NaOCl for 5 min. All specimens were cleaned in distilled water for 1 min and were dried thoroughly. No acid or NaOCl treatment was applied to the nonrestored teeth. The prepared and unprepared surfaces of the specimens were coated with a thin film of gold in a vacuum evaporator, Polaron Sc500 Sputter Coater (VG Microtech Inc., Tokyo, Japan). The prepared or unprepared pulp walls and resin–dentine interfaces of specimens were observed using an SEM (JSM-5600, JEOL Ltd., Tokyo, Japan) at $\times 550$ to $\times 1500$ magnification.

At the end of the leakage method, the results were calculated as a percentage of Lp (hydraulic conductance)

and analysed statistically using a Repeated Measurements Multivariate ANOVA, Friedman test, Wilcoxon signed rank test, Kruskal–Wallis of one way ANOVA and Mann–Whitney *U*-test.

Results

Repeated measurements multivariate ANOVA tests revealed significant differences amongst the Lp values of adhesive systems ($P < 0.05$).

All bonding systems, except Prime&Bond NT (PBNT) and Prompt L-Pop (PLP), showed statistically different leakage values between different time periods ($P < 0.05$). Leakage values of Clearfil SE Bond (CSEB) did not show any significant difference between the immediate and

Table 2 Mean leakage values (mean \pm SD) of materials and statistical analyzes according to time periods ($n = 15$) (Lp = $\mu\text{L min}^{-1}\text{cm}^{-2}\text{cmH}_2\text{O}^{-1}$)

Time period	Adhesive system				
	PBNT	PLP	CSEB	SMPP	EBSM
Immediate	1.33 \pm 0.78 ^a	1.70 \pm 1.53 ^a	2.95 \pm 0.98 ^a	2.87 \pm 1.66 ^a	3.24 \pm 1.63 ^a
24 h	0.96 \pm 0.70 ^a	0.87 \pm 0.61 ^a	2.33 \pm 1.12 ^{ab}	1.08 \pm 0.60 ^b	2.45 \pm 1.09 ^b
1 week	1.04 \pm 0.69 ^a	1.16 \pm 0.94 ^a	1.74 \pm 1.06 ^b	1.70 \pm 0.76 ^b	1.66 \pm 0.73 ^c
1 month	1.20 \pm 0.60 ^a	1.29 \pm 0.72 ^a	1.50 \pm 0.87 ^b	1.44 \pm 0.73 ^b	1.66 \pm 0.73 ^{bc}
Friedman test: <i>P</i> (significant)	0.209	0.100	0.001	0.001	0.000

Wilcoxon signed rank test statistics: values with the same letter in the same column are not significantly different at $P \leq 0.05$.

Table 3 Statistical analyses and comparisons (mean \pm SD) of adhesive systems in each time period ($n = 15$) ($L_p = \mu\text{L min}^{-1} \text{cm}^{-2} \text{cmH}_2\text{O}^{-1}$)

Adhesive system	Time period			
	Immediate	24 h	1 week	1 month
PBNT	1.33 ± 0.78^a	0.96 ± 0.70^a	1.04 ± 0.69^a	1.20 ± 0.60^a
PLP	1.70 ± 1.53^a	0.87 ± 0.61^a	1.16 ± 0.94^a	1.29 ± 0.72^a
CSEB	2.95 ± 0.98^b	2.33 ± 1.12^a	1.74 ± 1.06^a	1.50 ± 0.87^a
SMPP	2.87 ± 1.66^b	1.08 ± 0.60^b	1.70 ± 0.76^a	1.44 ± 0.73^a
EBSM	3.24 ± 1.63^b	2.45 ± 1.09^b	1.66 ± 0.73^a	1.66 ± 0.73^a
Kruskal–Wallis P (significant)	0.000	0.000	0.169	0.318

Mann–Whitney U -test statistics: values with the same letter in the same column are not significantly different at $P \leq 0.05$.

24-h time periods ($P > 0.05$). CSEB leaked significantly less after 1 week and 1 month than it did immediately ($P < 0.05$). After 24 h, 1 week and 1 month, both Scotch-bond Multi Purpose Plus (SMPP) and EBS-Multi (EBSM) leaked significantly less than they did immediately ($P < 0.05$; Table 2).

PBNT and PLP showed significantly less leakage immediately after restoration placement ($P < 0.05$). But, there were no statistically significant differences among the L_p values of SMPP, CSEB and EBSM ($P > 0.05$). After 24 h, PLP, PBNT and SMPP had significantly less L_p values than CSEB and EBSM ($P < 0.05$). After 1 week and 1 month, leakage values of all adhesive systems did not show any significant difference ($P > 0.05$) (Table 3).

SEM observation of the pulp chamber wall demonstrated that a dentine surface without smear layer was present in the nontreated group (Figs 3 and 4). However, NaOCl application removed the collagen fibrils, leaving the dentine surface smooth. Funnel-shaped, enlarged

dentine tubule orifices were also observed (Figs 5 and 6). In resin–dentine interfaces of specimens, no hybridization zone was seen (Figs 8–11). In one specimen, a zone resembling a hybrid layer was evident with penetration of resin tags (Fig. 7). In the SEM micrographs of self-etching dentine bonding systems, PLP and CSEB (Figs 8 and 10) appeared to have comparatively shorter resin tags than phosphoric acid containing dentine bonding systems, PBNT, SMPP and EBSM (Figs 7, 9 and 11).

Discussion

Bacteria, dyes, radioisotopes, light microscopic methods or SEM methods have been used to measure leakage around restorative materials. Dye methods are still the most frequently used approach to measure the sealing capacity of different restorative materials. The main problems with these methods are that they provide qualitative, rather than quantitative, information. These

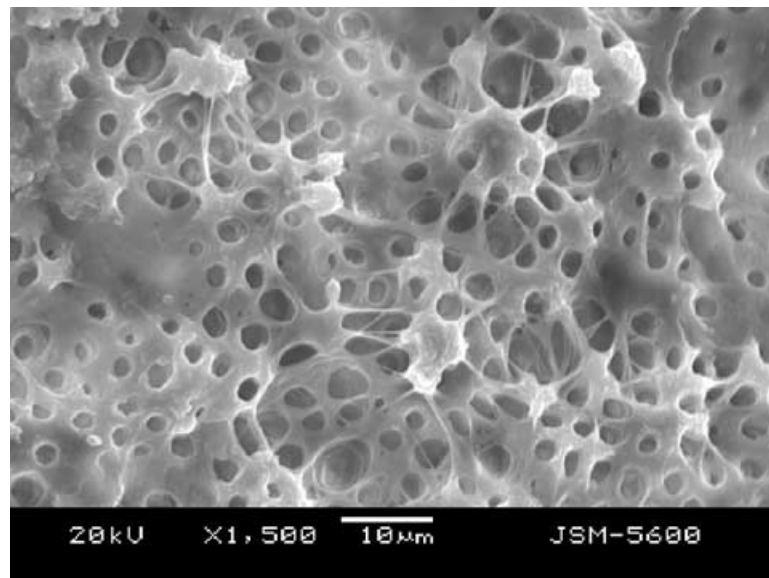


Figure 3 An SEM observation of mesial dentine wall without any smear layer in nontreated tooth.

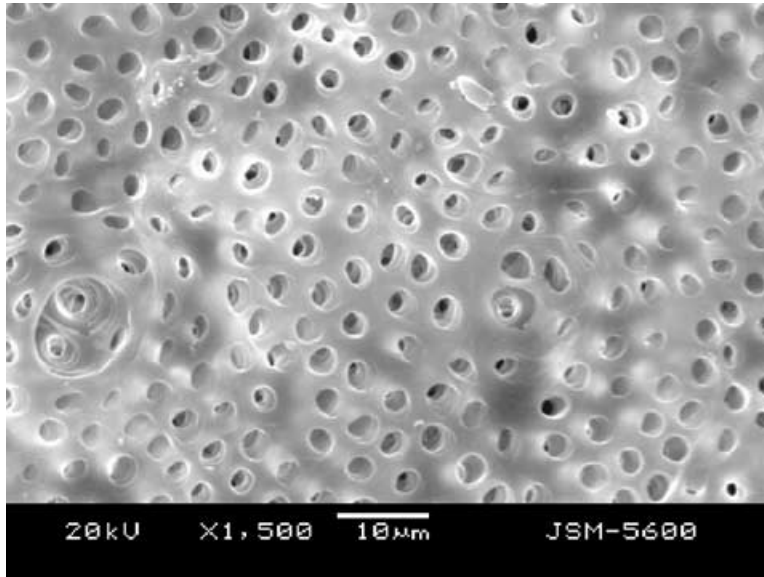


Figure 4 The application of NaOCl may have removed some collagen fibrils leaving the interdentine surface with a smooth appearance. Some funneling of the tubule orifices may also be observed.

methods can indicate the presence or absence of leakage but not the amount. It should be noted that the use of detector dyes in leakage studies may be problematic as a result of the difference in molecular weight between water and dye molecules. An interface may be hermetically sealed to dyes with high molecular weights (c. 200–300; Pashley *et al.* 1985, Pagliarini *et al.* 1996, Del Nero & Macorra 1999), but not to water with a molecular weight of 18. The lack of standardization, high level of variation and noncomparability of data in earlier

studies has led the relevance of leakage studies to be questioned. The use of fluid filtration systems have been recommended to enhance reliability, reproducibility and comparability (Wu & Wesselink 1993). This system, first described by Derkson *et al.* (1986), was designed to evaluate the sealing properties of temporary filling materials by Pashley *et al.* (1988b) and was modified by Wu & Wesselink (1993) for endodontic leakage studies. Wu *et al.* (1994) and Youngson *et al.* (1999) reported that the fluid filtration method was a more sensitive

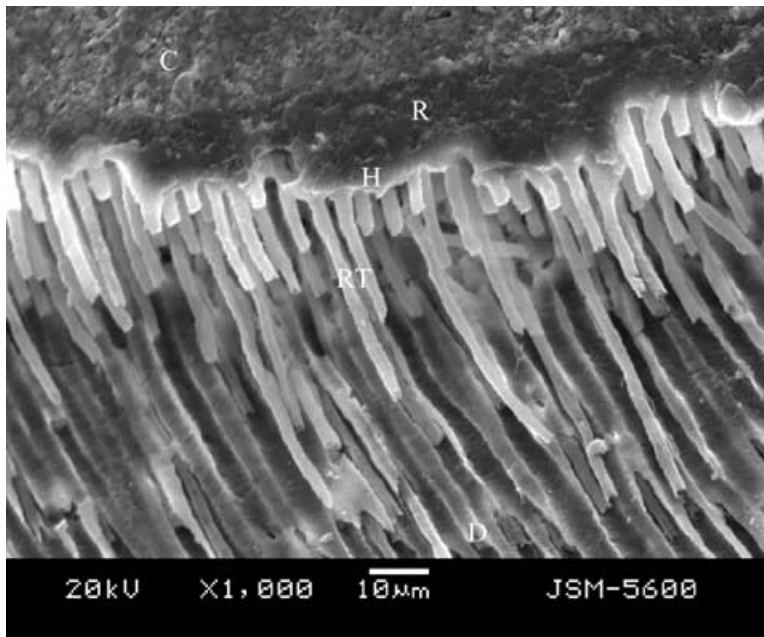
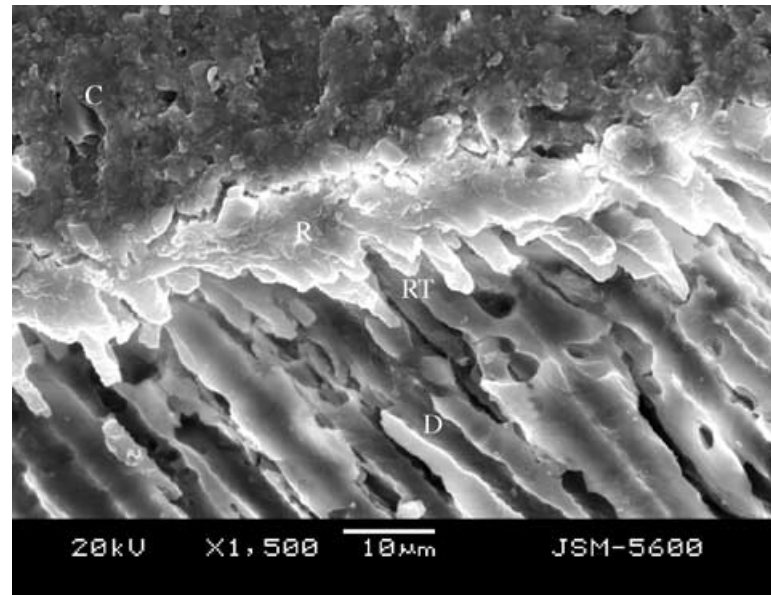


Figure 5 Cross-section of interface between PBNT and dentine substrate treated with 36% H_3PO_4 and NaOCl. A zone resembling hybrid layer is evident with penetration of resin tags. Many resin tags are clearly shown. C, composite; R, resin; H, hybrid layer; RT, resin tag; and D, dentine.

Figure 6 Cross-section of interface between PLP and dentine substrate treated with NaOCl. No hybrid layer is observed. Small resin tags are clearly observed. C, composite; R, resin; RT, resin tag; and D, dentine.

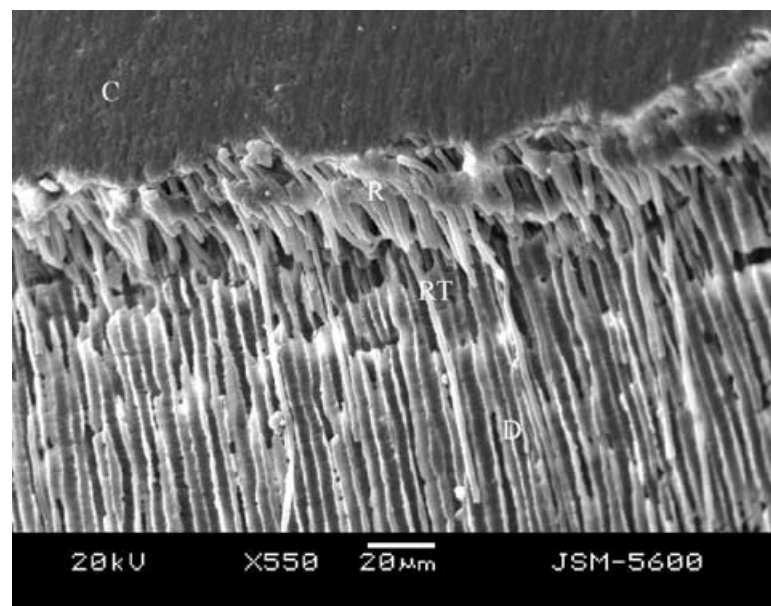


technique than the dye method. The fluid filtration method permits quantitative, nondestructive measurement of leakage in a longitudinal manner by time (Pashley *et al.* 1985; 1988a, Derkson *et al.* 1986). In this study, the change of leakage values with increasing period of time (immediately, 1 day, 1 week and 1 month) showed that longitudinal leakage studies are important in determining leakage values of materials.

Pulp chamber wall dentine is not prepared during endodontic procedure, and does not have a smear layer

(Figs 1 and 2). The sealing effectiveness of adhesive systems depends mostly on the structure of the collagen-rich predentine, and the number and permeability of the dentinal tubules. The application of NaOCl during endodontic therapy may irreversibly alter the physical characteristics of dentine (Chow 1983, Nikaido *et al.* 1999, Saleh & Ettman 1999). In the SEM observations, NaOCl application appeared to create a smooth surface, the intertubular dentine, suggesting some collagen fibrils were removed along with peritubular dentine at

Figure 7 Cross-section of interface between SMPP and dentine substrate treated with 35% H_3PO_4 and NaOCl. No hybrid zone is evident. Long resin tags are indicated by the arrows. C, composite; R, resin; RT, resin tag; and D, dentine.



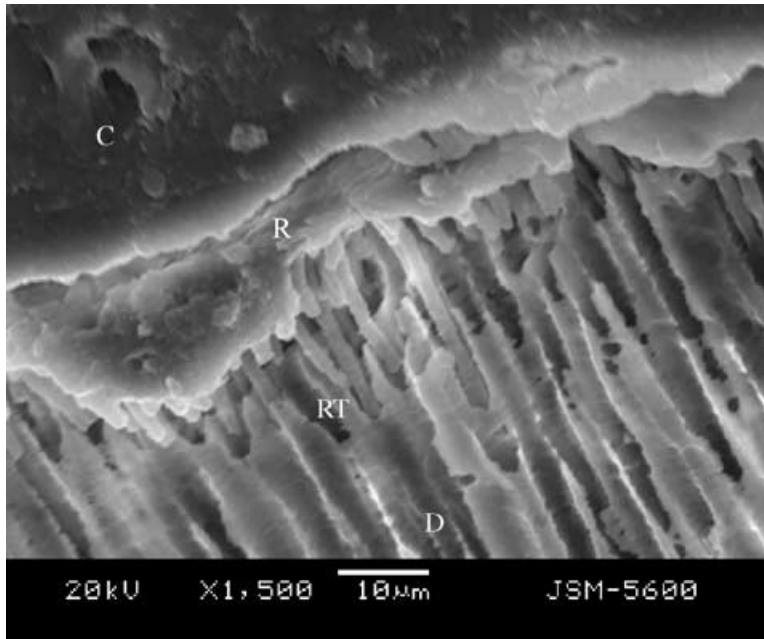


Figure 8 Cross-section of interface between CSEB and dentine substrate treated with NaOCl. No hybrid zone is evident. Penetration of resin tags into the tubules are clearly observed. C, composite; R, resin; RT, resin tag; and D, dentine.

the tubule orifices (Figs 3 and 4). In addition, the box shape of pulp chambers may also influence the sealing ability of the materials because of polymerization shrinkage caused by the C-factor (the ratio of bonded to unbonded surface area). The smaller the C-factor, the less the competition between the strength of the bond and the forces of polymerization contraction. The most unfavourable C-factor is found in box-like cavities

(Carvalho *et al.* 1996). In the example previously described of a box-like cavity, if each wall had the same dimensions, the C-factor would be 5/1 or 5.

In concurrence with previous studies (Bouillaquet *et al.* 2000, Belli *et al.* 2001, Escibano *et al.* 2001), in this study, none of the adhesive systems were capable of completely preventing the penetration of fluid across the bonded interface in every specimen. The leakage of fluid

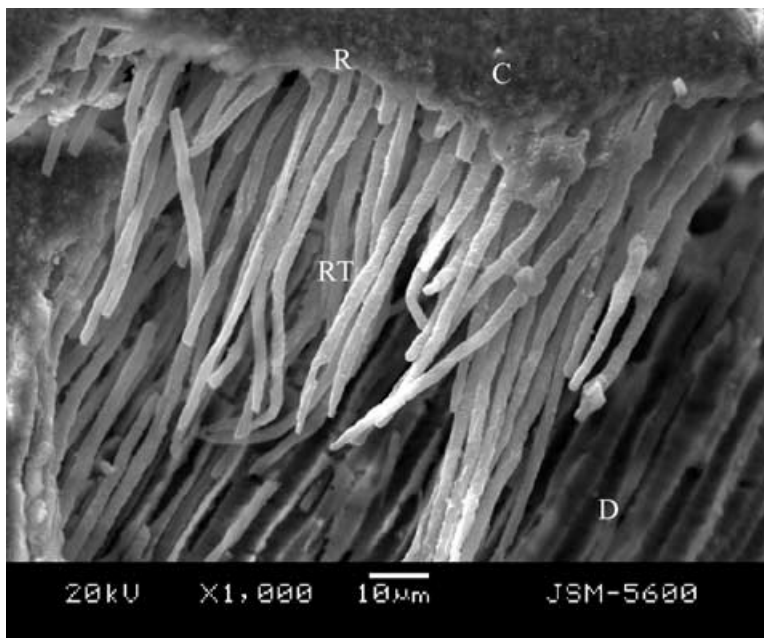


Figure 9 Cross-section of interface between EBSM and dentine substrate treated with 32% H₃PO₄ and NaOCl. No hybrid zone is evident. Many resin tags are clearly shown. C, composite; R, resin; RT, resin tag; and D, dentine.

may be because of the presence of fluid-filled channels around resin tags that create nanometre-sized porosities within the hybrid layer (Bouillaquet *et al.* 2000). Some reports have demonstrated that resin monomers may not penetrate uniformly into the demineralized dentine and may not completely infiltrate the exposed collagen network (Pashley *et al.* 1993, Sano *et al.* 1994). The polymerization contraction of composite resin materials and incomplete sealing of bonding systems to dentine surfaces are most likely responsible for the development of microgaps; the number of gaps depends on the polymerization contraction of composite resin materials (Zivkovic 2000, Al-Ehaideb & Mohammed 2001, Demarco *et al.* 2001). The leakage in the present study can be attributed to the polymerization shrinkage of composite resin materials.

Retief (1994) and Carvalho *et al.* (1996) reported that no dental bonding systems could prevent the development of marginal gaps at the dentine–restoration interface when evaluated 10 min after placement of restorations because of the polymerization shrinkage of the resin materials after curing. However, the hygroscopic expansion resulting from immersion of the materials in water or saline may cause a significant reduction in the dimension of marginal gaps. This may explain why higher leakage values were observed immediately after curing, and the absorption of water by the materials over the following time periods expanded the materials slightly, increasing the sealing integrity.

Conclusion

None of the materials had the ability to seal pulp chamber walls. PBNT and PLP were more successful than the other systems over the short term, but no differences in the sealing of the materials were observed over the long term. The use of these materials may be recommended for the restoration of pulp chambers of endodontically treated teeth. But, further studies of the effectiveness of bonding systems are required.

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