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Sealing Ability of New Adhesive Root Canal Filling Materials Measured by New Dye Penetration Method

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The sealing ability of new adhesive root canal filling materials was evaluated using a new dye penetration method. Twenty-eight single-rooted mandibular premolars were randomly divided into four groups of seven teeth each and filled by lateral condensation using one of these combinations: Resilon point with Epiphany sealer (RE); gutta-percha point with Sealapex sealer (GS); gutta-percha point with dentin activator and Superbond sealer (GDS); or gutta-percha point with Accel primer, dentin activator, and Superbond sealer (GADS). Amount of 0.06% methylene blue dye solution (MB) that leaked from the coronal portion to the apical area was measured with a spectrophotometer at one, four, eight, 15, and 30 days in an accumulative manner. The total amount of leaked MB on day 30 was significantly higher for GDS than the other combinations ($p<0.05$).

Keywords: Leakage, Sealer, Spectrophotometer

INTRODUCTION

Successful root canal treatment is achieved by hermetic obturation of the root canal space after canal cleaning and shaping. A root canal filling must seal the canal walls both apically and laterally to prevent further ingress of microorganisms or tissue fluids from percolating back into the root canal space[1].

The use of a root canal sealer is necessary for the hermetic sealing of root canal space. On this note, Hovland & Dumsha[2] demonstrated that there was significantly greater leakage when the root canal space was filled with a gutta-percha point alone *versus* a gutta-percha point and sealer.

With regard to dentin bonding agents, methyl methacrylate (MMA)-based resins are well known for their strong adhesion to dentin[3-5]. However, MMA-based resins exhibit several problems in terms of short working time[6,7], radioopacity[8], and lack of removability[6] when used for endodontic purposes. Consequentially, Superbond sealer (Sun Medical, Shiga, Japan) was recently developed to overcome these drawbacks. Hemmi *et al.*[9] suggested that Superbond sealer showed better tissue compatibility than zinc oxide-eugenol root canal sealers. Further, Baba *et al.*[10] reported that Superbond sealer was not affected by root canal irrigant during obturation.

With respect to endodontic filling materials, a thermoplastic synthetic polymer-based root canal filling material (Resilon, Resilon Research LLC, Madison, CT, USA) was introduced as an alternative to gutta-percha points[11,12]. It can bond to MMA-based resins since it contains a dimethacrylate resin. It is also capable of chemical coupling to resin sealers, such as Epiphany sealer (Pentron, Wallingford, CT, USA)[11,12], Real Seal sealer (SybronEndo, Orange, CA, USA)[13], and Next sealer (Heraeus Kulzer, Armonk, NY, USA)[14]. Epiphany sealer is a dual-cure resin composite sealer. The resin matrix is composed of Bis-GMA, ethoxylated Bis-GMA, UDMA, and hydrophilic difunctional methacrylates. The adjunctive use of self-etching adhesives and Epiphany sealer purportedly creates a 'Monoblock' between intraradicular dentin and root-filling material, which is said to be more resistant to both bacterial leakage[11] and root fracture[15] when compared with similar teeth root-filled with gutta-percha points and conventional sealers. In addition, Epiphany sealer shows good permeability[11,16] as well as high radioopacity[11], biocompatibility[11,17], and removability[11]. However, Gesi *et al.*[18] suggested that the interfacial strength achieved with a Resilon point and Epiphany sealer to intraradicular dentin was not superior to that of a gutta-percha point and conventional sealer. A possible explanation to these differing and contradicting dentin bonding results could be found in a report by Tay *et al.*[19], which showed that a cavity configuration factor existed in long narrow root canals, and which affected dentin bonding. Furthermore, according to Tay *et al.*[17], Resilon points were susceptible to both alkaline and enzymatic hydrolyses.

Various test methods have been developed to evaluate the sealing ability of filling materials, including dye penetration[20-23], radioactive isotopes[24,25], bacterial or bacterial metabolite leakage[16,26,27], and fluid filtration[28-30]. Although dye penetration methods are simple and easy, they require sample destruction during processing and do not permit evaluation of sealing ability over time. Thus, we developed a new non-destructive method using methylene blue dye solution and leveraging its absorbance to evaluate coronal leakage over time[31].

With the abovementioned, newly developed method to evaluate coronal leakage[31], the purpose of the present study was to examine the sealing ability of new adhesive root canal filling materials. The materials to be investigated were combinations of a Resilon point with Epiphany sealer or a gutta-percha point with Superbond sealer.

MATERIALS AND METHODS

Tooth specimens

A total of 32 human mandibular premolars with a single root canal were used in this study. The criteria for tooth selection were: straight, single-rooted teeth; complete root formation; no root caries; no fracture line; and a patent canal. The teeth were stored in distilled water after extraction. After removing the crowns with a low-speed diamond cutting machine (ISOMET, Buehler, Lake Bluff, IL, USA) under a water coolant, the root lengths were standardized to 12 mm. Following which, all soft tissue and calculus was removed from the teeth with a periodontal scaler.

The roots of two teeth were used as negative controls, with no root canal preparation. With the roots of the remaining 30 teeth, the working length was determined by passing a #15 K-file (Zipperer, München, Germany) into the canal until the tip of the file was just visible through the apical foramen. The canal was then instrumented with #15 to #40 K-files using a standardized technique, and irrigated with 2 ml of 6% NaOCl at each file change. Finally, the canal was prepared using rotary Ni-Ti ProFile® with a 0.04 taper (Tulsa Dental Products, Tulsa, OK, USA), until a working length was achieved with a master apical rotary file #7 (tip diameter: 0.465 mm). After root canal instrumentation, a #15 K-file was passed 1 mm beyond the apex to remove any dentinal plugs and ensure the patency of the foramen for dye penetration test. Then, the canal was immediately flushed with 2.5 ml of 15% EDTA (Showa Yakuhin, Tokyo, Japan) and 2 ml of 6% NaOCl. After irrigation with 3 ml of distilled water, the canal was dried with an air syringe and paper points (GC, Tokyo, Japan).

The roots of two prepared teeth were used as positive controls. The remaining 28 teeth were randomly divided into four groups (Groups A – D) of seven teeth each. All the roots, except for the controls, were filled using the lateral condensation technique. A master point was selected to fit the working length with tug-back for each canal. The selected master point was coated with sealer and gently seated at the working length. Each sealer was mixed according to the manufacturer's instructions. A spreader (NTD11T, Brasseler, Lemgo, Germany) was introduced to within 2 mm of the working length. Accessory points coated with sealer were laterally condensed until they could not be introduced more than 3 mm into the root canal. Excess points protruding from the coronal portion were cut off using a heated instrument. All the specimens were stored at room temperature for 24 hours after root canal filling.

1) Group A

A Resilon point was used for both the master and accessory points with Epiphany sealer. A self-etching primer (Epiphany primer, Pentron) was applied inside the canal with sponge pellets and gently dried with paper points. After root canal filling, the coronal surface was light-cured for 40 seconds using a standard light curing unit (DC Blue LEX, Yoshida, Tokyo, Japan) to ensure setting.

2) Group B

A gutta-percha point (GC) for the master point and fine-sized accessory points (GC) were applied with Sealapex sealer (SybronEndo) and treated by the lateral condensation method.

3) Group C

The same brand of gutta-percha point was used as described for Group B. According to the manufacturer's instructions, canal dentin was etched with a 10:3 citric acid-ferric chloride dentin activator (Sun Medical) by placing 1.0 ml of the solution into the canal with a small pipette for 30 seconds. The canal was then rinsed with distilled water, and dried with paper points and an air syringe. Superbond sealer was mixed according to the manufacturer's instructions at this blend ratio: monomer liquid/catalyst/powder = 0.112 ml/0.007 ml/130 mg.

4) Group D

Root canal space was treated with Accel primer (Sun Medical) using a pipette for 10 seconds, and then dried with paper points and an air syringe. The remaining procedure for root canal filling was the same as that described for Group C.

5) Control groups

For the positive controls, only root canal preparation without root canal filling was carried out. For the negative controls, no root canal preparation was performed, and the cut surface and orifice of the root were coated with paraffin wax.

Microleakage test

Figure 1 shows the device constructed for microleakage testing. A polyethylene tube with a tip diameter of 10 mm was attached to the coronal portion of each root using Superbond C&B (Sun Medical). A 0.2 ml aliquot of 0.06% methylene blue dye solution (Muto, Tokyo, Japan) was poured into the polyethylene tube. Height of the methylene blue dye solution in the tube was approximately 5 mm.

The assembly was then placed in a glass bottle with a rubber stopper that was not sealed, but which prevented the evaporation of 3 ml of distilled water in the glass bottle. Next, 2 mm of the root apex was immersed in the distilled water in the glass bottle.

The model was then maintained in an incubator that provided 100% humidity at 37℃ for the duration of the observation period. Water in the glass bottle was removed for each measurement and replaced with fresh distilled water. The amount of methylene blue in each water sample was measured by an absorbance at 630 nm using an Immuno-Mini NJ 2300 spectrophotometer (Nalge Nunc International, Rochester, NY, USA) at one, four, eight, 15, and 30 days. Distilled water was used as the standard solution.

Standard curve

To determine the concentration of each sample, a standard curve drawn on our previous report[31] was used. This was accomplished by creating 15 serial dilutions of 1% methylene blue solution and plotting the calculated absorbances *versus* the known concentrations. A coefficient of correlation (r^2) and a regression curve equation were then obtained as follows:

$$10^4 \cdot Y = X^3 - X^2 + 13 \cdot X + 0.4, \; r^2 = 0.99$$

where X is the absorbance and Y is the dye concentration. The concentration of each sample was extrapolated using this curve.

Statistical analysis

Amount of eluted dye was estimated by substituting the concentration of each sample into the above equation, and accumulated over all the time intervals. Amounts of methylene blue dye leakage and experimental time intervals were compared between the groups and analyzed statistically using two-way ANOVA. Results of day 30 were compared among all the groups using Fisher's PLSD test. Significance level was set at p=0.05.

RESULTS

Figure 2 shows the mean amounts and standard deviations of diluted dye in all the groups at the stipulated experimental time intervals. For all the experimental groups, leakage amount tended to increase and reach a plateau within days. In particular for the positive control group, methylene blue dye leakage increased rapidly from day 1 and reached a plateau. Moreover, its value was significantly higher than those of the other groups ($p<0.05$). As for the negative control group, no methylene blue dye was detected throughout the experiment.

As shown in Fig. 2, there were significant differences among the groups at each experimental time interval ($p<0.05$, two-way ANOVA). Statistical analysis revealed that the amount of leaked dye on day 30 was significantly higher for gutta-percha point with 10:3 citric acid-ferric chloride dentin activator and Superbond sealer (Group C) than for the other groups ($p<0.05$, Fisher's PLSD) (Fig. 3).

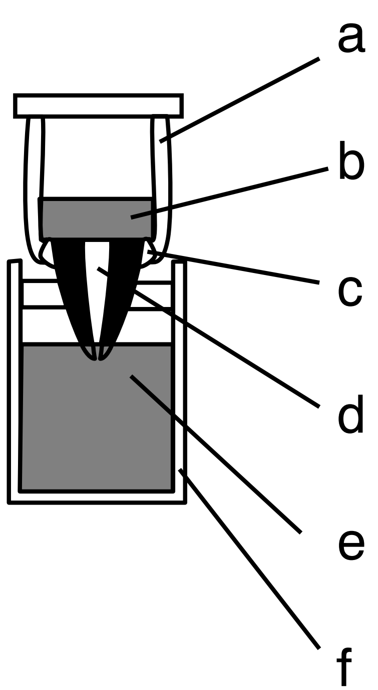

Fig. 1 Device for microleakage test: (a) polyethylene tube; (b) 0.2 ml of 0.06% methylene blue dye solution; (c) Superbond C&B; (d) root canal filling material; (e) distilled water; (f) glass bottle.

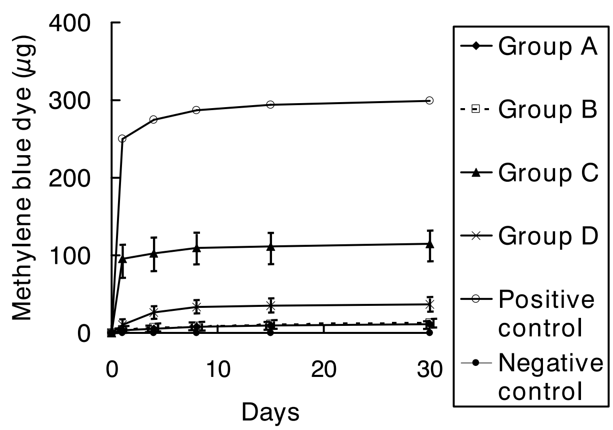

Fig. 2 Amounts of leaked methylene blue dye over time in the course of experiment according to the different types of root canal filling materials.

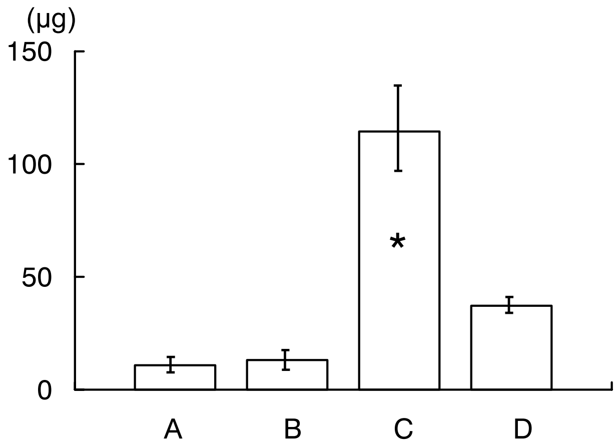

Fig. 3 Amounts of leaked methylene blue dye on day 30 (*: $p<0.05$, one-way ANOVA).

DISCUSSION

Conventional dye penetration studies are non-quantitative, and they typically involve destroying the specimens during evaluation. Consequentially, Derkson *et al.*[28] developed a fluid filtration system to measure the sealing efficacy of dentine-pulp complex by quantifying dentine permeability before and after filling a cavity with different materials. This method has since been popular for determining the sealing ability of endodontic filling materials[28-30]. However, Pommel & Camps[32] pointed out that while both pressure and measurement time are both very important factors that affect microleakage results, there was no standardization of materials and methods regarding pressure and measurement time range in the fluid filtration method. In addition, Camps & Pashley[23] commented that the fluid filtration system required more time for experimentation because the filtration values tended to decrease over time before reaching a plateau.

We selected an improved passive dye penetration method using a methylene blue dye solution and its absorbance to evaluate coronal leakage easily and longitudinally. Quantitative analysis of coronal leakage is possible by determining the concentration of methylene blue dye solution released into water in a glass bottle continuously over time. Some previous studies[33,34] have shown that dye diffusion was negatively affected by entrapped air, resulting in failure to penetrate the full extent of the void unless a vacuum was applied. However, other studies[35-37] have indicated that there was little or no difference in the amount of dye penetration regardless of whether or not a vacuum was applied before dye application. The clinical relevance of placing teeth in a vacuum may be questioned, since no such vacuum exists in the human body. Having taken into account these various considerations, a passive dye penetration method was eventually adopted for this study. Pressure of the methylene blue solution exuded from the tube on the coronal surface was approximately 0.02 N/cm^2, which was negligible compared to atmospheric pressure (9.81 N/cm^2). As such, this pressure was considered not to have any effect on our results.

With the methylene blue dye method, we previously evaluated the sealing permeability of various types of posts over a time period of 30 days[31]. The results revealed significant differences between post types and experimental time intervals, whereby a core build-up with glass ionomer cement showed more coronal leakage than resin cement over the observation period.

In the present study, we hypothesized that Groups A, C, and D would show less leakage than Group B due to the adhesive ability of the compounds. However, Group C with Superbond sealer showed significantly lower sealing ability than the other three groups. Superbond sealer consisted of a 4-META/MMA-TBB resin—and MMA-based resins are adversely affected by root canal irrigants such as NaOCl, root canal dressings, and root-filling materials[38,39]. To weaken the influence of any residual NaOCl on the root canal surface, reducing agents—such as ascorbic acid, sodium thiosulfate, and citric acid—have been used[38,40]. As for Group D, it involved the use of Accel as a primer after final root canal irrigation. Accel consisted of an aromatic sulfonate, water, and ethanol. It should be noted that aromatic sulfonate has similar effect as a NaOCl-reducing agent. As a result, the sealing ability of Group D was significantly higher than that of Group C. These findings thus indicated that the use of a primer to weaken the NaOCl influence might be necessary when 4-META/MMA-TBB resin-based sealers are used.

Epiphany sealer was marketed with an Epiphany primer. The Epiphany primer was a self-etching primer that contained a sulfonic acid-terminated functional monomer, HEMA, water, and a polymerization initiator. Sulfonic acid would thus weaken the influence of root canal irrigant. Furthermore, dentin preparation with the primer might have prevented shrinkage of the resin filling away from the dentin wall and aided in sealing the roots filled with the Resilon "Monoblock" System. This could be one key reason to account for the significant difference between Groups A and C.

Gaps between gutta-percha points and sealers, as well as between root canal walls and sealers, would be two main routes for coronal leakage. However, the results revealed no significant differences between Groups A and B. According to Tay *et al.*[41], the quality of an apical seal achieved with a Resilon point and Epiphany sealer was not superior to that obtained with a gutta-percha point and conventional sealer due to polymerization contraction that occurred between the root canal filling material and sealer. Furthermore, periapical dentinal tubules probably also had an influence on coronal leakage. The present findings thus suggested that both conventional and adhesive sealers could achieve equivalent results in sealing root canals. Nonetheless, more research into reducing leakage in roots is required.

CONCLUSIONS

Results of the present study revealed that coronal leakage increased over time for all the examined sealers, and that no root canal filling material was able to achieve a complete seal. It was also found that the sealing ability of a Resilon point with Epiphany sealer was significantly better than that of a gutta-percha point with 10:3 citric acid-ferric chloride dentin activator and Superbond sealer.

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