ORIGINAL RESEARCH

Influence of pH Change and Water Storage on the Sealing Ability of Two Resin-based Root-filling Materials

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ABSTRACT

Aim: To evaluate the influence of pH change and water storage up to 90 days on the sealing ability of two resin-based root-filling materials.

Materials and methods: Forty-four human mandibular singlerooted teeth were instrumented and filled with gutta-percha/ AH Plus or Resilon/Epiphany SE (n = 20 per group). Two teeth each were used as positive and negative controls. Specimens were set for 7 days under 100% humidity at 37°C. They were allocated into two subgroups (n = 10) according to whether they were tested immediately or stored for up to 90 days in water before testing. Sealing ability was evaluated by passive dye penetration. Absorbance at 630 nm (in µg/ml) was measured by spectrophotometry. The pH values were obtained in triplicate. Data were submitted to ANOVA by post-hoc Tukey's test (α = 0.05).

Results: Specimens filled with Resilon/Epiphany SE exhibited more leakage than specimens filled with gutta-percha/AH Plus at the immediate time point (p < 0.001). No differences were detected between the groups after storage, or between the materials with pH changes after 30, 60 and 90 days (p > 0.05).

Conclusion: Gutta-percha/AH Plus provided superior sealing at the immediate time point. Water storage and pH changes did not influence the sealing ability of tested materials.

Clinical significance: These results suggest that Resilon/ Epiphany SE sealer offered no apparent advantage over the more conventional gutta-percha/AH Plus sealer technique in terms of sealing ability.

Keywords: Endodontics, Gutta-percha, Hydrogen-ion concentration, Water storage.

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INTRODUCTION

The main purposes of root canal therapy are to eliminate microorganisms and their byproducts from the root canal system and to prevent reinfection.¹ Gutta-percha combined with a sealer is the most commonly used approach for root canal filling. However, this standard method does not provide a completely hermetic seal of the root canal system.²

Various endodontic sealers are currently in use, and studies have also investigated the use of resin-based sealers.³ Experience in restorative dentistry has shown that the adhesion of composite resins to dentin is improved by dentin-bonding agents,⁴ which create a micromechanical interlock between the dentin collagen and resin by forming a hybrid layer.⁵ Self-etch (SE) adhesive systems simultaneously promote demineralization and resin monomer infiltration, thereby reducing the number of steps compared to etch-and-rinse systems. However, the materials of SE systems have lower hydrogenion concentrations (pHs) than the acids used with etch-and-rinse adhesive systems.^{6,7}

Resilon (Resilon Research LLC, Madison CT, USA) is a synthetic endodontic material that was developed to replace gutta-percha. Resilon is used in combination with Epiphany SE, a root canal sealant (Pentron Clinical Technologies, Wallingford, CT, USA).⁸ This root canal filling system bonds to the root dentine, reportedly forming a 'resin monoblock' and providing an efficient seal.⁹ However, controversial results for the immediate-and long-term performances of the Resilon/Epiphany SE system have been obtained.¹⁰⁻¹³ Moreover, Resilon (polycaprolactone-based) is biodegradable under microbial attack¹⁴ and is susceptible to alkaline and enzymatic hydrolysis.^{15,16}

The present study was designed to compare the immediate- and long-term sealing abilities of two root canal filling systems: epoxy- (gutta-percha/AH Plus) and dimethacrylate resin-based sealers (Resilon/Epiphany SE). Three null hypotheses were tested: (1) gutta-percha/AH Plus and Resilon/Epiphany SE are not different in their immediate sealing abilities; (2) the sealing abilities of the systems are not affected by the use of a 90-day period of storage in water; and (3) the systems show no correlation between the sealing ability and the pH change of the storage water when the specimen is stored for up to 90 days in water.

MATERIALS AND METHODS

Forty-four mandibular single-rooted human premolar teeth with straight root canals and completely formed apices were selected. The teeth were stored in 0.2% sodium azide (NaN₃, E. Merck, Darmstadt, Germany) at 4° C until use.

Specimen Preparation

The crowns were removed at the amelo-cemental junction by a slow-speed machine (Isomet 1000 Precision Saw; Buehler Ltd., Lake Bluff, IL, USA) equipped with a diamond-impregnated copper disc (Extec Corp., Enfield, CT, USA) under water cooling. The root lengths were standardized to 13 mm. A size #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) was inserted into the root canal until it was seen at the apical foramen. The working length (WL) was established as 1 mm short of the apical foramen.

After coronal flaring was completed with Gates-Glidden burs (numbers 2-4, Dentsply, Maillefer), specimens were instrumented by the crown-down technique to the ISO size #50 K-file. Irrigation was achieved with 0.5% sodium hypochlorite (NaOCl) between each instrument. Afterwards, the apical foramen diameter was standardized with a #30 K-file. The smear layer was removed by flushing the specimen with 5.0 ml of 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes. Specimens were rinsed with saline solution (15 ml) and dried with paper points.

The teeth were randomly (http://www.random. org) assigned to two experimental groups (n = 20 per group). Four additional teeth were used as positive (n = 2) and negative (n = 2) controls. Endodontic sealers were prepared according to the manufacturers' instructions (Table 1). The cold lateral condensation (LC) filling technique was performed.

For the gutta-percha/AH Plus group, an ISO #50 master gutta-percha cone was lightly coated with AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) and placed into the canal to the WL. A size B finger spreader (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal at 1 mm short of the WL. The LC filling technique was used with accessory gutta-percha cones until the entire root canal was filled. Excess gutta-percha was removed with a heated plugger at 2 mm below the amelo-cemental junction and was compacted vertically.

For the Resilon/Epiphany SE group, LC was performed with Resilon cones. Epiphany SE sealer was used as described earlier. The coronal surface of the root filling was light-cured for 40 s (600 mW/cm²) with a lightcuring unit (Jet Lite 4000 Plus model; J. Morita Inc. Irvine, CA, USA). All teeth were stored in a vacuum chamber under 100% humidity at 37°C for 7 days.

For the positive controls, the root canals were left unfilled. Two coats of nail polish and a thick layer of sticky wax were consecutively applied to the external root surfaces, except for the apical 2 mm. For the negative controls, the root canals were filled, and the external surface, including the apical foramen, was totally coated.

The experimental group specimens were randomly allocated into two subgroups (n = 10 each) according to the storage conditions: immediate testing (immediate subgroup) or testing after storage for upto 90 days in deionized water (water storage subgroup). Specimens were stored individually in hermetically sealed vials containing deionized water and kept in an oven at 37° C.

Sealers (Batch number)	Manufacturers	Ingredients
AH Plus (0710000127)	Dentsply DeTrey, Konstanz Germany	Paste A: epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments.
		Paste B: amines, calcium tungstate, zirconium oxide, silica, silicone oil, UDMA.
Epiphany SE™ (153525)	Pentron Clinical Technologies LLC, Wallingford, CT, USA	Epiphany Sealer: BisGMA, UDMA, PEGDMA, EBPADMA, HEMA, AMPS and acidic methacylate resins, barium sulfate, silane-treated glass, silica, calcium hydroxide, hydroxylaptite, Ca-AI-F-silicate, bismuth oxychloride with amines, peroxide, photoinitiator, stabilizers and pigments. Resilon: polyester polymer, bioactive glass, bismuth oxide, barium sulfate, bifunctional dimethacrylate.

 Table 1: Batch numbers, manufacturers and ingredients of tested sealers

UDMA: Urethanethyl dimethacrylate; Bis-GMA: Bisphenol glycidyl methacrylate; PEGDMA: Poly (ethylene glycol) dimethacrylate; EBPADMA: Ethoxylated bisphenol A dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; AMPS: 2-acrylamido-2-methylpropane sulfonic acid

The storage water was changed monthly.¹⁷ To avoid thermocycling effects, the water was heated to 37°C prior to changing.¹⁸ Preservatives and antimicrobial agents were not used in the storage water in this study.

Microleakage Test

The nondestructive method to evaluate coronal leakage over time described by Ishimura et al¹⁹ was used in the present study. In specific, the tapered end of a 2 ml plastic tube (Eppendorf-Elkay, Shrewsbury, MA, USA) was cut. Dental roots were inserted individually into the tubes until they protruded through the ends. The root/tube interface was coated with epoxy resin (Durepoxi Henkel Ltda., São Paulo, SP, Brazil). Two coats of nail polish and a thick layer of sticky wax were consecutively applied to the external root surfaces, except for the apical 2 mm. A 0.2 ml aliquot of 0.06% methylene blue dye solution was poured into the Eppendorf tube. Two millimeters of the root apex were immersed into a glass bottle containing 3 ml of deionized water.

The testing apparatus was maintained in an oven under 100% humidity at 37°C for the duration of the observation time. At each measurement time, water in the glass bottle was removed and replaced with fresh deionized water. The sampled water was centrifugated at 5000 rpm for 10 minutes. For each sample, a 1 ml aliquot of the centrifugated water was used to measure the amount of methylene blue dye. The dye concentration (in μ g/ml) was determined from the absorbance at 630 nm by spectrophotometry (Spectrophotometer DU 800, Beckman Coulter, Inc., Fullerton, CA, USA) at 7, 14 and 28 days. Deionized water was used as the standard solution.

A calibration curve was drawn for the spectrophotometric analyses. Six serial dilutions of 1% methylene blue solution were tested, and the calculated absorbance was plotted against the known concentration. The correlation coefficient of the standard curve was $r^2 = 0.99$, and the equation for the regression curve was, $y \times 10^4 = x^3 - x^2 +$ $13 \times x + 0.4$, where x is the absorbance and y is the dye concentration. The concentration of each sample was extrapolated with this curve.

pH Evaluation

At baseline and after 30, 60, and 90 days of storage at 37°C, the pH values of the deionized water were determined by a digital pH meter (Twin pH, Horiba, Kioto, Japan). At the beginning of each test schedule, the device was calibrated with two standard solutions (pH values of 7.0 and 4.0), according to manufacturer's recommendations. The pH was tested three times for each sample, and the average value was calculated and recorded.

The amount of eluted dye was estimated by substituting the concentration of each sample into the equation, and the amount was summed over all of the time intervals. The data were statistically evaluated by two-way ANOVA, with microleakage as the dependent variable and the materials and storage conditions as factors. All post-hoc multiple comparisons were performed by Tukey's test. One-way ANOVA with repeated measures was used to compare the effect of materials on pH over time. The materials were considered as the main factor. A linear regression analysis was used to examine if a correlation existed between the microleakage and the baseline-toobservation mean difference in pH (mean difference between pH values at 30, 60 and 90 days and at baseline). The MINITAB statistical software program (Minitab Inc. Release 14 for Windows 2003, State College, PA, USA) was used for all analyses. Significance was set at the 5%.

RESULTS

Microleakage

All specimens in the positive control group leaked within 7 days. The negative control showed no leakage for the entire experimental period of 28 days. Table 2 presents the average microleakage results (mean and standard deviations) for the groups. Two-way ANOVA indicated that the microleakage results were influenced by the storage condition (f = 11.30, p < 0.05) and type of material (f = 299.76, p < 0.001). These two factors showed significant interactions (f = 322.97, p < 0.001). Therefore, the differences between the materials (gutta-percha/AH Plus and Resilon/Epiphany SE) were dependent on the effects of the storage conditions (immediate *vs* water storage subgroups; p < 0.001). Tukey's post-hoc test revealed no significant differences between the materials after 90 days of storage in water.

pH Measurements

The baseline pH values for deionized water were approximately 5.0, 5.5, and 5.0 at 30, 60, and 90 days, respectively. Table 3 displays the average pH values (mean and SD) after each experimental time among the groups. The filling material appeared to have no effect on the

Table 2: Mean values (standard deviations)					
of microleakage (mg/ml)					

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Groups (n)		Immediate	Storage condition	
			90-day in water	
	Gutta-percha/AH Plus (20)	0.582 (0.202) ^c	1.107 (0.090) ^b	
Resilon/Epiphany SE (20)		1.487 (0.092) ^a	1.089 (0.037) ^b	
	Different superscript letters of	mn show statistically		
	significant differences (p < 0	0.001)		

 Table 3: Mean (standard deviations) pH values in three times of observation

	Days			
Groups (n)	30	60	90	
Gutta-percha/ AH Plus (10)	6.43 (0.183)	7.19 (0.325)	7.36 (0.217)	
Resilon/Epiphany SE (10)	6.62 (0.162)	7.19 (0.233)	7.47 (0.258)	
(p > 0.05)				

baseline-to-observation mean difference in pH at the three observation times (p = 0.1172; one-way ANOVA); however, there was a time effect (p < 0.0001). The interaction of these two factors was not significant (p = 0.4969), which indicated that the effect of time on pH was identical for the two materials.

The baseline-to-observation mean differences in pH at 30 and 60 days were not different according to Tukey's post-hoc test (p = 0.0819), and both were different from the mean difference at 90 days (p < 0.0001). Graph 1 presents the mean differences in pH of the water over time. Linear regression analysis showed that there was no correlation between microleakage and the mean difference in pH from baseline over time (p > 0.05; Graph 2).

DISCUSSION

The first null hypothesis of the present study was rejected, because the sealing ability of gutta-percha/AH Plus was higher than that of Resilon/Epiphany SE at the immediate time point. The second null hypothesis was not rejected, because no difference in sealing ability was detected between the two materials after storage for 90 days in water.

The sealing ability of the root canal sealers was quantitatively tested by the passive dye penetration model, in which the absorbance of a 0.06% methylene blue



Graph 1: Mean differences of baseline pH values and after 30, 60 and 90 days (p < 0.0001)

dye solution is measured to evaluate coronal leakage.¹⁹ It is possible to determine the concentration of methylene blue dye solution released into water in a glass bottle continuously over 28 days. The major advantage of this method is its ability to measure microleakage without destroying the root specimens. To avoid anatomical variations and to standardize the leakage measurements, the specimen length and apical foramen diameter were kept identical.

The Resilon/Epiphany SE immediate subgroup showed poorer results than the gutta-percha/AH Plus immediate subgroup (p < 0.001). There are several possible reasons for this finding: (1) The root canal system has an unfavorable geometry for resin bonding.²⁰ (2) The extremely high configuration factor (C-factor) of the root canals maximizes the polymerization shrinkage stress along the root dentin-sealer interface, which might result in the debonding of sealer.²¹ In addition, the root canal walls cannot compensate for shrinkage stresses by elastic deformation.²¹ Therefore, it is not possible to achieve the gap-free monoblock that would be created by bonding the Epiphany SE sealer to both the Resilon and the radicular dentin.^{22,23}

The storage of specimens in water for 90 days did not influence the sealing ability of either of the root-filling systems (p > 0.05). This result is in agreement with one study that obtained measurements immediately after root filling and after storage for up to 90 days in artificial saliva with the fluid filtration technique.²⁴

Gutta-percha/AH Plus showed a trend of increasing microleakage over time. This sealing loss may be attributable to the lack of adhesion at the interface of the gutta-percha with the AH Plus sealer.^{25,26} Comparatively better sealing ability was obtained for Resilon/Epiphany SE. The reduced leakage in this group may be explained



Graph 2: Regression analysis of the correlation between microleakage (μ g/ml) and mean differences of baseline pH values and after 30, 60 and 90 days

by the water sorption property of methacrylate resinbased root canal sealers, such as Epiphany SE,²⁷ which leads to expansion.²⁸

The third null hypothesis of this study was not rejected. The pH change of the storage water over 90 days did not influence the microleakage of the sealers. The pH may have been enhanced by the leaching out of OH- ions from the sealer components, such as calcium hydroxide or calcium tungstate, into the water (Table 1), which would make the water more alkaline. The influence of this alkalinity on the properties of the composite can be explained by the interaction of the composite with the OH- ions during hydrolysis. Under the condition of excess hydroxyl ions, such as are present in water or saliva at pH 7.0,²⁹ accelerated degradation of the composite is expected.

The reasons for the loss of sealing capacity over time cannot be explained by the current evaluation. Mild SE adhesives (pH>2) reportedly activate latent matrix metalloproteinases without denaturing these enzymes and may adversely affect the longevity of bonded root canal fillings.³⁰ Furthermore, the decrease in bonding effectiveness may have been caused by the degradation of the interface components by hydrolysis after about 3 months.⁶ This situation would suggest the compromised sealing ability of the root canal filling over the long-term.

Our results should be interpreted carefully, because the storage time employed in this study was shorter than those used in previous studies (>1 year).^{4,31,32} Once water sorption occurs, composite materials are susceptible to solubility.²⁷ Dissolution of the AH Plus and Epiphany SE sealers occurs over long time periods and may permit gap formation between the dentinal walls and root filling materials.^{27,33} The resulting fluid movement through nanometer-sized voids along the collagen fibrils within the hybrid layer is detrimental to the bond integrity over time.³⁴

CONCLUSION

Under the conditions and limitations of the current study, the following conclusions may be made: (1) At the immediate time point, gutta-percha/AH Plus provided superior sealing ability compared to resilon/epiphany SE, (2) the sealing ability after storage in water for 90 days was similar between the gutta-percha/AH Plus and Resilon/Epiphany SE root filling systems and (3) changes in the pH of the storage water did not influence the microleakage of the tested materials.

CLINICAL SIGNIFICANCE

In view of the results it can be speculated that, clinically, a better immediate sealing ability could be expected with

gutta-percha/AH Plus. However, its ability was similar the Resilon/Epiphany SE root filling system up to 90 days.

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