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Evaluation of Chemical and Physical Properties of an Experimental Endodontic Sealer in Comparison with AH-26 and AH-Plus

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ABSTRACT

Introduction: This study aimed to assess the physical and chemical properties of an experimental endodontic resin sealer (Resil) compared with AH-26 and AH-Plus. **Methods and Materials:** In this *in vitro* experimental study, dimensional stability (by measurement of length; *n*=5), pH (using a pH meter; *n*=5), and antibacterial activity (by agar diffusion test; *n*=8) of Resil, AH-26 and AH-Plus were evaluated and compared using one-way ANOVA and Tukey's test. **Results:** All three groups showed significant expansion from day 1 to day 30 ($P<0.05$). The difference in the mean dimensional changes between the AH-Plus and experimental sealer was significant ($P=0.020$). Two hours after mixing, the pH of experimental sealer (Resil) was significantly lower than that of AH-Plus ($P<0.001$) and higher than that of AH-26 ($P<0.001$). Antibacterial activity of the experimental sealer before and after setting was significantly higher than that of the other two sealers ($P<0.001$). **Conclusion:** The results of this *in vitro* study showed that the experimental sealer had greater 1 to 30 days dimensional changes than the other two sealers. It had an alkaline pH, and showed superior antibacterial activity compared with AH-26 and AH-Plus. It may be possible to use this sealer in the clinic after animal studies because an epoxy resin based sealer with lower price and more appropriate properties is favorable.

Keywords: AH-Plus Sealer; Endodontics; Epoxy Resin AH-26; Root Canal Filling Material; Root Canal Sealer

Introduction

Complete filling of the prepared root canal system is an important component of successful root canal treatment [1]. Gutta-percha along with root canal sealers are the commonly used materials for obturation of the root canal system. Gutta-percha does not adhere to the root dentin; thus, sealers are used as bonding agent. Endodontic sealers are used to fill the gap between the root filling material and root canal walls [2]. Application of sealer along with gutta-percha is imperative for better adaptation of gutta-percha to root canal walls and sealing of dentinal tubules. An ideal sealer should have favorable properties such as optimal biocompatibility, no polymerization shrinkage, insolubility in oral and tissue fluids, and antimicrobial activity [3]. Many endodontic sealers are currently available in the market amongst which, AH-26 (Dentsply Maillefer, Ballaigues, Switzerland) is a commonly used epoxy resin sealer. Shrinkage is a major disadvantage of AH-26 sealer [4]. AH-Plus is a newer sealer commercially available in dental markets worldwide, which has the optimal properties of AH-26 without its shortcomings [4].

An ideal sealer has yet to be introduced to the market. Also, no domestically manufactured sealer is available with properties comparable to those of foreign-made sealers. Thus, search is ongoing for a new resin-based sealer. Despite the production of new sealers with mineral trioxide aggregate or silicon base and ceramic sealers with calcium phosphate base, resin sealers are still preferred in dental clinics [1].

In previous studies [5, 6], a new resin-based sealer (Resil) composed of calcium tungstate, zirconium oxide, aerosol, bismuth oxide, titanium oxide, hexamine and an epoxy resin was evaluated. The results showed that it had some superiorities over AH-26 sealer including shorter setting time and less cytotoxicity [5, 6]. Structurally, it was highly similar to AH-26 and AH-Plus. Also, Resil had acceptable flow, solubility and film thickness according to ANSI/ADA No.57 and ISO 6876 (2012) standards. Its radiopacity was higher than that of 3 mm of aluminum and was in accord with ANSI/ADA N0.57 and ISO 6876 (2012) standards [5, 6].

This study aimed to assess the chemical and physical properties of Resil compared with AH-26 and AH-Plus sealers.

Materials and Methods

This *in vitro* experimental study was performed on Resil in comparison with AH-26 and AH-Plus (Dentsply De Trey Gmbh, Konstanz, Germany). Sample size was calculated to be 5 samples in each group for assessment of dimensional changes and pH, and 8 samples in each group for assessment of antibacterial activity according to previous studies [7-10].

The sealers had to be homogenously mixed before testing. For Resil, two units of powder were mixed with one unit of liquid to obtain adequate consistency. The mixing technique for AH-26 and AH-Plus was in accordance with the manufacturers' instructions [2]. To ensure accurate mixing and complete setting of the samples, the following measures were taken: (I) after mixing, the sealers were assessed using the string-out test. A homogenous sealer should be stringed out from the surface of glass slab by 1.5 to 2.5 mm[5]; (II) after mixing, the sealers were assessed using the drop test. The sealer had to adhere to the spatula for 10-15 sec before dropping [2, 5]; (III) the reported setting time is 13 h for Resil, and 15 h and 8 h for AH-26 and AH-Plus, respectively [5]. Also, the samples were inspected for presence of void visually and those without voids were selected for the study. Samples that did not meet the above-mentioned criteria were excluded and replaced. The samples were then subjected to the following physical and chemical tests:

Dimensional stability

Teflon molds were used to fabricate samples with 12 mm height and 6 mm diameter. The mold was placed on a glass plate (1 mm in thickness, 25 mm in width and 75 mm in length). The glass plate wrapped with a fine cellophane sheet. The mold was then filled until a slight excess of material was observed at its upper end. A microscope slide also wrapped in cellophane was then pressed onto the upper surface of the mold. The assembled microscope slide, cellophane-wrapped slide, and the mold containing the material in the middle were then kept firmly joined with the aid of a C-shaped clamp. Five min after initial mixing, the assembly was incubated at 37°C and 95% humidity for 48 h [11]. The flat end of molds was then polished using 600-grit sand paper. The samples were then removed from the mold and their length was measured by a digital caliper with ±0.0001 mm accuracy (Mitutoyo, Tokyo, Japan). They were then stored in a 50-mL container containing 30 mL of deionized water at 37°C for 30 days. Next, they were dried on an absorbent paper and their length was measured again. The percentage of dimensional change was calculated using the formula: $\frac{(L_{30\ days}-L_0)}{L_0}$ Where $L_{30\ days}$ was the length of the sample after 30 days of storage and L_0 was the primary length of the sample. The mean value of dimensional changes of the five samples in each group was calculated and recorded as the dimensional change of the respective sealer [10, 12].

Alterations in pH

The samples were placed in 1-mm plastic tubes with 4 mm diameter. Each tube was immersed in a glass flask containing 10 mL of deionized water. The flask was then hermetically sealed after immersion of samples. The pH was measured at 2 and 72 h and 7 days after spatulation using a pH meter (3310; Genway, Staffordshire, UK). Before the test, the apparatus was calibrated at the standard solutions of pH 4.0, 7.0, and 12.0, respectively. At each measurement time point, the tubes were transferred to new flasks containing fresh solution, and the pH of the old solution was measured [13].

Antibacterial activity

The agar diffusion test was used for assessment of antibacterial activity. Microscopic assessment was carried out under aseptic conditions in a laminar flow chamber. Antibacterial activity was evaluated using standard strain *Enterococcus faecalis* (*E. faecalis*; ATCC 29212). The microorganisms were cultured in brain heart infusion broth (Merck, Darmstadt, Germany) at 37°C for 18 h. The bacterial suspension was prepared using 0.85% saline with 0.5 McFarland standard turbidity; 0.1 mL of the bacterial suspension was cultured on brain heart infusion broth in 20 plates using a Drigalsky's loop. Next, the agar was punched out to create four holes with 6 mm diameter and 4 mm depth (three holes for the sealers and one hole as control) [14]. The plates were then divided into two groups. Half of them were used for assessment of antibacterial activity before setting and the remaining half for assessment of antibacterial activity after setting of sealers. For the fabrication of set samples, Teflon molds were used to fabricate sealer samples with 5 mm height and 4 mm diameter. The molds were then stored at room temperature for 48 h. The samples were then removed from the mold and placed in the created holes [14].

Assessment of antibacterial activity before setting (fresh sealer)

As explained earlier, holes in half of the plates were filled with fresh sealers. They were then stored at room temperature for 2 h and incubated in anaerobic condition at 37°C for 48 h. The diameter of the growth inhibition zone around each hole was measured using a digital caliper with ±0.0001 accuracy (Mitutoyo, Tokyo, Japan) and reported in millimeters (mm) [14].

Assessment of antibacterial activity after setting

In the remaining plates, the set samples were placed in holes and incubated in anaerobic condition at 37°C for 48 h. The diameter of the growth inhibition zone around each hole was measured using a digital caliper and reported in millimeters (mm).

Bacterial suspension was not added to two plates in both the set and fresh sample plates. One of these control plates did not have sealer and was used to control the medium sterilization and the other plate contained sealer and was used to control the possible contamination of sealers.

Statistical analysis

The mean and standard deviation values were reported for the variables measured in the three sealer groups. One-way ANOVA was applied for the comparison of variables among the three groups. The Tukey's post-hoc test was applied for pairwise comparisons. Generalized linear model was used to control for the confounding factors. Data were analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) at .05 level of significance.

Results

Dimensional stability

Table 1 compares the three groups regarding dimensional changes for 1 to 30 days. The mean dimension of samples in all three groups significantly increased from day 1 to day 30 (P<0.05). Pairwise comparisons of dimensional changes revealed that only the difference between AH-Plus and Resil was statistically significant (P=0.020).

Changes in pH

Table 1 shows the changes in the mean pH in the three groups at 2 and 72 h and 7 days after spatulation. Pairwise comparisons of sealers at 2 h showed that Resil had significantly lower pH than AH-Plus (P<0.001) and higher than AH-26 (P<0.001). At 72 h and 7 days after spatulation, however, the mean pH of Resil and AH-Plus was not significantly different (P=1.00) and they both had a significantly higher pH than AH-26 (P<0.05). The pH of AH-Plus and Resil significantly decreased over time (P<0.05); but the reduction in the mean pH of AH-26 was only significant between 2 h and 7 days (P=0.024).

Antibacterial activity

Table 1 shows the mean diameter of the growth inhibition zone around the sealers. The mean antibacterial activity in all groups decreased after setting compared with their fresh state (before setting; P<0.001). Pairwise comparisons of antibacterial activity of sealers before and after setting showed that the antibacterial activity of Resil (both before and after setting) was significantly higher than that of the other two sealers (P<0.001). The antibacterial activity of AH-Plus (both before and after setting) was significantly lower than that of the other two sealers (P<0.05). Growth inhibition zone was not seen around any sample of AH-Plus after setting.

Discussion

In this study, the results showed that Resil experimental sealer had dimensional changes greater than those of other two sealers. It had an alkaline pH between the pH values of the other two sealers. It showed superior antibacterial activity compared with AH-26 and AH-Plus.

This study assessed the physical and chemical properties of Resil in comparison with AH-26 and AH-Plus. Selection of the latter two sealers was because of their high popularity among the resin sealers available in the market. Also, a previous study on characterization of Resil revealed that its chemical structure was similar to that of AH-26 and AH-Plus [5, 6]. Given that Resil experimental sealer is similar to AH-26 in terms of physical and chemical properties, it would be acceptable for clinical use, and if it resembles AH-Plus, it would be gold standard [15, 16].

A previous study on Resil showed that its setting time was shorter (around 11 h) and its cytotoxicity was lower than that of AH-26 [5, 6]. Assessment of dimensional changes of Resil, AH-26 and AH-Plus in our study indicated that all sealers underwent expansion from day 1 to day 30 the difference in the

Table 1. Properties of the sealers evaluated in this study

Groups	1 Day	30 Days	pH (2 h)	pH (72 h)	pH (7 days)	AB* (Before)	AB* (After)
AH-Plus	11.246 (0.323) ^{A1}	11.350 (0.417) ^{A2}	9.752 (0.218) ^{A1}	7.714 (0.186) ^{A2}	7.256 (0.215) ^{A3}	5.71 (0.488) ^{A1}	0.00 (0.000) ^{A2}
AH-26	11.648 (0.271) ^{A1}	11.858 (0.219) ^{A2}	7.148 (0.224) ^{B1}	7.004 (0.347) ^{B1}	5.730 (0.376) ^{B2}	20.57 (1.134) ^{B1}	3.29 (2.289) ^{B2}
Recil	11.323 (0.26) ^{A1}	11.618 (0.302) ^{A2}	80702 (0.265) ^{C1}	7.886 (0.251) ^{A2}	7.366 (0.182) ^{A3}	31.14 (4.337) ^{C1}	11.29 (2.690) ^{C2}

AB: Antibacterial activity; Different letter and number each group indicates statistically difference*

mean dimensional changes between the AH-Plus and Resil was significant. According to the ISO standard and ANSI/AFS specifications, sealers can maximally have 1% shrinkage or 0.1%expansion [17, 18]. The mean expansion of Resil during 30 days in our study was 0.29%±0.10%, which was significantly higher than that of AH-Plus, but had no significant difference with the mean expansion of AH-26 [1, 11]. The mean expansion of Resil and AH-26 was over the acceptable threshold of 0.1% according to the standards [11, 19]. Its high expansion can be explained by the water absorption of epoxy resin after polymerization. Possibly this high expansion of Resil and AH-26 must compensate for the shrinkage suffered by the resin-based sealer after polymerization [11].

Some other studies [7, 9-11, 18] also demonstrated that AH-26 and AH-Plus had setting expansion. According to Carvalho *et al.* [11] the expansion of AH-Plus compensates its polymerization shrinkage. Ideally, root filling materials and sealers should have acceptable dimensional stability or slight expansion. However, risk of root fracture as the result of stresses generated by the expansion of sealers always exists. It should be noted that other factors such as the modulus of elasticity of dentin and root filling material, and tensile strength of dentin also play a role in this respect [9]. Gutta-percha has low modulus of elasticity and decreases the stresses created by the expansion of sealer to some extent. On the other hand, shrinkage of sealer is less acceptable since it causes gap at the sealer/gutta-percha and sealer/root canal wall interface and leads to microleakage. Therefore, shrinkage of sealer imposes a greater risk for treatment failure compared with its slight expansion [9, 19, 20].

Several studies [21-23] showed that the pH of AH-26 and AH-Plus increased after setting, which was different from our findings. On the other hand, Silva *et al.* [24] reported that the pH of AH-Plus decreased from 7.34 to 7.07 within 4 weeks. One possible reason for the variability in the results is difference in methodologies. Faria *et al* .[23] and Duarte *et al.* [22] immersed the sealers for pH measurement before their setting while in this study and that of Silva *et al.,* [24] the pH of samples was measured after setting.

In the current study, the initial alkaline pH of the three sealers indicated the release of calcium and hydroxyl ions [25, 26]. A previous study showed that the cationic polyaddition reaction in AH-26 sealer starts after mixing of epoxy resin with amine [5]. They also showed presence of unreacted epoxy groups after completion of the setting time claimed by the manufacturer [5]. Thus, the setting reactions continue after completion of the setting time claimed by the manufacturer. Presence of acid hydrogen components such as bisphenol epoxy resin in the composition of resin sealers in this study may be responsible for the reactions with hydroxyl ions and subsequent gradual reduction in pH. The setting reaction of sealer components may gradually prevent the release of hydroxyl ions and result in gradual change in pH [26, 27].

Sealers should ideally possess antimicrobial activity. Several methods are used for assessment of antibacterial activity of materials, and agar diffusion test is among the most commonly used techniques for this purpose. However, it should be noted that the results of this test highly depend on factors such as shape, size, molecular weight, and diffusion properties of antimicrobial agents and volume and concentration of the material used as well as the incubation time and duration of exposure to the culture medium [28]. *E. faecalis* is often used for assessment of antimicrobial activity of dental materials since its role in endodontic treatment failure has been well documented [29]. Also, it is among the most resistant bacteria in the root canal system [30]. The current study assessed the antibacterial activity of sealers in fresh and set forms. Since the likelihood of diffusion of sealer in the agar is higher in fresh form, reduction in antibacterial activity after setting was expected in all three groups. Also, it should be noted that use of fresh sealer simulates the clinical setting when the root canal is filled. An interesting finding of this study was higher antimicrobial activity of Resil in both fresh and set forms, compared with AH-26 and AH-Plus. A previous study showed that AH-Plus sealer had no antibacterial activity after setting [4], which was in agreement with our findings. Heling *et al.* [31] and Moazami *et al.* [32] reported that the antibacterial activity of AH-26 sealer was higher than that of AH-Plus, which was in accordance with the current results. In a systematic review by AlShwaimi *et al.* [33] it was shown that AH-Plus and AH-26 had strong antibacterial activity in fresh state but their antibacterial activity significantly decreased after 2 to 7 days. Antibacterial activity of sealers should be evaluated along with their cytotoxic effects.

This study had some limitations. For instance, it had an *in vitro* design. In the oral environment, many factors affect the physical and chemical properties of sealers that cannot be simulated *in vitro*. Thus, generalization of results to the clinical setting must be done with caution. Future animal studies and then clinical trials are required to compare the properties of these sealers *in vivo*. Moreover, other properties of Resil experimental sealer such as its microleakage, discoloration and push-out bond strength should be evaluated in future studies.

Conclusion

Within the limitations of this *in vitro* study, the results showed that Resil experimental sealer had greater 1 to 30 days dimensional changes than those of other two sealers. It had an

alkaline pH between the pH values of the other two sealers. It showed superior antibacterial activity compared with AH-26 and AH-Plus. It may be possible to use this sealer in the clinic after animal studies because an epoxy resin based sealer with lower price and more appropriate properties is favorable.

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Conflict of Interest: 'None declared'.

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