Effect of Intracanal Medicament on the Sealing Ability of Root Canals Filled with Resilon

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Abstract

The purpose of this in vitro study was to investigate the effects of the use of calcium hydroxide as an intracanal dressing on the sealing ability of a thermoplastic synthetic polymer-based root filling (Resilon). Forty-seven single rooted teeth were decoronated and instrumented to ISO sizes 40. The teeth were randomly divided into three experimental groups of 15 roots each. Group 1 was immediately filled. Group 2 and group 3 had calcium hydroxide paste placed with lentulo-spiral filler. After 7 days, calcium hydroxide was removed from the canals with two different techniques: #15 K-file agitated irrigation with 17% Ethylenediaminetetraacetic acid (EDTA) (group 2) or ultrasonically agitated irrigation with 17% EDTA (group 3) for 2 min. All teeth were filled with Resilon points and the resin sealer (Ephiphany root canal sealant) using lateral condensation technique. Two teeth were immediately filled with Resilon master point size 40/0.04 without sealer to act as a positive control. A split chamber microbial leakage model using Streptococcus mutans was used and the leakage was evaluated daily for a period of 30 days. Overall, 6 of 44 (14%) of samples filled with Resilon points and the resin sealer had microbial leakage. Three samples in group 1 (21%), two samples in group 2 (13%), and one specimen in group 3 (7%) had bacterial leakage. Using the Fisher’s Exact test, there was no statistically significant difference in leakage between the groups with calcium hydroxide dressing and the group without calcium hydroxide ($p > 0.05$). Under the condition of this study, calcium hydroxide did not adversely affect the seal of the root-canal system filled with Resilon. 

Key Words

Bacterial leakage, calcium hydroxide, Resilon

Apical periodontitis is an inflammatory process on the periradicular tissues caused by microbes in the root canal system (1–4). The goal of endodontic treatment is to prevent and eliminate apical periodontitis. This goal is accomplished through disinfection of the root canal space and adequate root canal filling to prevent recontamination. Long term success of endodontic therapy depends on the level of root-canal disinfection (5, 6) as well as proper sealing of both root canal and access cavity with materials (7, 8).

The idea root filling material should entomb residual bacteria after instrumentation, seal the root canal space preventing re-infection from coronal leakage, and stop apical penetration of tissue fluids from reaching surviving bacteria in the root canal system (6). The conventional root canal filling materials and sealers fail to fulfill the above criteria (7, 9, 10–13). Recently, an innovative adhesive material, Resilon (Resilon Research LLC, Madison, CT), has become available for filling of root canal space. Resilon is a thermoplastic synthetic polymer-based root canal filling material, which contains bioactive glass and radiopaque fillers. Resilon has some handling properties as gutta-percha, and can be softened with heat or dissolved with solvents like chloroform for retreatment purposes. The sealer used specifically for Resilon is a dual curable dental resin composite sealer (Ephiphany Root Canal Sealant, Pentron Clinical Technologies, Wallingford, CT). This sealer forms a bond to the core filling material and the cleaned dentin wall, hence creating a monoblock (12, 13).

Previous studies have shown that roots filled with the Resilon system were resistant to bacterial penetration (12, 13). An in vitro study using the split chamber microbial leakage model showed that roots filled with Resilon were superior to the roots filled with gutta-percha in resisting bacterial penetration (12). Over a 30 day period, less than 13% of samples filled with Resilon had bacterial leakage while 73% to 93% of samples filled with gutta-percha had leakage depending on the organism and technique used (12). In another study, Shipper et al. demonstrated that the teeth filled with the Resilon system had less apical periodontitis compared to the teeth filled with gutta-percha and AH26 sealer using a dog model (13). In this study, the author reported 81% of the teeth filled with Resilon were free of periapical inflammation 14 wk after being challenged by coronal microorganisms while only 18% of the teeth filled with gutta-percha and AH26 sealer were free of inflammation (13). In addition to the superior sealing ability, Resilon has also been shown to increase resistance to tooth fracture when used as the root canal filling material compared to the teeth filled with gutta-percha (14).

Placement of intracanal medicaments such as calcium hydroxide is often recommended for disinfection of the root canal space in teeth with apical periodontitis (15–17). Intracanal medicaments are used to: (a) eliminate bacteria in the root canal; (b) prevent bacterial proliferation between appointments; (c) act as a physiochemical barrier, preventing root-canal reinflection and nutrient supply to the remaining bacteria; (d) and stimulate periapical tissues to heal (18). Calcium hydroxide is one of most widely used intracanal medications in endodontics today and remains the best medicament available to reduce residual microbial flora (19). It is a strong alkaline substance, which has a pH of approximately 12.5. In an aqueous solution, calcium hydroxide dissociates into calcium and hydroxyl ions that will lead to a lowered oxygen tension and an increase in the pH in the inflamed periapical tissues (20). With a high pH, calcium hydroxide has an excellent broad antimicrobial effect.

The effect on leakage of the root canal system is an important consideration when placing an intracanal medication. Several studies have investigated calcium hydroxide...
remnants left in a canal and the effect these remnants have on the sealing ability of the permanent filling material (21–25). These studies have shown that the apical seal may or may not be adversely affected depending on the type of sealers used. To date, no study has demonstrated whether intracanal placement of calcium hydroxide increases the leakage of a root canal system filled with Resilon. The purpose of this in vitro study was to determine the influence of calcium hydroxide intracanal medication and various techniques for its removal on the sealing ability of roots filled with the Resilon system using a split chamber microbial leakage model.

Materials and Methods

A total of 47 single-rooted human teeth were used in this study. The teeth were stored in 0.2% thymol in normal saline solution until use. The teeth were immersed in 5% sodium hypochlorite (NaOCl) for approximately 15 min to remove organic material from the root surfaces. Any remaining tissue was mechanically removed using a curette with attention not to damage the root surface.

Each tooth was decoronated to give approximately 16 mm of root length from the coronal surface to the apex of the root with high-speed handpiece and a multipurpose bur (Dentsply Maillefer, Tulsa, OK) using air and water spray. An operating microscope (Carl Zeiss Surgical, Inc., Thornwood, NY) was used to inspect the roots for cracks under ×5 magnification. Root canal patency was verified by placing a #15 K-file (Kerr, Romulus, MI) through the apical foramen. The working length was established by subtracting 1 mm from this measurement. Each canal was instrumented using a high torque motor at 300 rpm with 0.04 Profile Series 29 .04 taper nickel-titanium rotary instruments (Dentsply Tulsa Dental, Tulsa, OK) to the working length to ISO size 40 by the crown-down technique. A total of 15 ml of 1.25% NaOCl was used for irrigation during instrumentation with a syringe and a 27-gauge Monoject endodontic irrigation needle (Sherwood Medical, St. Louis, MO). Five milliliters of 17% Ethylenediaminetetracitic acid (EDTA) (Pulpdent Corp., Watertown, MA) rinses were used after instrumentation to remove smear layer. The root canals were dried with sterile paper points (Patterson Dental Supply, Inc., St. Paul, MN). All roots and instruments used from this point on were sterilized with ethylene oxide gas. The teeth were stored in 0.2% thymol in normal saline solution, enclosed in sealed tubes, and placed in an incubator for 7 days at 37°C. After removal from the incubator, the Cavit was removed and the calcium hydroxide paste was cleaned with 5 ml of 17% EDTA (Pulpdent Corp., Watertown, MA) and #15 K-files (Kerr, Romulus, MI) for 2 min. During irrigation, the file was placed to the working length and moved up and down 10 times.

Group 3

Calcium hydroxide treatment for 1 wk, calcium hydroxide removed by ultrasonically agitated irrigation with 17% EDTA, followed by root canal filling. Calcium hydroxide was placed in the root canals and stored as described in group 2. After removal from the incubator, the Cavit was removed and the calcium hydroxide paste was cleaned with ultrasonically agitated irrigation with 5 ml of 17% EDTA (Pulpdent Corp., Watertown, MA) for 2 min. A Satelec P-5 booster (Dentsply Tulsa Dental) set to power 3 with Satelec K25 (21 mm) K-files (Dentsply Tulsa Dental) was used for ultrasonic irrigation. The ultrasonic tip was placed 1 mm from the working length during the irrigation for 2 min.

Positive Control

Two teeth were treated as in group 1 but no sealer was used for root filling. All root canals were rinsed with 5 ml of 17% EDTA and dried with sterile paper points (Patterson Dental Supply, Inc., St. Paul, MN). A self-etching primer (Epiphany Primer; Pentron Clinical Technologies) was placed into the canal with sterile paper points saturated with the primer. Excess primer was then removed with paper points. All roots were filled with Resilon master point size 40/0.04, medium fine Resilon accessory points (Resilon Pentron, Wallingford, CT) and the resin sealer (Epiphany root canal sealant, Pentron, Wallingford, CT) using lateral condensation technique.

The teeth were stored in gauze that was dampened with storage medium, enclosed in sealed tubes, and placed in an incubator for an additional 7 days at 37°C to allow the sealer to set. The microbial leakage study was carried out as previously described by Shipper et al. (12). The microbial leakage model consisted of an upper chamber and a lower chamber was used (10). The upper chamber consisted of a Corning 15-ml polycarbonate centrifuge tube (Corning Inc., Corning, NY) 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. Approximately 4 mm of root remained in the upper chamber.

The upper chamber consisted of 10 ml of WC Broth (Wilkins-Chalgren), which was inserted into the lower chamber: 20-ml clear, scintillation vial (Wheaton, Millville, NJ). The lower chamber consisted of 15 ml of basal broth with phenol red indicator to which 1% sucrose was added. The vial contents were then filtered sterilized (0.2-μm pore size) and a quality check was performed during 2 days of shelveing.

<table>
<thead>
<tr>
<th>TABLE 1. Description of experimental groups</th>
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<tr>
<td>Ca(OH)₂ treatment for 1 week</td>
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<tr>
<td>Group 1 (N = 15)</td>
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<td>Group 2 (N = 15)</td>
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<td>Group 3 (N = 15)</td>
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Federal Republic of Germany). Teeth were stored in sterilized gauze that was dampened with storage medium (0.2% thymol in normal saline solution), enclosed in sealed tubes, and placed in an incubator for 7 days at 37°C. After removal from the incubator, the Cavit was removed and the calcium hydroxide paste was cleaned with 5 ml of 17% EDTA (Pulpdent Corp., Watertown, MA) and a #15 K-file (Kerr, Romulus, MI) for 2 min. During irrigation, the file was placed to the working length and moved up and down 10 times.
**Streptococcus mutans** (ATCC 10449) was grown in 10 ml of WC broth during 24 h. The bacteria were identified using selective media and morphology. After 48 h, **S. mutans** ATCC 10449 was added to 10 ml of WC broth adjusting the optical density to 0.2 OD_{660} \text{nm}. A total of 0.2 ml of the adjusted bacterial suspension was added to the upper chamber. Turbidity in the upper chamber was visually discernible within 24 h. On every second day, 9 ml of broth was removed from the upper chamber and replaced with fresh broth. The centrifuge cap was replaced to prevent evaporation and contamination. The specimens were placed in an incubator at 35 °C. Specimens were checked every 24 h over a 30 day period for a change in the color of the pH indicator in the lower chamber. A color shift from red to yellow would indicate a positive culture result (cell metabolism leading to acid production). The purity of the bacterial growth was confirmed by positive Gram-stained cocci in chains that could grow on the streptococci-selective MSB plate.

### Scanning Electron Microscope (SEM) Preparation

At completion of the leakage study, one specimen with microbial leakage was randomly chosen. The specimen was longitudinally sectioned so that the dentin-filling interface could be obtained. They were mounted onto a SEM specimen stub and coated with a gold/palladium film with a Polaron E5200 (Watford, Hertfordshire, England) sputter coater. The specimen was viewed with a JEOL JEM 6300 scanning electron microscope (Tokyo, Japan) at 15 kV accelerating voltage. Images were taken in digitized format.

### Statistical Analysis

The investigators examining leakage during the 30 days were blinded to all groups. Fisher’s Exact test was used to determine whether there was significant differences in the number of leakage between the groups with calcium hydroxide dressing (group 2 and 3) and the group without calcium hydroxide dressing (group 1) (p < 0.05). Difference between group 2 and group 3 was also examined using the Fisher’s Exact test (p < 0.05).

### Results

One specimen in group 1 was contaminated during the study and was discarded. Three out of 14 specimens in group 1 (21%) had microbial leakage, which occurred on day 1, 12, and 22. Two out of 15 specimens in group 2 (13%) had microbial leakage that occurred on day 1 and 12. Only one specimen out of 15 in group 3 (7%) had leakage on day 7. Overall, 14% of roots filled with Resilon had leakage. Both positive control roots leaked on day 1. The results are summarized in Table 2 and Fig. 1. Fisher’s Exact test showed there was no statistical difference between the groups with calcium hydroxide dressing (group 2 and 3) and the group without calcium hydroxide dressing (group 1) (p = 0.364). No statistical difference was found between group 2 and group 3 (p = 1.000).

Under SEM, bacteria were observed in between the dentin-filling interface of the longitudinally sectioned specimen with microbial leakage (Fig. 2). The gap of approximately 5 to 7 \( \mu \text{m} \) wide was found between the resin sealer and dentinal wall, which was filled with numerous microorganisms with spherical shape.

### Table 2. Number of specimens with microbial leakage and days at which microleakage occurred

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Leaks</th>
<th>Days</th>
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<tbody>
<tr>
<td>1. No calcium hydroxide treatment</td>
<td>(N = 14)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>2. Removal of calcium hydroxide with hand file and 17% EDTA</td>
<td>(N = 15)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>3. Removal of calcium hydroxide with ultrasonic files and 17% EDTA</td>
<td>(N = 15)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td>(N = 44)</td>
<td>6 (14%)</td>
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### Discussion

Long-term prognosis of root canal therapy is affected by the degree of root-canal disinfection (5, 6), as well as by the quality of coronal and root canal seal (7, 8). Calcium hydroxide is an effective intracanal medication for root canal disinfection when used for more than 1 wk (15–17). However, its interaction with sealer materials could potentially affect the sealing quality of root fillings. Several studies investigated the effects of calcium hydroxide intracanal medication on apical seal of roots filled with gutta-percha filling and different types of sealers (21–25). However, there is no study until now to show the effect of calcium hydroxide on the apical seal of roots filled with Resilon.

In this study, we used a split chamber microbial leakage model with **S. mutans**, as described by Shipper et al. (12), to evaluate apical leakage in roots filled with Resilon. No significant difference was found between groups medicated with calcium hydroxide and the control group without calcium hydroxide medication. There was no difference in the microbial leakage whether calcium hydroxide was removed by hand file agitation or ultrasonic agitation with 17% EDTA. Thus, our study showed that calcium hydroxide did not adversely affect the apical seal of the root canal system filled with Resilon. This is consistent with previous reports regarding the effects of calcium hydroxide on apical seal of roots filled with gutta-percha filling and different sealers (21, 24, 25). Porkaew et al. (21) evaluated methylene blue dye penetration of roots medicated with different calcium hydroxide preparations and the roots with no medication. All roots were filled with gutta-percha and Grossman’s sealer using lateral condensation. A significant decrease in leakage in the teeth medicated with calcium hydroxide was found. Wuerch et al. (24) evaluated the effect of 2% chlorhexidine gluconate or calcium hydroxide as an intracanal medication on the apical seal of the root canal system filled with gutta-percha filling and AH Plus sealer using a fluid-filtration device over 60 days. Neither 2% chlorhexidine gel nor calcium hydroxide paste adversely affected the apical seal. Çalışkan et al. (25) also showed there was no significant effect on microbial leakage in Resilon medicated roots.

![Percentage of specimens filled with Resilon that showed leakage over 30 days](image-url)
apical leakage when calcium hydroxide was used as an intracanal medication. On the other hand, Kim et al. (23) demonstrated that calcium hydroxide intracanal medication increases apical leakage of gutta-percha root fillings when a zinc oxide-eugenol sealer was used. It appears that the effect of calcium hydroxide intracanal medications on the apical seal is dependent on the type of sealer used.

There was 17% EDTA used to remove calcium hydroxide from the root canals because of its ability to form a stable complex with calcium (26). In addition, EDTA can effectively remove smear layer from the root canal dentin wall (27–30). This is important to allow the sealer to penetrate the dentinal tubules and thereby the adaptation of the root canal filling to the root canal wall is much improved (31, 32). In the case of the Resilon system, smear layer removal is crucial to allow the sealer to adhere to the dentin walls, and in turn the sealer closely adapts to the Resilon points, creating a monoblock system (12, 13).

Our data showed that the root canal system filled with Resilon has adequate seal. 7 to 21% of specimens showed microbial leakage over 30 days with overall of 14% leakage. This is consistent with a previous study (12). Shipper et al. (12) evaluated microbial leakage using \textit{S. mutans} and \textit{Enterococcus faecalis} in roots filled with Resilon or gutta-percha using either lateral or vertical condensation technique. All positive control groups (Resilon or gutta-percha filling without sealer) leaked within 24 h, showing the importance of sealer in sealing the root canal system. There was 73 to 93% of specimens filled with gutta-percha and AH26 sealer leaked over 30 days. In contrast, only 8 to 13% of specimens filled with Resilon points and Resilon sealer leaked. Overall, 12% of roots filled with Resilon leaked, which is similar to 14% leakage found in our study.

In this study, the earliest leakage occurred within 24 h. One sample from the control group without calcium hydroxide treatment and one sample from a group treated with calcium hydroxide leaked within 24 h after the microbial leakage experiment started. The remaining 4 samples had leakage between day 7 and day 22. Both positive control samples leaked within 24 h. In the previous study (12), Shipper et al. reported that the earliest leakage occurred in between day 5 and day 12 for the samples filled with Resilon points and the resin sealer. However, the samples filled with Resilon master point without sealer leaked within 24 h. It is likely that the earliest leakage found in our study could be a result of an experimental error. Some possible explanations are (a) inadequate root canal instrumentation, (b) insufficient amount of sealer used, allowing a gap between the filling material and dentin wall, (c) contamination on the surface of the root tip, and (d) undetected cracks that allowed bacteria to travel from the upper chamber to the lower chamber directly instead of through the root canal filling.

Because the Resilon system showed good sealing property, we were interested in what can potentially cause microbial leakage in teeth filled with Resilon. One specimen with leakage was randomly selected for SEM study to examine the dentin-filling interface. The gap was observed between the Resilon points and resin sealer. Numerous spherical microorganisms (\textit{S. mutans}) are seen in the gap.

Figure 2. High-power SEM (5000×) micrograph of a longitudinal section of a root with leakage. The root was filled with Resilon points and resin sealer. A gap is evident between the resin sealer and the dentin. Numerous spherical microorganisms (\textit{S. mutans}) are seen in the gap.
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