An Evaluation of Microbial Leakage in Roots Filled with a Thermoplastic Synthetic Polymer-Based Root Canal Filling Material (Resilon)

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The purpose of this study was to compare bacterial leakage using Streptococcus mutans and Enterococcus faecalis through gutta-percha and a thermoplastic synthetic polymer-based root filling (Resilon) using two filling techniques during a 30-day period. Teeth were decoronated, roots prepared to a length of 16 mm, and instrumented to ISO sizes 40 to 50. A total of 156 roots were randomly divided into 8 groups of 15 roots (groups 1–8) and 3 control groups (12 roots each). Roots were filled using lateral and vertical condensation techniques with gutta-percha and AH 26 sealer (groups 1 and 2) or with gutta-percha and Epiphany sealer (groups 3 and 4). Groups 5 and 6 were filled with Resilon and Epiphany sealer using the lateral or vertical condensation techniques. A split chamber microbial leakage model was used in which S. mutans placed in the upper chamber could reach the lower chamber only through the filled canal. Groups 7 and 8 were identical to groups 5 and 6 respectively; however, E. faecalis was used to test the leakage. Positive controls were filled with Resilon (12 roots) and gutta-percha (12 roots) without sealer and tested with bacteria, whereas negative controls (12 roots) were sealed with wax to test the seal between chambers. All but one positive control leaked within 24 h, whereas none of the negative controls leaked. Resilon showed minimal leakage (group 8: one leakage; groups 5–7: each with two leakages), which was significantly less than gutta-percha, in which approximately 80% of specimens with either technique or sealer leaked. Kruskal-Wallis test showed statistical significance when all groups were compared (p < 0.05). Mann-Whitney U test compared the respective groups and found Resilon groups superior to gutta-percha groups (p < 0.05).

Apical periodontitis is caused by intracanal bacteria (1–3). Prevention or healing of apical periodontitis involves a combination of disinfection of the root canal space through chemomechanical means (4, 5) and sealing both the root canal and access cavity with materials that will prevent reinfection (6, 7).

Presently, the requirements for instrumentation of the root canal that will result in predictable success are well established (8–10). However, once the canal has been optimally disinfected, the present filling materials and techniques fail in achieving the requirement of providing a suitable seal to further challenge by bacteria (6, 7). In fact, some have claimed that our success in endodontics is related more to the quality of the coronal restoration than to the filling of the canal even though the depth of the root filling is always more than that of the coronal restoration (7).

It seems that a root filling containing gutta-percha is the weak point in endodontic therapy (11–13). Torabinejad et al. (14) showed that when gutta-percha filled canals were challenged by bacteria, 50% allowed penetration through the entire length of the canal within 30 days. Shipper and Trope (15) showed that when using the FiberFill obturator (Pentron Clinical Technologies, Wallingford, CT; a resin fiber post with 5–8 mm of gutta-percha apically) and a resin bonding sealer, there was a 50% improvement in prevention of bacterial leakage compared with the standard gutta-percha techniques. Shipper and Trope (15) suggested that a resin core root canal filling that could bond to the root canal walls is desirable. The material should have an excellent apical fit, be bonded with a dentin-bonding system to a resin sealer, and the sealer itself should bond to the canal wall. Thus, should the coronal seal of the root-canal system be lost or broken, another barrier to the coronal bacterial challenge may be achieved with a bonded filling material. The only drawback to this concept is the ability to retreat such a root canal filling.

Many different materials have been proposed as root canal fillings, but none have replaced gutta-percha, which is universally accepted as the “gold standard” filling material. All bonding agents and resins studied to date had problems in working properties, radiopacity, and retreatability (16–18).

Resin filling materials have steadily gained popularity and are now accepted both for anterior and posterior teeth (19). In addition, as the bonding systems have improved so have the resistance to bacterial penetration of the materials (20). If resin were to be used in the entire length of the canal, a material that adequately seals the canal may have been found. Resilon (Resilon Research LLC, Madison, CT) is a thermoplastic synthetic polymer-based root canal filling material. Based on polymers of polyester, Resilon

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contains bioactive glass and radiopaque fillers. It performs like gutta-percha, has the same handling properties, and for retreatment purposes may be softened with heat or dissolved with solvents like chloroform. Similar to gutta-percha, there are master cones in all ISO (International Organization for Standardization) sizes and accessory cones in different sizes are available. In addition, Resilon pellets are available, which can be used for the backfill in the warm thermoplasticized techniques. The sealer, Epiphany Root Canal Sealant (Pentron Clinical Technologies) is a dual curable dental resin composite sealer.

The success of root-canal treatment is dependent on the development and maintenance of the seal of the root-canal system. Leakage tests are an accepted method to compare the seal of filling materials, even though a universally acceptable model does not exist (21).

The purpose of this study was to examine the resistance to bacterial penetration of this new resin filling material (Resilon). Streptococcus mutans or Enterococcus faecalis were tested during a 30-day period through gutta-percha and Resilon using two filling techniques: lateral and warm vertical condensation or a continuous wave of condensation (System B).

MATERIALS AND METHODS

A total of 156 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.

The crowns were removed and the coronal surfaces of the root were prepared perpendicular to the long axis of the root with a high-speed handpiece and a multipurpose bur (Dentsply Maillefer, Tulsa, OK) using air and water spray. The length of all roots was prepared approximately 16 mm from the coronal surface to the apex of the root. An operating microscope (Carl Zeiss Surgical, Inc., Thornwood, NY) was used to inspect the roots for cracks under >5 magnification. The working length was established with #15 K-file (Kerr, Romulus, MI) 0.5 to 1 mm short of the minor foramen. The #15 K-file was passed through the apical foramen of the canal before and after instrumentation to ensure patency. A total of 15 ml of 1.25% NaOCl was used for irrigation between instruments with a syringe and a 27-gauge Monoject endodontic irrigation needle (Sherwood Medical, St. Louis, MO). Five milliliters of 17% EDTA rinses were used during and after instrumentation to remove the smear layer and decrease coronal leakage.

The instrumentation was performed with a crown-down technique in the coronal two thirds of the root canal with Profile Series 29 .04 Taper nickel-titanium rotary instruments (Dentsply Tulsa Dental, Tulsa, OK) and preparation of the apical third with .04 Taper nickel-titanium hand instruments (Dentsply Tulsa Dental) to an ISO size 50. The root canals were dried with sterile paper points (Patterson Dental Supply, Inc., St. Paul, MN) before filling.

The roots were randomly divided into 8 groups of 15 roots each and 3 control groups as follows.

Group 1. Lateral condensation of gutta-percha with AH 26 sealer (S. mutans; 15 roots). These roots were filled with gutta-percha and AH 26 sealer (Dentsply Maillefer) using a cold lateral condensation technique. An ISO size 50 master gutta-percha cone (Kerr) was coated with AH 26 sealer and placed into the root canal to working length. A size 30 finger spreader (Dentsply Maillefer) was then inserted into the canal to a level approximately 1 mm short of working length. Lateral condensation with fine accessory gutta-percha cones (Kerr) was performed until the root canal was filled. Sticky wax was softened in an open flame and painted over the root surface to seal it except the apical 2 mm and coronal orifice, which were left free of sticky wax. S. mutans was used to test leakage.

Group 2. Vertical condensation of gutta-percha with AH 26 sealer (S. mutans; 15 roots). A gutta-percha master cone (Obtura Spartan, Fenton, MO) was fitted apically and vertically thermoplasticized using a continuous wave of condensation technique (System B, Analytic Endodontics, Orange, CA). A backfill with Obtura II (Obtura Spartan) gutta-percha was performed. AH 26 sealer was used with the placement of the master cone. Sticky wax was prepared as in group 1. S. mutans was used to test bacterial leakage.

Group 3. Lateral condensation of gutta-percha with Epiphany sealer (S. mutans; 15 roots). Roots were prepared the same as group 1, but Epiphany sealer was applied with a lentulo spiral filler (Dentsply Maillefer). S. mutans was used to test bacterial leakage.

Group 4. Vertical condensation of gutta-percha with Epiphany sealer (S. mutans; 15 roots). Roots were prepared the same as group 2, but Epiphany sealer was used. S. mutans was used to test bacterial leakage.

Group 5. Lateral condensation of Resilon with Epiphany sealer (S. mutans; 15 roots). After instrumentation, a self-etching primer (Epiphany Primer; Pentron Clinical Technologies) was placed into the canal with a thin needle. Excess primer was then removed with paper points (Patterson Dental Supply, Inc.). Roots were filled with lateral condensation of Resilon and Epiphany sealer. Sticky wax was prepared as in group 1. S. mutans was used to test bacterial leakage.

Group 6. Vertical condensation of Resilon with Epiphany sealer (S. mutans; 15 roots). The roots were prepared with the primer as in group 5. Roots were filled with a master cone of Resilon and Epiphany sealer using the continuous wave of condensation (System B) technique and backfilled with Resilon in an Obtura II gun (Obtura Spartan). Sticky wax was prepared as in group 1. S. mutans was used to test bacterial leakage.

Group 7. Lateral condensation of Resilon with Epiphany sealer (E. faecalis; 15 roots). Roots were prepared the same as group 5 except E. faecalis was used to test leakage.

Group 8. Vertical condensation of Resilon with Epiphany sealer (E. faecalis; 15 roots). Roots were prepared the same as group 6 except E. faecalis was used to test leakage.

Positive control filled with Resilon (12 roots). Roots were filled with representative samples of techniques and bacteria but without sealer. Sticky wax was prepared as in group 1.

Positive control filled with gutta-percha (12 roots). Roots were filled with representative samples of techniques and bacteria but without sealer. Sticky wax was prepared as in group 1.

Negative control (12 roots). Roots were filled with representative techniques, materials, and bacteria but without sealer. Sticky wax completely covered the surface of the root as well as the canal orifice coronally.

Teeth were stored in gauze that was dampened with storage medium, enclosed in sealed tubes, and placed in an incubator for 14 days at 37°C to allow the sealer to set. The microbial leakage
model consisted of an upper chamber and a lower chamber as described by Torabinejad et al. (14). 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.

**Groups Tested with Streptococcus mutans**

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 sheling.

*S. mutans* (ATCC 10449) was grown in 10 ml of WC broth during 24 h. The bacteria were identified using selective media and morphology, i.e. Gram-stained on colonies harvested from MSB (mitis/salivarius/bacitracin) agar plate. After 48 h, *S. mutans* ATCC 10449 was added to 10 ml of WC broth adjusting the optical density to 0.2 OD_{660} 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 for a change in color of the pH indicator from red to yellow, which indicated a positive result (metabolism as acid production), every change in color of the pH indicator from red to yellow, which added 2 ml of an overnight culture of streptomycin-resistant streptomycin in the lower chamber. To the upper chamber was reach approximately 3 mm into 3 ml sterile TSB with 2 mg mL⁻¹ streptomycin. The bacteria and medium in the upper chamber were replaced with freshly grown cultures twice weekly. Bacteria penetrating along the root filling were detected by turbidity observed in the lower chamber. On observation of turbidity in the lower chamber, the seal was broken, and the nature and purity of the organism growing there confirmed by Gram stain, cultural morphology, and streptomycin resistance.

**Groups Tested with Enterococcus faecalis**

*E. faecalis*, strain A197A, was adapted to and maintained on TSB with 2 mg mL⁻¹ streptomycin and used as a test organism. The filled root specimens were aseptically mounted with sticky wax to conical 15-ml polycarbonate centrifuge tubes that had the tip portion cut off to accommodate the coronal face of the specimens. The wax sealed off the bottom part of the tube, which then served as the upper chamber in the final mount, in which the tube was introduced into and sealed to the neck of a flat-bottomed 20-ml, clear, scintillation vial. The tip of the root was mounted to reach approximately 3 mm into 3 ml sterile TSB with 2 mg mL⁻¹ streptomycin in the lower chamber. To the upper chamber was added 2 ml of an overnight culture of streptomycin-resistant *E. faecalis* in TSB with 2 mg mL⁻¹ streptomycin. The bacteria and medium in the upper chamber were replaced with freshly grown cultures twice weekly. Bacteria penetrating along the root filling were detected by turbidity observed in the lower chamber. On observation of turbidity in the lower chamber, the seal was broken, and the nature and purity of the organism growing there confirmed by Gram stain, cultural morphology, and streptomycin resistance.

### Scanning Electron Microscope Preparation

At completion of the leakage study, one Resilon and one gutta-percha specimen was randomly chosen. Each 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. Specimens were viewed with a JEOL JEM 6300 scanning electron microscope (Tokyo, Japan) at 15kV accelerating voltage. Images were digital.

The investigators examining leakage during the 30 days were blinded to all groups. Statistical analysis was performed using the Kruskal-Wallis test for nonparametric data to determine whether there were significant differences between groups. Pairs of groups were compared using the Mann-Whitney *U* test (p < 0.05).

### RESULTS

One specimen in group 3 and one in group 4 (groups with gutta-percha fillings and Epiphany sealer) were contaminated during the study and were discarded. The median, minimum, and maximum days of leakage and mean rank for each leakage group are shown in Table 1. Statistical analysis using the Kruskal-Wallis test revealed significant differences among the experimental groups (p < 0.05). The Mann-Whitney *U* test was used to compare the respective groups, and the Resilon groups were found to be superior to the gutta-percha groups with respect to the number and rate that the specimens in each group leaked (p < 0.05).

All but one positive control leaked within 24 h (one specimen from the Resilon group), whereas none of the negative controls leaked. Resilon showed minimal leakage (group 8: 7% or one leakage; groups 5–7: each with 13% leakage or two leakages), which was significantly less than gutta-percha, in which 73% to 93% of specimens with either sealer or technique leaked (Fig. 1). All Resilon and Epiphany sealer groups leaked significantly less than all groups in which AH 26 was used as a sealer (gutta-percha and Resilon and AH 26).

There was no statistical difference in leakage between Resilon groups using *S. mutans* and *E. faecalis* (groups 5–8). However, Resilon groups with *E. faecalis* leaked earlier than Resilon groups with *S. mutans* (Table 1).

### SEM Findings and Figures

The dentin-filling interface of the longitudinal sections showed a uniform gap in the gutta-percha specimen (Fig. 2). This gap was approximately 10 micrometers wide and was between the resin sealer that penetrated the dentin (resin tags) and the gutta-percha filling (Fig. 3). There was no gap at low-power SEM between the Resilon filling and the dentin (Fig. 4). A decalcified specimen at high-power SEM (Fig. 5) showed no gap between the Resilon and Epiphany sealer, and also showed the resin tags that had penetrated the dentin.

### DISCUSSION

Although the potential for an extremely high success rate is accepted for endodontic procedures (22), population studies indicate that in most metropolitan areas, success is achieved in approximately 50% of cases (7, 23). Thus, there is great room for
improvement. Conceptually, endodontic therapy is very simple—after optimal cleaning of the canal, a filling material should be placed that will entomb remaining bacteria and block additional bacteria from entering from the oral cavity.

Techniques are available to ensure that the practitioner is able to clean the canal to a level that will ensure a very high degree of success (22). However, filling of the canal with gutta-percha even by the most technically proficient operator will not result in a seal that is dependable during the long term. In fact as stated earlier, the coronal restoration is more likely the reason for our success during the long term than the gutta-percha fill. Few would argue that gutta-percha should be replaced by a material that better seals the canal.

In this study, *S. mutans* and *E. faecalis*, which are facultative anaerobic bacteria, were used to test leakage through the root-canal system. Facultative bacteria are predominant in failed previously treated canals (24–26), with the most frequently isolated bacteria being enterococci (27). Thus, these bacteria seem relevant to clinical practice.

As shown in numerous in vitro studies, gutta-percha fillings leak at an alarming rate (11, 12, 14, 15). Thus, it would be advantageous to replace it with a filling material that prevents leakage at all levels of the root-canal system.

Resilon, a thermoplastic synthetic polymer-based root canal filling material, has been developed, which has the same handling properties of gutta-percha. Based on polymers of polyester, Resilon contains bioactive glass, bismuth oxychloride, and barium sulfate. The overall filler content is approximately 65% by weight. Epiphany sealer is a dual curable dental resin composite sealer. The resin matrix is a mixture of BisGMA, ethoxylated BisGMA, UDMA, and hydrophilic difunctional methacrylates. It contains fillers of calcium hydroxide, barium sulfate, barium glass, and silica. The total filler content in the sealer is approximately 70% by weight. Forty seconds of light will cure the coronal 2 mm of the canal, whereas the entire filling will self-cure in approximately 15 to 30 min. Unlike previous resin filling materials that could not be effectively removed from the canal (16, 18), Resilon can be softened and dissolved like gutta-percha with solvents like chloroform.

In this study as in previous ones, gutta-percha allowed bacterial penetration in a high proportion of cases (8, 11, 14). The Resilon groups with self-etch primer and resin sealer resisted bacterial penetration to both test bacteria. In addition, lateral condensation

### Table 1. Minimum, median and maximum days at which microleakage occurred and the mean rank of comparisons among the experimental groups (p < 0.05)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Minimum (days)</th>
<th>Median</th>
<th>Maximum (days)</th>
<th>Mean Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lateral GP-AH26 sealer (<em>S. mutans</em>)</td>
<td>15</td>
<td>4</td>
<td>8</td>
<td>31</td>
<td>35.50&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Vertical GP-AH26 sealer (<em>S. mutans</em>)</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>31</td>
<td>47.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3. Lateral GP-Epiphany sealer (<em>S. mutans</em>)</td>
<td>14</td>
<td>4</td>
<td>5</td>
<td>31</td>
<td>29.00&lt;sup&gt;0&lt;/sup&gt;</td>
</tr>
<tr>
<td>4. Vertical GP-Epiphany sealer (<em>S. mutans</em>)</td>
<td>14</td>
<td>3</td>
<td>6</td>
<td>31</td>
<td>27.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5. Lateral Resilon-Epiphany sealer (<em>S. mutans</em>)</td>
<td>15</td>
<td>12</td>
<td>31</td>
<td>31</td>
<td>83.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6. Vertical Resilon-Epiphany sealer (<em>S. mutans</em>)</td>
<td>15</td>
<td>12</td>
<td>31</td>
<td>31</td>
<td>83.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7. Lateral Resilon Epiphany sealer (<em>E. faecalis</em>)</td>
<td>15</td>
<td>8</td>
<td>31</td>
<td>31</td>
<td>83.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8. Vertical Resilon-Epiphany sealer (<em>E. faecalis</em>)</td>
<td>15</td>
<td>5</td>
<td>31</td>
<td>31</td>
<td>83.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Statistical differences are expressed by different letters. The larger the number in minimum, median, maximum, and mean rank the less the microleakage. The number 31 represents no leakage.

Fig 1. Percentage of specimens in each group that showed leakage during 30 days. Roots filled with Resilon™ and Epiphany™ sealer leaked significantly less than all other roots (p < 0.05).
and vertical condensation of softened Resilon were equally effective in resisting bacterial penetration.

The amount of leakage between S. mutans and E. faecalis was not statistically different through the Resilon filling. Only one specimen leaked in vertical condensation of Resilon using E. faecalis, whereas the other three Resilon groups had two leakages each.

Both positive controls (Resilon or gutta-percha groups) without sealer leaked within 24 h. We specifically tested Resilon without sealer, because some have suggested that these materials will adapt so closely to the canal wall that sealer may not be necessary. The results show, as with gutta-percha, the sealer is critical for the seal provided by this new material.

Seventeen percent EDTA was used during and after instrumentation to remove the smear layer and decrease coronal leakage (28). The adherence of the sealer to the dentin walls is a function of smear-layer removal (Figs. 3 and 5). High bond strengths cannot be achieved unless the smear layer is removed (29). The bond of the smear layer to the underlying dentin is relatively weak, approximately 5 MPa (28), and cannot withstand the shrinkage associated with the curing of resins. The resins pull the smear layer from the dentin and provide an avenue for microleakage.

In addition to 17% EDTA, Epiphany Primer was applied to the dentin walls of the root canals that were to be filled with Resilon. Epiphany Primer is self-etch primer that contains sulfonic acid terminated functional monomer, HEMA, water, and polymerization initiator. The preparation of the dentin through these chemical agents may prevent shrinkage of the resin filling (Fig. 4) away from the dentin wall and aid in sealing the roots filled with Resilon (Fig. 1; Table 1).

The excellent sealing capability of Resilon may be attributed to the “mono-block” which is created by the Resilon filling closely adapting to the Epiphany sealer and in turn the Epiphany sealer adhering to the dentin walls. In contrast, the high-power SEM micrograph showed how the gutta-percha filling pulled away from the AH 26 sealer, whereas the sealer remained against the dentin wall with its resin tags penetrating the dentin tubules (Fig. 3). This gap (Figs. 2 and 3) between gutta-percha and sealer may create an avenue for microleakage, which may explain the rapid rate of
leakage in the gutta-percha specimens (Table 1). Thus, the seal between the core material and the sealer seems to be critical for the resistance to leakage seen in the Resilon/Epiphany groups.

It should be appreciated that in this experimental model even very minimal penetration of bacteria into the lower chamber broth will result in a “failure” when the bacteria multiply in the medium. This minimal leakage may be easily handled by the body’s defenses without a clinical failure resulting. Therefore, it is not possible to make a direct correlation between these results and the in vivo situation (21). However, because such overwhelming evidence exists of the poor performance of gutta-percha as a filling material, the vast superiority shown by the new material in this study is promising and should be tested in in vivo models.

Dr. Trope has a financial relationship with the manufacturer of Resilon™.

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References