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# Ultrastructural Evaluation of the Apical Seal in Roots Filled with a Polycaprolactone-Based Root Canal Filling Material

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## Abstract TOP

This in vitro study compared the ultrastructural quality of the apical seal achieved with Resilon/Epiphany and gutta-percha/AH Plus. Single-rooted extracted human teeth were prepared using a crown-down technique, debrided with NaOCl and EDTA, and obturated with either Resilon/Epiphany or gutta-percha/AH Plus. They were examined for gaps along canal walls using SEM, and for apical leakage using a transmission electron microscopy (TEM). SEM revealed both gap-free regions, and gap-containing regions in canals filled with both materials. TEM revealed the presence of silver deposits along the sealer-hybrid layer interface in Resilon/Epiphany, and between the sealer and gutta-percha in the controls. It is concluded that a complete hermetic apical seal cannot be achieved with either root filling materials.

**Article Outline**



**Figures/Tables**



Improvements in adhesive technology have fostered attempts to reduce apical and coronal leakage by bonding to root canal walls. Total-etch adhesives have been tested with resin cements as alternative root filling materials (1, 2). Those results showed that dentin adhesives significantly reduced apical leakage. Self-etching primers have also been used for bonding to root canal dentin (3-5). As epoxy resin sealers do not copolymerize with metharcrylate resin-based adhesives, a methacrylate resin sealer was developed with a self-etching primer that resulted in improvements in the apical seal (6) and adhesion to root dentin (7).

Recently, a new thermoplastic, filled polymer (Resilon; Resilon Research LLC, Madison, CT) has been introduced that has the potential to challenge gutta-percha as a root filling material (8, 9). The thermoplasticity of Resilon is because of polycaprolactone (10), a biodegradable polyester with a moderately low melting point (11), while its bondability is derived from the inclusion of resin with methacryloxy groups. This filling material also contains glass fillers, and barium chloride as fillers, and is capable of coupling to resin sealers, an example of which is Epiphany (Pentron Clinical Technologies, Wallingford CT). Epiphany Root Canal Sealant is a dual-curable resin composite containing a new redox catalyst (12), that enables optimal auto-polymerization under acidic environments (13, 14).

As there is no single, universally accepted model of leakage testing in endodontics (15), the objective of this study was to examine the ultrastructural quality of the apical seal in Resilon filled versus gutta-percha filled root canals that were created with the same compaction technique, using a silver tracer penetration protocol that has been employed in leakage evaluation of adhesive-bonded coronal dentin (16).

## Methods TOP

Twenty-four single-rooted extracted human teeth were collected under a protocol reviewed and approved by the IRB of the Medical College of Georgia. The teeth were stored in a 0.5% chloramine T at 4°C and were used within 1 month after extraction.

### Experimental Design TOP

Access cavities were prepared under an operating microscope using tapered diamond burs in a high-speed handpiece under water cooling. Canal length was determined by passing a size #15 Flex-o-file (Dentsply Maillefer, Tulsa, OK) into the root canal until its tip was visible from the apical foramen. The working length was established 1-mm short of the apex. Instrumentation was performed with a crown-down technique, using Profile nickel-titanium rotary instruments (Dentsply Maillefer). All canals were prepared to ISO size 35, 0.06 taper. Canal patency was maintained with an ISO size 15 Flex-o-file. The canal was irrigated between each instrument with 3 ml of 2.6% sodium hypochlorite (NaOCl) using a 27-gauge irrigation needle. Irrigant was seen passing through the patent foramen. Three milliliters of 17% EDTA was used for 1 min after the completion of instrumentation and the final rinse of NaOCl, followed by 3 ml of distilled water for 1 min because of root canal obturation. The root canals were dried with multiple paper points and randomly divided into two groups of 10 teeth each.

### Group 1: Warm Vertical Compaction of Resilon with Epiphany Sealer TOP

Epiphany self-etching primer was introduced into the root canal with a micro-brush and excess primer was removed with paper points. A nonstandardized Resilon master cone was tried-in to within 1 mm of working length. Epiphany sealer was then placed into the root canal using a lentulo spiral, and the Resilon root canal filling material was downpacked using the continuous wave condensation technique (System B, SybronEndo, Orange CA) at a reduced temperature of 150°C and a power setting of 10 as recommended by the manufacturer. Backfilling was performed with Obtura II (Spartan, Fenton, MO) using 23 gauge needle tips at a temperature of 140°C. After backfilling, the coronal surface of the root filling was light-cured for 40 s with a LED light-curing unit (L.E. Demetron 1, Sybron Kerr) to polymerize the surface of the dual-cured methacrylate sealer.

### Group 2: Warm Vertical Compaction of Gutta-Percha with AH Plus (control) TOP

A nonstandardized gutta-percha master cone (DiaDent) was tried-in to within 1 mm of the working length. AH Plus resin root canal sealer (Dentsply Maillefer) was placed into the root canal with the master gutta-percha cone. Root canals were obturated using the continuous wave condensation technique at 200°C and then backfilled.

After obturation of the root canals, access cavities were restored with a total-etch dentin adhesive and hybrid resin composite. Following light-curing, restored teeth were stored in distilled water at 37°C for 24 hr.

### Environmental Scanning Electron Microscopy TOP

Two Resilon and two gutta-percha specimens were randomly chosen for environmental scanning electron microscopy (ESEM) examination. Each specimen was sectioned longitudinally to expose the filling-root dentin interface. The exposed surfaces were polished with 1200-grit silicon carbide paper, and brought into relief by etching with 10% phosphoric acid for 15 s and deproteinizing in 5% NaOCl for 10 min. After rinsing with water, the specimens were placed on the cooling stage of an ESEM (Philips XL-30 ESEM-FEG; Eindhoven, The Netherlands) and examined wet and without coating at 20 kV.

### TEM TOP

The remaining ten teeth from each group were used to examine apical leakage using the silver tracer penetration technique (16). Postoperative radiographs were first taken of the teeth. They were then coated with nail polish except for the apical 2 mm of the root surface. After drying of the nail polish, the teeth were immersed in 50 wt% ammoniacal silver nitrate, at a pressure of 30 kPa (0.3 atm) for 3 hr (17).

The silver-impregnated teeth were rinsed and placed in photodeveloper to reduce the silver ions into metallic silver. One-half of each tooth was processed without further demineralization, and the other half was completely demineralized in EDTA. Specimens were fixed, dehydrated, and embedded in epoxy resin (18). There were 90-nm thick sections containing areas with or without leakage prepared using an ultramicrotome. Undemineralized sections were examined without further staining. Demineralized sections were stained with uranyl acetate and lead citrate. All sections were examined using a TEM (Philips EM208S) at 80 kV.

## Results TOP

FE-ESEM revealed excellent coupling of Resilon to Epiphany sealer, despite the presence of both gap-free (Fig. 1 *A*) and gap-containing (Fig. 1 *B*) regions along the sealer-dentin interface within the same tooth. Profuse resin tag formation was evident in gap-free regions (Fig. 1 *A*), but resin tags were either sparse or completely absent in the gap-containing regions (Fig. 1 *B*). Likewise, both gap-free (Fig. 1 *C*) and gap-containing regions (Fig. 1 *D*) could be identified in control gutta-percha specimens. Separation of the gutta-percha from AH Plus sealer was frequently observed (Fig. 1 *D*).

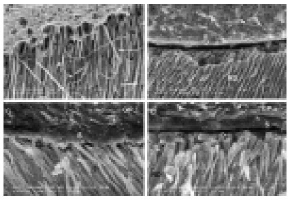

**Figure 1.** FE-ESEM micrographs of wet, nondehydrated sectioned specimens of vertical condensation filled roots, showing the variability of gap formation. (*A*) A specimen that was filled with Resilon bonded with the self-etching resin system Epiphany, taken at a level 4 mm from the apex. No gap could be seen between the filled resin sealer (S) and the root dentin. Numerous resin tags (pointer) extended into the dentinal tubules. (*B*) Another region from the opposite side of the Resilon/Epiphany-filled root canal, taken 4 mm from the apex. A gap (arrow) could be seen between the S and the root dentin (RD). No resin tags could be identified in dentinal tubules. (*C*) A control specimen that was filled with gutta-percha (GP) and AH Plus, taken at a level 4 mm from the apex. No gap could be discerned between the sealer (S) and the root dentin. Numerous resin tags (pointer) were present. (*D*) Another region from the same tooth, showing separation of the GP from the sealer S, and the presence of a gap (arrow) between the sealer and the RD, despite the presence of resin tags (pointer).

TEM micrographs of Resilon confirmed the presence of electron-dense fillers and bioactive glass fillers within the polymer matrix (Fig. 2 *A*). The latter could be identified by the presence of a siliceous hydrogel layer (19) around the periphery of the particles (Fig. 2 *B*).

**Figure 2.** A TEM micrographs of the Resilon obturating material. (*A*) Highly electron-dense fillers (F) and less electron-dense bioactive glass fillers (G) were present within the polyester matrix. (*B*) At a higher magnification, a hydrogel layer (H) could be seen along the periphery of the bioactive glass. (*C*) Radiograph and longitudinal section of a representative specimen that was filled with Resilon/Epiphany and then immersed in a 50 wt% ammoniacal silver nitrate trace solution to examine leakage within the filled root canal. (D) Undemineralized, unstained TEM micrograph taken at a level of 6 mm from the apex, from the region labeled A in *C* where a seal was apparent (i.e. without visible silver deposits). A 1 to 2 µm thick, hybrid layer (H) was created by the self-etching primer along the surface of the root dentin (RD). The dentinal tubules were patent, and contained some of the smaller filler particles of the resin sealer (S) in the tubular orifices (pointer). The rest of the tubules were occupied by the unfilled self-etching primer (asterisks), forming resin tags that corresponded with those depicted in Fig. 1A. (*E*) The corresponding demineralized, stained TEM section, confirming the presence of a stained hybrid layer on the dentin surface (H) and along the periphery of the dentinal tubules (arrow). The filled resin S extended into the patent tubules (pointer), blending with the primer (asterisk) to form resin tags. RD: laboratory demineralized root dentin. (*F*) A high magnification view of Fig. 2E, showing the presence of normal banded collagen fibrils (arrow) immediately beneath the stained hybrid layer.

After immersion of the filled roots in $AgNO_3$, leakage was observed in 9 of 10 Resilon-filled, and all gutta-percha-filled root canals. The leakages in both groups were confined to the apical 4 mm, and occurred on either one or both sides of the root fillings. Apical leakage observed in longitudinal sections of the same tooth is shown in Fig. 2 *C*. Heavily filled Epiphany resin sealer was seen along the patent dentinal tubular orifices, blending with the unfilled, self-etching primer beneath to form resin tags (Fig. 2 *D*). Silver was absent from bonded interfaces, and a 1 to 2 µm thick hybrid layer was observed along the root dentin surface as well as around dentinal tubules. The existence of the hybrid layer around the periphery of dentinal tubules was confirmed in demineralized, stained sections (Fig. 2 *E*). Banding of collagen fibrils beneath the hybrid layer indicated that they were not denatured by the heat generated during warm vertical compaction (Fig. 2 *F*).

Undemineralized, unstained TEM sections from leakage-exhibiting regions of the Resilon-filled root canals revealed heavy silver deposits between the methacrylate resin sealer and root dentin. In areas that exhibited severe leakage, continuous bands of silver were present along the interface. When no resin tags were present, the dentinal tubules were also filled with silver deposits (Fig. 3 *A*). In areas that exhibited moderate leakage, discontinuous bands of silver were present along the interface, with dentinal tubules occupied by heavily filled resin sealer (Fig. 3 *B*).

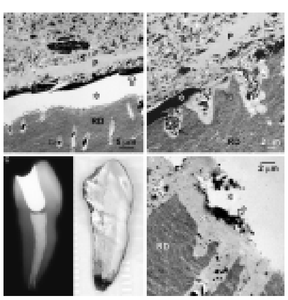

**Figure 3.** Undemineralized, unstained TEM micrographs illustrating the variability of silver nitrate leakage in the Resilon/Epiphany and gutta-percha/AH Plus specimens. (*A*) A section taken from the region labeled B in Fig. 2C the Resilon/Epiphany filled specimen in *A*, where leakage was visible to the naked eye in the form of silver deposits. A thick band of silver (open arrow) was present between the sealer (S) and the root dentin (RD). The section separated during ultramicrotomy, resulted in an empty space (asterisk). No resin tags were present and the dentinal tubules were filled with silver deposits (open arrowhead). A layer of unfilled primer (P) was observed within the bulk of the filled resin sealer. (*B*) Another section of the Resilon/Epiphany filled specimen, taken from the region labeled C in Fig. 2C. There was partial bonding (pointer) and partial gap formation (asterisks), despite the penetration of adhesive S into the dentinal tubules (arrow). Such nonuniform bonding suggests a three-dimensional leakage pattern within the filled root canal. P: unfilled primer. (*C*) Postoperative radiograph and longitudinal section of a gutta-percha filled root canal showing the occurrence of leakage along the cement-dentin interface (open arrowheads). (*D*) TEM micrograph of the region indicated by the left open arrowhead in *C*. The unbonded gutta-percha separated from the section during ultramicrotomy, leaving behind an empty space (asterisk). Silver remnants (open arrow) were present on top of the sparsely filled endodontic sealer S. The sealer penetrated the patent dentinal tubules to form resin tags. There was no hybrid layer on the surface of such control RD.

Apical leakage observed in a longitudinal section of the same tooth is shown in Fig. 3 *C*. Undemineralized, unstained sections showed leakage between epoxy resin sealer and unbonded gutta-percha (Fig. 3 *D*). No hybrid layer was present and resin tags were observed in dentinal tubules that consisted of the sparsely filled epoxy resin sealer.

## Discussion TOP

Examination of fully hydrated specimens by ESEM is essential for differentiating genuine gaps between root filling and dentin that are susceptible to leakage of microorganisms and antigens, from potential artifactual gaps created after vacuum desiccation in conventional SEMs (20). Although it is possible to illustrate gap-free regions from selective regions of the filled canal walls, the overall ESEM and TEM results indicated that creation of a consistent hermetic apical seal in the apical 4 mm of the root canals was not realized for either filling material. This highlights the importance of establishing a coronal seal after endodontic treatment (21, 22). An immediate coronal seal may be created in Resilon-Epiphany by light-curing the dual-curable sealer.

There was excellent coupling of Resilon to Epiphany sealer, unlike gutta-percha, where there was no chemical adhesion to AH Plus sealer. While the goal of creating a monobloc of material between a root canal filling material and a sealer certainly does have merit (23, 24), this goal may be prevented by different weak links in the two filling systems that can only be observed at the TEM level. In gutta-percha-filled canals, the weak link occurred between gutta-percha and sealer, leaving an intact layer of sealer on the surface of dentin and within dentinal tubules (Fig. 3 *D*).

Unlike gutta-percha specimens, the weak link in Resilon-filled root canals resided along the sealer-dentin interface. These separations were not caused by the inability of self-etching primer to reach the apical portion of the canals, as a layer of unfilled primer was clearly evident (Fig. 3 *A*). The self-etching primer was applied to the root canal after using EDTA and water as the final rinse, thus minimizing the compromising effect of NaOCl on primer/resin sealer polymerization (25). These gaps were probably created by rapid polymerization contraction of the methacylate-based resin sealer. Root canals have high cavity configuration factors that contribute to polymerization stresses created by resin-based materials along root canal walls (26). Polymerization of the Epiphany sealer may also be promoted by heat generated during continuous wave-warm vertical compaction, preventing relief of polymerization stresses by slow flow. The manufacturer's instruction for immediately light-curing the coronal root filling to create a coronal seal may also limit flow of resin sealer for stress relief (27). Moreover, manipulation of the partially polymerized sealer during condensation may disrupt developing bonds between self-etching primer and root dentin.

It may be concluded that the quality of apical seal achieved with the new polycaprolactone-based root filling material and methacrylate-based sealer is not superior to gutta-percha and a conventional epoxy-resin sealer. Another area of concern is the biodegradability of Resilon, in the event that a hermetic seal is not established. Whereas gutta-percha is a relatively inert material, polycaprolactone is biodegradable under microbial attack (11). It is well known that lipases released by bacteria can cleave the ester bonds of polycaprolactone (28-30). Further studies should be performed to confirm the hydrolysis of Resilon by salivary/bacterial enzymes.

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