# Efficacy of Two Contemporary Single-cone Filling Techniques in Preventing Bacterial Leakage

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## Abstract

This in vitro study evaluated the sealing efficacy of three root-filling systems/techniques in preventing bacterial leakage. Instrumented single-rooted root segments were filled with (1) warm vertical compaction with gutta-percha/AH Plus; (2) single-cone technique with ActiV GP; and (3) single-cone technique with Gutta-Flow. A dual-chamber leakage model using *S. mutans* as a microbial marker was used for leakage evaluation. Bacterial penetration was monitored over a 100-day period. Leakage was recorded when turbidity was observed in the lower chamber. Gutta-percha warm vertical compaction exhibited the best seal with bacterial leakage observed in only 16.7% of the specimens between 59 and 100 days. All ActiV GP specimens leaked between 7 and 100 days; 50% of the Gutta-Flow specimens leaked between 22 and 100 days. The two contemporary single-cone techniques did not insure a durable apical seal against bacterial leakage. A warm vertical compaction technique using thermoplasticized gutta-percha and AH Plus sealer appears to be more effective in minimizing bacterial leakage. *(J Endod 2007;33:310–313)*

## Key Words

ActiV GP, AH Plus, bacterial leakage, coronal seal, Gutta-Flow, single-cone

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Filling of root canals optimally in three dimensions after cleaning and shaping is paramount in preventing re-infection of the root canal space (1–6). Single-cone techniques performed with conventional sealers have been perceived to be less effective in sealing root canals than the gutta-percha warm vertical compaction technique (7). However, noncompaction, single-cone filling of root canals has recently been revived (8, 9) with the introduction of greater taper master cones that closely match the geometry of nickel–titanium instrumentation systems (10). The advent of contemporary root canal sealing systems that claim to create bonds along the sealer–gutta-percha interface by modifications of the sealer or the root-filling material may also support the use of a single-cone obturation technique (11). Limited information, however, is available on the sealing quality of these new single-cone root fillings as compared with that of gutta-percha warm vertical compaction.

Although the use of dyes, radioisotopes, fluid filtration, bacteria, and endotoxin penetration techniques have been used to evaluate the seal of endodontic materials (12–14), the bacteria leakage model has been advocated as a more clinically relevant model (15). Thus, the purpose of this study was to compare the apical seal of two new single-cone filling systems vs. a gutta-percha warm vertical compaction technique using a bacterial leakage model. The null hypothesis tested was that there are no differences in the apical seal of root canals filled with the three endodontic filling techniques.

## Materials and Methods

Fifty-six extracted human maxillary anterior teeth, stored at 4°C in 0.9% sodium hypochlorite (NaCl) containing 0.02% $NaN_3$ to prevent bacterial growth, were decoronated to obtain approximately 17-mm long root segments. The experimental design consisted of three experimental groups ($n = 12$). Three root segments were used for each of the initial positive and negative control groups. An additional 14 roots were used as subsequent weekly positive controls to ensure the viability of the bacteria inoculation during the 100-day period of bacterial leakage evaluation. Cleaning, shaping, and filling were performed under an operating microscope (Global Surgical Corp., St. Louis, MO, USA). Each experimental group contained 25% round canals and 75% oval-shaped canals, as determined by the use of bucco-lingual and mesio-distal radiographs, and examination under the operating microscope. Radiographs were also used for instrumentation length determination and quality control of the root fillings. Canal patency was achieved with an ISO #15 Flex-o-file (Dentsply Maillefer, Ballaigues, Switzerland). Instrumentation was performed to 0.5 mm short of the radiographic apex with a crown-down technique using EndoSequence 0.06 taper nickel titanium rotary instruments (Brasseler USA, Savannah, GA, USA) to ISO size #40. The root canals were alternately rinsed with 17% EDTA and 5 mL of 6.15% NaOCl between instrumentation, with the former used as the final rinse (10 mL) before filling of the instrumented root canals.

### Gutta-percha Warm Vertical Compaction Group

Each canal was filled with an epoxy resin–based sealer (AH Plus, Dentsply Caulk, Milford, DE, USA) and a prefitted gutta-percha master cone (EndoSequence 0.06 taper, Brasseler USA). The gutta-percha was down-packed with a System B heat source (SybronEndo, Orange, CA, USA) and the canal was back-filled using Obtura II (Spartan

Fenton, MO, USA). The canal orifice was filled with a provisional restoration (Cavit, 3M ESPE, Seefeld, Germany).

### ActiV GP Single-Cone Group

Each canal was fitted with a single ISO size #40, 0.06 taper, ActiV GP (Brasseler USA) glass-ionomer filler-coated gutta-percha master cone. The master cone was coated with the ActiV GP glass-ionomer sealer and slowly inserted into the canal to its working length. A layer of ActiV GP sealer was placed on top of the master cone to create a coronal seal, as recommended by the manufacturer. Each canal orifice was similarly filled with a Cavit provisional restoration.

### Gutta-Flow Single-Cone Group

Gutta-Flow sealer (Colténe-Whaledent, Altstätten, Switzerland) was triturated and dispensed into the root canal using the "Canal Tip" provided by the manufacturer. The polydimethylsiloxane-based Gutta-Flow sealer contains fine gutta-percha powder and nano-silver particles. Each canal was fitted with an ISO #40, 0.06 taper, standard Endo-Sequence non-glass-ionomer filler-coated gutta-percha master cone (Brasseler USA). The master cone was coated with the sealer and slowly inserted into the canal. Backfilling of the Gutta-Flow sealer was performed by reinserting the "Canal Tip" between the master cone and canal walls. Excess gutta-percha was removed with a heated instrument followed by the placement of a Cavit provisional restoration.

### Positive and Negative Controls

Seventeen roots were used as positive controls by filling the canal space with a single size #40, 0.06 taper master gutta-percha cone in the absence of any sealer. For the negative controls, three root segments were filled as in the gutta-percha warm vertical compaction group, dipped in molten sticky wax, and further covered with nail varnish.

Radiographic documentation was performed to ensure the quality of the root filling. The filled root segments were stored for 7 days at 37°C and 100% relative humidity to allow the sealers to set completely before leakage evaluation with a bacterial leakage model.

### Bacterial Leakage Evaluation

*Streptococcus mutans* (ATCC 25175) was cultured with a fresh sterile medium of Todd Hewitt broth (30 g/L; pH 7.4; Difco, Detroit, MI, USA). The culture (0.5 mL of bacteria in 10 mL of medium) was incubated at 37°C in the presence of $CO_2$ for 24 hours. After incubation, the culture was spiral-plated to ensure colony uniformity. Gram stain revealed Gram-positive cocci with visible chaining. Weekly additions of 1 mL of the bacteria culture to 40 mL of fresh medium were done and incubated overnight. This oral streptococci culture ($7.9 \times 10^8$ CFU/mL) was used to inoculate the upper chamber of the samples.

A 20-mL liquid scintillation vial (Fisher Scientific, Pittsburgh, PA, USA) with a polyethylene-lined plastic top was modified to create a dual-chamber device. A hole was created in the center of each plastic top for the fitting of a 1,000-$\mu$L disposable polyethylene pipette (Fisherbrand Redi-Tip, Fisher Scientific). The junction between the pipette and the hole was secured with epoxy-based glue and sealed with nail varnish.

The provisional restoration was removed from each root segment. The tip of each pipette was cut until it fit snugly to the coronal third of the filled root segment. The root/pipette interface was secured with sticky wax. Nail varnish was applied on the root surface to 2 mm from the apex. Each root/vial assembly was sterilized by a 12-hour cycle in an ethylene oxide gas sterilizer. After sterilization and degassing, 10 mL of sterile Todd Hewitt broth was aseptically placed into the vial to a level of nearly 2 mm above the root end (Fig. 1). The vials were incubated at 37°C for 24 hours. After inspection of the turbidity of the broth to ensure the sterility of the assemblies, 0.5-mL aliquots of the oral streptococci culture were used to inoculate the upper chambers. The chamber openings were covered with sterile Parafilm and the assemblies were incubated in $CO_2$ at 37°C for up to 100 days. The medium in each glass vial was monitored daily for signs of turbidity, which would indicate that leakage had occurred throughout the 17-mm length of the filled root canal. Two examiners, blind with respect to determining turbidity changes of the experimental groups, were designated for the turbidity assessment.

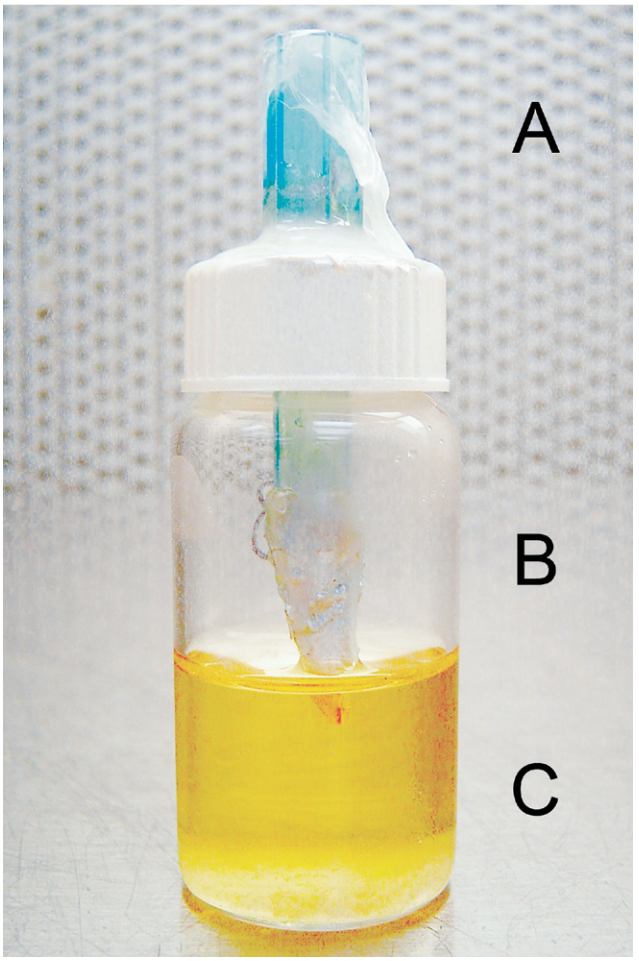


**Figure 1.** The dual-chamber bacterial leakage assembly used in the study. A modified scintillation vial with a disposable polyethylene pipette adapted around the root segment forms the upper chamber (*A*) of the assembly containing the bacterial suspension. Sticky wax and nail varnish covering the root surface except for the apical 2 mm of the root (*B*). The lower chamber (*C*) contains 10 mL of Todd Hewitt broth. The apical 2 mm of the root segment is suspended in Todd Hewitt broth.

Gram staining of the bacterial culture from both the lower and upper chambers was performed to avoid false positive results. The bacterial culture in the upper chamber was aseptically replaced with the use of disposable Pasteur pipettes every 7 days to ensure viability. A new positive control was added at weekly intervals ($n = 14$) to the remaining specimens under investigation to assess the viability of the freshly inoculated bacteria.

### Statistical Analysis

The Kaplan–Meier method was used to estimate "survival curves" for each experimental group; the "death" of a specimen was said to have occurred at the time at which bacterial leakage first became evident. The

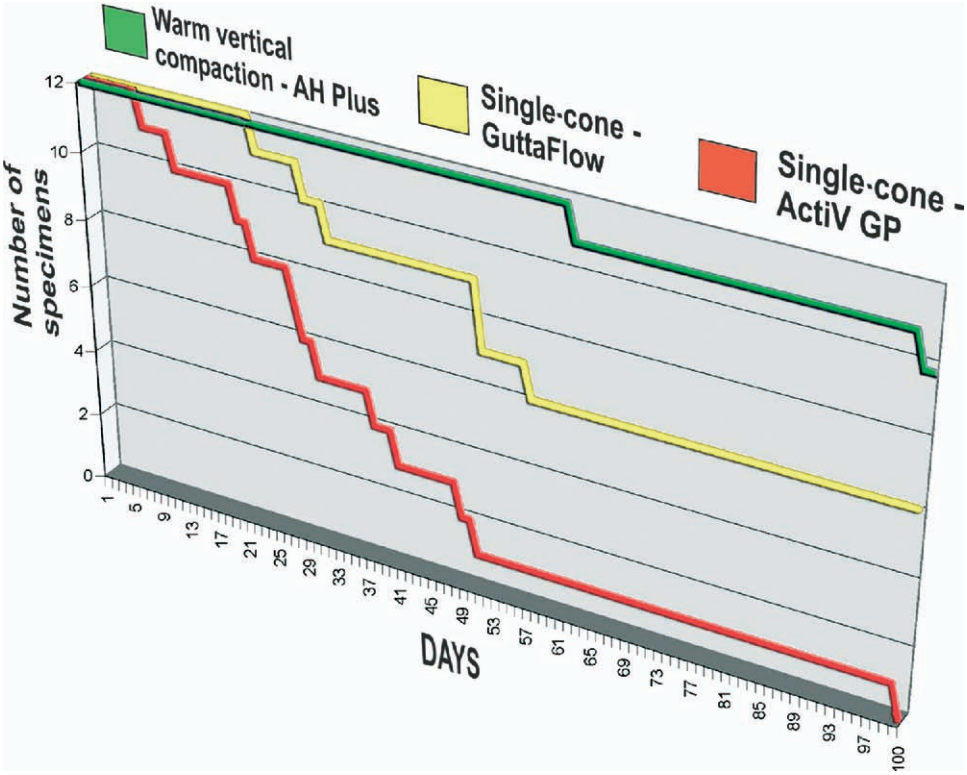


**Figure 2.** Bacterial leakage "survival curves" in the three experimental groups during a 100-day period. A specimen was classified as "dead" at the first appearance of bacterial leakage.

log-rank test was used to compare the survival curves of the three groups, using a significance level of 0.05. The Bonferroni method was used to account for multiple comparisons when comparing the survival curves of each pair of experimental groups, using a significance level of $0.05/3 = 0.0167$.

## Results

All positive controls showed turbidity in the lower chambers within 24 to 48 hours. No leakage was observed in the negative controls during the entire 100-day period. Significant differences were observed among the experimental groups ($p < 0.001$), with the gutta-percha warm vertical compaction group achieving better seal against bacterial leakage than both the ActiV GP ($p < 0.001$) and Gutta-Flow ($p < 0.001$) groups. The "survival curve" of Active GP did not differ significantly from that of Gutta-Flow ($p = 0.054$).

The gutta-percha warm vertical compaction group exhibited no leakage for the first 2 months. The first appearance of turbidity occurred at 62 days, with leakage observed in 2 of 12 specimens (16.6%) after 100 days. During the first 2 months, 11 of 12 of the specimens (91.6%) from the ActiV GP group leaked, with the first specimen leaking at 7 days. All specimens in the ActiV GP group leaked after 100 days. For the Gutta-Flow group, 6 of 12 specimens (50%) leaked within the first 2 months, with the first specimen leaking at 22 days. The number of leaked specimens remained at 50% until the end of the study at 100 days (Fig. 2).

## Discussion

In the absence of an intact coronal seal, re-contamination of the entire length of filled root canal by bacteria or their by-products may occur (16, 17). The bacterial leakage model used in this study is similar to that reported by Wolanek et al. (16). *S. mutans* was chosen because of its potential role in re-infecting filled root canals (18). The results of this study warrant rejection of the null hypothesis that there are no differences in the apical seal of root canals filled with the three endodontic filling techniques. The differences in bacterial leakage among the experimental groups may be attributed, in part, to the variation in sealer thickness in the three filling techniques (19). Because root shape may be an important factor in leakage of single-cone obturation techniques, equal percentages of round and oval-shaped canals were included in the three experimental groups. Although the penetration depths of heat carriers in a warm vertical compaction technique preclude optimal plasticizing of the gutta-percha master cones along the apical third of root canals (20, 21), a thinner sealer interface may be expected along the middle and coronal thirds of the canals when compared with single-cone techniques. Even with the advent of greater taper master cones that closely match the geometry of nickel–titanium rotary instruments in contemporary single-cone techniques (10), a larger volume of sealer should still be anticipated, given the existence of canal fins, anastomoses, and cul-de-sacs, and in the presence of oval-shaped canals (22).

The polydimethylsiloxane-based Gutta-Flow sealer is claimed to expand slightly during setting, enhancing its adaptation to root dentin walls (9). This phenomenon may be beneficial in the apical third of a root canal where the master cone has a better fit to the shaped canal. Although radiographic evidence of voids or incomplete filling were absent from all the tested specimens, the presence of microscopic voids (9) or gaps between dentinal walls and sealer in the presence of thicker sealer layers could have resulted in a less durable apical seal when this system was evaluated for bacterial leakage.

Endodontic sealers may deteriorate on exposure to oral fluids (23). Although glass-ionomer cements (GICs) bond chemically to dental hard tissues (24), they also exhibit rapid water sorption and leaching

of water-soluble components (25, 26). ActiV GP uses glass-ionomer filler-coated gutta-percha cones that, according to the manufacturer, are bondable to intraradicular dentin by the use of a glass-ionomer sealer, creating a "monoblock" within the root canal space (27). The results of this study indicate that such an objective was not successfully accomplished because bacterial leakage occurred as early as 7 days and all specimens leaked after 100 days. These results suggest that the ActiV GP sealer, like other self-curing glass-ionomer cements and resin composites, may have undergone shrinkage during its setting phase (28), creating gaps between the sealer and root dentin that permit rapid bacterial penetration. This issue may be the consequence of the unfavorable cavity geometry encountered in root canals (29). The presence of irregular-shaped canals that are not optimally filled with a single-cone approach provides another possible reason for rapid bacterial penetration (30).

Within the limits of this in vitro study, it may be concluded that the two contemporary single-cone root-filling techniques do not ensure a durable apical seal against bacterial leakage when compared to a gutta-percha warm vertical compaction technique. Different techniques for endodontic leakage evaluation often yield conflicting results (31). Wu et al. (32) demonstrated the lack of correlation between bacterial penetration and fluid transport in root canal fillings. Similarly, Barthel et al. (33) found no correlation between bacterial and dye leakage. Although a bacterial leakage model appears to be more clinically relevant compared with a fluid filtration model, it is not possible to directly correlate the amount of leakage to the clinical outcomes of endodontic treatments (34), in that the amount of leakage that is clinically significant to result in endodontic failure is unknown (35). Thus, the efficacy of contemporary single-cone filling endodontic techniques should be further evaluated in randomized clinical trials.

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