# An In Vitro Evaluation of Human Saliva Penetration in Obturated Root Canals with Different Pairs of Root Canal Filling Materials

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**ABSTRACT**

**Statement of Problem:** Gutta-percha presents no adhesion to the tooth structure and sealers. Ideally, it should be replaced by a material that offers better sealing in the entire length of the root canal.

**Purpose:** The aim of this study was to compare the coronal microleakage of a root canal filled with different pairs of Gutta-percha (GP), Resilon, Epiphany and AH26.

**Materials and Method:** For this study, 130 mandibular premolars were chosen. After decoronation and preparation of the root canals of the teeth, they were divided into four experimental groups with 30 teeth each and 2 control groups. Group one was obturated with GP and AH26 sealer, group two with GP and Epiphany sealer, group three with Resilon tips and Epiphany sealer, and group four with Resilon tips and AH26 sealer. All the groups were obturated, using cold lateral condensation. Micro-leakage was tested using a two-chamber bacterial method. The data were subjected to statistical analysis, using a Kaplan-Meier test.

**Results:** The bacterial microleakage test showed no significant difference between groups ( $p$ =0.1718).

**Conclusion:** It is concluded that, in vitro, the epiphany obturation system is as good as gutta-percha sealed with AH26 when compared over 90 days of saliva storage.

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## Introduction

Successful endodontic therapy depends on a complete chemomechanical preparation of the root canal system and a three-dimensional filling that provides a complete sealing of the spaces previously occupied by the dental pulp [1]. Because gutta-percha presents no adhesion to the tooth structure, ideally it should be replaced by a material that offers better sealing in all root thirds [1-2]. Epiphany obturation system is a new material that was developed to replace gutta-percha and traditional sealers for root canal obturation. The epiphany obturation system consists of three main items: the core material, the sealer, and its bonding agent.

Resilon core material is a thermoplastic synthetic polymer-based (polyester) root canal core material that contains bioactive glass, bismuth oxychloride, and barium sulfate. The sealant (Epiphany Root Canal Sealant, Real seal Root Canal Sealant) is a dual-curable dental resin composite sealer. Resilon bonding agent is a self-etching primer that contains sulfonic

acid-terminated functional monomer, HEMA, water, and polymerization initiator [3]. Many investigators have compared the sealing ability of this system to the others with different methods. Some studies [4-8] have assessed the apical sealing ability of the new resin-based Epiphany-Resilon root canal filling system, and compared this with the sealing abilities of different pairings of gutta percha and sealers. It was found that there were no significant differences between Epiphany-Resilon combination and all the other groups. In assessment of the coronal seal, Shemesh et al [9] did not find any significant difference in the coronal leakage between the two vertically compacted filling materials, Resilon with Epiphany sealer and gutta-percha with AH26 along the coronal region of the root fillings, using both glucose penetration and fluid transport model.

Kaya, et al [10] compared the coronal sealing ability of gutta-percha and thermoplastic synthetic polymer-based systems along the root canals, using a recently introduced glucose penetration model. They found that Gutta-percha and AH Plus combinations allowed similar patterns of glucose penetration to Resilon Epiphany combinations**.**

Stephen et al [11] compared the coronal sealing ability of Resilon Epiphany to Gutta-percha and Roth or AH Plus sealers using fluid filtration. Resilon/ Epiphany was not better than gutta-percha/Roth or gutta-percha/AH Plus at sealing root canals.

To simulate the clinical condition, a dog model was used by Shipper et al [2] to assess and compare in vivo the efficacy of gutta-percha and AH26 sealer versus Resilon and Epiphany filled roots in preventing apical periodontitis subsequent to coronal inoculation with oral microorganism. At the 14-week post-coronal inoculation, mild inflammation was observed in 82% of the roots filled with Gutta-percha and AH26 sealer. This was statistically more than the roots filled with Resilon, Epiphany primer and the sealer (19%), and the roots in the negative control group.

However, preliminary studies of Resilon have shown remarkable promise, such as a decrease in the amount of the leakage of the root canals treated using Resilon when compared to GP [2, 12-14] and an increase in the fracture resistance of endodontically treated roots [15]. But in the above-mentioned studies, there were no significant differences between resilon and other obturation materials in the coronal seal.

The aims of this in-vitro study were to assess the human saliva penetration in obturated root canals with different pairs of Gutta-percha, Resilon, Epiphany and AH26.

**Materials and Method**

In this experimental study, there were 130 single canal human teeth without any anatomical abnormality or calcification extracted for orthodontic or periodontal purposes were used. The specimens were cleaned of all periodontal attachments and kept in an aqueous solution of 0.5% Chloramine T for no longer than 6 months. The coronal parts of the teeth were removed to produce the specimens of approximately 15 mm. The canals were prepared in a crown down sequence using protaper (Dentsply Maillefer, Ballaigues, Switzerland) instruments according to manufacturer's protocol and irrigation with 5% NaOCl was performed.

Apical patency was verified after root canal preparation using a size 10 K-File (Maillefer, Ballaiges, Switzerland). 5cc EDTA 17% was used as the last irrigant before rinsing the canal with sterile water.

The teeth were randomly assigned to four experimental groups (30 teeth in each group), and five teeth were assigned to the positive and negative control groups. Group one was obturated with GP (Maillefer, Ballaiges, Switzerland) and AH26 (Dentsply Maillefer, Ballaigues, Switzerland) sealer, group two with GP and Epiphany sealer (Pentron, Wallingford, CT), group three with Resilon tips and Epiphany sealer (Pentron, Wallingford, CT), and group four with Resilon tips and AH26 sealer.

All the groups were obturated, using cold lateral condensation technique. Five roots as positive contr-

ols were obturated with single gutta-percha cones without any root canal sealer. The remaining five roots were obturated with Gutta-percha and AH26 sealer, using lateral condensation technique. The entire external surface (including the apical foramen) of each root in this latter group was coated with a double layer of nail varnish. These roots served as negative controls. The manufacturer's instructions were followed meticulously during the obturation process, as Resilon and Epiphany are technique sensitive materials.

The radiographs were used to check the adequacy of root canal fillings. Bacterial microleakage was tested using the two-chamber method described by Torabinejad et al [16] and Barthel et al [17] (Figure 1).

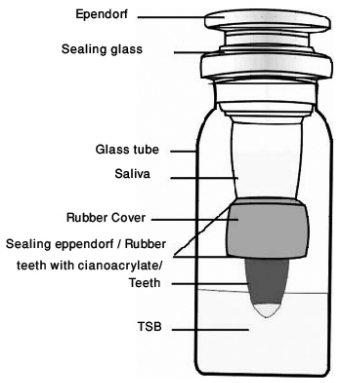


**Figure 1** Setup of model design

The experimental specimens were covered with nail varnish excluding the coronal and apical 1 to 2 mm. The specimens were attached to a 5mm cut off the conical ends of polyethylene 1.5mm Eppendorf tubes (Sigma, Aston Manor, Johannesburg, SA) with the apical portion of the specimen protruding through the tube. The space between the tube and specimen was sealed using PVC solvent weld cement (Latrochem, Honeydew, Johannesburg, SA) and two layers of nail varnish. The Eppendorf tubes were then attached to an opening in the screw cap of Polyethylene specimen containers (Sigma, Aston Manor). The prepared two-chamber systems were sterilized overnight using ethylene oxide. After sterilization, under a laminar airflow hood, sterile trypticase soy broth (Selecta-Media, Ferndale, Johannesburg, SA) was poured into the lower chamber of each specimen to a level of 2 to 3 mm above the apical foramen of each filled root and the chamber was sealed.

The specimens were incubated aerobically at 37°C for 3 days to verify sterility. The saliva collected every other day from the students was carefully placed in the upper chambers. Vitality of bacteria in saliva during 2 day intervals was confirmed by a pilot study. All specimens were kept at 37°C and the lower chambers were checked daily for 3 months for the occurence of turbidity. The number of days it took for the broth to become turbid was recorded as an indicator of entire root canal recontamination. The data were subjected to statistical analysis using a Kaplan-Meier test.

## Results

All the positive control specimens leaked within 24 hours and none of the negative control ones did so within 90 days. The samples in the experimental groups that became positive within 24 hours were found to have leakage through the latex tubing. These 6 samples were discarded.

Seven canals obturated with GP, Epiphany (Group 2) were contaminated within an average of 23.57 days, ranging from 5 to 46 days. Also, 14 canals obturated with Resilon, AH26 (Group 4) were contaminated within an average of 28.5 days ranging from 4 to 76 days and six canals obturated with GP, AH26 (Group 1) were contaminated within an average of 31.33 days ranging from 8 to 58 days. Finally, 10 canals obturated with Resilon, Epiphany (Group 3) were contaminated within an average of 30.4 days ranging from 11 to 49 days (Table 1).

The sequence of the experimental groups which showed more contaminated samples was as follows: Resilon, AH26> Resilon, Epiphany> GP, Epiphany> GP, AH26. The mean and the standard deviation values for the time of contamination in the four experimental groups are shown in Table 2.

**Table 1** Number of days from inoculation to bacterial microleakage

| Specimen Number | GP Epiphany | Resilon AH26 | GP AH26 | Resilon Epiphany |
|---|---|---|---|---|
| 1 | 90+ | 4 | 90+ | 90+ |
| 2 | 90+ | 14 | discarded | 90+ |
| 3 | 14 | 90+ | discarded | 90+ |
| 4 | 90+ | 90+ | 90+ | 12 |
| 5 | 90+ | 42 | 90+ | 25 |
| 6 | 46 | 90+ | 90+ | 90+ |
| 7 | 90+ | 90+ | 90+ | 90+ |
| 8 | 90+ | 15 | 14 | 90+ |
| 9 | 90+ | 25 | 90+ | 42 |
| 10 | 28 | 90+ | 56 | 90+ |
| 11 | 33 | 29 | 90+ | 33 |
| 12 | discarded | 90+ | 90+ | 49 |
| 13 | 90+ | 90+ | 58 | 90+ |
| 14 | 90+ | 29 | 90+ | 90+ |
| 15 | 22 | 19 | 90+ | 26 |
| 16 | 90+ | 90+ | 90+ | 90+ |
| 17 | 90+ | 28 | 19 | 24 |
| 18 | discarded | 90+ | 90+ | 90+ |
| 19 | 90+ | 90+ | 90+ | 90+ |
| 20 | 90+ | 90+ | 90+ | discarded |
| 21 | 17 | 35 | 90+ | 90+ |
| 22 | 90+ | 12 | 90+ | 48 |
| 23 | 90+ | 90+ | 8 | 90+ |
| 24 | Discarded | 90+ | 33 | 90+ |
| 25 | 5 | 90+ | 90+ | 90+ |
| 26 | 90+ | 64 | 90+ | 90+ |
| 27 | 90+ | 90+ | 90+ | 11 |
| 28 | 90+ | 90+ | 90+ | 90+ |
| 29 | 90+ | 7 | 90+ | 34 |
| 30 | 90+ | 76 | 90+ | 90+ |

**Table 2** The mean and the standard deviation values for time of contamination in four experimental groups

| Groups | N | Mean | Std. Deviation |
|---|---|---|---|
| GP Epiphany | 7 | 23.5714 | 13.50132 |
| Resilon AH26 | 14 | 28.5000 | 20.70210 |
| GP AH26 | 6 | 31.3333 | 21.53756 |
| Resilon Epiphany | 10 | 30.4000 | 13.39320 |
| Total | 37 | 28.5405 | 17.36311 |

Although GP, AH26 group showed the least contaminated samples, Kaplan-Meier test showed no significant statistical difference between the groups ( $p$ =0.17). The mean of the days of contaminated samples in all the groups was compared as well. Moreover, the Kaplan-Meier test showed no significant difference between the mean of the days.

## Discussion

A bacterial microleakage model was used to compare the sealing ability of Resilon to that of GP. During the bacterial microleakage test, all the positive control specimens leaked within 24 hours, indicating the ability of the saliva to penetrate the prepared root canals. Also, none of the negative control specimens leaked within 90 days, indicating that the seal created between the two-chambers of the systems was efficient. During the bacterial microleakage test, there was no statistically significant difference between the results obtained when using GP or Resilon with different pairs of sealer. These results are in agreement with the findings of the studies using similar in vitro systems [18].

In contrast, Shipper et al [19] compared lateral and vertical condensations of Gutta-percha with AH26 to those of Resilon/Epiphany when exposed to Streptococcus mutans. They found that Resilon/ Epiphany leaked significantly less than the Gutta-percha groups. Shipper et al [1] also compared the bacterial leakage of lateral and vertical condensations of gutta-percha with AH26 and Resilon/Epiphany. The study used Streptococcus mutans and Enterococcus faecalis. Again, the Resilon/ Epiphany groups leaked significantly less than the Gutta-percha groups.

The media used in these studies lacked the proteins, enzymes or bacterial populations, and the other products normally found in the natural saliva. The use of human saliva in our study provided these elements. However, it is a static model and does not simulate clinical conditions.

To simulate clinical condition, a dog model was used by Shipper et al [2] to assess and compare, in vivo, the efficacy of Gutta-percha and AH26 sealer versus resilon and Epiphany filled roots in preventing apical periodontitis subsequent to coronal inoculation with oral microorganism. At the 14-week postcoronal inoculation, mild inflammation was observed in 82% of the roots filled with Gutta-percha and AH26 sealer. This was statistically more than the roots filled with Resilon with Epiphany primer and sealer (19%) and

those in the negative control. In this study, a cotton pellet soaked in the dog's dental plaque was placed in the access cavity and the teeth were sealed with Glass Ionomer filling material. The microorganisms which were inoculated in the pulp chamber were limited and had a tendency toward anaerobic bacteria. However, in our study the saliva refreshed every other day to preserve vital bacteria and fresh contents of the saliva in contact with the canal obturation material.

Furthermore, the solubility of the set material, when determined in accordance with ANSI/ADA Specification 57 [20], should not exceed 3% by mass. AH Plus (0.21%) was within the recommended range, whereas Epiphany (3.41%) showed a higher value than the ANSI / ADA recommendations [21].

A further investigation was conducted to determine the EpiphanyTM components released during the test period. The deionized distilled water used for solubility test of Epiphany TM root canal sealer was submitted to atomic absorption spectrometry and showed an extensive calcium release (41.46 mg/ $L^{1}$). Calcium ion release has been shown to favor a more alkaline pH of the environment leading to biochemical effects that culminate in the acceleration of the repair process [22]. This high calcium release by EpiphanyTM sealer could explain the reduced apical periodontitis observed clinically [3] and its intraosseous biocompatibility [23], but in long term this solubility may lead to material disintegration and more leakage.

## Conclusion

Our study did not support the manufacturer's claim that the Resilon/Epiphany System resists leakage significantly better than Gutta-percha-based obturations. Resilon/Epiphany acted statistically equal to Gutta- percha with AH 26 obturations. Future long-term clinical studies are needed to determine if outcome studies support the use of this material.

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