



## MICROLEAKAGE OF GUTTA PERCHA AND RESILON USED WITH EPOXY RESIN SEALERS. A GLUCOSE FILTRATION INVESTIGATION

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### ABSTRACT

**Aim:** To compare the seal obtained by gutta-percha and Resilon root filling material with 3 different epoxy resin sealers using the glucose filtration method.

**Materials and Methods:** The roots of 96 extracted human permanent single rooted teeth were instrumented and randomly divided into two equal groups according to the used core material whether gutta-percha or Resilon. Each group was further subdivided into three subgroups according to the sealer used, group IA (n=15): gutta-percha+AH Plus, group IB (n=15): gutta-percha+MM-Seal, group IC (n=15): gutta-percha+EZ Fill, group IIA (n=15): Resilon+AH Plus, group IIB (n=15): Resilon+MM-Seal, group IIC (n=15): Resilon+EZ Fill. In the positive control group (n=3), the root canals were obturated using gutta-percha without any sealer; while in the negative control group (n=3), the root canals were obturated using gutta-percha and sealer but the external surfaces of the roots were completely

coated with sticky wax, including the root canal orifice and apical foramina. The glucose filtration model was used to quantify the microleakage at 1, 2, 3 and 4 weeks.

**Results:** The amount of microleakage increased with time in all experimental groups. There was no statistically significant difference between gutta-percha and Resilon throughout the experiment period when used with AH Plus. Moreover, no significant difference existed when the core materials were used with MM-Seal at the 1st and 2nd week and with EZ Fill at the 1st week only. However, Resilon showed higher glucose leakage value than gutta-percha in the rest of results. AH Plus showed higher mean glucose concentration value than MM-Seal and EZ Fill throughout the experiment period.

**Conclusion:** None of the materials tested prevented leakage. Once started, the amount of leakage increased steadily over time, regardless of the root filling material used. The sealing ability of gutta-percha was similar to Resilon when used with AH Plus sealer. Resilon showed less sealing ability than gutta-percha when used with MM-Seal and EZ Fill sealers. AH Plus allowed more glucose penetration than MM-Seal and EZ Fill sealer.

**KEY WORDS:** AH Plus, EZ Fill, glucose filtration, gutta percha, MM Seal, resilon

## INTRODUCTION

The aim of root canal treatment is to eliminate bacteria and their by-products, and to prevent re-contamination of the root canal space. Long term success after treatment is dependent on the development and maintenance of a bacteria-tight seal along the length of the root canal. Efforts to provide endodontic sealer that have the ideal physical and biological properties are ongoing. Epoxy resin-based sealers have proven their capability in this respect in many in vitro and in vivo investigations.<sup>(1,2)</sup> Various test methods have been described to evaluate the quality of seal as dye penetration,<sup>(3)</sup> radioactive isotopes test,<sup>(4)</sup> bacteria or bacterial metabolites leakage test,<sup>(5,6)</sup> electrochemical technique,<sup>(7)</sup> and fluid filtration.<sup>(8)</sup> However, the published reports often reach different or even conflicting conclusions. As pointed out by Wu and Wesselink, there was a high level of variation in these results and it was difficult to draw firm conclusions as to which filling technique or material was the best in sealing the root canal system.<sup>(9)</sup> The glucose filtration test was suggested in 2005 by Xu et al.<sup>(10)</sup> It measures the filtration rate of glucose molecules that pass along the root canal filling. The present study was conducted to evaluate the sealing ability of epoxy resin sealers using the glucose leakage test.

## MATERIALS AND METHODS

### Sample selection and preparation

Ninety six single rooted teeth had their lengths standardized to 13-15 mm by removing the coronal portion at the cemento-enamel junction.

All roots were prepared using manual ProTaper files to size F5. The canals were irrigated after each instrument with 6 mL of 5.25% (NaOCl) using a 27-gauge needle that was placed 1 mm short of the working length. Prior to obturation, all canals received a final flush with 1 ml 17% EDTA for 1 minute followed by 6 ml 5.25% NaOCl for 30

seconds to remove the smear layer. The canals were then rinsed with 3 ml normal saline to remove any residues and to avoid the prolonged effect of irrigating solutions.

### Classification of the samples

The prepared root canals were randomly divided into two main groups according to the used core material whether gutta-percha or Resilon. Each group was further subdivided into three subgroups according to the sealer used, group IA (n=15): gutta-percha+AH Plus, group IB (n=15): gutta-percha+MM-Seal, group IC (n=15): gutta-percha+EZ Fill, group IIA (n=15): Resilon+AH Plus, group IIB (n=15): Resilon+MM-Seal, group IIC (n=15): Resilon+EZ Fill. In the positive control group (n=3), the root canals were obturated using gutta-percha without any sealer. In the negative control group (n=3), the root canals were obturated using gutta-percha and sealer but the external surfaces of the roots were completely coated with sticky wax, including the root canal orifice and apical foramina.

### Obturation of root canals

A standard amount of 0.05ml of each tested sealer was used in each canal. The sealers were mixed according to manufacturer instructions and placed into the root canal using the master cone. The master points (size 50 of either gutta percha or Resilon) were placed into the root canal to full working length. Lateral compaction technique was performed using a size 30 finger spreader to 1 mm short of the working length. Size 25 accessory cones were inserted into the canal until the spreader could not penetrate more than 2 to 3 mm. The excess was removed with a heated instrument and the filling was compacted vertically. The external surface of the specimens of experimental and positive control groups were coated with transparent nail varnish and sticky wax, except for the orifice and the apical 3 mm. The filled root specimens were stored in an incubator at 100% humidity at 37°C for 1 week to allow the sealers to set completely.

### Glucose filtration test

Leakage was quantified using glucose filtration test. A 15 cm long plastic pipette with 1 mm diameter was glued to the coronal part of the root then glued to the end of an eppendorf vial. A hole was created in the screw cap of the glass bottle through which the plastic tube was passed. A seal was obtained in all junctions by using glue and sticky wax. The assembly was then placed in a glass bottle which contained 3 ml of 0.2% solution of  $\text{NaN}_3$  (apical reservoir) and at least 2mm of the root end immersed in the solution. A 22 gauge needle was inserted through the screw cap to equalize the exterior and interior pressure (Figure 1). The prepared glucose solution (1mol/L) was injected in the plastic pipette. The model was then transferred to an incubator that provides 100% humidity at a temperature of  $37^\circ\text{C}$  for the duration of observation periods. The leaked glucose that passed through the obturated canal was collected in the glass bottle. The concentration of leaked glucose was measured at 1, 2, 3 and 4 weeks using spectrophotometer.

### Statistical analysis

Kruskal-Wallis test was used to evaluate the significance of the difference. Pair-wise comparisons were performed using the Mann-Whitney U test. The significance level was set at  $P \leq 0.05$ .

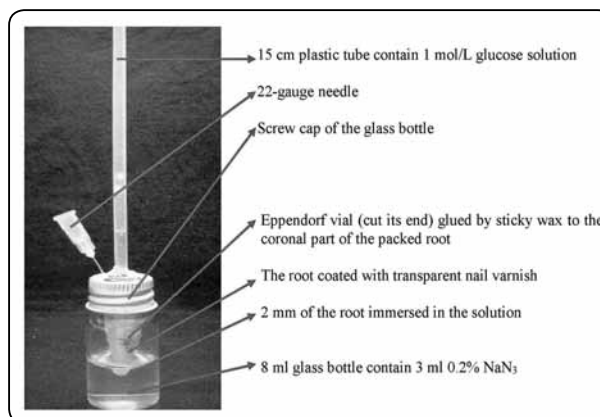


Fig. (1) Glucose filtration set up

## RESULTS

### I. Changes by time within each group

Friedman's test showed a statistically significant difference between the four periods ( $P$ -value  $< 0.001$ ).

#### Group IA (Gutta-percha+AH Plus) (Table 1)

There was statistically significant increase in leakage values from week 1 to week 2, from week 2 to week 3 and from week 3 to week 4.

#### Group IB (Gutta-percha+MM-Seal) (Table 1)

There was no statistically significant increase in leakage values from week 1 to week 2. However, there was gradual increase in leakage from 2<sup>nd</sup> to 4<sup>th</sup> week which was of statistical significance.

TABLE (1) Mean, standard deviation (SD) values and results of Friedman's test for comparison between leaked glucose concentration (mg/dl) at the different time periods in gutta-percha groups

Sealer	Week 1		Week 2		Week 3		Week 4		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
AH Plus (Group IA)	19.3	7.5	25.8	18.9	32.6	21.3	39.9	24	<0.001*
MM-Seal (Group IB)	10.8	7.8	11.8	8.7	15.5	9.7	21.4	11.2	<0.001*
EZ-Fill (Group IC)	6.6	5.1	9.9	5.4	15.6	10	17.7	10.4	<0.001*

\*: Significant at  $P \leq 0.05$

**Group IC (Gutta-percha+EZ-Fill) (Table 1)**

There was significant increase in leakage values from week 1 to week 3. The increase in glucose penetration later on was of no statistical significance.

**Group II (Resilon): (Table 2)**

There was statistical significant increase in leakage values from week 1 to week 2, from week 2 to week 3, and from week 3 to 4 when Resilon was used with all of the tested sealers.

**II. Comparison between the core materials (Gutta-percha&Resilon)****1) With AH Plus (Table 3)**

There was no statistically significant difference between the mean leakage value of gutta-percha and Resilon throughout the experiment period ( $P>0.05$ ).

**2) With MM-Seal (Table 4)**

There was no statistically significant difference between the mean leakage value of gutta-percha and Resilon at the 1<sup>st</sup> and 2<sup>nd</sup> week ( $P>0.05$ ). At the 3<sup>rd</sup> and 4<sup>th</sup> week, gutta-percha showed significantly lower mean leakage value than Resilon.

**TABLE (2) Mean, standard deviation (SD) values and results of Friedman's test for comparison between leaked glucose concentration (mg/dl) at the different time periods in Resilon groups**

Sealer \ Period	Week 1		Week 2		Week 3		Week 4		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
AH Plus (Group IIA)	15.3	7.3	22.3	11.8	40	9.3	44.7	10.5	<0.001*
MM-Seal (Group IIB)	4	2.1	15.3	8.8	29.6	12.9	34	13.7	<0.001*
EZ-Fill (Group IIC)	5	4.1	18.7	6.6	38	8	42.2	6.5	<0.001*

\*: Significant at  $P \leq 0.05$

**TABLE (3) Mean, standard deviation (SD) values and results of Mann-Whitney U test for comparison between leaked glucose concentrations (mg/dl) with different core materials using AH Plus sealer**

Material \ Period	GP		Resilon		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
Week 1	19.3	7.5	15.3	7.3	0.654
Week 2	25.8	18.9	22.3	11.8	0.571
Week 3	32.6	21.3	40	9.3	0.221
Week 4	39.9	24	44.7	10.5	0.571

\*: Significant at  $P \leq 0.05$

**TABLE (4) Mean, standard deviation (SD) values and results of Mann-Whitney U test for comparison between leaked glucose concentrations (mg/dl) with different core materials using MM-Seal sealer**

Material \ Period	GP		Resilon		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
Week 1	10.8	7.8	4	2.1	0.054
Week 2	11.8	8.7	15.3	8.8	0.385
Week 3	15.5	9.7	29.6	12.9	0.025*
Week 4	21.4	11.2	34	13.7	0.017*

\*: Significant at  $P \leq 0.05$

### 3) With EZ-Fill sealer (Table 5)

There was no statistically significant difference between the mean leakage value of gutta-percha and Resilon at the 1<sup>st</sup> week only. At the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week, gutta-percha showed significantly lower mean leakage value than Resilon ( $P \leq 0.05$ ).

TABLE (5) Mean, standard deviation (SD) values and results of Mann-Whitney U test for comparison between leaked glucose concentrations (mg/dl) with different core materials using EZ-Fill sealer

Material Period	GP		Resilon		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
Week 1	6.6	5.1	5	4.1	0.240
Week 2	9.9	5.4	18.7	6.6	0.002*
Week 3	15.6	10	38	8	<0.001*
Week 4	17.7	10.4	42.2	6.5	<0.001*

\*: Significant at  $P \leq 0.05$

TABLE (6) Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between leaked glucose concentrations (mg/dl) of different sealers with gutta percha

Sealer Period	AH Plus		MM-Seal		EZ-Fill		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
Week 1	19.3	7.5	10.8	7.8	6.6	5.1	0.037*
Week 2	25.8	18.9	11.8	8.7	9.9	5.4	0.016*
Week 3	32.6	21.3	15.5	9.7	15.6	10	0.004*
Week 4	39.9	24	21.4	11.2	17.7	10.4	<0.001*

\*: Significant at  $P \leq 0.05$

TABLE (7) Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between leaked glucose concentrations (mg/dl) of different sealers with Resilon

Sealer Period	AH Plus		MM-Seal		EZ-Fill		P-value
	Mean (mg/dl)	SD	Mean (mg/dl)	SD	Mean (mg/dl)	SD	
Week 1	15.3	7.3	4	2.1	5	4.1	0.026*
Week 2	22.3	11.8	15.3	8.8	18.7	6.6	0.258
Week 3	40	9.3	29.6	12.9	38	8	0.114
Week 4	44.7	10.5	34	13.7	42.2	6.5	0.097

\*: Significant at  $P \leq 0.05$

### III. Comparison between the sealers

#### 1) With gutta-percha (Table 6)

There was a statistically significant difference between the three sealers thought out the experiment period. Pair-wise comparisons showed that AH Plus had the highest mean leaked glucose concentration. While no statistically significant difference existed between MM-Seal and EZ Fill.

#### 2) With Resilon (Table 7)

Only at the 1<sup>st</sup> week was the leakage of AH Plus significantly greater than the other two sealers. No statistically significant difference existed between MM-Seal and EZ Fill. At the rest of experimental periods, although AH Plus exhibited higher glucose concentration values, this was of no statistical significance.

## ■ DISCUSSION

Epoxy resin based sealers are glue like materials and known for their adhesive properties. They were used in the present study because they showed a better performance in relation to sealing than non resin-based ones.<sup>(4,11,12)</sup> It is believed that such good performance is due to their low solubility<sup>(13,14)</sup> and dimensional stability.<sup>(15)</sup>

Shemesh et al.<sup>(16)</sup> suggested that the glucose leakage method can be seen as a further development of the fluid transport concept. In this model, the tooth is continuously subjected to the pressure of the glucose solution in the coronal chamber.<sup>(16)</sup> This would eliminate any entrapped air within the root filling. The smaller molecular size of the glucose tracer, chemical stability and the stricter test may be seen as more relevant to clinical outcome.<sup>(10)</sup> The possibility of quantitative measurements, the availability of the materials and equipment, simplicity, reproducibility, and sensitivity of the test may be added to its advantages.<sup>(10,16)</sup>

In the present study, a continuous increasing rate of leakage occurred from the first day onward in all of the experimental groups. This seemed logical as the coronal pressure was applied continuously from the first day to the end of the test.<sup>(10,16)</sup> In fact, this was in agreement with other glucose microleakage studies.<sup>(10,16,17)</sup>

Throughout the experiment period, no statistically significant difference was found between gutta-percha and Resilon when they were used with AH Plus. This was not in agreement with the result obtained by Onay et al.<sup>(18)</sup> and Hirai et al.<sup>(19)</sup> This might be due to the fact that they used the fluid filtration method as a tool for assessing leakage. However, the results obtained by Kaya et al.<sup>(17)</sup> were not in agreement with those of the present study although they used the same method of evaluation at the same experimental periods. The difference is more likely related to the sample preparation protocol.

Resilon and gutta-percha showed similar glucose leakage values when used with MM-Seal and EZ Fill root canal sealers at the beginning of the test only. Later on, Resilon showed higher glucose leakage value than gutta-percha. This was in agreement with Onay et al.<sup>(20)</sup> Gutta percha has been shown to be dimensionally stable<sup>(21, 22)</sup> while Resilon shrank about 0.5%.<sup>(23)</sup> The literature is lacking concerning the dimensional changes of MM-Seal and EZ Fill. However, some studies tested their surface porosity,<sup>(24)</sup> bond strength<sup>(25)</sup> and solubility.<sup>(26)</sup>

MM-Seal and EZ Fill root canal sealers displayed more effective sealing than AH Plus. To our best knowledge, this is the first study that evaluated and compared the microleakage of Resilon and gutta-percha with those sealers. The poor performance of AH Plus may be attributable to silicon oils present in its composition and not present in MM-Seal and EZ Fill sealers according to the information supplied by the manufacturers. This silicon oil may affect the sealing properties.<sup>(27-30)</sup> EZ Fill exhibited high bond strength in comparison with AH Plus.<sup>(1)</sup> The bond strength of root canal sealers to dentin was important for maintaining the integrity of the seal in root canal filling,<sup>(31)</sup> and adhesion of a root canal sealer to both gutta-percha and root dentin would be desirable in obtaining a hermetic seal.<sup>(31,32)</sup> Moreover, it has been shown that smear layer removal adversely affects the seal of AH Plus,<sup>(33-35)</sup> but the effect on the sealing ability of EZ Fill and MM Seal has no reference in the literature.

The different results among the sealers may also be due to different mixing methods. AH Plus is a hand-mixed 2-paste sealer, while MM-Seal and EZ Fill are syringe-mixed pastes. The syringe mixed sealers, may allow better control of the ratio of mixed components with relatively less inclusion of air porosity, which facilitate the use of a sealer with optimal physical properties.<sup>(24, 36)</sup> This may consequently influence the sealing ability.<sup>(37)</sup> In contrast, Bodrumlu et al.<sup>(38)</sup> and Roggendorf et al.<sup>(39)</sup> found that the apical sealing ability of MM-Seal

and EZ Fill was similar to that of AH Plus. In these studies, dye penetration tests were used to assess leakage and the type of samples and preparation protocols were different.

## CONCLUSIONS

Therefore, it was concluded that, none of the materials completely sealed the root apex in vitro. Once started, the amount of leakage increased steadily over time, regardless of the root filling material used. The sealing ability of gutta-percha was similar to Resilon when used with AH Plus sealer. Resilon showed less sealing ability than gutta-percha when used with MM-Seal and EZ Fill sealers.

AH Plus root canal sealer had less sealing ability than MM-Seal and EZ Fill sealer. The glucose leakage test allows a long term quantitative assessment of the sealing ability of various filling materials. However it needs a lot of time for the preparation of specimens.

Further investigations are required to evaluate the sealing ability and physical properties of MM-seal and EZ Fill root canal sealer.

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