Evaluation of the Sealing Ability and Antibacterial effect of Guttaflow; a new Root Canal Filling/Sealer Material using Enterococci


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ABSTRACT

Interest in the microbiological profile of post-treatment apical periodontitis has increased considerably during the last few years. It is generally believed that the major cause of treatment failure is the survival of microorganisms in the apical portion of the root-filled tooth. Enterococcus faecalis (E.faecalis) is the most drug resistant organism of these bacteria, and can survive in root canals for 12 months even under nutrient-deprive conditions. Aim: The study aimed to evaluate the sealing ability and the antibacterial effect of a new root canal sealer/filling material (Guttaflow) and comparing it with other sealing materials (Topseal and gutta-percha). Materials & Methods: To test the sealing ability of the tested materials, we used Enterococcus faecalis cultured in Brain Heart Infusion (BHI) (bacterial leakage study) and methylene blue dye (dye extraction study). Sixty four caries free, extracted human anterior teeth were cut to the cementoenamel junction, prepared in a crown down technique, and divided into 2 parts (32 for each study); one part for bacterial leakage study and the other part for dye extraction study. Then each 32 teeth were subdivided into three groups (n=10) and 2 control teeth. Group I: obturated with a cold lateral compaction using Gutta-percha and topseal sealer. Group II: obturated with cold lateral compaction using Gutta-percha and Guttaflow as a sealer. Group III: obturated with single cone technique using Gutta-perch and Guttaflow as filling/sealer. The antibacterial effect of the tested materials (against Enterococcus faecalis cultivated on Brain Heart Infusion Agar) were evaluated by measuring the zone of inhibition around discs made of the materials. Results: There was no statistically significant difference between the presence of coronal bacterial leakage among the three groups. For the dye study there was no statistically significant difference between Group II and Group III which showed low values of dye leakage. This was followed by Group I having the least absorbance (lowest dye leakage), with a significant difference between the three groups. Topseal showed the statistically significant highest mean inhibition zone (0.8±0.2). There was no statistically significant difference between Gutta percha and Guttaflow which showed the statistically significantly lowest mean values. Key words: Enterococcus faecalis, bacterial leakage study, Topseal, Guttaflow and Gutta-percha.

INTRODUCTION

It is now well established that the etiology of periradicular periodontitis is microbiological. Microorganisms most commonly infect the root canal system by ingress from the oral cavity through dental caries or defective restorations. The dentine-pulp complex of the tooth may react in different ways to the presence of microorganisms, but irreversible inflammatory changes may be ultimate. Enterococcus faecalis is the dominant species present in post-treatment apical periodontitis. It is the most frequently isolated species and is usually also the predominant isolate in the canal. Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. The analysis of the sealing ability of the different root canal fillers and cements has received much attention. In vitro methods have been primarily used to estimate the quality of the sealing, generally by evaluating the microleakage that allows tracers to penetrate along the obturated root canal. The tracers most often used are dyes, radioisotopes, bacteria, or bacterial by-products. The use of bacteria as tracers seems to give the most clinically relevant demonstration of microleakage associated with a root canal system. Many bacterial strains have been used to evaluate marginal leakage, but results are sometimes contradictory probably because they may depend on the bacterial strain used.
Everyday new obturating materials are introduced in the market so; the sealing ability of each material should be well investigated. Gutta-percha is the most commonly used obturating material and is the standard to which other obturating materials are compared. (10)

Guttaflow is a new material formed of fine powdered gutta-percha dispersed in Roekoseal sealer. A question has been raised about the behavior of this material regarding the leakage specially being used with not very popular single cone technique.

**Aim of the study**

The aim of the study was to evaluate the sealing ability and the antibacterial effect of a new root canal filling material (GuttaFlow) and comparing it with Gutta-percha and Topseal.

**MATERIALS & METHODS**

**I- Materials:**

<table>
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<tr>
<th>Table (1): Composition of the materials and their manufactures</th>
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<tr>
<td><strong>Materials</strong></td>
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<tr>
<td>Topseal</td>
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<td>Dentsply/Maillefer, Baillaigues, Switzerland</td>
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<td>GuttaFlow</td>
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<td>Coltene-whaledent Langenau, Germany</td>
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<tr>
<td>Gutta-Percha</td>
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<td>Aceonedent, Korea</td>
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**II- Method:**

**1- Selection of samples:**

Sixty four caries free, extracted human anterior teeth with full developed apices were used in this study. Any tooth that had a crack or defect was discarded. Teeth were cleaned from any calculus or periodontal tissue.

**2- Preparation of root canals:**

Access cavities were made. The canals were irrigated using a freshly prepared solution of Sodium Hypochlorite (NaOCl). Apical patency was verified after root canal preparation using k file size 10 (Dentsply, Maillefer, Ballaigues, Switzerland). A final rinse using NaOCl was performed and the canals were dried using paper points (Dentsply, Maillefer, Ballaigues, Switzerland).

**Group 1:**

The root canals were obturated using cold lateral condensation technique with gutta-percha (Aceonedent, Korea) and Topseal (Dentsply, Maillefer, Ballaigues, Switzerland) resin sealer.

**Group 2:**

The root canals were obturated using cold lateral condensation of gutta-percha and gutta-flow (Coltene- Whaledent, Altstätten, Switzerland) as sealer. This group was obturated as group 1.

**Group 3:**

Root canals were obturated using single cone gutta-percha and gutta-flow (Coltène-Whaledent, Altstätten, Switzerland).

**Control group:**

4 teeth were used as control 2 positive and 2 negative controls. The positive controls were prepared but left unobturated and the negative controls were not prepared.

**Leakage study:**

The 64 teeth were divided into 2 groups, 32 were used in the bacterial leakage and 32 were used in the dye extraction (10 from each group and 2 controls).

**a) coronal bacterial leakage:**

For the bacterial leakage, the teeth were autoclaved at 120°C for 20 minutes before obturation to make sure that no bacteria exist in...
Thirty teeth were prepared and divided into the previously mentioned 3 groups. After obturation the teeth were kept in 100% humidity for 48 hours to ensure the sealers were set. The external surface of the roots were covered by 2 layers of nail varnish except for the coronal 1mm and the apical 3mm to ensure that there is no leakage from any lateral canal.

For the positive control the tooth was not obturated and not covered by nail varnish and for the negative control the tooth was not instrumented and covered completely by nail varnish except for the coronal access. The tapered end of 0.5 ml Eppendorf plastic tubes were cut off and the obturated roots were inserted into the tubes until the roots protruded through the end. The part of the tube above the obturated root is called upper chamber Fig (1).

The junction between the tube and the root was sealed with cyanoacrylate and another 2 layers of nail varnish were applied to the roots except the apical 3mm.

The entire system was sterilized overnight in ethylene oxide gas. Then the root ends were inserted in sterile glass tubes filled with sterile Brain Heart Infusion Broth (lower chamber), so that approximately 2 to 3mm of the root was immersed in the sterile BHI broth Fig (2).

This will ensure that the only route for bacteria from the upper chamber to the lower chamber was through the obturated root canal.

The whole system was incubated at 37˚C for 2 days to ensure sterilization of the system. Any system that showed any sign of turbidity in the BHI was discarded.

Selected strain of *Enterococcus faecalis* was cultured in BHI broth at 37˚C for 24 hours. The bacterial suspension was adjusted to be equivalent in density to \(1 \times 10^8\) CFU/ml by using opacity standard (McFarland standard 0.5).

An inoculum of the above broth culture was inoculated into the upper chamber using a sterile bacteriological loop. The tubes orifices were sealed with sterile parafilm and the systems were incubated at 37˚C for 30 days. The inoculum of *E. faecalis* in BHI was changed with fresh inoculum every 5 days and the tubes were observed daily for any sign of turbidity in the sterile BHI broth in the lower chamber.

Any turbid BHI broth in the lower chamber was cultivated on BHI agar plates. Growing colonies on plates were identified; either *E. faecalis* or any other contaminant. If turbidity was due to *E. faecalis*, which indicated that bacteria passed through the canal to the sterile BHI broth in the lower chamber (bacterial leakage occurred through obturated root canal). Clear and turbid BHI broth in lower chamber are shown in Fig (3).
Fig (3): showing negative control tube (clear BHI broth in lower chamber) and positive control tube (turbid BHI broth in lower chamber).

b) Dye extraction study:
The other 32 teeth were divided into the same groups as the bacterial study. The methylene blue dye (2% buffer) was placed in the coronal access of the roots and was left 24 hours. The roots were placed under running tap water for 30 minutes to remove any residue of methylene blue and then the varnish was removed with a Parker blade # 15. The roots were then placed in vials containing 1ml concentrated nitric acid (69 wt %) for 3 days. Then the vials were centrifuged (Universal 16R; Hettich Zentrifugen, Tuttlingen, Germany) at 14,000 rpm for 5 minutes. Two hundred microliters of the supernatant from each sample was transferred and sample absorbance was read by a spectrophotometer (SLT Spectra II; Labinstruments A-5082, Salzburg, Austria) at 550nm using concentrated nitric acid as blank.

4-The antibacterial study:
The agar plate diffusion procedure was used to observe the antibacterial effect of Gutta-percha, Gutta-flow and Topseal against E. faecalis. Discs of each material were fabricated in sterile metal rings of 6mm inside diameter and 1.5mm height under aseptic conditions Fig (4). All specimens were exposed to ultraviolet light for 2 hours for each surface to prevent any contamination. E. faecalis used was (American type culture # 29212 ATCC 29212) commonly found in root canal and periapical infection. Colonies of the E. faecalis were picked up by the loop and then suspended in BHI to make suspension equivalent in density to opacity standard (McFarland standard 0.5) (1 x 10^8 CFU/mL). Sterile swab was immersed into the suspension and squeezed from excess fluid against the side of the tube and then rubbed over plate of BHI agar, plates were incubated overnight at 37°C. The antibacterial activity of each material tested was determined by measuring the diameter of zones of inhibition in millimeters. Five agar plates were tested for each material and all assays were repeated two times to ensure reproducibility.

Fig (4): Showing the metal rings

Statistical analysis
Qualitative data were presented as frequencies and percentages. Chi-square (x^2) test was used to compare between the groups. Quantitative data were presented as mean and standard deviation (SD) values. One way ANOVA (Analysis of Variance) was used to compare between means. Duncan’s test for pairwise comparisons was used to determine significant differences between means when ANOVA test is significant. The significance level was set at P ≤ 0.05. Statistical analysis was performed with SPSS 15.0® (Statistical Package for Scientific Studies) for Windows

RESULTS

Comparison between the presence of coronal bacterial leakage in the three groups by weeks: Fig (5)

During the first week: the percentage of coronal leakage for group I and III was 60% and
for group II was 10% with a statistically significant difference between the three groups.

*At the second week* the percentage of coronal leakage was 60% for group I and 70% for Group II and 90% for Group II, while for the third week the percentage of coronal leakage was 100% for the three groups. There was no statistically significant difference between presence of coronal leakage among the three groups.

Comparison between presence of coronal bacterial leakage in each group through different times: Fig (6)

*In Group I,* the percentage of coronal leakage at the first and second weeks was 60%, while at the third week the percentage of coronal leakage was 100%. There was no statistically significant change in the percentage of leakage between first and the second week and between them and the third weeks.

*In Group II,* the percentage of coronal leakage at the first week was 10% and 70% at the second week, with a statistically significant increase in the percentage of leakage. The percentage of coronal leakage was 100% at the third week, there was no statistically significant change in the percentage of leakage between second and third week.

*In Group III,* it was noticed that the percentage of coronal leakage at the first week was 60%, at the second week was 90%, while the percentage of coronal leakage was 100% at the third week, with non statistically significant change in the percentage of leakage.
Dye extraction study
Each sample absorbance was read by a spectrophotometer (SLT Spectra II; Labinstruments A-5082, Salzburg, Austria) at 550nm using concentrated nitric acid as blank. One way ANOVA (Analysis of Variance) was used to compare between means. Duncan’s test for pair-wise comparisons was used to determine significant differences between means when ANOVA test is significant.

Group I have the least absorbance with the mean 0.44±0.1, the maximum value was 0.837 and the minimum value was 0.132. Group II have the mean 0.74±0.2, the maximum value was 0.995 and the minimum value was 0.419. Group III have the mean 0.76±0.1, the maximum value was 0.988 and the minimum was 0.561.

The positive control having the highest mean absorbance 1.08±0.2 and the negative control having the lowest mean absorbance 0.04 ±0.01 (lowest dye leakage).

There was no statistically significant difference between Group II and Group III which showed lower values, followed by Group I. There was a statistically significant difference between the three groups. Figure (7).

Figure (7): Mean and standard deviation values of absorbance in the three groups

Antibacterial effect
It was found that Topseal showed the highest mean inhibition zone (0.8±0.2) the maximum inhibitory zone was 1mm while the minimum inhibition zone was 0.5mm.

While Guttapercha showed no zone of inhibition, the mean inhibition zone was zero.

The GuttaFlow also had no antibacterial effect which showed no inhibition zone.

Topseal showed a statistically significant difference with Guttapercha and GuttaFlow, while there was no statistically significant difference between Guttapercha and GuttaFlow. Table (2) figures (8-10).

Table (2): Comparison between mean inhibition zones for three materials:

<table>
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<tr>
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<th>Topseal</th>
<th>Guttapercha</th>
<th>Guttaflow</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.8 ± a</td>
<td>0 ± b</td>
<td>0 ± b</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>0</td>
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P-value: <0.001*

*: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Duncan’s test
Fig (8): showing a zone of inhibition around the disc of Topseal.

Fig (9): showing no zone of inhibition around the Gutta-percha disc

Fig (10): showing no zone of inhibition around the Gutta Flow disc

DISCUSSION

Guttaflow is a new material formed of fine powdered gutta-percha dispersed in Roekoseal sealer. A question has been raised about the behavior of this material regarding the leakage specially being used with not very popular single cone technique.

To evaluate the sealing ability of any material different techniques are available as bacteria, bacterial toxins, dye, radioisotopes and fluid filtration.\(^{(1)}\)

In this study bacteria leakage and dye extraction were used for many reasons. When bacteria are used, they are more representative of the clinical situation and give accurate results than dye penetration because of many factors as: ionic charge, PH, temperature and ability of the viable microbes to change their size and to move actively, duplicate and grow which cannot be represented by the aqueous dye solution. Also, small dye molecules can penetrate where bacteria cannot, which is not clinically right.\(^{(12)}\)

Choosing the *E. Faecalis* in this study was based on many factors. *E. Faecalis* is a part of normal oral flora in humans and frequently found in mixed infections. It is also one of the most commonly isolated microbes from the root canal.\(^{(13)}\)

The number of microorganisms that had caused turbidity was not measured as the purpose was only to prove that some bacteria can penetrate the root canal fillings. The fact that in some specimens only one microorganism could leak and cause turbidity and in another case more microorganisms could penetrate through the root filling.\(^{(14)}\)

In the bacterial coronal leakage study there was no significant difference between the three groups. This result is in agreement with Miletic et al.\(^{(14)}\)

The comparison between the presence of coronal bacterial leakage in the three groups by weeks was recorded and in the first week there was a statistically significant difference between the three groups. Group I and III showed equal percentages of coronal leakage. The two groups showed higher percentage of leakage than Group II. During the second and the third weeks there was no statistically significant difference between the three groups.

The dye extraction study showed that group I using the resin sealer topseal and the Gutta-percha with the cold lateral compaction technique has the least absorbance and the least leakage among the three groups. This result may be due to the resin sealer (epoxy resin) have adaptation and adhesion to the canal walls and lack of solubility these properties are due to the resin component present in the material which is absent in the Guttaflow. This result is in agreement with Monticelli et al.\(^{(15-16)}\) who studied the sealing ability of two contemporary single cone obturation systems Guttaflow and Active GP compared with an epoxy resin based
sealer, and this study also proved that the two techniques gave more leakage than the epoxy resin based sealer.

Our results are in agreement with Timpawat et al. (13), but not in agreement with De-Deus et al (17) who found that Roekoseal and Gutta-flow gave better sealing than the resin sealer, and with Cobankara et al (18) who found that different sealers showed better sealing than the resin sealer.

It is important to use several techniques for the evaluation of the sealing ability of any material because sometimes as in this study the two techniques give different results. The bacterial study found no significant difference between three sealers while the dye study found significant difference between the same three groups.

This is in agreement with Barthel et al (19) who found no correlation between the results obtained from bacterial and dye leakage study.

The antibacterial effect of the three materials Gutta-percha, Guttaflow and topsel was investigated. Sometimes there are remnants of bacteria or bacterial toxins present in the root canal so this antibacterial action of the filling material is important to minimize the infection and lead to endodontic treatment success. (20-21)

The antibacterial effect of the topsel may be due to the presence of bisphenol diglycidyl ether, which is a component of the resin based materials, and also these materials release formaldehyde in their polymerization process. These components present in the resin based sealers gave them the antibacterial effect that was absent in the Gutta-percha and Guttaflow. The result is in agreement with Cobankara (21) et al. but our result is not in agreement with Mohammadi and Yazdizadeh (22) who studied the antibacterial effect of GuttaFlow and AH-26 resin sealer against Staphylococcus aureus. They found that the resin sealer had higher activity than GuttaFlow. This may be due to the difference in bacteria, technique, time and ingredients of the tested material which can affect the results of the microbiological study as concluded by Cobankara et al. (21)

Conclusions and recommendations:

There was no different effect on bacterial leakage between sealers and techniques by time. The chemical composition of the sealer material had an influence on the antibacterial effect. The technique of obturation affects the sealing ability of the materials. It is recommended to restore immediately the teeth after the endodontic treatment.

REFERENCES


Title: Determining the Effect of Using the Antibacterial Material on the Leakage of Root

Abstract: The leakage of root is one of the most important reasons for the failure of root treatment. Therefore, the aim of this study was to investigate the effect of a new antibacterial material on the leakage of root in root canals infected with E. faecalis and to compare its effect with that of a commercially available material.

Methods: The study included 32 roots divided into three groups: Group A: E. faecalis and a commercially available material, Group B: E. faecalis and the new antibacterial material, and Group C: E. faecalis without any treatment. Root canals were filled with gutta percha and AH Plus root canal sealer in all groups. The leakage of root was measured using a dye leakage test and compared between the groups.

Results: The leakage of root was significantly lower in Group B compared to Group A and Group C. No significant difference was found between Group A and Group C.

Conclusion: The new antibacterial material showed a significant reduction in the leakage of root compared to the commercially available material and was equivalent to no treatment.