Comparison of Apical Sealing Ability of Two Phases of Gutta–Percha: A Bacterial Leakage Model

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INTRODUCTION
One major concern of endodontists is to prevent microleakage after root canal filling, in order to prevent bacterial invasion to the root canal space. That is because microorganisms play a significant role in pulpal and periapical diseases [1, 2].

Torabinejad et al. [3] reported that in coronally unsealed endodontically treated teeth, saliva penetration was seen after 30 days regardless of the obturation technique (lateral versus vertical condensation). Trope et al. [4] explained that endotoxins could penetrate through an obturated root canal in less than 21 days, if the coronal seal is not achieved.

Abstract
Objectives: The aim of this study was to compare apical sealing ability of alpha and beta phases of gutta-percha by means of bacterial leakage model.

Materials and Methods: Fifty single-rooted human premolars were selected. The root canals were prepared with Mtwo rotary instruments up to apical #35.04. Forty teeth were randomly divided into two groups (n= 20). The root canals were obturated by alpha phase (G1) and beta phase (G2) of gutta-percha and AH26 sealer, respectively, with warm vertical compaction technique. Ten teeth served as positive (n=8) and negative (n=2) control groups. Then, the specimens were sterilized with ethylene oxide gas. Bacterial suspension of Enterococcus faecalis (E. faecalis) in 0.5 McFarland concentration was prepared. All teeth were mounted in plastic vial caps containing Muller Hinton broth and then exposed to bacterial suspension of E. faecalis every three days up to 31 days. The number of days required for the contamination of the entire root canals was recorded. The data were analyzed using Mann Whitney U test.

Results: There were no significant differences in bacterial leakage between the G1 and G2 groups (P>0.05). Negative controls revealed no microbial leakage; whereas positive controls showed gross microbial leakage.

Conclusion: Despite better thermal conduction and adaptability of alpha phase of gutta-percha, our study revealed no significant difference in bacterial leakage between alpha and beta phases of gutta-percha in warm vertical compaction.

Keywords: Dental Leakage; Gutta-Percha; Dental Seal

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For the first time, Bowman used gutta-percha as a root filling material and mentioned that one of the practical ways to limit microorganisms in the root canal system is to achieve a three dimensional root canal filling [5]. This method can deprive the remaining microorganisms after cleaning and shaping of the canal system. There are two phases of gutta-percha for root canal filling. The most popular material for root canal filling is the beta phase of gutta-percha. The other is the thermoplastic alpha phase introduced by Johnston. It is stated that gutta-percha in alpha phase has special characteristics such as less shrinkage, lower viscosity and more adherence than the beta phase [5-7]. Controversy exists regarding which phase of gutta-percha can provide a better apical seal. Gutmann et al. [8] compared the radiographic quality of obturation with alpha and beta phases of gutta-percha (Thermafil versus lateral condensation method). They reported that the quality of the alpha phase of gutta-percha was significantly better. Wolcott et al. [9] reported that more gutta-percha was seen in the lateral canals when alpha phase gutta-percha coated rigid carrier technique was used; whereas more sealer was present when using cold lateral condensation and these differences were statistically significant. Pommel and Campes [10] revealed that after one month, microbial leakage in Thermafil method and vertical condensation technique was significantly less than that in lateral condensation and single cone methods. On the other hand, De Deus et al. [11] showed that there was no statistically significant difference in the sealing ability of lateral condensation, warm vertical condensation and Thermafil methods in a period of 100 days. Gutmann et al. [12] showed that there was no statistically significant difference between Thermafil and lateral condensation when evaluating dye microleakage after 24 hours, seven days and five months. Bakhtiar et al. [13] compared the apical seal of lateral condensation, Thermafil and one step technique by assessing microbial leakage. They described that there was no significant difference among the groups after 60 days. Controversy exists regarding the apical seal provided by gutta-percha phases. It should be noted that in all previous studies, the alpha phase of gutta-percha was used by the Thermafil method [9-13] but the aim of this study was to compare the apical sealing ability of alpha and beta gutta-percha cones by using warm vertical condensation method.

**MATERIALS AND METHODS**

In this experimental study, 50 extracted human premolars with a single root canal were selected. The teeth, which had been previously treated endodontically, had more than one root canal, caries, calcification, fracture, internal or external root resorption, curvature and open apex were excluded from the study. Radiographs were taken from the buccolingual and mesiodistal aspects for evaluating the aforementioned criteria. All samples were kept in 5.25% sodium hypochlorite for 24 hours. Periodontal ligament residues were also removed. Access cavity was prepared in all samples. A #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to measure the canal’s diameter (if #15 K-file was loose, the tooth was excluded from the study). All samples were prepared with Mtwo rotary files (VDW, Munich, Germany) up to #35.04. Irrigation was performed between each two subsequent files with 5.25% hypochlorite. Then, the canals were dried with paper points (Meta Bio-med Co. Ltd., Seoul, Korea). The teeth were divided into three groups, according to the obturation technique used:

1-Obturation with alpha phase gutta-percha (VDW, Munich, Germany) (n=20).
2- Obturation with beta phase gutta-percha (VDW, Munich, Germany) (n=20).
3-Control groups with no obturation.

For obturation, a #35.04 gutta-percha master cone and AH26 sealer were used by means of

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BeeFill® Pack II (VDW, Munich, Germany). After obturation, gutta-percha was cut with a #30 plagger (Dentsply Maillefer, Ballaigues, Switzerland) and compressed. Subsequently, a radiograph was taken to evaluate the quality of filling. All samples were cut 10 mm coronal to the apex with a diamond disc (D+Z, Bern, Switzerland). They were incubated at 37°C in a fully saturated condition for 72 hours.

Preparing samples for evaluation of microleakage:
The outer surfaces of the samples other than the apical 2 mm were covered with two layers of nail varnish for prevention of bacterial leakage from the accessory and lateral canals except for the apical foramen. Samples were mounted and fixed in the plastic vial caps and sealed with cyanoacrylate glue; then, they were sterilized with ethylene oxide gas for 24 hours. Afterwards, Mueller Hinton Broth (Merck, Darmstadt, Germany) was added to the vials in such a way that the apical 2 mm of the roots was in contact with the medium. Enterococcus faecalis (ATCC2012) was cultured on blood agar medium. Then, a bacterial suspension of E. faecalis in 0.5 McFarland concentration was prepared. All obturated samples were exposed to the bacterial suspension of E. faecalis every three days coronally. Samples in the control group were considered as negative controls (n=2) and were not exposed to bacterial suspension; the remaining control samples (n=8) served as positive controls and were exposed to the bacterial suspension. The number of days required for the contamination of the entire root canals was recorded. The turbidity of the Mueller Hinton broth was assessed for up to 31 days.

The Mann Whitney U test was performed for data analysis. P ≤0.05 was considered statistically significant.

RESULTS
Distribution of bacterial leakage time in both groups is presented in Table 1. The negative control samples (without bacteria and no canal filling) showed no turbidity after 24 hours of incubation. These samples remained bacteria-free for the whole month. The positive control samples (with bacteria and no canal filling) showed 100% turbidity after 24 hours. All samples in groups G₁ and G₂ were infected with E. faecalis after 30 days. The mean time of bacterial leakage in G₁ and G₂ was 12.05±9.85 and 10.5±7.8 days, respectively. The difference between the two groups was not statistically significant in this respect (P=0.74).

DISCUSSION
The proper obturation of the root canal system is a key factor to achieve successful results in endodontic treatments [9]. Inadequate apical and coronal seal is the most important reason for endodontic treatment failure [6, 9, 14]. Although beta phase gutta-percha is the most commonly used obturation material, the alpha phase gutta-percha can flow better in the root canals and fill more lateral canals; thus, it can provide a better apical seal [7, 8, 9, 12].

In this in vitro study, we used bacterial leakage model that is more similar to clinical situation than other microleakage evaluation methods. Barthel et al. [15] compared bacterial microleakage with dye penetration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error Mean</th>
<th>Median</th>
<th>Minimum - Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leakage</td>
<td>Alpha</td>
<td>20</td>
<td>12.05</td>
<td>9.57</td>
<td>2.14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td>20</td>
<td>10.50</td>
<td>7.80</td>
<td>1.75</td>
<td>10</td>
</tr>
</tbody>
</table>
with microorganisms, dye penetration is not a proper method to simulate the clinical setting. Since E. faecalis is responsible for one-third of endodontic treatment failures, in the present study, we used E. faecalis suspension [16-19]. AH26 was used as a sealer for root canal filling in our study. Limkangwalmongkol et al. [20] used dye penetration method to assess the microleakage and they described that sealing ability of AH26 was better than that of Tubliseal, Sealapex and Apexit.

Zhang et al. [21] reported that alpha phase gutta-percha with warm vertical compaction technique moved significantly more into the lateral canals and depressions than the beta-phase gutta-percha. Meyer et al. [22] compared shrinkage of alpha and beta phases of gutta-percha. They showed that beta phase gutta-percha had more shrinkage and led to higher level of microleakage.

In this study, we did not observe any turbidity in Muller Hinton broth medium in the negative control group, which means nail varnish and cyanoacrylate glue prevent bacterial leakage from other parts of the root, except for the apical foramen. Also, we observed complete turbidity in the positive control group.

Results of the current study showed that there was no statistically significant difference in apical bacterial leakage between alpha and beta phases of gutta-percha.

CONCLUSION
In this in vitro study, we concluded that the type of gutta-percha crystallization had no impact on its apical sealing ability.

REFERENCES