INTRODUCTION

The main goal of root canal treatment is the elimination of the root canal bacteria, the residual bacteria after cleaning and shaping procedures obligates to have a root canal obturating material and sealers with antibacterial properties. The antimicrobial activity of root canal sealers gives them superiority especially in cases of recurrent infections. Many in-vitro studies investigated the antibacterial activity of many sealers with various methods (1). Agar diffusion test (ADT) is one of the most commonly used methods for evaluation of the antibacterial activity. E-faecalis is such a microbial species which was found as a major cause of persistent root canal infection and was detected...
in the peri-apical lesions (2-5) also E-faecalis has the ability to invade into dentinal tubules and resist most of the chemicals used in root canal treatment (6). In this research the antibacterial effect of three different sealers was evaluated against E-faecalis.

MATERIALS AND METHODS

Material
In this study, the following materials were used:

a) Bacterial isolates
A total of 7 Enterococcus faecalis isolates were included in this study.

b) Substances
1- Ceraseal (bio-ceramic sealer)
2- AD Seal (Resin based sealer).
3- Zinc oxide and eugenol sealer.

c) Media
The following media were used in this study

Brain-Heart Infusion Broth
This medium was used to prepare the suspension of Enterococcus faecalis.

Brain-Heart Infusion Agar
This medium was used to test the effect of the different substances on the growth of Enterococcus Faecalis using the diffusion agar method.

Methods

a) Preparation of Brain-Heart Infusion Broth
Thirty seven grams of the medium were suspended in one liter of distilled water. Heating with frequent agitation ensured good mixing & dissolution. The suspension was then boiled for one minute until complete dissolution. It was later dispensed into appropriate containers and sterilized at 121°C for 15 minutes. The prepared medium was stored at 2-8°C. For best results, the medium was used on the same day.

b) Preparation of Brain-Heart Infusion Agar
Fifteen grams of agar powder were added to 1 liter BHI broth & then heated to dissolve agar before dispensing into appropriate containers. Autoclaving was then performed for 15 min at 121°C to ensure adequate sterilization. The mixture was then poured into Petri dishes & left to cool & solidify.

c) Preparation of Enterococcus Faecalis Suspension
A sterile swab was used to transfer bacterial growth from the primary culture into the BHI broth bottle & mixed well to form a homogenous suspension.

d) Classification of samples
1- 21 samples were classified according to the tested material into 3 groups
2- Group 1: consisted of 21 holes filled with Ceraseal
3- Group 2: consisted of 21 holes filled with AD Seal
4- Group 3: consisted of 21 holes filled with Zinc oxide and eugenol sealer

Each group were further classified into 3 subgroups according to the observation period.

Subgroup A: Consisted of 7 holes evaluated after one day
Subgroup B: Consisted of 7 holes evaluated after three days
Subgroup C: Consisted of 7 holes evaluated after seven days
e) Implantation of the E-faecalis in the agar plates

A sterile cotton swab was dipped into the suspension and excess fluid was removed by turning the swab against the inside of the tube. The inoculum was evenly spread over the entire surface of dry BHI agar plates by swabbing in three different directions.

f) Mixing and application of the tested substances

Three holes of 4 mm diameter were made on the agar surface by a metal punch leaving about 10-15 mm away from the edge of the petri dish, and these holes were separated from each other by a distance not less than 20 mm to avoid overlapping zones of inhibition.

Each hole contained one of the tested materials and marked as following

Hole number I for Ceraseal, hole number II for AD Seal and hole number III for ZnO&E

All the agar plates were incubated at 37°C in aerobic conditions for the required observation periods.

g) Method of evaluation

After one day observation period the area of microbial growth inhibition (lack of bacterial colonization) around the holes were measured at the largest diameter with a poly gauge millimeter ruler.

The agar plates were then re-incubated to take the measurements of the inhibitory zones after 3 days and then after 7 days.

h) Statistical analysis

Data were analyzed by SPSS software (version 16.0, SPSS, Chicago, IL, USA). Data in each group were compared by the ANOVA and Kruskal-Wallis tests. Also the Dunnett’s test was performed to compare the results between two groups. The level of significance was set at 0.05.

RESULTS

A) Group one (Ceraseal):

Showed effect on growth of the bacterial strain with a zone of inhibition of 16 mm that did not increase over 7 days

B) Group two AD Seal:

Showed no area of inhibition of bacterial growth among the three observation periods.

C) Group three ZnO&E:

Showed no area of inhibition of bacterial growth among the three observation periods.

TABLE (1) The diameter of the inhibitory zone of tested materials .

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Ceraseal</th>
<th>AD Seal</th>
<th>ZnO&amp;E</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>One day</td>
<td>16 mm</td>
<td>0 mm</td>
<td>0 mm</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3 days</td>
<td>16 mm</td>
<td>0 mm</td>
<td>0 mm</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>7 days</td>
<td>16 mm</td>
<td>0 mm</td>
<td>0 mm</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P≤ 0.05 is considered significant.

DISCUSSION

E-faecalis is considered the most resistant species that can survive in the root canal system even after the endodontic treatment (7). This persistent species is the main cause of endodontic failure. It is advantageous for the endodontic sealers to have the property of bactericidal or at least the bacteriostatic activity which may help to eliminate the residual bacteria which was not eliminated during the chemo-mechanical preparation of the root canal system which can improve the success rate of the endodontic treatment (8,9). This study was conducted to evaluate the antibacterial activity of Bio-ceramic based sealer compared to resin based sealers and zinc oxide and eugenol based sealer
using agar diffusion method (ADT). ADT depends on the solubility and physical properties of the antimicrobial component of the sealer\(^{10}\). The size of the inhibitory zone is dependant on the toxicity of the material to a particular strain of the bacteria and the ability of the tested material and its ability to diffuse through the used medium\(^{13}\). The results based on the comparison of the effect of duration on the anti bacterial activity of each tested sealers and comparing the anti bacterial property of different sealers in the same observation period. For resin seal, sealer and zinc oxide and eugenol based sealer they exhibited no anti-bacterial activity which was not changed by time. These findings were in agreement with Wainstein etal.\(^{7}\). This may be related to its lack of solubility and diffuse-ability of this sealer. On the other hand the Bioceramic sealer showed significantly larger inhibitory zone after one day observation period that remained steady among the whole observation periods. This finding approved the antibacterial effect of the bioceramic sealer which is related to the rapid ionic exchange with the release of calcium ions and hydroxyl ions\(^{11,12}\). This reaction is responsible for the strong alkaline medium surrounding the bioceramics that allows its antibacterial effect this finding was in agreement with Singh et al\(^{13}\).

**CONCLUSION**

It has been concluded that Bioceramic sealer has higher inhibitory effect on E-faecalis than both Resin and ZnO&E sealers.

**REFERENCES**


