Antibacterial activity of three endodontic sealers with various bases

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ABSTRACT

Purpose: The antibacterial activity of three endodontic sealers, a Ca(OH)2 based sealer (apexit) a Zoe based sealer (Drofill) and a resin based sealer (AH26) was assessed on the growth of an anaerobic bacteria (peprostreptococcus spp.) using the Agar diffusion test (ADT).

Materials and Method: Thirty brain heart diffusion agar plates were incubated with peptostreptococcus anaerobics. Each plate was divided into two separate areas. In one area, 0.1ml droplet of a given fresh sealer and in the other area a dry material of the same sealer were placed, such that each plate had two areas with the same sealer in two forms of fresh and dry (set form). There were ten plates for each of the three sealers. The plates were placed into anaerobic jars and incubated at 37°C. After varying periods, zone of inhibition of bacterial growth were observed, measured and compared by t and Paired T-test.

Results: There was no statistically significant differences between the activity of AH26 and drofill in the fresh form (P>0.05). However there were significant differences between (the two sealers in set form) (P<0.05) Drofill was more antibacterials in the set form than AH26. All sealers were more active when fresh than ages.

Conclusion: It is likely that the eugenol in the Dorifill and the formaldehyde in the AH26 are responsible for their greater antimicrobial activity.

INTRODUCTION

Root canals are very irregular in shape, thus the elimination or reduction of microbes from the complex, three dimensional root canal system, especially the dentinal tubules is one of our main problems in endodontic therapy.

The closed root canal network can serve as an incubator for bacterial growth. Due to the low oxygen content of a closed root space anaerobic bacteria, especially are given an ideal atmosphere to live, grow, and ultimately activate the vast immunological defense systems that result in host destruction.

It is widely accepted that bacteria are the ultimate cause of root canal treatment failure.1,2,3

The bacteria residing within the canal system can proliferate and divide, so, reinfect the endodontically treated teeth and interfere the healing process of the periapical tissue, therefore various substances has been recommended to eliminate microorganisms as endodontic irrigants and intra canal medicaments.

When endodontic therapy is required, a solid or semisolid root canal filling material
alone cannot provide an exact fitness. Root canal sealers are used in endodontics to help filling any voids that not filled by root canal filling material.

Several requirements of an ideal sealer have been described, one of them is that a sealer should be antibacterial,\(^\text{(2,4)}\) considering the importance of this subject and the contradictory data gained by previous studies.

Alkhatib et al showed that Grossman sealer and then AH\(_{26}\) were the most effective sealers. The next effective sealers were tubliseal and sealapect.\(^\text{(5)}\)

Another study showed that sealapect and tubliseal had the highest antimicrobial activity.\(^\text{(6)}\)

In 1999, Michael and et al evaluated the antimicrobial effect of three sealers with Ca(OH)\(_2\) base and one with ZOE base and the Roth sealer (ZOE based) showed significant more antimicrobial effect than other sealers, and sealapect was the most effective after Roth.\(^\text{(2)}\)

In this study, the antibacterial activity of three endodontic sealers Apexit (Ca(OH)\(_2\) based), Drofill (ZOE based), AH\(_{26}\) (resin based) would be compared on peptostreptococcus anaerobes in order to achieve a higher success rate for endodontic treatment in clinic.

**MATERIALS & METHODS**

In this experimental study was an three group of sealers, AH\(_{26}\) (dentsly), Dorifill (Dorident), Apexit (Ivoclar vivadent) were prepared according to the manufacturer's instructions into two subgroups as: fresh prepared sealer and set form sealers. The organism used was peptostreptococcus anaerobes. For this study, the sample was taken from the pus of an acute oral abscess from the maxillofacial department of Shariati Hospital. The samples were collected by aspiration using a sterile syringe and were immediately transferred to the microbiologic department of Pastur Institute.

The samples were cultures on proper mediums like Brucella Blood agar (Difco, Detroit-M1). For separating the existing (facultative and obligative) anaerobic bacteria, they were incubated in an aerobic and anaerobic condition at 37\(^\circ\)C.

After 24 hours the essential examinations were done on the formed bacteria colonies. First, a smear was prepared from those colonies, they were identified by gram strain and examined microscopically. gr+ Gram positive cocci were seen which were double or more with short chains, one after another.

These bacteria proliferates very well in an anaerobic condition (%5 CO\(_2\), 85% N\(_2\), 10% H\(_2\)). After performing diagnostic biochemical test like Endol and nitrate, examining the effect of the diagnostic Discs Kanamycin and colsitin and finally observing it's resistance against the two antibiotics, we concluded that the obligative anaerobic bacteria is peptostreptococcus anaerobius.

**Procedures:**

Our technique was ADT (agar diffusion test). 0.1ml from every prepared sealer was used.\(^\text{(2)}\) Every sealer was used in two form:

A: The sealer 48 hours after mixing when it got hard (set sealer) was applied on the culture plate.

B: The fresh prepared sealer, immediately after mixing was applied on the culture plate.

In order to have a hemogen microorganisms growth, a 0.5gr/lit McFarland microbial suspension was prepared using 24 hrs cultured bacteria according to the following instructions.

4 or 5 colonies from the indicated organisms were solved in Brucella broth liquid medium. Until the opacity of the achieved suspension was equal the standard %5 Mc Farland one. Plates containing Brucella Blood agar were examined to be sure that they are decontaminated. 0.5\(^\text{cc}\) microbial suspension was poured in each plate containing a medium and was spread with a sterile swab. After 4-5minutes the excessive water was sucked by a syringe.

10 plates were considered for each sealer was applied (0.1\(^\text{cc}\) fresh sealer, 0.1\(^\text{cc}\) set sealer). All the procedures were done under the Laminar air flow appliance and under complete sterile condition.
10 plates belonging to one type along with palladium as catalyst for absorbing the possible remaining oxygen were put in an anaerobic jar.

The air was evacuated in three steps from three jars and the gases N₂ 85% and CO₂ 5% and H₂ 10% were injected into the jar.

Then, the anaerobic jars were put into the incubator at 37°C.

The plates were evaluated at 24h, returned incubation, evaluated another time at 7 days for size of the zone of inhibition.

Positive control, Negative control:
As a positive growth control 2 plates contained just the organism to ensure that the bacterial life cycle does not become inactive before the last 7-day observation.

As a negative control, sealer was placed on 4 plates only with pieces (0.1ml) from three different types of sealers to control the possible effect of the sealer on the plate.

Reading the size of zone of inhibition:
The plates were examined and evaluated for growth inhibitory zones around each sealer as evidenced by lack of bacterial colonization (clearing of agar) adjacent to each sealer over 360 degrees.

The most uniform segment of zone of inhibition was measured with an endodontic millimeter ruler measuring from the outermost edge of the sealer to the end of the zone of inhibition for a total of 20 measurements to evaluate each sealer.

A mean zone of inhibition was then determined for each sealer. Wider zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved sealers.

Data collected were statistically analyzed by comparing the means of the zones of inhibition for each sealer (six groups).

The independent and paired t-test were used for statistical analysis with a significant level of P<0.05.

RESULTS

For every group, 10 samples set sealer and 10 fresh prepared, were considered. So, we had 60 pieces sealer in all of the groups.

The zone of inhibition was measured for every sealer after 24h and after 7 days with a ruler (mm) according to Kirby and Bauer methods. There were 10 samples for every sealer in every group, in order to obtain the final diameter of the zone of inhibition for every subgroup the data was averaged 24 hours after incubating the plates. The zones were measured afterward. All of the measurements belonging to the 60 samples in 30 plates were recorded. The tested plates were again put in anaerobic condition in the incubator for 6 days (7 days collectively after culturing the bacteria).

After measuring the zone of inhibition at 7 days and comparing with results at 24h, no differences were seen in the size of the zones. The fresh prepared Apexit sealer showed little antibacterial activity. That means the average zone of inhibition measured for 10 samples was 3.78 mm in diameter. But the set Apexit sealer had no antibacterial activity in any sample.

However around the two other sealers (AH26 and Dorifill) in both two forms set and fresh prepared a significant zone of inhibition was seen.

In this study SPSS ver.10 program was used after calculating statistical descriptive data in order to examine the normality of the data.

The Kolmogrov-smirnov test was used. For comparing the average of the fresh prepared and set sealers of AH26 and Dorifill, the t-test (as paired and independent) were used. The variances were tested by the Leven's statistical test to assess if they are homogen. The average diameter of zone of inhibition for every subgroup (total 6 subgroups) was measured and you see them in table 1.

Table 1. The mean diameter of zone of inhibition in the 6 subgroups.

<table>
<thead>
<tr>
<th>Sealers name</th>
<th>Mean diameter of zone of inhibition mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh prepared AH26</td>
<td>41.500</td>
</tr>
<tr>
<td>Dorifill</td>
<td>40.0500</td>
</tr>
<tr>
<td>Apexit</td>
<td>3.78</td>
</tr>
<tr>
<td>Set Sealer AH26</td>
<td>23.2500</td>
</tr>
<tr>
<td>Dorifill</td>
<td>26.7000</td>
</tr>
<tr>
<td>Apexit</td>
<td>0</td>
</tr>
</tbody>
</table>
As you see in the table, there was a great difference between the average diameter of the zone of inhibition of a fresh prepared Apexit with the two others and this sealer in set form had no antibacterial activity.

Because of the great differences seen in the zone of inhibition of Apexit in compare with the others, the sealer was omitted from the statistical analysis of this study.

The two sealers AH26 and Dorifill were evaluated and compared with each other (fresh prepared and set form). For considering the antibacterial effect and the results are noted here.

The Leven's test was done and at last we noticed that the variances for fresh prepared AH26, Dorifill were equal (P>0.05).

It was no significant differences between the fresh form of the two sealer (AH26 and Dorifill). When these two sealers were compared, statistical analysis showed significant difference between them (P=0.001 <0.05), thus antibacterial activity of Dorifill in set form was higher than AH26 in set form.

In order to investigate the difference of sealer's antibacterial effect in fresh prepared and set form, t-test analysis was done as a paired sample test.

When fresh prepared AH26 and set form AH26 were analyzed, a highly significant difference (P<0.001) was observed between the antibacterial characteristics of the two forms of Fresh and Set AH26 sealers. Thus, the antibacterial activity in fresh prepared sealer AH26 was very higher than in set form.

In comparing fresh prepared with set form Dorifill, there was a great significant difference (P<0.001) between two forms of Dorifill sealer, thus, antibacterial activity in fresh prepared form Dorifill was very higher than the set form.

The positive growth controls exhibited obvious significant growth at 24h that completely covered Blood agar plates. The growth appeared maximal at 48-h observation and was viable throughout the experimental period.

On negative control plates, after 24-h and 7 days around fresh prepared AH26 and fresh prepared and set form Dorifill we observed RBC hemolysis on Blood agar in plates except the Apexit groups and set form AH26.

This phenomenon (RBC hemolysis) was observed in control and in experimental groups.

**DISCUSSION**

The present agar diffusion study compared the size of zones of inhibition produced by sealers against peptostreptococcus:

The size of zones of inhibition depends on the toxicity of the particular substance (sealer in this instance) for the bacteria and ability of the substance to diffuse through the test medium (agar). The diffusibility of a substance is a function of its hydrophilicity or hydrophobicity. Size of molecules and the rate of release from the insoluble matrix in which it is bound.\(^2\)

Al-Khatib and others were the first researchers who investigated the antibacterial activity of endodontic sealers.\(^5\) After them, many investigators used similar models to evaluate sealer's antibacterial effect.\(^2,6,7\)

Considering the importance of the subject and lacking enough studies, we decided to evaluate the antibacterial effect of different endodontic sealers with an in vitro experimental model. Because of the variable sealers used in endodontic treatments, we chose the three sealers AH26, Dorifill Apexit with different bases for this study.

As it was said, the obligated anaerobies microorganisms are very prevalent pathogens in oral infections and infected root canals. Between them obligative anaerobic gr+ positive coccies called peptostreptococcus species are important bacetria involving pulps. Sandquist was the first one who considered peptostreptococcus one of the prevalent bacteria in causing inflammed pulp with symptoms and periapical lesion.\(^8\)

Gomes and et al reported that these microorganism are one of the important factors to cause infection and pain in pulp and periapical lesions.\(^9\)

Lane and et al (2001) concluded that peptostreptococcus was very high in pulp necrosis.\(^10\) In this study, hemolysis was also
seen around set Dorifill sealer on the medium but no change was observed around the set AH<sub>26</sub> sealer. The reason could be the remaining eugenol molecules in set Dorifill sealer and the decrease formaldehyde in set AH<sub>26</sub> sealer.

Al-Khatib examined the antibacterial effect of Grossman's sealer (zoe base), AH<sub>26</sub> and selapex (Ca(OH) base) with ADT technique, only in the fresh prepared form, on the anaerobic microorganisms bacterioids endodontalis. They concluded that: AH<sub>26</sub> > Grossman > Selapex and the zone of inhibition for none of them changed after 7 days.

In this study, the antibacterial activity of the sealer with zoe base was more than the one with Ca(OH)<sub>2</sub> base, which is similar to our results.

But they reported that the antibacterial effect of AH<sub>26</sub> sealer was more than the sealer with zoe base which is not similar with our results. The reason for these differences could be due to the type of the sealer with zoe base and the type of anaerobic bacteria. In their study, Grossman's sealer and the anaerobic bacteroid Black pigmented parphyramones Endodontalis was used, but we chosen Dorifill sealer and the anaerobic peptostreptococcus.

The different defense patterns of the bacteria and the various additional materials used in the sealer with zoe base can be reason that we had different conditions.

Abdulkader (1990) has also investigated about the antibacterial effects of Roth, tubliseal and Apexit sealers on the anaerobic bacteria peptostreptococcus micros with the ADT technique. This investigator reported all of sealers had antibacterial effects and overall the antibacterial effect of the sealers with zoe base in more than those with (Ca(OH)<sub>2</sub> base, and sealers with zoe base produced zones of hemolysis around themselves on the gelous agar, but around the apexit sealer there was no hemolysis and no change of agar color. Their results and are in accordance.

Heling(1996) studied about the antibacterial effects of different types of endodontic sealer in dentin tubules of fresh extracted centrals. He examined the sealers AH26, EWT (zoe bases) and Selapex on the Enterococcus faecalis and results were as follows:

AH<sub>26</sub> had the most antibacterial activity and the selapex's antibacterial effects had increased after 4 days.

The achieved results contradicted with ours because he used a different technique in his study. The microorganisms were cultured in dentin tubules and after removing them from medium containing bacteria, then they applied sealers on the mediums and after incubation at different time periods. Infected dentin powders were prepared by using a bur. The achieved sample was poured in a liquid medium and its optical density was measured so they were able to count the bacteria in solution.

However, our technique was the ADT technique which is based on the diffusion of the effective material in the appropriated specific gelous medium for the bacterial growth and the only reason for the bacteria death is the antibacterial effect of the sealer. In Heling's study the tooth samples were in contact with the sealers and out of the medium for 24-48 hours. Lack of adequate nutrition and the time passed had also a role in the death of microorganism on the other hand, the tested bacteria in this research was E. Faecalis which is one of the facultative anaerobics with a different defense pattern in compare to peptostreptococcus.

Michel compared the antibacterial effect between three sealers with Ca(OH)<sub>2</sub> base and one sealer with zoe base on streptococc Milleri microorganisms with the ADT technique. The zone of inhibition was evaluated in the period of 24 and 48 hours and 7 days after culturing the indicated bacteria and these results were achieved.

The sealer Roth (zoe base) in compared with Ca(OH)<sub>2</sub> base had high antibacterial activity and after 24 hours in all groups the diameter of the zone on inhibition remained unchanged. The results from the above study is also similar to our results.

Siqueria investigated the antibacterial effect of Grossman's sealer, EWT sealer, AH<sub>26</sub> plus sealer (resin base) and sealer
AH26 (resin base) on two anaerobic bacteria porphyromonas gingivalis, prevotella a nigrescence with ADT technique. All the sealers had antibacterial effect on the used anaerobic bacteria and there was no significant difference between the sealers with zoe base and resin base considering their antibacterial effect.

The results confirmed the results of our study.

CONCLUSION

The antibacterial effect of the two sealers AH26 and Dorifill in fresh form is more than this effect for the same sealers in set form. This could be due to the decrease of humidity, the release of eugenol and formaldehyde molecules (P<0.001).

The antibacterial effect of the Dorifill sealer in set form is more than AH26 sealer in the same form that is probably because of the remaining eugenol molecules.

We concluded that the antibacterial activity of the sealers used in this study were Dorifill > AH26 > Apexit.

REFERENCES