An evaluation of the Antimicrobial Activity of Five Endodontic Sealers on three bacterial species.
(In vitro study)

Dr. Amer Abbass Muhammed  B.D.S., M.Sc. (Conservative).
Dr. Abdul-Kareem Jasim Al-Azzawi  B.D.S., M.Sc. (Conservative).
Dr. Abbas Sabri Al-Mizraqchi  B.B.S., M.Sc., Ph.D. (Microbiology).

Abstract

To evaluate the antimicrobial action of five endodontic sealers after 24 hr and 48 hr.

The sealers studied were Canason, Acroseal, Epiphany, AH-Plus and MTA, while the microorganisms used were Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis. Agar diffusion method on Muller Hinton agar was employed; forty five petriplates with 25 ml of Muller Hinton agar were inoculated with 0.1 ml of the experimental suspension. Five cavities, each one measuring 5 ml in diameter and 4 ml in depth, were made in each agar plate using cork pooper and then completely filled with the product to be tested.

Canason containing eugenol and formaldehyde proved to be the most effective against all microorganisms tested. This was followed by Epiphany, Acroseal and AH-Plus which showed antibacterial activity on all tested microorganisms higher than that of MTA which showed the least action on all tested microorganisms. No antimicrobial activity was seen after 48 hrs.

All the sealers evaluated in this study showed different inhibitory effect against all bacterial strains.

Introduction

Root canal therapy is an invaluable measure to preserve teeth that would otherwise need to be extracted. With advancing technology, a better understanding of root canal anatomy, and improved materials, root canal therapy is achieving an increasingly high overall success rate (1). However, bacteria inside the root canal system have a significant impact on this success rate. When a tooth is infected prior to treatment, the success of root canal therapy drops to 86%, which is a compromise from the 96% success rate of root canal treated teeth without apical periodontitis (2).

A few bacterial species, predominantly facultative anaerobes are responsible for causing apical periodontitis observed in root canal failure (3). These microorganisms that have leaked into the canal after its obturation or from bacterial not eliminated during therapy (4). Since removing all bacteria in the canal prior to obturation has proven to be difficult even after chemomechanical preparation (5).

Bacteria may also persist within the dentinal tubules or lateral canals after
root canal obturation and may repopulate the former root canal (6). At the final stage of root canal obturation, the remaining bacteria should be destroyed to get successful results. Endodontic sealer has the ability to do this action as one of the requirements of good sealer cement as determined by Grossman (7). Endodontic Sealer remains for a long time inside the root canal, and it may reach bacteria located in inaccessible regions of the root canal system (8).

Endodontic treatment can be aided by clarification of the antibacterial susceptibility of the pathogenic bacteria present inside the infected pulp, to these endodontic sealers which have different antibacterial activities against various microorganisms presents inside diseased pulp. These differences in antimicrobial activities are attributed to their chemical constituents and additives incorporated within the sealers. The most desirable chemical would be the one that combines maximum antibacterial effect with minimum toxicity. Therefore, one has to choose the one which combines antimicrobial effect with low toxic effect (9,10).

Materials and method

Three standard bacterial strains obtained from the clinical laboratories of the medical city in Baghdad were used in this study which were Streptococcus viridans, which obtained an a chocolate agar media, Staphylococcus aureus, which obtained on a tryptone soya blood agar media and Enterococcus faecalis, which obtained on a tryptone soya blood agar media. The microorganisms were identified - in the central health laboratories-ministry of health in Baghdad by a combination of colonial pigmentation, colonial morphology, haemolysis on a tryptone soya blood agar, cell morphology (microscopic morphology) and biochemical tests. A total of 75 samples were used in this study which were divided into 5 groups consisted of 15 plate for each group, 15 plates inoculated with Streptococcus viridans containing 5 types of sealers as group 1, 15 plates inoculated with Staphylococcus aureus containing 5 types of sealers as group 2, 15 plates inoculated with Enterococcus faecalis containing 5 types of sealers as group 3, 15 plates with 5 types of sealers without any bacteria as a negative control group and 15 plates with inoculums without any sealer as a positive control group.

The tests for the three types of bacteria (Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis) were done with Agar Diffusion method. Five sealers were used in this study which was Acroseal (Septodont), Canason (Voco), Epiphany (Pentron), AH-plus (Dentsply) and MTA (Angelus). 4 to 5 pure colonies of each bacterial strain were taken by a sterile loop. These colonies were inoculated in 10ml of BHI broth in a small screw cap tubes. Incubation of these tubes was done for 24 hour at 37 °C. Turbid suspensions were noticed at the next day. 5 ml of a sterile 0.85% normal saline solution in screw cap tubes were prepared. Bacterial strains were individually inoculated into the tubes and the suspension was adjusted visually to match the turbidity of a McFarland 0.5 scale. This number of standard contains approximately $1.5 \times 10^8$ ml of bacterial cell density.

A 9 cm diameter plates with 25 ml of Mueller Hinton Agar media in each were prepared. A sterile spreader was used to inoculate the microorganisms from the prepared normal saline tubes inoculated with microorganisms which had been fit to 0.5 McFarland standard. With an adjustable micropipette 0.1 ml
of each bacterial suspension was added to the surface of the plates which were inoculated by spreading the suspension in three directions, and a final spreading was done over the outer rim of the plate. After that, the plates were allowed to dry for 3-5 minutes. Within 15 minutes, after inoculation of the plates, five wells measuring 4 mm in depth and 5 mm in diameter were made in each agar plate using cork poorer. Each was filled completely with the five types of sealers after being mixed according to the manufacturer’s instructions. The plates were preincubated in culture media at environmental temperature for two hours before incubation to allow dissociation and diffusion of sealers. The plates were incubated at 37 °C for 24 hours in the incubator (11). The agar plates were examined for bacterial inhibition zones at the next day. With a scientific ruler (with accuracy of 0.5 mm) the diameter of these zones were measured by passing the ruler through the center of the wells. Inhibition zones were recorded at 24 and 48 hours for each sealer for each bacterial strain.

Results

Effect of the five sealers on Streptococcus viridans:
From Fig 1 it’s clear that Canason exhibited the highest mean of inhibition zone value (31.800), followed by Epiphany, Acroseal, AH-plus with values of (13.900), (13.800), (9.300) respectively. The least mean value of the antibacterial action of Streptococcus viridans was shown by MTA with a mean of (5.930). Also, it can be seen that there are no changes in the antibacterial action of all endodontic sealer after 48 hours.

Effect of the five sealers on Staphylococcus aureus

From Fig.3 it's clear that Canason exhibited the highest mean of inhibition zone value (33.800) followed by Epiphany with a mean of (16.50). AH-plus came after Epiphany in its antibacterial activity against Staphylococcus aureus with a mean of (11.870) and higher than Acroseal with a mean of (11.670). The least mean value of antibacterial activity against Staphylococcus aureus was shown by MTA with a mean value (11.530).

Effect of the five sealers on Enterococcus faecalis

Fig.5 shows that Canason had the highest antibacterial activity with the mean value of (34.00) while the lowest mean was shown by MTA (5.670). The other sealers where ranging in between as Epiphany, Acroseal and AH-plus with a mean values of (16.77), (10.13) and (8.70) respectively.

Effect of each of the five sealers on the three bacterial strains.

Statistical analysis of data by using analysis of variance ANOVA was done which showed that there was a statistically high significant difference between each type of the five endodontic sealers in their antibacterial action against the three types of bacteria as shown in table 1.

Discussion

Establishing the spectrum of activity of any antimicrobial agent is useful for improving the infection control process. In general, there are three in vitro techniques that have been used for this purpose – the dilution method which yields a quantitative result for the amount of antimicrobial agent that is needed; the agar diffusion method, which gives an inhibition zone around the well containing the agent and that could be related to its effect, and the direct exposure method, which
An evaluation of the Antimicrobial Activity of Five … Vol.: 6 No.: 3 2009

provides qualitative information about the substances. The method of measuring antimicrobial activity used here was to determine the size of the zone of bacterial growth inhibition around the specimen. This size of this zone will depend on at least two major factors. The first is the toxicity of the components of the material under study. The second is the diffusibility of any toxic factors released from the specimen. This diffusibility is a function of the hydrophilicity or hydrophobicity of the substances being released and the rate of which these substances are released from the matrix of the specimen under study (12).

However, great care was taken to keep the plates for 2 hrs at room temperature to allow the diffusion of the agents through the agar and then incubated at 37°C under appropriate gaseous condition (11). In the present study different sealers showed varying effects on different bacteria. Based on aforementioned factors, Canason endodontic sealer had the highest mean value among the others in inhibiting Streptococcus viridans growth. The paraformaldehyde, which is incorporated in the cement, is a potent antibacterial agent with a low molecular weight and low surface tension. These properties determined the higher penetrability and spreading of this endodontic sealer (13). It seems probable that release of formaldehyde from Canason was the source of its antibacterial activity. Formaldehyde is a phenolic compound which has a strong antibacterial activity in vitro (14). Furthermore, Eugenol incorporated with Canason potentiated the high antibacterial action of it on Streptococcus viridans. Eugenol is a chemical essence of oil of clove, it has been used in endodontics primarily as anodyne and also as an antibacterial agent in higher concentration (10^-2-10^-3 mol L^-1) (15). Eugenol is also lipophilic, affecting the lipid in cell membrane and increasing the cell membrane permeability so this may be another mechanisms by which eugenol could have antibacterial activity (16). Canason was more effective in inhibiting the growth of Staphylococcus aureus than Streptococcus viridans since Streptococcus viridans is less sensitive to eugenol than Staphylococcus aureus (17). Canason had too the highest level between other sealers in inhibition of Enterococcus faecalis growth for the same reasons mentioned before.

Epiphany sealer is a dual-curable resin composite containing a new redox catalyst, developed with a self-etching primer, and a new thermoplastic filled polymer (Resilon) in place of the gutta percha. Epiphany sealer came in the second stage in inhibition of bacterial growth for Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis. A possible explanation for the high antibacterial activity of this sealer could be that water diffusion leads to erosion of the composite resin material causing release of unreacted monomers (18).

Since epiphany is a dual curable methacrylate resin sealer and based on a mixture of bisphenol A-glycidyl methacrylate (BisGMA), urethane dimethacrylate (UDMA) and hydrophilic difunctional methacrylate, another reason for the high antibacterial activity of this sealer could be the residual monomers which were shown to be the main components released from cured dental composite materials (19) in addition to amine and epoxy resin components of the sealer (20). The oxygen inhibition layer of the surface of any polymerizing resin leaves an uncured monomer layer which could be another reason (21). Although epiphany and AH-plus are epoxy resin sealers Epiphany showed
more antibacterial activity than AH-plus especially against *Staphylococcus aureus* and *Enterococcus faecalis*. This may be due to the difference in solubility between the two sealers. Versiani et al. (2006) found that AH-plus solubility was within the normal range, whereas Epiphany showed higher value than the ANSI/ADA 2000 (22). Also there was an extensive calcium release from Epiphany which has been shown to favor a more alkaline pH of the environment. This high calcium release by Epiphany sealer could be another reason to explain the high level of antibacterial activity (23). Acroseal sealer which is a calcium hydroxide based endodontic sealer showed an antibacterial activity for all types of bacteria in different amount. This antibacterial activity is probably due to one of the following mechanisms. The first is the damage of the bacterial cytoplasmic membrane. As well as CaOH sealers depend on ionization that release hydroxyl ions causing an increase in pH, A pH > 9 may reversibly or irreversibly inactivate cellular membrane enzymes of the microorganisms, resulting in a loss of biological activity of the cytoplasmic membrane (24). Or leading to the destruction of the phospholipids or nonsaturated fatty acids that result in a loss of cytoplasmic membrane integrity (25). The second is protein denaturation. The alkalinezation provided by calcium hydroxide induces the breakdown of ionic bond that maintains the tertiary structure of protein. These change frequently result in the loss of the biological activity of the enzyme and disruption of the cellular metabolism. Structural proteins may also be damaged by hydroxyl ions (26). The third is Damage to the DNA. Hydroxyl ions react with the bacterial DNA and induce the splitting of the strands. Genes are then lost. Consequently, DNA replication is inhibited and the cellular activity is disarranged. Free radicals may also induce lethal mutations (27). It has been suggested that the ability of calcium hydroxide to absorb carbon dioxide may contribute to its antibacterial activity specially facultative and obligate anaerobic (28). Another reason to inhibit bacterial growth may be because of the presence of amines in the epoxy base of Acroseal as a new calcium hydroxide based sealer (29). Acroseal Calcium hydroxide sealer showed an antibacterial activity less than epiphany and Canason for several reasons. It may had been due to it’s low diffusibility in agar and due to the buffering ability of the artificial media which reduced it’s high pH and lowering it’s antibacterial activity (30). The low solubility and diffusibility of CaOH as well as the dentine buffering ability, may make it difficult to reach an increase in the pH of eliminating bacteria located within dentinal tubules the same as in agar diffusion method (31). Acroseal, appears to have lower solubility than other calcium hydroxide sealers, probably because of it’s epoxy resin component which may be another reason to its decreased antibacterial activity (29). In the present study, the preincubation in culture medium at environmental temperature for 2 hrs before incubation allowed dissociation and diffusion of the sealer evaluated in agar medium for short period of time, and influenced the results of calcium hydroxide based sealer, thus providing evidence of antimicrobial activity (11). The high significant difference of Acroseal antibacterial activity between *Streptococcus viridans*, *Staphylococcus aureus* and *Enterococcus faecalis* may be due to difference in the optimum pH for growth for each bacteria. Because *Streptococcus viridans* grows easily in neutral or slightly acid media (pH =...
6.8-7.0), the inhibition caused by this material is more than that on *Staphylococcus aureus* which need an optimum pH for growth between 7 and 7.5 (32). Low antibacterial activity of Acroseal sealer on *Enterococcus faecalis* may due to ability of this bacteria to tolerate very high pH values varying from 9 to 11 (33).

AH-plus sealer which is a new resin based sealer showed an antibacterial activity lower than that of Acroseal on all types of bacteria. This lower antibacterial activity could probably be due to its low contents of water-soluble toxic compounds such as formaldehyde and short sitting time which may induce milder antibacterial activity (34). Or it could be due to minute amount of formaldehyde from the sealer or by the release of the amine and epoxy resin components of the sealer (30) since AH-plus sealer based on polymerization reaction of epoxy resin amines (35).

MTA sealer showed the least antibacterial action. This sealer is marketed in grey-coloured (GMTA) and white-coloured (WMTA) preparation; both are 75% Portland cement, 20% bismuth oxide and 5% gypsum by weight. In recent years, the use of the white-coloured preparation has become more popular. The main component of GMTA formula are tricalcium Oxide, tricalcium silicate, bismuth oxide, dicalcium oxide, Tricalcium aluminate, tetracalcium aluminoferrite and calcium sulphate dehydrate. The WMTA preparation, however, lack tetra calcium aluminoferrate (36). MTA has a soluble fraction mainly composed of calcium hydroxide and the water in contact with MTA had a high alkaline pH, ranging from 11.94 to 11.99 (37). It’s therefore possible that MTA, releasing calcium hydroxide, possesses antimicrobial activity (38).

There is probably no absolute way of determining the effectiveness of any sealer via in vitro studies. The results of such antibacterial tests may not highly correlate with in vivo data, however, its’ save to say that, if a test material consistently induces a strong antibacterial effect in the sensitivity tests, it is very likely also to exert antibacterial action in living tissue. The most desirable endodontic sealer would be one that combines maximal antibacterial effect with minimal toxicity. Therefore, one has to chose the one which combines a reasonably high antibacterial effect with a low toxic effect.

**Conclusion**

Canason showed the highest antimicrobial activity against all microorganisms used in this study, while Acroseal, Epiphany and AH-Plus showed antibacterial activity lesser than that of Canason. The least antimicrobial activity was showed by MTA against all microorganisms used in this study. There were a high significant differences in the antibacterial activity of the five endodontic sealers on *Streptococcus viridans*, *Staphylococcus aureus* and *Enterococcus faecalis*. There were no significant differences in the antibacterial activity of the Acroseal, AH-Plus and MTA on *Staphylococcus aureus*. There was no difference in the zones of inhibition between the 24 and 48 hrs time period for all types of microorganisms used in this study. So it is advisable according to this result not to depend on the antimicrobial activity of the sealer alone in the treatment of infected root canal.

**References**

1- Friedman S, Mor C. The success of endodontic therapy-healing and
29- Eldeniz AU, Erdemir A, Kurtaglu F. Comparative evaluation of pH and


---

Fig. 1 Comparism between the mean of inhibition zones of endodontic sealers produced against Streptococcus viridans after 24 hours

Fig.2 Agar diffusion method of Endodontic sealer on Streptococcus viridans on Mueller Hinton Agar media
Fig. 3 Comparism between the mean of inhibition zones of endodontic sealers produced against *Staphylococcus aureus* after 24 hours

Fig. 4 Agar diffusion method of Endodontic sealer on *Staphylococcus aureus* on Mueller Hinton Agar media

Fig. 5 Comparism between the mean of inhibition zones of endodontic sealers produced against *Enterococcus faecalis* after 24 hours
Fig. 6 Agar diffusion method of Endodontic sealer on Enterococcus faecalis on Mueller Hinton Agar media

Table 1 ANOVA test show the difference among the five endodontic sealers on the three types of bacteria

<table>
<thead>
<tr>
<th>Endodontic sealers</th>
<th>F-test</th>
<th>P-value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroseal</td>
<td>87.32</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Canason</td>
<td>22.85</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Epiphany</td>
<td>47.83</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>AH plus</td>
<td>91.25</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>MTA</td>
<td>373.7</td>
<td>0.000</td>
<td>HS</td>
</tr>
</tbody>
</table>

*HS: Highly significant difference at level P < 0.001*