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In vitro evaluation of the Antimicrobial Activity of four resin based Endodontic Sealers on three bacterial species

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Abstract

Aim: The aim of this in vitro study was to evaluate the antimicrobial action of four resin based endodontic sealers after 24 hrs.

Materials and Method: The freshly mixed sealers were AH26, AH-plus, Epiphany and Topseal. They were prepared according to manufacturer's instruction and placed in prepared wells of 40 agar plates inoculated with *Streptococcus viridans*, *Staphylococcus aureus* and *Enterococcus faecalis.* (10 samples for each microorganism). Four cavities, each one measuring 5 ml in diameter and 4 ml in depth, were made in each agar plate using cork poorer. Agar diffusion method on Muller Hinton agar was employed, and zones of inhibition were measured after 1day.

Results: AH26 containing proved to be the most effective against all microorganisms tested. This was followed by Epiphany. Topseal which showed antibacterial activity on all tested microorganisms slightly higher than that of AH-Plus which showed the least action on all tested microorganisms.

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

Keywords: sealers, endodontic, antimicrobial action, microorganism.

Introduction

The main goal of endodontic therapy is prevention and control of root canal infection. The initial control is set by biomechanical preparation. Biomechanical preparation of a root canal, irrigation, intracanal medication eliminates the greatest amount of microorganisms and their by-products from the canal [(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

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have leaked into the canal after its obturation or from bacteria that 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].

The proliferation and growth of remaining intra-canal microorganisms may destroy periapical tissues and results in periapical pathosis. Furthermore, if the access cavity is not sufficiently sealed, bacteria may penetrate into an obturated root canal within few days; persisting or re-infecting bacteria may induce or sustain apical periodontitis. Therefore, endodontic filling materials should be antibacterial/antimicrobial. Adding anti-microbial agents to root canal sealers is a method that can add antimicrobial properties to the sealers [6].

Endodontic treatment can be aided by clarification of the antibacterial susceptibility of the pathogenic bacteria present inside the infected pulp, to these endodontic sealers that 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 that combines antimicrobial effect with low toxic effect [7, 8].

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*, *Staphylococcus aureus*, and *Enterococcus faecalis*. The microorganisms were identified - in the central health laboratories - ministry of health in Baghdad by a combination of colonial pigmentation, colonial morphology, hemolysis on a tryptone soya blood agar, cell morphology (microscopic morphology) and biochemical tests, 30 samples were used in this study which were divided into 3groups consisted of 10 plate for each group, 10 plates inoculated with *Streptococcus viridans* containing 4 types of sealers as group1. 10 plates inoculated with *Staphylococcus aureus* containing 4 types of sealers as group2, 10 plates inoculated with *Enterococcus faecalis* containing 4 types of sealers as group 3, 5 plates with 4 types of sealers without any bacteria as a negative control group and 5 plates with inoculums without any sealer as a positive control group.

The tests for the three types of bacteria (*Streptococcus viridian*, *Staphylococcus aureus* and *Enterococcus faecalis*) were done with Agar Diffusion method. Four sealers were used in this study which were AH26 (Dentsply), AH-plus (Dentsply), Epiphany (Pentron) and Topseal. (Dentsply) (Figure 1)

Four to five 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 were done for 24 hour at 37 C^{o}. 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 were adjusted visually to match the turbidity of a McFarland 0.5 scale. This number of standard contains approximately 1.5×10^{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 that had been fit to 0.5 McFarland standards. With an adjustable micropipette, 0.1 ml of each bacterial suspension was added to the surface of the plates that 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, four 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 four 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 ^{o}C for 24 hours in the incubator [9]**.** 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 sealers for each bacterial strain.

Results

Effect of the four sealers on *Streptococcus viridans:*

From Figure (2), it is clear that AH26 exhibited the highest mean of inhibition zone, followed by Epiphany. The least mean value of the antibacterial action of *Streptococcus viridans* was shown by Topseal followed by AH-Plus.

Effect of the four sealers on *Staphylococcus aureus*:

From Figure (4) it is clear that AH26 exhibited the highest mean of inhibition zone followed by Epiphany, Topseal came after Epiphany in its antibacterial activity against *Staphylococcus aureus* .The least mean value of antibacterial activity against *Staphylococcus aureus* was shown by AH-Plus.

Effect of the four sealers on Enterococcus faecalis:

Figure (6) shows that AH26 had the highest antibacterial activity, while the lowest mean was shown by Topseal and AH-Plus. Epiphany sealer was ranging in between.

Discussion

ADT (agar diffusion test) is the most commonly used method for evaluating antimicrobial activity of dental materials [10, 11]**.**

The results of this method are influenced by the contact between a material and agar, the possibility of material diffusion into agar (depends on the setting time), agar viscosity, incubation, temperature etc. The main drawback of this method is that it cannot differentiate bactericidal from bacteriostatic effect of a material. Test results are influenced not only by material toxicity, but also by the possibility of dissolving the material in the water component of agar and the diffusion that depends on material solubility and setting time. Highly diffusible material can produce a large growth inhibition zone. Many authors have agreed that this method can be used to compare materials and show which one has the greater antimicrobial effect in the root canal [12].

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 [13]. 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 [9].

AH26 endodontic sealer had the highest mean value among the others in inhibiting *Streptococcus viridans* growth. AH26 also contains hexamethylenetramine that decomposes into ammonia and formaldehyde in an acid environment. The formaldehyde that released from hexamethylenetetramine, 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 [14, 15].

The antimicrobial effect of resin-based sealers may be related to bisphenol A diglycidyl ether that was identified as a mutagenic component of the resin based material. In addition, formaldehyde release in the polymerization process may also assist its antimicrobial properties [16]. Formaldehyde is a phenolic compound that has a strong antibacterial activity in vitro [17]. AH26 was more effective in inhibiting the growth of *Staphylococcus aureus* than *Streptococcus viridans* since *Streptococcus viridans* is less sensitive to formaldehyde than *Staphylococcus aureus* [18]. AH26 had 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 [19]. 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 [20] in addition to amine and epoxy resin components of the sealer [21]. The oxygen inhibition layer of the surface of any polymerizing resin leaves an uncured monomer layer that could be another reason [22]. 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. [23].

Also, there was an extensive calcium release from Epiphany that 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 [24].

AH26 and AH-Plus are basically the same material. The difference between them lies in the presence of silicone and aerosol in the formula as well as the elimination of formaldehyde release from the latter material [24].

AH-plus and Topseal sealer which are a new resin based sealers showed an antibacterial activity lower than that of other sealers 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 that may induce milder antibacterial activity [25]. On the other hand, 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 [21] since AH-plus and Topseal sealers based on polymerization reaction of epoxy resin amines [26].

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 choose the one that combines a reasonably high antibacterial effect with a low toxic effect [7].

Conclusion

Epiphany showed antibacterial activity lesser than that of AH26 that showed the highest antimicrobial activity against all microorganisms used in this study .The least antimicrobial activity was showed by both Topseal followed by AH-plus against all microorganisms used in this study. Therefore, it is advisable according to these results not to depend on the antimicrobial activity of the sealer alone in the treatment of infected root canal.

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Figure 1: AH26, AH-plus, Epiphany and Topseal sealers

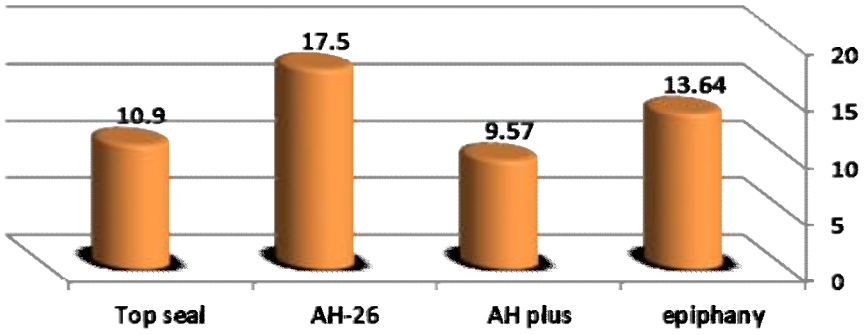

Figure 2: Comparison between the mean of inhibition zones of endodontic sealers produced against *Streptococcus viridans* after 24 hours

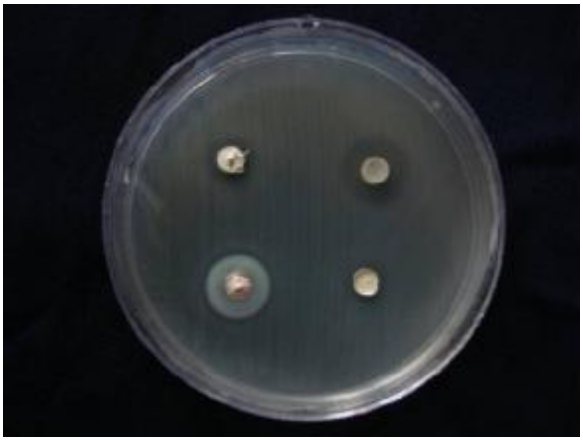

Figure 3: Agar diffusion method of Endodontic sealer on *Streptococcus viridans* on Mueller Hinton Agar media

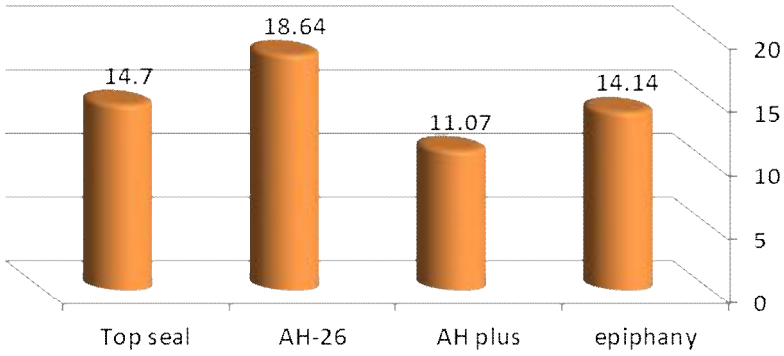

Figure 4: Comparism between the mean of inhibition zones of endodontic sealers produced against *Staphylococcus aureus* after 24 hours

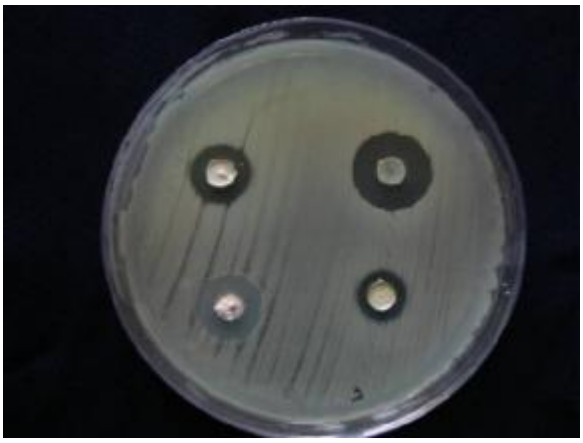

Figure 5: Agar diffusion method of Endodontic sealer on *Staphylococcus aureus* on Mueller Hinton Agar media

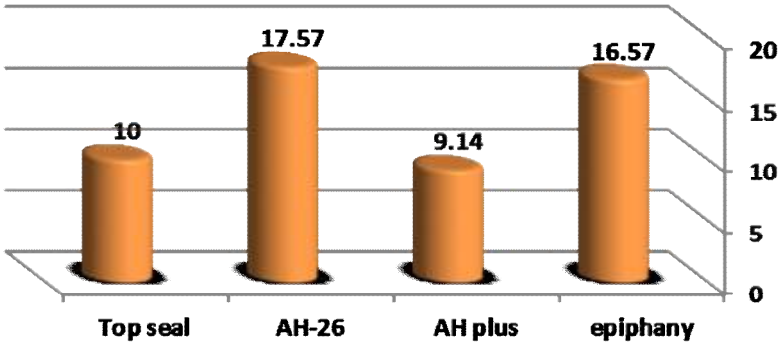

Figure 6: Comparison between the mean of inhibition zones of endodontic sealers produced against *Enterococcus faecalis* after 24 hours

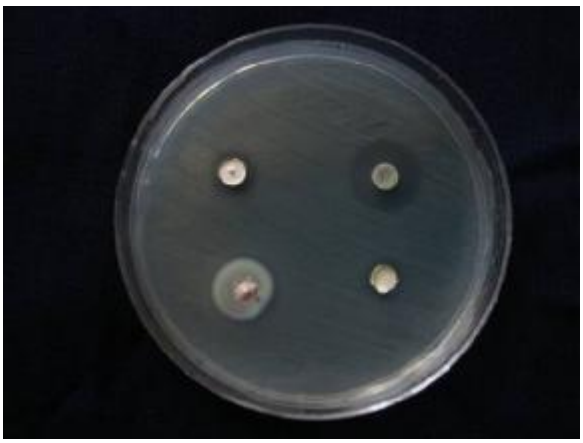

Fig. 7 Agar diffusion method of Endodontic sealer on *Enterococcus faecalis* on Mueller Hinton Agar media.