Evaluation of antibacterial activity of three endodontic sealers against three bacterial strains isolated from root canal. An in vitro study

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Evaluation of antibacterial activity of three endodontic sealers against three bacterial strains isolated from root canal. An in vitro study

Haitham Younis Mohammed, Hadeel Mizher Younis, Hiba younis Khalaf

1College of dentistry, Tikrit University, Iraq
2College of dentistry, Tikrit University, medical microbiology,
3college of veterinary, Tikrit University, medical microbiology

Abstract. This study evaluated in vitro antibacterial action of three endodontic sealer (zinc oxide Zn, carbon hydroxide CaOH and polyoxymethylene Dexamethason PD) against three bacterial strains commonly found in root canal infection which includes Serratia merasences ,Enterococcus feacalis, and Staphylococcus aureus by using agar diffusion method on muller hinton agar and distilled water used as control material. Thirty petriplates(10 petriplates for Serratia merasences ,10 petriplates for Enterococcus feacalis, and 10 petriplates for Staphylococcus aureus ) with 25ml of muller hinton agar were inoculated with 0.1 ml of tested suspensions of tested bacteria(Serratia merasences ,Enterococcus feacalis, and Staphylococcus aureus). Four cavities each one measuring 5ml in diameter and 4 ml in depth, were made in each muller hinton agar plates using cork poorer and then filled completely with experimental product. After incubation at 37c for 24hrs the zone of bacterial growth inhibition produced around the well was measured in mm in diameter. In this study carbon hydroxide proved to be the most effective sealer against three tested bacteria Serratia merasences ,Enterococcus feacalis, and Staphylococcus aureus, this is followed by PD sealer which also showed antimicrobial activity against the three tested microorganisms higher than zinc oxide which showed the least action on three tested bacteria. In Conclusion : All the three endodontic sealers evaluated in this study showed different inhibitory effect against the three tested bacterial strains (Serratia merasences ,Enterococcus feacalis, and Staphylococcus aureus)

1. Introduction
Endodontic therapy is invaluable measurement to protect or preserve teeth that could otherwise need to be extraction. With advancing methodology a best understanding of root canal structure anatomy, and used improved materials, endodontic therapy is achieved an increasingly high at all success rate [1]. However, microorganisms in the root canal have significant impact on this successful rate. When the tooth is exposure to microorganisms and infected prior to use treatment ,the success of endodontic therapy drops to 86%, which is consist from the 96%success rate of endodontic treated teeth without apical periodontal disease [2].The endodontic infection is of multimicrobial nature with predominance of facultative anaerobic microorganisms, which are leak within the root canal after obturation [3].Since removing all microorganisms in canal prior to obturation difficult proven even after chemomechanical preparation[4]. Bacteria may also persist inside root canal system like lateral canal, secondary canal,apical foramen,dental tubule, accessory canal apical cementum surface and extraradicular infection[5,6]. In the last stage of endodontic obturation, the staying bacteria should be destroyed to obtain successful results. Root canal sealers have ability to do these action like one of requirements of
best sealer cement as showed by Grossman [7]. Root canal sealer stays for a long period inside the canal and it may be reach microorganism present inaccessible sites of endodontic system [8]. Therefore, endodontic sealer should be able to eliminate remaining pathogens ,neutralize their toxic products and prevent reinfection to create good environment for treatment process to proceed[9]. Root canal treatment also,can be helped by clarification of antibacterial sensitivity of pathogenic microorganism found inside the infected pulp , to these root canal sealers which have different antimicrobial actions against different bacteria present inside infected pulp.This various antimicrobial actions are due to their chemical composition and additive incorporated inside sealers. The most common chemical could by one that have effect with minimum toxicity. Therefore ,the one antibacterial effect with the least toxic effect[10,11]. Agar diffusion method has been very widely used for such study[9,12-19].

2. Materials and Methods
Fifty patients were examined as the sample of this study. They were referred to the Laboratory of Microbiology, Department of Microbiology, College of Medicine ,Tikrit University after examining them in dental teaching in College of Dentistry of the same University . The diagnosis of root canal disease was made through a clinical examination , so patients fulfilling the criteria to be diagnosed as endodontic infection. The evaluation of endodontic criteria were root canal obturation length, root canal obturation density (homogeneity), and root canal obturation taper. Also, an X-ray of the involved tooth was taken (Figure 2). Clinical examination and diagnosis in each case take place by a dentist. The involved tooth was isolated under a rubber dam. The field was disinfected with tincture of iodine and the access cavity was prepared with a sterile round bur. On gaining access to the pulp, a sterile reamer/file/broach was inserted into the root canal up to the apical foramina and root canal content were obtained for culture[20]. Root canal contents were inoculated on brain heart infusion broth ,then inoculated on brain heart infusion agar ,blood agar and MacConkey agar and placed in an incubator at 37 degree C for 18 – 24hrs. Colony characteristics were noted in case of any growth and identification was done by Grams staining and by standard biochemical reactions[5] For the agar diffusion test. thirty petriplates(10 for Serratia merasences ,10 for Enterococcus faecalis, and 10 for Staphylococcus aureus ) with 25ml of muller hinton agar were inoculated with 0.1 ml of suspension of tested bacteria . Four cavities each one measuring 5ml in diameter and 4 ml in depth ,were made in each muller hinton agar plates using cork poorer and then it filled completely with cao,zn ,and PD. 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 incubation of the plates at 37c for 24hrs the zone of tested bacterial growth inhibition produced around the well was measured in (mm) in diameter. Statistical analysis of data by using analysis of variance ANOVA was done. P value of ≤ 0.05 was regarded as statistically significant difference between each type of the three endodontic sealers in their antibacterial action against the three types of bacteria. Bar chart used to present the data.

3. Results
Effect of the three sealers on Serratia merasences: From Table 1 it's clear that carbon hydroxide exhibited the highest mean of inhibition zone value (33.0), followed by PD with values of (12.310). The least mean value of the antibacterial action of Serratia merasences was shown by zinc oxide with a mean of (6.770).

Effect of the three sealers on Staphylococcus aureus: From Table 3 it's clear that carbon hydroxide exhibited the highest mean of inhibition zone value (31.400) followed by PD with a mean of (17.260). The least mean value of antibacterial activity against Staphylococcus aureus was shown by zinc oxide with a mean value (7.090).

Effect of the three sealers on Enterococcus faecalis: Table 5 shows that carbon hydroxide had the highest antibacterial activity with the mean value of (30.244) while the lowest mean was shown by zinc oxide (5.370). The PD sealers where with a mean values of (14.980).

Effect of each of the three sealers on the three bacterial strains: In this study carbon hydroxide proved to be it is the most effective sealer against three tested bacteria Serratia merasences,
Enterococcus feacalis, and Staphylococcus aureus with mean (33.31, 30.24) respectively. This is followed by PD sealer which also showed antimicrobial activity against the three tested microorganisms higher than zinc oxide which showed the least action on three tested bacteria Serratia merrasences, Enterococcus feacalis, and Staphylococcus aureus with mean (12.31, 17.26, 14.68) respectively. Statistical analysis of data by using analysis of variance ANOVA was done which showed that there was a statistically high significant difference (p-value < 0.05) and between each type of the three endodontic sealers in their antibacterial action against the three types of bacteria and F-value (1565.21, 638.89, 814.38) for Serratia merrasences, Staphylococcus aureus, and Enterococcus feacalis respectively as shown in Figure 1, and Tables 2, 4 and 6.

Table 1: Comparison between means of inhibition zone of endodontic sealers produced against Serratia merrasences

| Sealers | N | \( \text{mean} \pm \text{SD} \) | CI (95%) |
|---------|---|-----------------|--------|
| CaO     | 10| 33.000±0.776    | (32.283; 33.717) |
| PD      | 10| 12.310±1.252    | (11.593; 13.027) |
| ZnO     | 10| 6.770±1.222     | (6.053; 7.487)   |

Pooled StDev = 1.10504

SD: Standard deviation, N: number of tested bacteria ZnO: zinc oxide, CaOH carbon hydroxide, and PD: polyxymethylene Dexamethasone. CI: confidence limited

Table 2: Analysis of Variance of inhibition zone of endodontic sealers produced against Serratia merrasences

| Source   | DF | Adj SS   | Adj MS | F-Value | P-Value |
|----------|----|----------|--------|---------|---------|
| Sealer   | 2  | 3822.60  | 1911.30| 1565.21 | 0.000005|
| Error    | 27 | 32.97    | 1.22   |         |         |
| Total    | 29 | 3855.57  |        |         |         |

Adj SS: Adjustment sum square, Adj MS: Adjustment mean square

Table 3: Comparison between means of inhibition zone of endodontic sealers produced against Staphylococcus aureus

| Sealers | N | \( \text{mean} \pm \text{SD} \) | CI (95%) |
|---------|---|-----------------|--------|
| CaO     | 10| 31.400±1.612    | (30.409; 32.391) |
| PD      | 10| 17.260±1.509    | (16.269; 18.251) |
| ZnO     | 10| 7.090±1.456     | (6.099; 8.081)   |

Pooled StDev = 1.52744

SD: Standard deviation, N: number of tested bacteria ZnO: zinc oxide, CaOH carbon hydroxide, and PD: polyxymethylene Dexamethasone. CI: confidence limited
Table 4: Analysis of variance of inhibition zone of endodontic sealers produced against *Staphylococcus aureus*

| Source   | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|----------|----|---------|---------|---------|---------|
| Sealer   | 2  | 2981.15 | 1490.57 | 638.89  | 0.0007  |
| Error    | 27 | 62.99   | 2.33    |         |         |
| Total    | 29 | 3044.14 |         |         |         |

Adj SS: Adjustment sum square, Adj MS: Adjustment mean square

Table 5: Comparison between means of inhibition zone of endodontic sealers produced against *Enterococcus fecalis*

| sealers | N | means± SD | CI (95%)          |
|---------|---|-----------|-------------------|
| CaO     | 10| 30.244±1.969 | (29.320; 31.168) |
| PD      | 10| 14.980±1.192  | (14.103; 15.857) |
| ZnO     | 10| 5.370±0.622   | (4.493; 6.247)   |

Pooled StDev = 1.34849

SD: Standard deviation, N: number of tested bacteria, ZnO: zinc oxide, CaOH: carbon hydroxide, and PD: polyxymethylene Dexamethasone. CI: confidence limited

Table 6: Analysis of variance of inhibition zone of endodontic sealers produced against *Enterococcus fecalis*

| Source | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|--------|----|---------|---------|---------|---------|
| Sealer | 2  | 2961.79 | 1480.90 | 814.38  | 0.00008 |
| Error  | 26 | 47.28   | 1.82    |         |         |
| Total  | 28 | 3009.07 |         |         |         |

Adj SS: Adjustment sum square, Adj MS: Adjustment mean square

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![Graph showing inhibition zones of Staphylococcus aureus, Enterococcus fecalis, and Serratia marcescens for various sealers](image)
Figure 1: Comparison between the mean of inhibition zones of three endodontic sealers produced against *Staphylococcus aureus*, *Enterococcus feacalis* and *Serratia merrasesces*.

Figure 2: X-ray for patient with endodontic infection (The dark area around the entire root of the tooth above indicates the presence of a large infection)

4. Discussion
Measuring the activity of antibacterial agent is used for improving the infection control program. Generally, there are three in vitro techniques that have been used for this purpose – the dilution method which give a quantitative result for the amount of antibacterial agent that is needed; the agar diffusion method, which gives an inhibition zone around the well containing the sealer and that could be related to its effect, and the direct exposure method, which provides qualitative information about the used materials. The method of measuring antibacterial action used here was to determine the size of the zone of bacterial growth inhibition around the sealer. The size of this zone will depend on two most factors. The first is the toxicity of the components of the sealer under study. The second is the diffusibility of any toxic factors released from the sealer. This diffusibility is a function of the hydrophilicity or hydrophobicity of the material being released and the rate of which these materials are released from the matrix of the sealer 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 37oc under appropriate gaseous condition [11]. In the present study different sealers showed varying effects on different bacteria. Based on mentioned factors, Carbon hydroxide endodontic sealer had the highest mean value among the others in inhibiting *Serratia merrasesces* growth. 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 [21] Or leading to the destruction of the phospholipids or nonsaturated fatty acids that result in a loss of cytoplasmic membrane integrity [22]. The second is protein denaturation. The alkalineization 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 [23]. 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 [24]. It has been suggested that the ability of calcium hydroxide to absorb carbon dioxide may contribute to its antibacterial activity especially facultative and obligate anaerobic [25]. Another reason to inhibit bacterial growth may be because of the presence of amines in the epoxy base of carbon hydroxide as a
new calcium hydroxide based sealer [26]. Calcium hydroxide sealer showed an antibacterial activity less than PD and Zinc oxide for several reasons. It may had been due to its low diffusibility in agar and due to the buffering ability of the artificial media, which reduced, it’s high pH and lowering its antibacterial activity [27]. 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 [28]. In the present study, the pre incubation 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 [10]. There is probably no absolute method of determining the effectiveness of any sealer through 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.

5. Conclusion
In this study carbon hydroxide proved to be the most effective sealer against three tested bacteria serratia merasences, Enterococcus feacalis, and staphylococcus aureus followed by PD and Zn respectively. In general there are all the three endodontic sealers evaluated in this study showed different inhibitory effect and highly significant against the three tested bacterial strains (serratia merasences, Enterococcus feacalis, and staphylococcus aureus).

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