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In vitro evaluation of the antibacterial effects of MTA- Fillapex and BIO-C® sealer at different time intervals

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ABSTRACT

Failure of endodontic treatment is closely associated with bacterial infection, the study aimed to evaluate the antibacterial properties of MTA- Fillapex and BIO-C against two bacterial species Enterococcus faecalis (E. faecalis) and Staphylococcus aureus (S. aureus). In this *in vitro* study, two endodontic sealers were used to evaluate antibacterial properties by using an agar diffusion test (ADT) and direct contact test (DCT). The width of the growth inhibition zone after 24 hours was reported in (ADT) as the endodontic sealers' effectiveness. In DCT, the survival of microorganisms was assessed at various periods following the exposure of the bacterial suspension to the sealers for (20 and 40 min) at (1 day, 7 days and 14 days). Colony-forming unit (CFU) counts were made. In ADT, the zones of microbial growth inhibition in BIO-C sealer caused by *E. Facealis* were relatively larger than that created due to *S. Auerous* bacteria with mean 0.781 mm and 0.538 mm respectively. Thus, this difference turned out to be significant ($p<0.001$). In DCT At both time points tested, no significant difference was found among control cases against the tested microbes ($p > 0.05$). In terms of sealers, BIO-C had the most potent antimicrobial properties. It showed significant inhibition against both *E.faecalis* and *S. Auerous* on day 1 and the first week of contact times tested. Moreover both BIO-C and MTA Fillapex sealers perform worthy antibacterial activity up to 1 week and BIO-C sealers show better antibacterial efficacy against *E. faecalis* than MTA Fillapex sealers.

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Introduction

Failure of endodontic treatment is closely associated with a bacterial infection. Therefore, the primary goal of endodontic therapy is the elimination of microorganism and their products inside the root canal system(1, 2). Unfortunately, there are various bacterial species inside the root canal system, Enterococcus faecalis (E. faecalis) has been detected commonly in peri apical lesions on the other hand Staphylococcus aureus (S. aureus) has been detected in early diagnosis of root canal infection (3). To eliminate the microorganism and bacterial infective substance inside the canal several clinical protocols were performed including chemo mechanical root canal system preparation (4), but even after all these clinical protocols, evidence suggests that residual microorganism remains inside the root canal system interfering with healing and repairing tissue processes(5-7). Sealing the root canal system is considered as the main procedure after chemo-mechanical preparation to prevent the survival of residual microorganisms, therefore the anti-microbial properties of sealing material are a golden standard for any type of root canal sealer (8, 9).

The BIO-C® sealer, a tri-calcium silicate-based sealer material which has many advantages; it is considered as a bioactive and biocompatible material that induces the formation of hard tissue (hydroxyapatite) in bone and teeth by highly releasing calcium ions. In addition to anti-microbial properties that came from the alkaline ph of sealer after application with adequate flowability (10-12).

Moreover, root canal sealer based on MTA, such as MTA-Fillapex (Angelus, Londrina, PR, Brazil), It has resinous components, and according to their maker, it has high radiopacity, is simple to handle, and has a long working period. (13, 14).

Many in vitro studies have been conducted to assess the antibacterial activity of endodontic materials using various methodologies. In the present study, the antimicrobial activity of MTA- Fillapex and BIO-C® sealers were evaluated by agar diffusion test (ADT) and direct contact test (DCT) (15-18). Endodontic sealers can have varying inhibitory effects based on their composition, together with the assessment technique and test durations applied. One of the most often utilized approaches is ADT. (8). Nevertheless, this approach has a shortcoming in that it is affected by diffusion and the physical features of the test specimens. The direct contact test is frequently used to examine the antibacterial effect of root canal cements and endodontic filling products. The quantitative test is intended for the examination of insoluble compounds in typical configurations(19). Therefore, the current study aimed to evaluate the antibacterial properties of MTA- Fillapex and BIO-C® against two bacterial species (*E. faecalis* and *S. aureus*) by both ADT and DCT at different time intervals.

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Materials and Methods

In this in vitro observational study two endodontic sealers were used: an MTA based sealer (MTA- Fillapex; Angelus, Londrina, PR, Brazil) and a new calcium silicate-based sealer (BIO-C®sealer; Angelus, Londrina, PR, Brazil). In line with the manufacturer's instructions, the sealers were made.

The test was done under strict aseptic conditions in the Department of Microbiology (Rizgary hospital, Erbil, Iraq). Antimicrobial activities of the sealers were evaluated against S. aureus (ATCC-25923), E. faecalis (ATCC-29212). The microorganisms were cultivated on blood agar at 37 °C for 2 days. Then the suspensions were prepared in matching with 0.5 McFarland Standard turbidity (about 1.5 10^8 CFU/ml).

Using double-layered plates, an agar diffusion test (ADT) was performed. The first layer was 50 ml Muller Hinton agar (MHA- Merck; Germany). All four freshly mixed sealers were placed in four (6 mm diameter and 4 mm deep) wells. were punched at equidistant points in ten plates. All plates were left incubating for one day at 37 °C. As the second layer, standardized 0.5 ml McFarland bacterial suspensions were seeded into 20 ml of the Muller Hinton agar. The prepared plates were incubated at 37 °C for 24 hours and A suitable ruler was used to measure the inhibition zones surrounding each well to a precision of 0.5 mm. For each sealer, the measured zone's average diameter was calculated.

The modified direct contact test (DCT) was done to examine the antibacterial action of the root canal sealers that have already been reported (19). In the current investigation, all sealers were applied in sterilized flat bottom capped tubes. In the bottom of each tube, 0.5 g of the experimental sealers were placed. Sealers were classed as three groups (1-d, 7-d, and 14-d) samples after being let to set for 1, 7, and 14 days before testing in a humid atmosphere at 37 °C.

On the surface of each sealer, a 50 mL bacterial suspension (1.5 108 CFU/ml) was gently placed. As controls, normal saline solutions without the sealer were used. After incubating the plates in a humid environment at 37 °C for 20 and 40 minutes, 450 µL of sterile saline was added to each tube. The bacterial suspensions were transferred after 2 minutes of gentle mixing using a pipette and by culturing 50 µL of aliquots onto MHA plates after 10-fold repeated dilutions in sterile saline, the bacteria's survivability in the solutions was evaluated. Following 48 hours of incubation at 37 °C, colonies on the plates were enumerated and (CFU/mL) was calculated. All tests were carried out in duplicate.

Statistics analysis

In the Agar diffusion test; The diameter of inhibition zones was measured for each group. The descriptive statistic showed the mean and stander deviation of each group. The difference between groups were analyzed by t-test. In the direct contact test; CFU counts were measured and the chi-square test was used to reveal the statistical differences between groups.

Results

Agar Diffusion Test

Table 1 shows the descriptive statistics of the impact of bacteria activities on both sealer groups BC Sealer and MTA Sealer. On BIC C sealer, the zones of microbial growth inhibition caused by *E. Facealis* were relatively larger than that created due to Staph Auerous bacteria with mean 0.781 mm and 0.538 mm respectively. Thus, this difference turned out to be statistically significant with ($p<0.001$) On the other hand, the MTA sealer type showed a large significant difference of inhibition zones against Staph Auerous by 12 mm mean values while with *E. Facealis* activity it was 0.250 mm.

Figure 1 displays the effectiveness of both activities clearly and shows inhibition zones on MTA sealer created by Staph Auerous microbes were very larger than that on BC sealer and also larger than E. Facealis activity on both sealers.

Table 2 explores the test difference of mean values of each test microbes effect on both sealers and both activities demonstrated inhibition zones differently and significantly with mean differences 0.531 mm and 11.462 mm, and ($p<0.001$) less than significant level 0.05 in both E. Facealis and Staph Auerous activities.

Direct Contact Test

To distinguish the effectiveness of antimicrobial activity on the used bacteria, a chi-square test of dependency

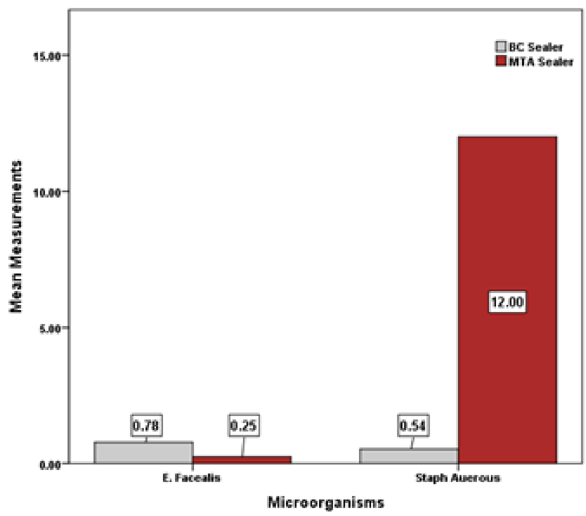

Figure 1. Mean values demonstrations.

Table 1. Descriptive statistics of inhibition for BC and MTA Sealers against microbes.

Sealer Groups	Bacteria Type	N	Mean ± SD	Mean Difference	T. Value (P-Value)
BIC C Sealer	E. Facealis	8	0.781 ± 0.116	0.244	4.318 (p<0.001)
	Staph Auerous	8	0.538 ± 0.109		
MTA Sealer	E. Facealis	8	0.250 ± 0.053	-11.750	-43.855 (p<0.001)
	Staph Auerous	8	12.000 ± 0.756		

Table 2. Statistical Test Comparison of bacterial activity separately against each sealer.

Bacteria Type	Sealer Groups	N	Mean Difference (SE)	T. Value (P-Value)
E. Facealis	BIC C Sealer	8	0.531 (0.045)	11.739 (p<0.001)
	MTA Sealer	8		
Staph Auerous	BIC C Sealer	8	-11.462 (0.270)	-42.447 (p<0.001)
	MTA Sealer	8		

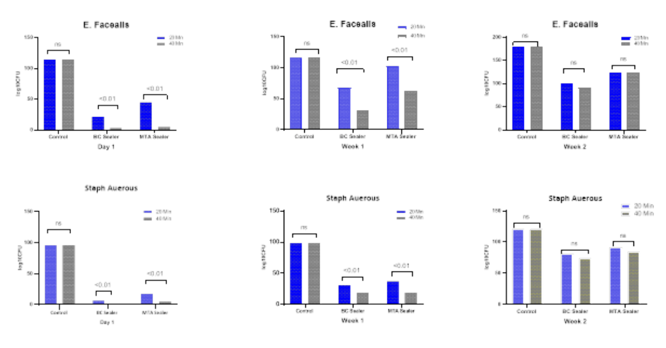

Figure2. Survival of microbes after direct contact test for 20 and 40 min with different sealers at different ages.

was implemented and Figure 2 depicts the antimicrobial activity of tested endodontic sealers made from a modified direct contact test. At both time points tested, no significant difference was found among control cases against the tested microbes ($p > 0.05$). In terms of sealers, BC had the most potent antimicrobial properties. It showed significant inhibition against both *E. faecalis* and Stapha Auerous at day 1 and the first week of contact times tested. MAT and BC sealer's antimicrobial effect deteriorated significantly over time. They had no antimicrobial effect after the second week of setting. Furthermore, both sealers seemed to have a similar impact on the Stapha Auerous bacteria at 20 min and 40 min for week one and week two-time points.

Discussion

The anti-bacterial properties of endodontic sealer are considered one of the ideal properties of optimal endodontic root canal sealer. The main function of endodontic sealer inside the root canal is to seal the canal by filling the space and gap between the core material and root canal wall.

In this study, the bioceramic sealer with MTA-based sealer was used due to their anti-microbial properties for both of them (11). *E. faecalis* and *S. Aureus* microorganisms were used as standard organisms for anti-microbial tests in addition that the *E. faecalis* was mainly associated with persistent endodontic treatment failure. It has the ability to penetrate and survive in the dentinal tubules alone or with other microorganisms in the canal space (20, 21). *S. aureus* mainly associated with primary endodontic infection also was isolated in cases of endodontic reinfection (22).

Accordingly; the success of endodontic treatment is directly related to the long-term anti-microbial activity of sealer against persistent endodontic infections.

Two types of antimicrobial test were used in this study; agar diffusion test and direct contact test the agar diffusion test only determine the capacity of sealer diffusion with anti-microbial properties, while the direct contact test is considered as a more anti-microbial-reliable test.

In the agar diffusion test, bio-ceramic sealer showed the strongest antimicrobial activity against *E. faecalis* but was weaker against S. Aureus when compared with MTA sealer which showed brilliant anti-microbial activity against S. Aureus about 12mm. These findings are in accordance with Rathod RK et al.(23) reported that BC sealer showed the least antimicrobial activity against S. aureus in agar diffusion test among tested sealers and Munitić MŠ et al. (24) showed that The premixed BC Sealer had better antibacterial efficacy than the MTA Fillapex sealers against *E. faecalis*. This can be due to that MTA sealers contain MTA and salicylate resin while bioceramic sealers contain mainly calcium silicate and calcium phosphate accordingly the flowability of bio-ceramic sealer was more than MTA Sealer and the anti-microbial activity directly related to diffusion and surface contact of sealer with alkaline PH that affects protein, lipid and DNA of bacteria causes the death of bacteria. MTA contains resin that has a direct effect on *S. Aureus* than *E.faecalis* may resist more, while *E. Faecalis* are directly affected by alkaline pH and hydroxyl release which was more in the bio-ceramic sealer.

In the direct contact test, all samples demonstrated a lower number of CFUs but bacteria were not completely eradicated in any group this result can be explained due to the type of bacteria used in the present study can affect the antibacterial efficacy of sealer. In most *in vitro* studies, the antibacterial effect of endodontic sealer was evaluated by using planktonic bacteria. However, because planktonic bacteria do not accurately represent a true clinical condition in infected root canals, the use of older bacterial biofilms with higher resistance has been suggested (16, 17, 24, 25).

In the present study, the anti-microbial action of both sealers decreased over time. After 2 weeks both sealers showed similar results as had no antimicrobial activity. These results are similar to the study of Bukhari and Karabucak, (26) that conclude BC Sealer was superior antimicrobial capacity for up to 2 weeks in killing *E. faecalis.* Besides opposite to the study Ustun et al. (27) that found bioceramic sealer to have antibacterial activity against *E. faecalis* using a time-kill assay and MTA fillapex were the only sealer that bactericidal at 30 days against *E. faecalis*.

In one day and one weeks bioceramic sealer act in a better way, which may be due to the sealer containing an amount of oxide compounds that potentially have antimicrobial activity These findings are consistent with previous researches conducted on bioceramic sealers have significant antibacterial efficacy against *E. faecalis* up to one week (19, 26, 28, 29)

Also in another study, Mangat et al.(29) compared the antimicrobial activity of Bioceramic BC sealer and MTA fillapex sealer against *E. faecalis* at distinct time intervals from 1day to 1 week and concluded that Bioceramic sealer showed better antimicrobial activity than MTA Fillapex and Apexit in both (ADT) and (DCT). In another word bioceramic sealer had longer activity than MTA sealer this result may be associated with Ca+2 ion and with setting reaction more calcium hydroxide formed which makes the environment more alkaline (19)

Within the limitation of the study, it can be concluded that both BIO C and MTA Filapex sealers perform worthy antibacterial activity for up to 1 week and Bio C sealer shows better antibacterial efficacy against *E. faecalis* than MTA fillapex sealer.

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Interest conflicts

The authors state that they have no conflicts of interest.

Author's contribution

The manuscript is equally liable to all writers.

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