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Comparison of the Antibacterial Effect of AH26, Adseal and Beta RCS Root Canal Sealers Against *Enterococcus Faecalis*, an *in Vitro* Study

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ABSTRACT

Objectives: Antibacterial activity against endodontic pathogens is a desirable feature for root canal sealers. The objective of this study was to compare the antibacterial effect of three resin-based endodontic sealers (AH26, Adseal, and Beta RCS) against *Enterococcus faecalis* in vitro.

Materials and Methods: The antibacterial properties of the sealers were assessed against *E. faecalis* using agar diffusion test (ADT) for fresh state (N=10) and direct contact test (DCT) for freshly-mixed and set states of the materials (N=10). In ADT, the diameter of the zones of inhibition was measured after 24h of contact. In DCT, the colony-forming units of the bacteria were counted after 30 minutes and 180 minutes of exposure. The results were analyzed with two-way ANOVA and independent sample t-test. $P<0.05$ was considered significant.

Results: Regarding DCT results, all test materials indicated an antibacterial effect, both in freshly-mixed and set states. The highest antibacterial effect was related to Adseal, whereas the lowest was observed in Beta RCS. There was a significant difference between all study groups (different sealers, setting states, and contact times; $P<0.001$), except for freshly-mixed AH26 and Adseal at 180 minutes ($P>0.05$). According to ADT, AH26 and Adseal represented the widest and the smallest inhibition zones, respectively ($P<0.001$).

Conclusion: Within the limitations of this *in vitro* study, AH26, Adseal, and Beta RCS showed antibacterial effects against *E. faecalis* in both freshly-mixed and set states. The antibacterial effect increased over time in all of the studied sealers.

Keywords: Anti-Bacterial Agents; Bacteria; Epoxy Resin-Based Root Canal Sealer; Root Canal Filling Materials

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INTRODUCTION

Remaining root canal infection is the leading cause of apical periodontitis and endodontic treatments are mainly aimed at treating and preventing this disease. Therefore, eradication of endodontic pathogens and their by-products from the root canal system plays an integral part treatment success [1]. Although root canal treatment can significantly reduce infection, it is impossible to completely eliminate the microorganisms and their byproducts from the root canal system [2]. Therefore, in addition to various methods of chemomechanical disinfection of the root canal system, sealers with antibacterial properties can further reduce root canal infection, helping to achieve the primary goal of endodontic treatment.

Enterococcus faecalis, a facultative gram-positive microorganism, is one of the most common bacterial species observed in failed root canal therapy cases [2] and over one-third of the root canals with persisting periapical lesions [3]. This bacteria is resistant to endodontic disinfection procedures and

intra-canal medications such as calcium hydroxide [4]. In addition, it has been shown that *E. faecalis* is able to colonize root canals space and form intracanal biofilm as a monoculture [5].

Root canal sealers are mainly aimed to fill the spaces between the core filling materials and the root canal walls, irregularities in root canal space, lateral and accessory canals, and spaces between lateral gutta-percha cones in lateral compaction technique [6]. The presence of antibacterial properties in sealers is an added advantage that can help eliminate the remaining bacteria from the root canal after cleaning and disinfecting procedures. Antibacterial properties of root canal sealers have been widely investigated in the literatureStudies have demonstrated that the antibacterial properties observed during the polymerization process of epoxy resin sealers can be attributed to the release of bisphenol diglycidyl ether and formaldehyde [7,8]. AH26 is an epoxy-resin sealer with high antibacterial properties against *E. faecalis* [9,10]. However, in previous studies, the antibacterial effect of silver-free AH26 has only been investigated by the agar diffusion method. Adseal is another commonly used epoxy resin-based sealer. It is provided in a dual syringe with base (epoxy resin and calcium phosphate) and catalyst (amines and bismuth subcarbonate). This sealer has demonstrated lower antibacterial properties compared to AH26 in its fresh state [9]. However, the comparison between fresh and set states of these sealers has not been investigated. Beta RCS is a newly introduced resin-based root canal sealer. Preliminary studies on biocompatibility, flowability, radiopacity, solubility, and film thickness of this material corresponded to ISO 6876 for endodontic sealers and was comparable to AH26 [11]. Although faster setting of Beta RCS has been claimed by the manufacturer, the antibacterial properties of this material compared to the other commonly used sealers has not been investigated.

The aim of this experimental study was to compare the antibacterial effect of AH26, Adseal, and Beta RCS sealers in both freshly-mixed and set states against *E. faecalis* using agar diffusion test (ADT) and direct contact test (DCT).

MATERIALS AND METHODS

In this experiment, the antibacterial activity was studied using the standard strain of *E. faecalis* (ATCC 11700) obtained from Pasteur Institute (Tehran, Iran) cultivated in brain heart infusion (BHI) agar for 48h. The inoculum was adjusted to match the turbidity equivalent to 10^5 CFU/ml for further investigations.

Equal amounts of AH26 (De Trey, Dentsply, Konstanz, Germany), Adseal (Meta Biomed, Korea), and Beta RCS (Beta Dent, Tehran, Iran) sealers were prepared according to the manufacturers' recommendations (N=10). AH26 and Beta RCS were mixed separately on sterile glass plates under aseptic conditions in 2/1 powder to liquid ratio. Adseal was injected on a separate plate as well and mixed thoroughly. Freshly-mixed samples were immediately subjected to the experiment while set specimens were used after 72h of incubation at 37°C and 95% relative humidity to ensure complete setting. The main composition of the tested sealers is represented in Table 1.

Table 1. The composition of the tested sealers

Sealer	Company	Components	Composition
Beta RCS	Beta Dent, Tehran, Iran	Powder	Bismuth oxide, methenamide
		Resin	Bisphenol-A-diglycidylether
AH26-silver free	De Trey, Dentsply, Konstanz, Germany	Powder	Bismuth oxide, methenamide
		Resin	Bisphenol-A-diglycidylether
Adseal	Meta Biomed Co. Ltd., Korea	Base	Bismuth subcarbonate, zirconium oxide, glycol salicylate, calcium phosphate, epoxy oligomer resin, ethylene
		Catalyst	Bismuth subcarbonate, tri ethanolamine, calcium phosphate, zirconium oxide, calcium oxide, poly aminobenzoate

In order to evaluate the antimicrobial properties, agar diffusion test (ADT) was used for the freshly-mixed samples and direct contact test (DCT) was used for both freshly-mixed and set specimens.

Agar diffusion test:

The bacteria were seeded on 25 sterile Petri dishes containing Müller–Hinton agar using swabs saturated in the bacterial suspension. Then, 5mm depth and 3mm diameter wells were punched, and the freshly-mixed sealers were poured into the wells (N=10). Wells without the addition of any sealer were considered as the control group (N=10). A total of 40 punched wells were examined and each group contained 10 wells. The Petri dishes were incubated at 37°C and 95% humidity for 24h. The measurement of the inhibition zones surrounding each well was conducted by employing a ruler to determine their respective diameters.. The mean diameter of the measured zone of inhibition was analyzed statistically to assess and compare the antimicrobial activity of the tested sealers.

Direct contact test:

The equivalent of 90 mg of the mixed sealers was poured into 24-well plates (N=10). In the freshly-mixed groups, 60 µl of bacterial suspension (10^5 CFU/ml) was added immediately to the wells. In the set groups, the plates were incubated for 72h, then the bacterial suspension was added. A well without addition of any sealer was considered as the control group (N=10). The plates were incubated at 37°C for 30 (freshly-mixed samples) and 180 minutes (freshly-mixed and set samples). One-hundred µl aliquots of the suspension were cultured onto sabouraud agar plates and incubated for 24h at 37°C. A total of 90 plates were used for the experimental groups and the sample size for each group was 10. Afterwards, the colony-forming units (CFU) were counted. The percentage of bacterial reduction was calculated by subtracting this amount from the CFU of the control groups multiply by 100 and devided by the CFU of the control.

Statistical analysis:

Mean±standard deviation (SD) was calculated, and a one-way ANOVA (followed by post hoc tests) were used for analyzing the results of ADT and DCT in 30 minutes contact time, since there was only one independent variable (sealer type) and one dependent variable (the diameter of inhibition zone and percentage of bacterial reduction, respectively).

In the DCT, percentage of bacterial reduction was reported as mean±SD, assuming normal distribution of data. To evaluate the effect of sealers and the setting status in 180 minutes, two-way ANOVA was used. To compare the effect of sealers and the contact time in the freshly mixed state, two-way ANOVA (followed by least significant difference pairwise comparison) was used, which due to the significant interaction between these two variables, the comparison between the 30 minutes and 180 minutes of contact time for each group was done with the t-test. All of the data were analysed using SPSS software, Version 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). P-values less than 0.05 were considered to be statistically significant.

RESULTS

Agar diffusion test:

The comparison of the inhibition zones of the studied sealers in freshly-mixed state is shown in Table 2. AH26 (37.00±2.26), Beta RCS (19.60±0.70), and Adseal (8.40±0.84) showed diameters of the inhibition zones in a decending order. The difference in antibacterial effect between all three sealers was statistically significant (P<0.001).

Direct contact test:

The means and standard deviations of the percentage of CFU reduction are represented in Table 3. Pairwise comparisons of the study groups are presented in Table 4. As shown in Figure 1, all the three sealers, indicated antibacterial effects against *E. faecalis* in both freshly-mixed and set states. Increasing the contact

Table 2. Comparison of the inhibition zones of the investigated sealers in their freshly-mixed state

Comparison	MD	95% CI	P
AH 26 vs. Beta RCS	17.4	15.79-19.01	<0.001
Beta RCS vs. Adseal	11.2	9.59-12.81	<0.001
AH 26 vs. Adseal	28.6	26.99-30.21	<0.001

MD: mean difference; CI: confidence interval

Table 3. Percentage of bacterial colony reduction in contact (minutes) with the studied sealers

Sealer	Contact time (minutes)	Condition (N=10, each)	
		Fresh	Set
AH26	30	34.07±2.08	-
	180	99.99	94.28±0.94
Beta RCS	30	21.63±1.29	-
	180	98.29±0.68	91.34±2.01
Adseal	30	49.6±2.91	-
	180	99.99	98.76±0.7

time from 30 minutes to 3h led to a significant increase in antibacterial effects in freshly-mixed states of all three sealers (P<0.001). Among all study groups, Adseal indicated the highest antibacterial effect, whereas the lowest antimicrobial efficacy was observed in Beta RCS sealer. Exept for AH26 and Adseal which were equally capable of eliminating *E. faecalis* during a 180-minute contact (P>0.05), there was a significant difference between all study groups (different sealers, setting states, and contact times; P<0.001).

Irrespective of the sealer type, the setting state (mean difference, 4.63; 95% confidence interval, 4.12 - 5.14) and contact time (mean difference, -64.32; 95% confidence interval, -65.14 - -63.51) significantly (P<0.001, both) afftected the percentage of bacterial reduction.

DISCUSSION

Total sterilization of the root canal system is not an achievable goal even following use of advanced cleaning and shaping methods, irrigants, and intra-canal medicaments [12]. The residual microorganisms can lead to failure of endodontic treatment [12]. Therefore, using a root canal sealer with antibacterial activity may help eliminate the residual microorganisms and increase the success of the treatment [13]. The aim of this experimentation was to to compare the antibacterial effect of three resin-based root canal sealers.

The presence of resistant bacteria in root canal system of endodontically treated teeth with post-treatment apical periodontitis is well documented in the literature [3]. In particular, *E. faecalis* is among the most resistant species leading to failure of root canal treatment [3]. In the present study, the standard strain of *E. faecalis* was selected to evaluate the antibacterial properties of the sealers.

Sealers in freshly-mixed and set states may exhibit different properties. In a study of Huang et al. [14] antibacterial activity of four endodontic sealers was evaluated in both freshly-mixed and set states. The result of this study demonstrated that most of the antibacterial efficacy of resin-based sealer is diminished after setting. Therefore, it is recommended to evaluate the properties of sealers both in freshly-mixed and set forms [15].

ADT and DCT are commonly used methods to evaluate the antibacterial activity of dental materials in a laboratory setting. Given that root canal sealers can affect the residual bacteria after endodontic treatment by diffusing to the dentinal tubules and periradicular areas, it seems that the ADT is a suitable method

Table 4. The comparison of the percentage of bacterial colony reduction of the investigated sealers

Condition	Comparison	Mean Difference	P	95% Confidence Interval	
				Lower Bound	Upper Bound
Freshly mixed with 30 minutes contact time	AH26 vs. Beta RCS	12.44	<0.001	10	14.88
	AH26 vs. Adseal	-15.53	<0.001	-17.97	-13.09
	Beta RCS vs. Adseal	-27.97	<0.001	-30.41	-25.53
Freshly mixed with 180 minutes contact time	AH26 vs. Beta RCS	1.7	<0.001	1.1	2.3
	AH26 vs. Adseal	0	1	0.00	0.00
	Beta RCS vs. Adseal	-1.7	<0.001	-2.3	-1.1
Fully set with 180 minutes contact time	AH26 vs. Beta RCS	2.94	<0.001	1.45	4.43
	AH26 vs. Adseal	-4.48	<0.001	-5.97	-2.99
	Beta RCS vs. Adseal	-7.42	<0.001	-8.91	-5.93

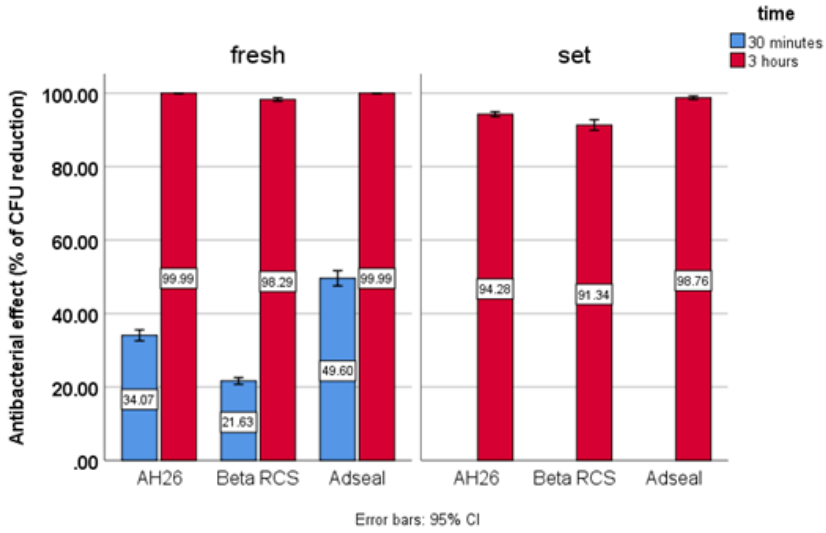

Fig. 1. Antibacterial activity of AH26, Beta RCS, and Adseal sealers by means of direct contact test. The values are reported as the percentage of CFU reduction compared to the control group. There was a significant difference between all study groups (P<0.001), with Adseal showing the highest and Beta RCS showing the lowest CFU reduction. The only exception was for freshly-mixed AH26 and Adseal at 180 minutes contact time (P>0.99).

to simulate the clinical application of these materials. In addition, it is recommended that DCT be used as an adjunct to ADT to cover its shortcomings. ADT is a quantitative method in which all the bacteria in the sample have a chance to contact the tested material [16]. Additionally, considering the point that DCT is independent of the diffusion and flowability properties of the tested material, it is suitable for evaluating the materials in the set state. In the present study, the antibacterial properties of the sealers were evaluated in freshly-mixed and set states using both ADT and DCT. In a study by Huang et al.[14] in 2019, the antibacterial effect of different sealers was evaluated and compared using DCT and ADT. While AH Plus showed no antibacterial effect in ADT against *E.faecalis*, DCT results showed complete inhibition of bacterial growth after 1 h contact time. In another study, AH26 showed a lower antibacterial effect than Endoflas using DCT, whereas when assessed with ADT, AH26 could produce a larger inhibition zone than Endoflas [16].

The results of the present study showed a strong antibacterial effect of AH26 sealer, in both freshly-mixed and set states. These findings were in agreement with the previous studies [9, 10, 17]. High antibacterial properties of AH26 can be attributable to the release of formaldehyde from the sealer during setting reaction. . In addition, it has been stated that the antibacterial properties of AH26 can be related to the release of Bisphenol A diglycidylether and the un-polymerized components (i.e., epoxide and amine) as setting reaction by-products [14]. In ADT, The results of the current investigation indicate that freshly-mixed AH26 had the largest zone of inhibition which can be related to its lower initial viscosity. As the flowability of the tested material affects the results of ADT [18], this might be attributed to the lower initial viscosity of this sealer compared to the other studied sealers. This finding was in accordance with the previous study evaluating the antibacterial properties of AH26 using ADT [10].

Adseal is another epoxy-resin root canal sealer. This sealer showed the least antibacterial effect using ADT method. This result was in accordance with previous studies, which have shown a lower antibacterial effect of Adseal compared to other resin-based sealers [19, 20]. However, higher antimicrobial efficacy was observed using DCT in comparison with the other studied sealers. This was in contrast with results of a study by Ehsani et al. [9] who demonstrated that Adseal has lower antibacterial effect compared with AH26. This difference might be related to different exposures of the specimens with the bacterial suspension. In the study of Ehsani et al.[9]

the sealers were poured into a microtube, and then the bacterial suspension was added, whereas in the current investigation the sealers were spread on the buttom of 24-well plates. Therefore, limited contact area of exposure between the sealer and bacterial suspention can be influential in discrepancy of the obtained results. In addition, the lower solubility of Adseal compared to AH26 as described by Song et al. [21] can exacerbate the effect of this condition. The difference between the results of ADT and DCT might be attributed to the different setting times and different flowability of the cement, as AH26 has a longer setting time (9-15h according to the manufacturer) and higher flowability [22] compared to Adseal (45 mins according to the manufacturer). High phosphorus release by Adseal as demonstrated by Abu Zeid et al. [23] can be responsible for its antibacterial effect as shown by DCT. It has also been shown by Lorencová et al [24] that phosphorus release from materials could exert antibacterial effects on gram-positive in vitro.

The present study showed that the inhibition zone of Beta RCS was smaller than that of AH26 and greater than that of Adseal in freshly-mixed state. Therefore, the setting time can be influential on the antibacterial properties of the material in agar diffusion test [18]. Similarly, shortest setting time examined by Adseal could have resulted in limited diffusion on agar plates indicating limited antibacterial action, whereas moderate and high antibacterial properties of Beta RCS and AH26 according to ADT can be responsible for their moderate and high microbial inhibition halo, respectively Beta RCS showed the lowest antibacterial activity in freshly-mixed and set states at 30min and 180min time points using DCT. This might be due to less releaseable material from the surface of Beta RCS, which needs further investigation in future studies.

In freshly-mixed and set states, all of the studied sealers resulted in more than 90% bacterial reduction after 180min direct contact time. This finding was in agreement with the results of a previous systematic review [8] in which resin-based sealers showed strong antibacterial effects against *E.faecalis*. This effect was attributed to the release of bisphenol diglycidyl ether and formaldehyde during the polymerization process[8].

CONCLUSION

Within the limitations of this *in vitro* study, AH26, Adseal, and Beta RCS showed antibacterial effect against *E. faecalis* in both freshly-mixed and set states. The antibacterial effect increased with time in all studied sealers.

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CONFLICT OF INTEREST STATEMENT

None declared.

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