Original Article

Investigation of Formaldehyde Release and Antibacterial Properties of BETA RCS Sealer Compared with Common Sealers (ADSEAL and AH26)

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ABSTRACT

Background: In cases where microbial infection is present, reducing the bacterial load within the root canal system is a critical factor for successful root canal treatment. In such situations, the antibacterial properties of root canal sealers may further contribute to improved endodontic outcomes. Formaldehyde (FA) is an effective antimicrobial agent, and its release from resin-based endodontic sealers has been well documented. This release contributes to the antibacterial properties of some endodontic sealers.

Purpose: This study aimed to investigate and compare the antibacterial properties and FA release rate of the Iranian BETA RCS sealer with the common sealers ADSEAL and AH26.

Materials and Method: In this fundamental experimental study, three sealers of BETA RCS, ADSEAL, and AH26 were evaluated. To measure FA release, 6 samples of each AH26 and BETA RCS sealers were prepared by mixing 0.2 g of liquid with the specified powder ratio, and 6 ADSEAL samples were performed by combining 0.2 g of base with the appropriate catalyst ratio, following the manufacturer's instructions. The samples were then analyzed at 3 time points of (time zero), 48 h, and 1 week. To investigate the antimicrobial properties of the sealers, *Enterococcus faecalis* and *Streptococcus mutans* were employed. The samples cultured in Plate Count Agar - PCA medium, incubated at 37 ^{o}C for 24 h and the formed-colonies were counted.

Results: The obtained results revealed that AH 26, ADSEAL, and BETA RCS sealers released FA, and the highest amount at all three times was related to AH26 sealer, while the lowest amount was correlated with ADSEAL. In all three sealers, the highest amount of FA release occurred after 48 h. The greatest antibacterial effect on *Enterococcus faecalis* and *Streptococcus mutans* was associated with AH26, followed by ADSEAL and BETA RCS, respectively.

Conclusion: Regarding the obtained outcomes, AH26 had the highest amount of FA released. Additionally, the anti-bacterial activity of AH26 demonstrated a stronger effect compared to the other two sealers, with ADSEAL showing acceptable results and BETA RCS exhibited the least antibacterial efficacy.

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Introduction

The success of primary endodontic treatment relies on the triad of mechanical debridement, disinfection, and obturation. Obturation is considered one of the most critical components of this clinical procedure [1]. Root canals are typically filled with a suitable sealer, a key

component of root canal obturation, to establish a liquid-tight seal. Endodontic sealers are commonly used to seal dentinal tubules, creating a homogenous interface between the dentinal walls and the obturation material, thereby contributing to the complete obturation of the canal space [2-3]. The initial functions of sealers include filling voids and irregularities within the root canal, eliminating residual bacteria following cleaning, and promoting disinfection through their germicidal action. Inadequate sealing can allow microorganisms to penetrate, leading to reinfection and ultimately resulting in treatment failure [4]. Therefore, the properties of the sealer are crucial for achieving effective sealing, as microleakage may occur at the sealer/dentin interface or between the sealer and filler core [5]. The most common root canal sealers are classified based on their composition or setting reactions uses. The most common classifications include zinc oxide eugenol (ZOE)-based sealers (Tubliseal, Grossman), non-eugenol sealers (epoxy resins; AH26), Diaket, calcium hydroxide-based (CRCS), and other types (resin, glass ionomer, bioceramic, MTA, and silicone-based sealers) [6-7].

AH26 is an epoxy resin that was initially developed as a single-filler material and has been widely utilized due to its favourable handling characteristics [8]. It exhibits excellent flow, adapts well to dentin walls, and provides sufficient working time and has highly toxic when freshly prepared; however, this toxicity decreases rapidly during the setting process. After 24 h, the cement demonstrates one of the lowest toxicity levels among endodontic sealers [9].

Beta RCS is a newly developed resin-based root canal sealer. Preliminary studies have assessed its biocompatibility, flowability, radiopacity, solubility, and film thickness, demonstrating that its properties comply with ISO 6876 standards for endodontic sealers and are comparable to those of AH26 [10-11].

ADSEAL sealer is a resin-based endodontic sealer known for its insolubility in tissue fluids and excellent biocompatibility. Cytotoxicity assays indicate that ADSEAL promotes increased mesenchymal cell proliferation and shows good compatibility with human periodontal ligament cells [12].

Formaldehyde (FA) is a chemical compound historically utilized in various endodontic sealers, primarily for its beneficial properties in root canal treatments. It is widely recognized as a potent disinfectant [13]. Certain materials, such as N2 (Indrag-Agsa, Bologna, Italy) and Riebler paste (Amubarut; Wera Karl, Biesingen, Germany), contain paraformaldehyde release FA upon hydrolysis. FA exerts its antimicrobial effect by cross-linking and denaturing microbial proteins and nucleic acids, which disrupts enzyme function, damages cell membranes, and inhibits DNA replication, ultimately causing bacterial cell death. This chemical action accounts for the rapid antimicrobial activity observed, although the effect may be limited over time due to dilution and binding within dentin [14-15]. In the case of AH26, the disinfectant component is methenamine, which hydrolyzes to produce ammonia and FA [16]. However, the long-term disinfectant efficacy of FA released from root canal sealers appears to be limited.

Enterococcus faecalis (*E. Faecalis*) is a versatile pathogen that significantly contributes to persistent endodontic infections following initial treatment and frequently appears in high numbers in root canal failures. Several traits enable its persistence including genetic polymorphisms, invasion into dentinal tubules, adaptation to adverse conditions, resistance to antimicrobial treatments, and notably, biofilm formation [17-18]. Evidence suggests that FA released from epoxy resin–based sealers such as AH26 enable to diffuse into dentinal tubules, imposing antibacterial effects beyond the sealer–dentin interface. Due to the ability of *E. faecalis* to penetrate dentinal tubules to depths of several hundred micrometers and persist via biofilm formation, such diffusion may contribute to antimicrobial efficacy, although it cannot be considered the sole mechanism of action [19].

Streptococcus mutans (*S. mutans*) is a Gram-positive, facultative anaerobic bacterium that plays a significant role in the development of dental caries [20]. Therefore, increasing the antimicrobial properties of sealers used in root canal therapy could effectively control periapical infections by targeting microorganisms like *E. faecalis*. The direct contact test (DCT) method is used to evaluate the antimicrobial properties of materials by assessing the direct interaction between microorganisms and the material being tested [20].

This study aimed to investigate the antimicrobial properties of AH26, ADSEAL, and BETA RCS against *S. mutans* and *E. faecalis* by using the DCT method

along with measuring FA production at three different time points using high-performance liquid chromatography (HPLC) techniques.

Materials and Method

Sample preparation and measurements

This experimental study (Ethical code: IR.AJAUMS.REC.1402.155) evaluated three commercially available root canal sealers: BETA RCS (BetaDent, Iran), ADSEAL resin sealer (Meta Biomed, South Korea), and AH26 resin sealer (Dentsply, Germany). To FA measurement, six samples each of AH26 and BETA RCS were prepared by mixing 0.2 grams of liquid with the appropriate proportion of powder according to the manufacturer's instructions. Similarly, six samples of ADSEAL sealer were performed by combining 0.2 grams of base with the required amount of catalyst. Moreover, one standard sample (positive control) and one negative control sample were selected. The samples were analyzed at three time points: immediately after mixing (time zero), after 48 h, and 1 week later.

Formaldehyde measurements

FA release was quantified using a validated 2,4-dinitrophenylhydrazine (DNPH) derivatization method followed by HPLC. DNPH was selected due to its rapid and stable reaction with FA. Quantitative analysis was performed using an HPLC device (Unicam-Crystal 200, UK) equipped with a photodiode photodiode detector, with detection carried out at 365 nm. FA levels were measured at each experimental time point [21].

Antibacterial Property Investigation

The antibacterial properties of the applied sealers were assessed using the DCT. Sample size was determined using G*Power software, resulting in six samples per sealer. A total of 43 laboratory samples were examined on the tested plates. In first and second groups, 6 samples each of sealers were tested against *S. mutans* and *E. faecalis*, respectively. The third and fourth group contains 3 positive control samples from *S. mutans* and *E. faecalis*, respectively. The fifth group was a negative control without sealer or bacteria. Standardized bacterial suspensions were prepared from fresh cultures and adjusted to 0.5 McFarland turbidity (≈1.5×10^8 CFU/mL). Each sealer was placed in sterile microtubes, followed by the addition of nutrient broth and bacterial suspension. After incubation, bacterial viability was assessed by serial dilution and plate counting. Aliquots were cultured on Plate Count Agar (PCA) medium (Merck, Germany) and incubated for 24 hours at 37 °C. Colony-forming units (CFUs) were counted, and antibacterial efficacy was determined by comparing CFU counts between test and control groups.

Statistical analysis

The data obtained were analyzed using SPSS software (version 27) (IBM Corporation, USA) and were assessed using both descriptive and inferential statistical methods. Descriptive statistics, including tables, graphs, frequency distributions, means, and standard deviations, were employed to summarize the data. Additionally, the results were reported as Mean ± SEM (Standard Error of the Mean). For further analysis, a Kruskal-Wallis test was conducted with a significance level set at $p < 0.05$. Since the data did not meet the assumptions required for parametric tests, including normal distribution and homogeneity of variance. Therefore, a non-parametric approach was considered more appropriate. The Kruskal–Wallis test allows for the comparison of multiple independent groups without assuming normality and is suitable for analyzing ordinal or non-normally distributed continuous data, such as FA release rates and bacterial colony counts. Consequently, this method provided a robust and reliable means of identifying statistically significant differences among the tested sealers.

Results

Figures 1a-c depict the final chromatograms generated by the HPLC, illustrating the FA release rates from the AH26 sealer at three distinct time points: immediately (time zero), after 48h, and 1 week. The data reveal that the AH26 sealer released an average of 8.3mg/g FA at time 0, 71.1mg/g at 48h, and 21.8mg/g at 1 week. Hence, the highest amount of FA release was observed at 48 h.

As can be observed from Figures 2a-c, the FA amount from the ADSEAL released 2.5 mg/g at time zero, a significantly higher amount of 21.9 mg/g at 48 h and then decreased to approximately 7.2 mg/g at 1 week. Similarly, the highest FA release was observed at the 48h, followed by a gradual decrease (p Value= 0.001).

In accordance with Figures 3a-c, the FA release rates from the BETA RCS sealer obtained 5.4 mg/g at time zero, a significantly lower amount of 46.1 mg/g at 48 h,

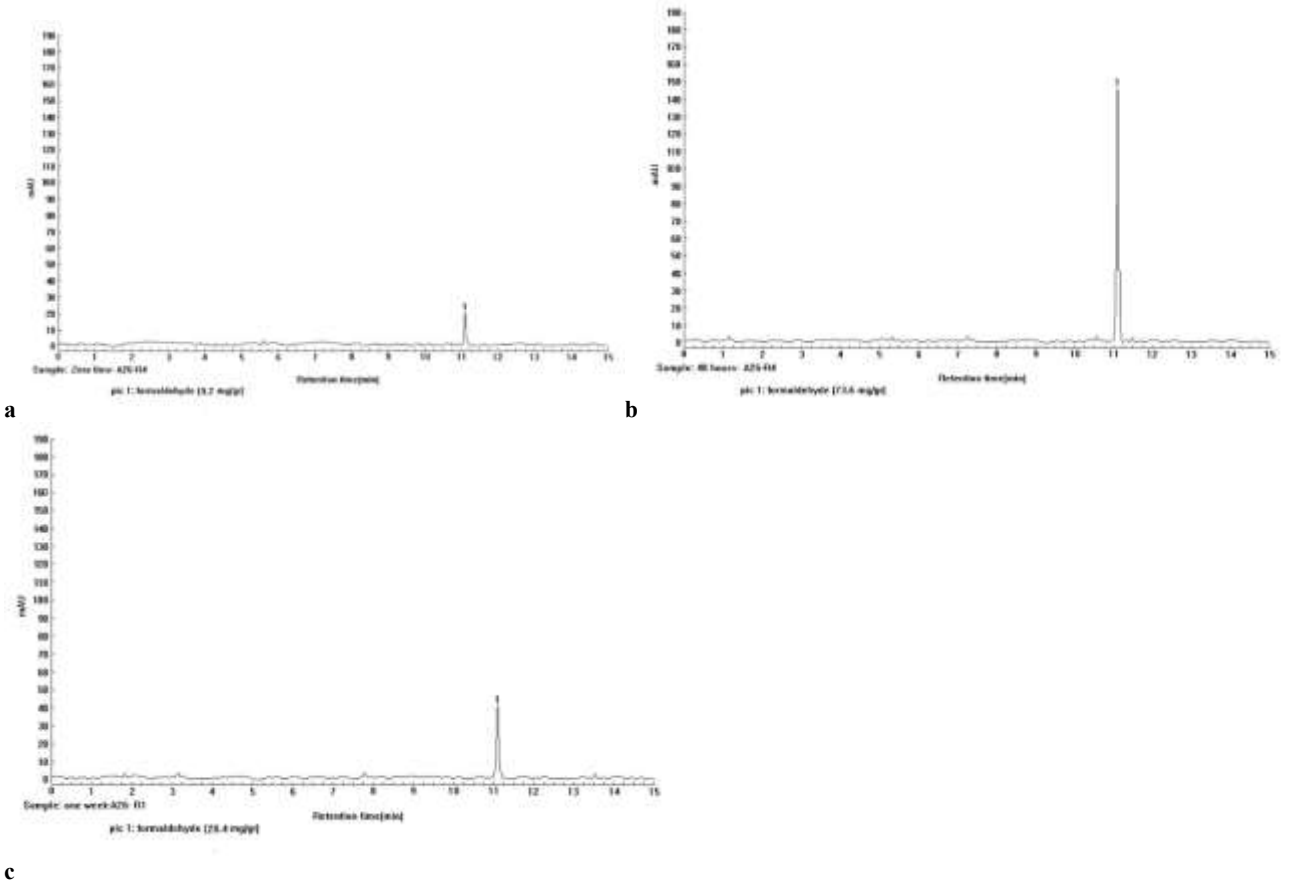


Figure 1: High-Performance Liquid Chromatography (HPLC) chromatogram of AH26 Sealer at time, **a:** zero, **b:** After 48 h, **c:** After 1 week (fourth repetition)

Table 1: The Mean and standard deviation of formaldehyde (FA) release at time zero, 48 h, and 1 week

Sealer	Time	Mean	Standard Deviation	*p* Value
AH26		8.3	0.7	
ADSEAL	Zero	2.5	0.5	0.001
BETA RCS		5.4	0.4	
AH26		71.1	1.84	
ADSEAL	48 h	21.9	1.85	0.001
BETA RCS		46.1	1.89	
AH26		21.8	1.8	
ADSEAL	1 week	7.2	0.5	0.001
BETA RCS		15.5	1.9	

Table 2: Mean and standard deviation of colonies formed by Enterococcus faecalis (*E. faecalis*) and Streptococcus mutans *(S. mutans)* in three sealers

Bacteria	Sealer	Mean	Standard Deviation	*p* Value
E. faecalis	**Control**	2.8×10^{21}	1.8×10^{20}	0.001
	AH26	1.39×10^{3}	1548.39	
	ADSEAL	4.37×10^{3}	726.69	
	BETA RCS	1.5×10^{4}	3065.33	
S. mutans	Control	4.2×10^{21}	4×10^{20}	<0.001
	AH26	3.01×10^{3}	781.20	
	ADSEAL	6.88×10^{3}	966.55	
	BETA RCS	3.5×10^{4}	2750.33	

and 15.5mg/g at 1 week. The highest FA release was related to 48h.

The average FA release concentration in different sealers at three times: zero, after 48h, and 1 week in mg/g represented in Figures 4. The results of Kruskal-Wallis test which was employed to compare the FA release rates of AH26, ADSEAL, and BETA RCS sealers indicated a statistically significant difference among all three sealers in terms of FA release at three time points (Table 1). As can be seen at time zero, 48h, and 1 week, the FA release rate showed significant differences among the three sealers ($p = 0.001$), with the following order: AH26 > BETA RCS > ADSEAL.

Figures 5 reveals the average colonies formed by *E. faecalis* and *S. mutans* in three sealers in ml/CFU. According to the statistical analysis using the Kruskal-Wallis test for investigating the antibacterial properties of sealers, significant differences were observed in the colony counts of *S. mutans* ($p< 0.001$) and for *E. faecalis* ($p= 0.001$) among AH26, ADSEAL, and BETA RCS sealers (Table 2). In both tests, AH26 sealer exhibited the lowest number of colony formations and the ADSEAL

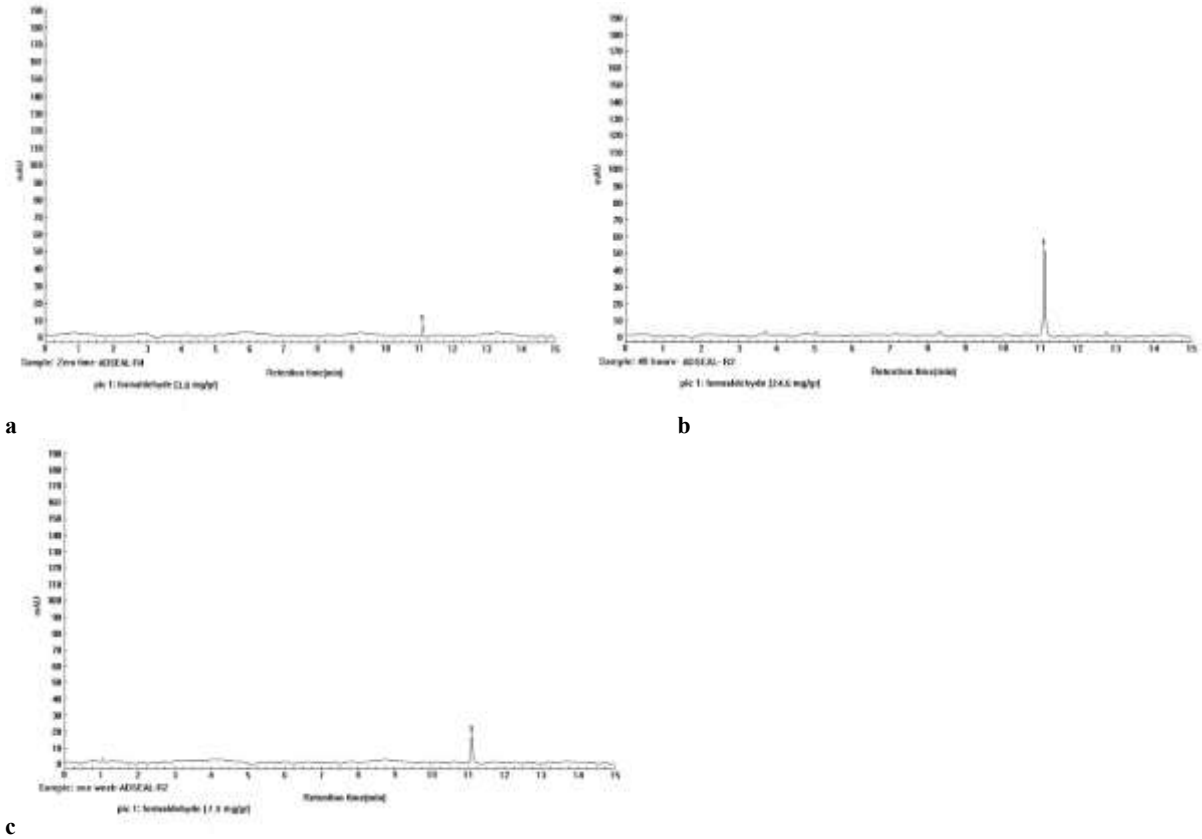


Figure 2: High-Performance Liquid Chromatography (HPLC) chromatogram of ADSEAL Sealer at time, **a:** zero, **b:** After 48h, **c:** After 1 week (fourth repetition)

and BETA RCS sealers followed in sequence. The absence of microbial growth on plates containing the negative control group confirmed the efficacy of the sterilization process.

Discussion

The success of root canal treatment hinges on multiple factors. While thorough canal debridement is crucial, appropriate obturation is equally vital for the efficacy of endodontic treatment [22]. The utilization of sealers during root canal obturation is indispensable for achieving optimal outcomes in endodontic procedures. These materials serve several critical functions including act as lubricants, facilitating the insertion of the master cone into the canal, filling voids and irregularities between accessory cones and the root canal wall and prevent the formation of vacant spaces within the root canal system [23]. Hence, sealers play a crucial role in establishing a hermetic seal- defined as an airtight and impermeable barrier- that is essential for successful endodontic treatment. The choice of sealer can impact the long-term prognosis of the treated tooth, with some materials demonstrating superior properties in terms of biocompatibility, adhesion, and antimicrobial efficacy [24]. Most dental materials used in root canal treatment can release various substances into their physiological environment. This release raises concerns about potential genotoxicity, mutagenicity, and carcinogenicity of these materials. In root canal treatment, FA can spread into the apical foramen and lateral canals, damaging the periodontal ligament and surrounding tissues [6].

In this study, AH26, ADSEAL, and RCS BETA sealers were evaluated for FA release amount and antibacterial properties against two bacterial strains of *S. mutans* which is a gram-positive, aerobic or facultative anaerobic microorganism recognized as a major etiological agent of plaque formation and dental caries and *E. faecalis* is a gram-positive, anaerobic microorganism that, despite constituting a small portion of the flora in untreated root canals, plays a significant role in the development of peri-root lesions following root canal treatment. Also, our investigation demonstrated that AH 26, ADSEAL, and BETA RCS sealers exhibited FA release at three distinct intervals: (zero time), 48 h, and 1

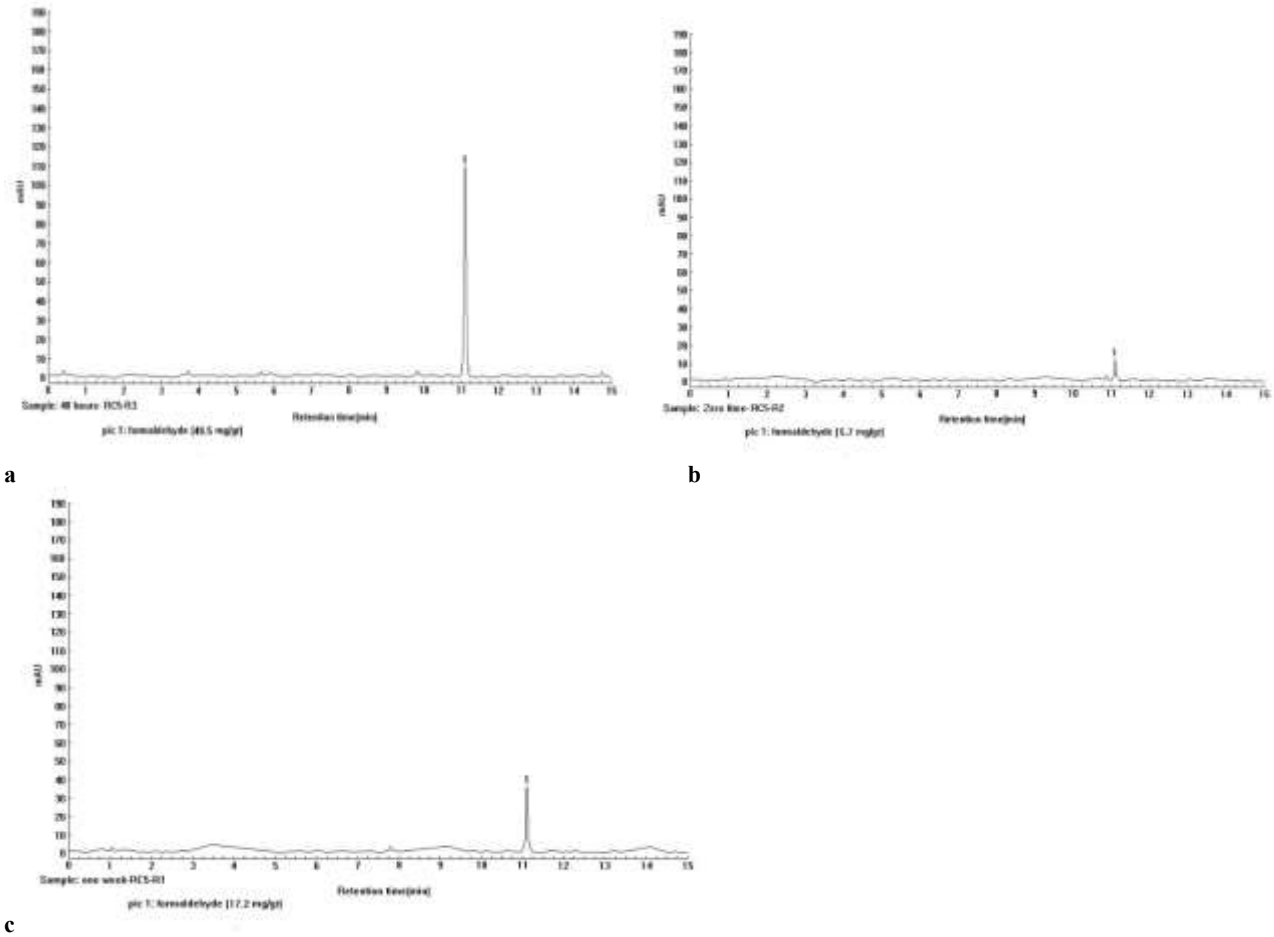


Figure 3: High-Performance Liquid Chromatography (HPLC) chromatogram of BETA RCS Sealer at time, **a:** Zero, **b:** After 48 h, **c:** After 1 week (fourth repetition)

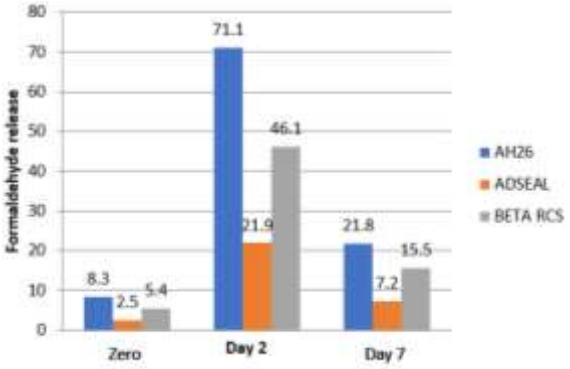
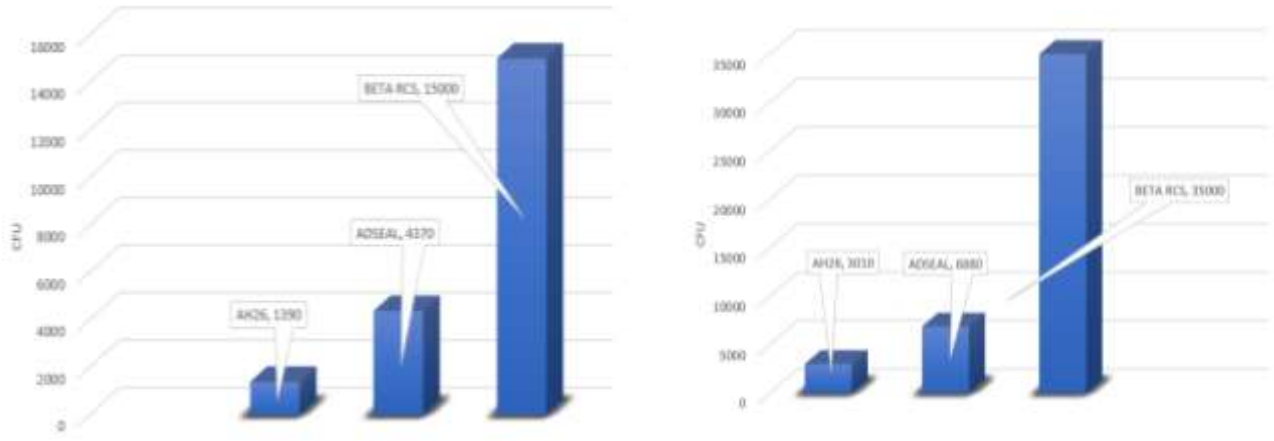


Figure 4: Average formaldehyde (FA) release concentration in different sealers at three times: zero, After 48 h, and After 1 week in mg/g

week. AH26 sealer consistently displayed the highest FA release rate across all three time points. For all three sealers, the highest FA release was observed at the 48 h. These findings align with the research conducted by *Spångberg et al.* [25] who reported a decrease in the antimicrobial activity of AH 26 sealer after 48 h. This phenomenon was attributed to the gradual release of FA from the sealer until it reached its final set state, which corroborates our observations regarding FA release patterns.

The predominant microbes in endodontic infections of untreated teeth are primarily obligate anaerobes [26]. However, aerobic microorganisms, though present in lesser quantities, can infiltrate the root canal during treatment. Once they gain entry, these aerobic species can persist even after the canal has been sealed, exploiting the altered conditions within the canal environment where the original microbial agents responsible for the initial infection have been eradicated [27]. This adaptation enables them to proliferate and potentially incite secondary infections [28]. The acquired results from our study represent that the AH26 sealer had the highest antibacterial effect among other sealers, followed by the ADSEAL sealer. In contrast, the RCS-BETA sealer demonstrates the lowest antibacterial efficacy against *E. faecalis* and *S. mutans*. Ghabraei *et al.* [11] compared the



a **b**

Figure 5: a: The average colonies formed by Enterococcus faecalis *(E.faecalis)*, **b:** Streptococcus mutans (*S. mutans)* in three sealers in ml/CFU

antibacterial properties of three resin-based endodontic sealers of AH26, ADSEAL, and Beta RCS against *E. faecalis* in an *in vitro* setting. They outcomes exhibited the antimicrobial efficacy of AH26, ADSEAL, and Beta RCS against *E. faecalis* in both their freshly mixed and set states which is consistent with the results of our study.

Khedmat *et al.* [29] indicated that all three root canal sealers including AH26, ADSEAL, and Beta RCS exhibited antibacterial activity against *E. faecalis*, but their effectiveness was not equal. Among them, AH26 showed the strongest antibacterial effect, which is mainly attributed to the release of FA during its setting reaction. ADSEAL demonstrated a moderate antibacterial effect, while Beta RCS showed the weakest activity against *E. faecalis*, which is supports our findings.

In a study conducted by Gomes *et al.* [30], the antimicrobial properties of five endodontic sealers of Endofil, Indomethasone, Indomethasone N, AH26, and AHPlus were evaluated against *Candida albicans*, *Staphylococcus aureus*, *E. faecalis*, *Streptococcus sanguis*, and *Actinomyces naeslundii*. The findings revealed that none of the sealers were able to completely inhibit microbial growth. Additionally, the antimicrobial activity of each sealer diminished over time and was influenced by the susceptibility of the microorganisms to the sealers. These outcomes align with the results observed in our current study.

The study by Koch *et al.* [31] investigated FA release from three different types of root canal sealers: phenolic resin, epoxy resin, and para-formaldehyde cement containing zinc oxide eugenol. Their findings suggest that lower FA release compared to freshly mixed samples at 48 h. This finding contrasts with our results, which may be attributed to discrepancies in experimental conditions such as variations in temperature and humidity as well as differences in the specific sealers utilized between their study and ours. Moreover, Koch *et al.* [32] examined the release rates of FA from three different root canal sealers: AH26, N2, and Amburat. Their findings indicated that the FA release rate from AH26 was higher than that of the other sealers, which aligns with our outcomes.

In the study of Dastorani *et al,* [33] the cytotoxicity and tissue response of three sealers AH Plus, ADSEAL, and Beta RCS were compared and it was found that ADSEAL exhibited better biocompatibility than Beta RCS. Based on the results of the present study, which showed that FA release from ADSEAL was lower than that from Beta RCS, it can be inferred that the level of FA release may be related to the biocompatibility of the sealers. However, further research is needed to confirm this hypothesis.

Conclusion

Based on the results obtained in this study, the AH26 sealer exhibited the highest FA rate and the FA release rate for all three sealers increased over time (up to 48 h). The obtained results from anti-bacterial activity demonstrated that the AH26 sealer had a stronger effect compared to the other two sealers. Given the high FA release amount of the AH26 sealer and its superior antibacterial

properties relative to the other sealers, it can be concluded that FA significantly influences the antimicrobial characteristics of sealers. Interestingly, despite higher FA releases from the BETA RCS sealer, its antibacterial properties were inferior to those of ADSEAL. This suggests that while FA is a critical factor affecting antibacterial properties, other factors also warrant further investigation.

Acknowledgements

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Ethical Approval

There is none to be disclosed.

Competing interests

The authors declare no conflict of interest in regard to this article.

Availability of data and materials

Data are available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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