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**Comparison of Antimicrobial Activity of
Root Canal Sealers: An In Vitro Study**

Comparison of Antimicrobial Activity of Root Canal Sealers: An In Vitro Study

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Abstract

Background and objective: The antimicrobial activity of root canal sealers may contribute to the eradication of remaining microbes in root canal. The aim of this study was to investigate antimicrobial effect of bioceramic sealer (NeoSEALER® Flo), resin sealer (ADSEAL Plus), and MTA based sealer (MTA- Fillapex).

Materials and methods: The present study aimed to comparatively verify the sealers antimicrobial activity against microorganisms. *Enterococcus faecalis* (*E.faecalis*) and *Candida albicans* (*C.albicans*) were submitted to the agar diffusion test (ADT) and modified direct contact test (MDCT), at 1hour, 1day, and 1week interval.

Results: In ADT, zone of inhibition (ZOI) were measured in millimeters. In MDCT, bacterial suspensions were exposed to the sealers, and colony-forming unit (CFU) counts were recorded to assess microbial survival. In ADT, At the 1hour and 1week interval, all sealers demonstrated no antibacterial activity against *E.faecalis* (ZOI=0.0mm), its only observed at the 1day, with NeoSEALER® Flo exhibiting the strongest activity (ZOI=13.19±0.37), followed by MTA Fillapex (12.19±0.37) and ADSEAL Plus (8.81±0.37). In MDCT, NeoSEALER® Flo showed higher antibacterial properties, decreased at 1week interval, followed by MTA Fillapex and ADSEAL Plus. In ADT and MDCT, MTA Fillapex showed highest and most consistent antifungal activity across all intervals and increased with time interval, followed by NeoSEALER® Flo, except for ADSEAL Plus, decreased with time interval.

Conclusion: All sealers showed antifungal activity against *C.albicans* at all intervals, none of the sealers maintained antibacterial activity against *E.faecalis* after 1week. Indicated a temporary antibacterial effect, which diminishes over time.

Keywords: Antibacterial effect, Antimicrobial effect, Growth inhibition, Root canal sealers, Time interval.

Introduction

Endodontic treatment involves the optimum shaping and debridement of the canal system, removing microorganisms and their byproducts, promoting healing at the apex, and preventing the development of apical lesions (1). The primary pathogenic agents in pulpal and periapical infections are microorganisms and their metabolites. Therefore, eliminating these microbiological organisms from the diseased root canal system is the primary goal of endodontic treatment (2).

E. faecalis and *C. albicans* are microbial species commonly associated with persistent root canal infections and are often found in treatment-resistant cases (3). These microorganisms have the ability to invade dentinal tubules, survive extended periods of nutrient deprivation, and resist most of the chemicals used in root canal procedures. Additionally, *E.faecalis* is the most frequently encountered bacterium in endodontically treated teeth, present in up to 90% of cases (4, 5).

The main aim of endodontic treatment is to eradicate microorganisms from the root canal system and to prevent them

from causing infection or re-infection in the root canal or surrounding periapical tissues (4). Endodontic treatment failure is often directly related to microbial infection (6). *E. faecalis* is commonly associated with different forms of periradicular diseases, such as endodontic treatment failure and ongoing infections (7).

Sealing the root canal system is a crucial step following chemo-mechanical preparation to prevent the survival of any remaining microorganisms. As a result, the antimicrobial properties of the sealing material are considered a gold standard for any root canal sealer (8). Various endodontic sealers have been developed and are now available for routine clinical use. These sealers have different compositions, which can lead to variations in their physicochemical characteristics and antimicrobial properties (3).

Bioceramic sealers have gained significant popularity in endodontic treatments recently. Their calcium silicate composition provides biocompatibility and bioactivity, allowing them to form an apatite layer when they come into contact with tissue and bond chemically with dentin. The micromechanical interlock between the bioceramic sealers and root dentin ensures the stability of the sealer-dentin interface, even when subjected to functional stress (9). Many in vitro studies have been performed to evaluate the antibacterial effectiveness of endodontic materials using a variety of techniques (10, 11).

An example of a calcium silicate sealer (bioceramic) is NeoSEALER® Flo, a premixed bioactive bioceramic root canal sealer known for its excellent handling properties. It is made from a combination of tantalite, calcium aluminate, tricalcium silicate, dicalcium silicate, calcium sulfate, tricalcium aluminate, polyethylene glycol, and a resin-free formulation (9). This biocompatible and antimicrobial sealer maintains dimensional stability. The manufacturer highlights several benefits, including being resin-free, bioactive, and promoting the formation of hydroxyapatite on the dentin surface. By releasing calcium and hydroxide ions, this improves dentinal tubule closure and encourages healing (5).

ADSEAL Plus (Meta Biomed Co, Cheongju, Korea) is an epoxy resin-based root canal sealer. It provides excellent sealing capability and strong adhesion to dentin, sets quickly while allowing for an adequate working time, and offers good radiopacity. This sealer is easy to mix, does not alter the color of the tooth, and remains stable in bodily fluids without disintegrating (12,13).

MTA-Fillapex (Angelus, Londrina, Brazil) is the first generation of MTA-based root canal sealers, formulated with salicylate resin, diluting resin, natural resin, calcium tungstate, bismuth oxide, nanoparticulate silica, pigments, and MTA, forming a colloidal gel that solidifies. It has an alkaline pH, exhibits antibacterial properties, and demonstrates suitable physical characteristics for use as an endodontic sealer (14).

This study aimed to evaluate the antimicrobial effectiveness of three different sealers: bioceramic sealer (NeoSEALER® Flo), resin sealer (ADSEAL Plus), and MTA-based sealer (MTA-Fillapex). Nevertheless, these three sealers' antimicrobial qualities were not assessed collectively. The agar diffusion test (ADT) and the modified direct contact test (MDCT) were used to measure the antimicrobial activity at various time points (1 hour, 1 day, and 1 week) against *E. faecalis* and *C. albicans*.

2 Materials and methods

2.1. Microorganisms

The microorganisms used in this study, *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC-10231), were provided by the pathology laboratory, Medya Diagnostic Center.

2.2. Materials and Study Design

For this study, three obturating materials were selected as experimental materials, with their composition and manufacturer details provided in Table 1

Table 1: The commercial name, compositions, manufacture and properties of the sealer materials used in the study

No	Material	Composition	Manufacturer
1	NeoSEALER® Flo	Tricalcium silicate (<25%) and dicalcium silicate (<10%) as bioactive components, and calcium aluminate (<25%), calcium aluminum oxide (grossite) (<6%), tricalcium aluminate (<5%) and tantalite (50%) as radiopacifier	Avalon Biomed CO., LTD. Korea.
2	ADSEAL Plus	13,5 g dual syringe containing 4,5 g of catalyst (bismuth subcarbonate and amines) and 9 g of base (calcium phosphate and epoxy resin).	(Meta Biomed Co, Cheongju, Korea) No:13/1-1 06800 Çankaya/Ankara TURKEY
3	MTA-Fillapex	Resins (salicylate, diluting, natural), radiopaque bismuth, nanoparticulated silica, mineral trioxide aggregate, pigments	(Angelus/ Londrina/ Parana/ Brazil)

Each group was then subjected to two different antimicrobial activity tests: the agar diffusion test (ADT) and the modified direct contact test (MDCT). These tests were conducted under strict

aseptic conditions in the Department of Microbiology at Rizgary Hospital, Erbil, Iraq. The antimicrobial effectiveness of the sealers was assessed against *E. faecalis* and *C. albicans* at various time

intervals (1hour, 1day, and 1week).

2.3. Materials Preparation

For NeoSEALER® Flo sealer: Available in pre mixed 2.2g Syringe and packaged with exclusive auto- mixing Flex Flo Tips™ to extrude the material on a mixing pad.

For ADSEAL Plus sealer: comes in a 13.5-gram dual syringe containing 4.5 grams of catalyst (bismuth subcarbonate and amines) and 9 grams of base (calcium phosphate and epoxy resin). Just combine the catalyst and base in a 2:1 ratio. The material is extruded on a mixing pad with a dispense tip by pressing the plunger. The base and catalyst are guaranteed to be dispensed by the dual syringe. To create a uniform mixture, the sealer is then combined with the included plastic spatula.

For MTA-Fillapex sealer: The material is extruded on a sterile mixing pad using a 4 g dual syringe with automixing tips. The base and catalyst are equally mixed in a 1:1 ratio thanks to the dual syringe and mixing tip.

2.4. Antimicrobial Study Design

Agar Diffusion Test

Microorganism strains were cultured in brain heart infusion broth (BHIB) for a duration of 24 hours at 37°C. Procedures were carried out following the guidelines of NCCLS. Following this, bacterial suspensions were extracted from the broth cultures and standardization of suspensions was done with a spectrophotometer adjusted to a 0.5 McFarland scale ($\sim 1.5 \times 10^8$ CFU/mL). For *E. Faecalis*, blood agar was utilized, while *C. Albicans* was cultured on Muller Hinton agar.

All three freshly mixed sealers were placed in three (6 mm diameter and 4 mm deep) wells, described as follows: group I: NeoSEALER® Flo sealer, group II: ADSEAL Plus sealer, and group III: MTA-Fillapex sealer. Before placing the material into the well of each petri, it will have mixed concordant to the instruction of the manufacturer until a homogeneous consistency is obtained. All obturating materials were punched at equidistant points in plates according to their respective groups. Furthermore, all petri was stored in an incubator at 37°C for 24 hours. After incubation, the zones of microbial growth inhibition (ZOI) were assessed at the radical zone using a sliding caliper on a millimeter scale with a precision of 0.02 mm, the formula developed by Levinson and recorded at intervals of one hour, one day, and one

week (15).

Modified Direct Contact Test (MDCT)

A 96- well flat bottom microtiter plate. After preparing each sealer according to the manufacturer's instructions. Each microtube vertically held with ultimate care to avoid the flowing of material to the well bottom where it could impede the path of light through the microplate well leading to false readings. Then the MDCT was performed for each sealer. Each endodontic sealing material was used before setting or freshly mixed and after setting (1hour, 1day and 1week).

In each experimental group, 20 mg of the experimental sealers were placed in the bottom of each tube. For sealer aging, microplates store in sterile Phosphate-Buffered Saline (PBS) solution at 37°C with 95% atmospheric humidity for 1hour, 1day, and 1week to simulate oral conditions. During the week-long aging process, the physiological serum was replaced every 24 hours. After each aging period, on the surface of each sealer, 10 μ L of a microorganism suspension containing approximately 10^6 bacteria were gently added to each microplate. In the control group, positive controls consisted of wells without sealer coating, adding 20 μ L of microbial suspension, while for negative control sealers without bacterial suspension. After that, the microplates were incubated for an hour at 37°C in a humid environment (16).

Throughout this time, the microbes immediately interacted with the exposed surface of the sealers. Using a micropipette, 180 μ L of sterile saline was introduced into each well and mixed for 2 minutes, bacterial suspensions were aliquoted at 20 μ L and subjected to a series of tenfold serial dilutions in sterile saline. 20 μ L portion were extracted and plated onto Brain Heart Infusion (BHI) culture medium to assess bacterial viability. After incubating the plates at 37 °C for 48 hours, colony-forming units per milliliter (CFU/mL) were enumerated to quantify surviving bacteria as illustrated in Fig. 1. All procedures were conducted in triplicate to ensure reproducibility (17, 18).

The logarithmic values of CFUs were calculated for all test specimens. Bacterial reduction was then quantified using the following equation:

$$\text{Bacterial reduction} = \log_{10} (\text{CFU}_{\text{control}}) - \log_{10} (\text{CFU}_{\text{treated}})$$



Fig. 1. Microplate for MDCT, each with 20 mg of the experimental sealers at 1hour, 1day and 1week.

2.5. Statistical Analysis

The dataset's conformity with a normal distribution was evaluated by conducting both the Shapiro–Wilk and Kolmogorov–Smirnov normality tests. The Data were collected and analyzed was performed by SPSS for Windows, version 27 (IBMCorp.,

Armonk, NY, USA).

The Kruskal–Wallis test and one-way ANOVA were used to evaluate descriptive statistics and mean differences between groups. For pairwise comparisons and group differences, the Mann–Whitney U-test was employed. Kruskal–Wallis test was

used to determine if there is a significant difference among 1st hour, 1st day and 1st week for each group, using SPSS version 27 (IBM Corp., Armonk, NY, USA). The significance level was set at $p < 0.05$.

3. Results

The effect of three endodontic sealers on *E. Faecalis* and *Candida Albicans* was investigated and were assigned to the following different groups: NeoSEALER® Flo, ADSEAL Plus; and MTA-Fillapex sealer; compared against the no-sealer control group. The effect was investigated after 1 hour, 1 day, and 1 week of setting.

3.1. Agar Diffusion Test

Table 2: The mean values of the inhibition zones of the tested materials in mm on *E. faecalis*.

Groups	Time	NeoSEALER® Flo	ADSEAL Plus	MTA-illapex
NeoSEALER® Flo	1	-	0.000	0.032
ADSEAL Plus	1	0.000	-	0.018
MTA-Fillapex	1	0.032	0.018	-

At the 1-day mark, the Kruskal-Wallis Test showed a highly significant difference across all sealer groups ($p < 0.00$). Mann-Whitney U-test for multiple comparisons were also used to compare the study sealers. Highly significant differences ($p <$

Table 2 displays the microbiological analysis results from agar diffusion tests conducted on all materials tested against *E. faecalis*. The data showed that NeoSEALER® Flo sealer consistently produced the largest zone of inhibition (ZOI) (13.19 ± 0.37), at the one- day interval, followed by MTA Fillapex (12.19 ± 0.37) and ADSEAL Plus sealer (8.81 ± 0.37) produced the smallest ZOI. All groups, on the other hand, demonstrated a zero inhibition zones at 1 hour and 1-week interval and was excluded from statistical analysis. The differences in ZOI between the sealants were statistically significant, as confirmed by the Kruskal–Wallis test ($p < 0.001$) (Table 2).

0.01) were observed between NeoSEALER® Flo, ADSEAL Plus ($p = 0.00$) and ADSEAL Plus, MTA-Fillapex ($p = 0.018$), except for a significant difference ($p < 0.05$) found between MTA-Fillapex and NeoSEALER® Flo ($p = 0.032$) (Table 3).

Table 3: Intergroup pairwise comparisons of agar diffusion test of sealers against *E. faecalis*.

Groups	Time	NeoSEALER® Flo	ADSEAL Plus	MTA-Fillapex
NeoSEALER® Flo	1 day	-	0.000	0.032
ADSEAL Plus	1 day	0.000	-	0.018
MTA-Fillapex	1 day	0.032	0.018	-

The efficacy of the sealer evaluated against *C. albicans* at three different time periods varied significantly, according to Kruskal–Wallis statistical analysis ($p < 0.001$). For one hour, one day, and one week, MTA-Fillapex produced the highest average ZOI (14.38 ± 0.64 , 17.81 ± 0.23 , and 44.38 ± 0.64 mm), followed by

NeoSEALER® Flo (13.63 ± 0.58 , 24.63 ± 0.64 mm) for one day and one week, respectively, at all three time points. At the same intervals, ADSEAL Plus produced the least ZOI averages (7.44 ± 0.56 , 6.81 ± 0.26 mm). (Table 4).

Table 4: Descriptive statistics zone of inhibition of sealers against *C. albicans*

Sealer	Time	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
NeoSEALER®	1 hour	8	7.75	0.38	0.13	7.43	8.07	7.50	8.50
ADSEAL Plus			11.13	0.64	0.23	10.59	11.66	10.50	12.00
MTA-Fillapex			14.38	0.64	0.23	13.84	14.91	13.50	15.00
NeoSEALER®	1 day	8	13.63	0.58	0.21	13.14	14.11	12.50	14.50
ADSEAL Plus			7.44	0.56	0.20	6.97	7.91	6.50	8.00
MTA-Fillapex			17.81	0.65	0.23	17.27	18.36	17.00	19.00
NeoSEALER®	1 week	8	24.63	1.25	0.44	23.58	25.67	23.50	27.50
ADSEAL Plus			6.81	0.26	0.09	6.60	7.03	6.50	7.00
MTA-Fillapex			44.38	0.64	0.23	43.84	44.91	43.50	45.00

All sealer groups showed highly significant differences ($p < 0.001$) against *C. albicans*, according to test across group comparisons.

Furthermore, significant differences ($p < 0.001$) were found in pairwise intergroup comparisons between NeoSEALER® Flo and MTA-Fillapex at 1-hour intervals and ADSEAL Plus and MTA-

Fillapex at 1-day intervals. Other groups likewise showed a significant difference ($p < 0.05$) (Table 5).

Table 5: Pairwise intergroup comparisons of agar diffusion test of sealers against *C. albicans*.

Sealer	Time	NeoSEALER® Flo	ADSEAL Plus	MTA-Fillapex
NeoSEALER® Flo	1 hour	-	0.02	0.00
ADSEAL Plus		0.02	-	0.02
MTA-Fillapex		0.00	0.02	-
NeoSEALER® Flo	1 day	-	0.02	0.02
ADSEAL Plus		0.02	-	0.00
MTA-Fillapex		0.02	0.00	-
NeoSEALER® Flo	1 week	-	0.02	0.02
ADSEAL Plus		0.02	-	0.00
MTA-Fillapex		0.02	0.00	-

A statistical analysis of the three time intervals showed significant differences for all sealers when comparing 1 week with both 1 hour and 1 day ($p < 0.001$). However, when comparing 1 hour to 1 day,

significant differences were observed for both NeoSEALER® Flo ($p = 0.02$) and MTA-Fillapex ($p = 0.02$), while for ADSEAL Plus, non-significant difference was observed ($p = 0.14$) (Table 6).

Table 6: Intergroup pairwise comparisons of agar diffusion test of sealers against *C. albicans* at three time intervals (1 hour, 1 day, and 1 week).

Groups	NeoSEALER® Flo	ADSEAL Plus	MTA-Fillapex
1hour- 1day	0.02	0.14	0.02
1hour- 1week	.000	0.00	0.00
1day- 1week	0.02	0.00	0.02

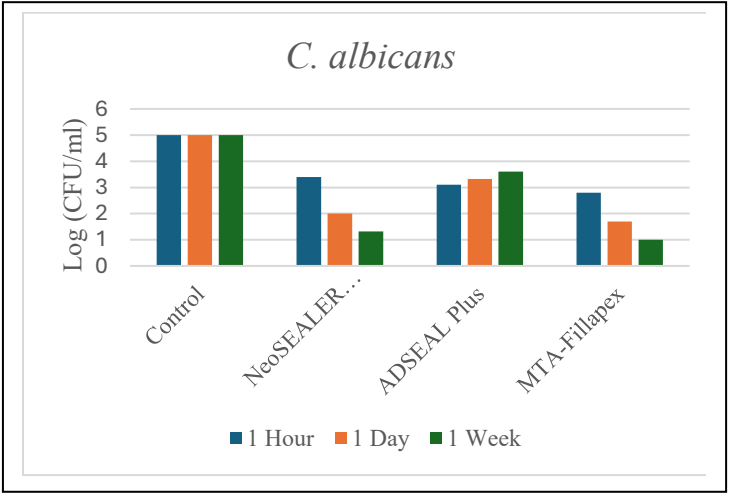
3.2. Modified Direct Contact Test

The assessment of microbial reduction of endodontic sealers was performed in MDCT, illustrated in (Table 7) (Fig. 2.). At all-time points assessed, the control groups showed no statistically significant differences in microbial survival ($p > 0.05$). Among the sealers tested, the bioceramic (NeoSEALER® Flo) exhibited the

strongest antibacterial effect, followed by MTA-Fillapex and ADSEAL Plus, significantly reducing populations of *E. faecalis* at day1 and declined substantially over time. However, MTA-Fillapex showed the highest antifungal activity against *C. albicans* followed by NeoSEALER® Flo and ADSEAL Plus and increased with time interval.

Table 7: Mean and standard deviation values of log microbial reduction for different sealers

Microbes	Sealer	Time	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P
						Lower Bound	Upper Bound			
<i>E. faecalis</i>	NeoSEALER® Flo	1 hour	4.84	0.15	0.66	4.66	5.02	4.64	5.04	0.001
	ADSEAL Plus		4.23	0.23	0.10	3.94	4.51	3.99	4.59	
	MTA-Fillapex		4.44	0.11	0.05	4.30	4.57	4.34	4.59	
	NeoSEALER® Flo	1 day	5.34	0.16	0.07	5.14	5.53	5.19	5.59	0.001
	ADSEAL Plus		4.74	0.15	0.07	4.56	4.92	4.54	4.89	
	MTA-Fillapex		4.94	0.08	0.04	4.84	5.03	4.84	5.04	
	NeoSEALER® Flo	1 week	2.54	0.20	0.09	2.29	2.79	2.34	2.84	0.001
	ADSEAL Plus		1.74	0.18	0.08	1.51	1.97	1.49	1.94	
	MTA-Fillapex		2.24	0.14	0.06	2.06	2.41	2.04	2.44	
	Control		7.84	2.59	0.49	6.46	9.20	6.28	8.96	
<i>C. albicans</i>	NeoSEALER® Flo	1 hour	3.80	0.11	0.05	3.67	3.93	3.70	3.95	0.000
	ADSEAL Plus		4.10	0.15	0.07	3.91	4.28	3.90	4.25	
	MTA-Fillapex		4.40	0.09	0.04	4.28	4.51	4.30	4.55	
	NeoSEALER® Flo	1 day	5.20	0.38	0.17	4.73	5.67	4.70	5.70	0.001
	ADSEAL Plus		3.88	0.22	0.10	3.61	4.15	3.70	4.25	
	MTA-Fillapex		5.50	0.22	0.10	5.22	5.77	5.30	5.85	
	NeoSEALER® Flo	1 week	5.88	0.04	0.02	5.83	5.93	5.85	5.94	0.001
	ADSEAL Plus		3.60	0.10	0.04	3.48	3.72	3.50	3.75	
	MTA-Fillapex		6.20	0.13	0.06	6.03	6.37	6.00	6.35	
	Control		7.20	0.97	0.43	6.00	8.40	6.06	8.19	



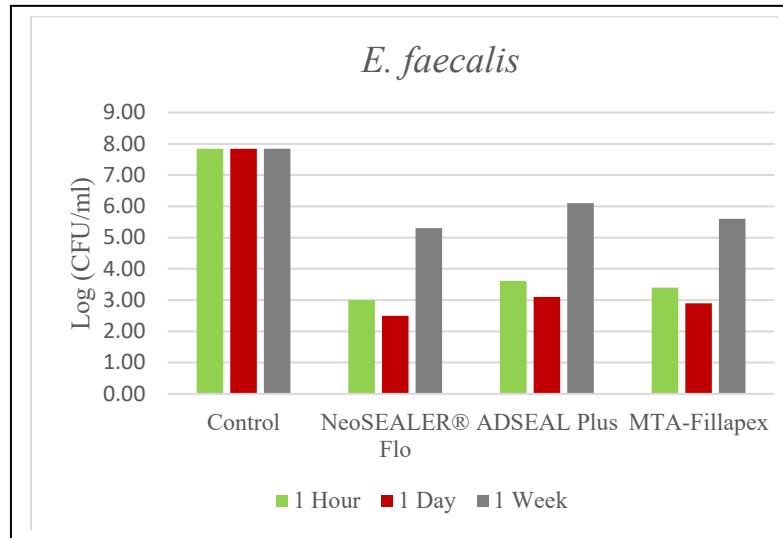


Fig. 2. Survival of microbes of tested sealers at different time interval in MDCT compared to control group.

The highest and lowest antimicrobial properties of sealers at time interval in both ADT and MDCT showed in **Fig. 3**.

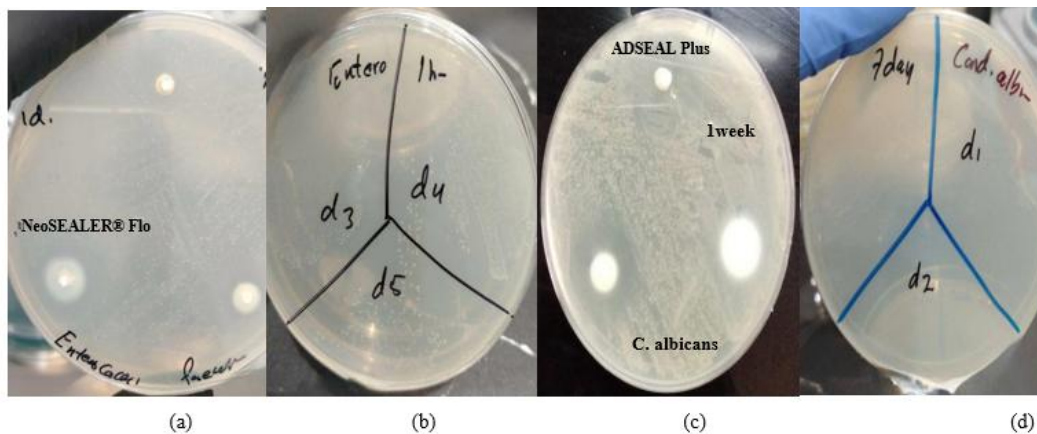


Fig. 3. Antimicrobial testing. (a) Highest ZOI in ADT for NeoSEALER® Flo at 1day against *E. faecalis* (b) Highest CFU count in MDCT for ADSEAL Plus at 1hour against *E. faecalis*; (c) Lowest ZOI for ADSEAL Plus at 1week against *C. albicans*; (d) Lowest CFU count in MDCT for MTA-Fillapex at 1week against *C. albicans*

4. Discussion

ADT and MDCT are widely utilized in vitro methodologies for assessing the antimicrobial efficacy of dental materials. Considering that root canal sealers exert their antimicrobial influence by diffusing into dentinal tubules and periradicular tissues, thereby targeting residual microbial populations post-endodontic therapy the ADT appears to be an appropriate and relevant assay for evaluating their antibacterial potential (12). Moreover, given that the MDCT operates independently of the material's diffusibility and flow characteristics, it is particularly well-suited for assessing antimicrobial efficacy once the material freshly mixed and fully set (19).

In present study, the antimicrobial activity of three different type of sealer NeoSEALER® Flo, ADSEAL Plus and MTA-Fillapex sealers against *C. albicans* and *E. faecalis* was evaluated in both freshly mixed and fully set conditions through the application of both the ADT and MDCT, thereby providing a comprehensive analysis of their bactericidal and fungicidal performance across different states of material maturation.

At present, there exists a paucity of published research specifically addressing the antifungal efficacy of NeoSEALER® Flo and ADSEAL Plus against *Candida albicans*, its antifungal properties remain even more inadequately investigated and largely uncharacterized in existing research. Considering the complexity of fungal infections and their resistance to endodontic procedures. While NeoSEALER® Flo, a bioceramic-based endodontic sealer, is comparatively less documented in terms of its antibacterial activity particularly when evaluated alongside MTA- Fillapex, especially against *E. faecalis*, and there is no studies evaluate antibacterial effect of ADSEAL Plus with other dental sealers.

A few studies (17, 18) have explored the antibacterial properties of NeoSEALER® Flo against *E. faecalis*, but with other type of sealers. NeoSEALER® Flo showed the highest bacterial reduction against *E. faecalis* throughout all intervals in MDCT compared to other sealers, while showed a reduction in their antibacterial activity with time after setting. The enhanced antibacterial efficacy of NeoSEALER® Flo against *E. faecalis* is primarily ascribed to its bioceramic formulation, which sustains

an elevated pH while continuously releasing calcium and hydroxide ions over a prolonged duration. This sustained ionic release fosters a highly alkaline microenvironment that is detrimental to bacterial viability, thereby positioning NeoSEALER® Flo as a superior candidate for endodontic applications targeting the eradication of persistent and resistant microbial infections (17).

Sebastian et al. (2024) also reported that NeoSEALER Flo demonstrate superior antibacterial properties against *E. faecalis* which reduces with time in a study of MDCT on three sets of sealers: Freshly mixed sealers, sealers that were 1-day old, and sealers that were 7-day old, these findings are consistent with our study's results, the same finding was observed in study done by Mangat et al. (2020).

However, the antifungal result of NeoSEALER® Flo against *C. albicans* increased with time interval according to both ADT and MDCT, may be due to that NeoSEALER® Flo are initially alkaline (high pH), a property known to exert antifungal effects, more antimicrobial properties compared to ADSEAL Plus, but less than MTA-Fillapex. To the best of our knowledge, no previous study has evaluated the antimicrobial effect of NeoSEALER Flo bioceramic sealer on any other microorganisms (18).

In this study ADSEAL Plus, an epoxy resin-based endodontic sealer, demonstrates a moderate spectrum of antimicrobial activity against *E. faecalis* and *C. albicans*. Although it exerts measurable inhibitory effects, its antimicrobial potency is comparatively inferior to NeoSEALER® Flo and MTA Fillapex. Furthermore, the bacteriostatic efficacy of ADSEAL Plus increased during 1hour and 1day, while progressively attenuates with the passage of time in MDCT, while its efficacy appears only in 1day in ADT. The results align with the findings of Simsek and Kanik (2021), who observed the antimicrobial effect of Adseal at 24h, and 48h, and Shakouie et al. (2012) showed that at 7day old adseal didn't show antibacterial effect against *E. faecalis* and study of Ghabraei et al. (2024).

The antimicrobial efficacy of ADSEAL Plus, is primarily can be attributed to several factors related to the bioactive properties of its constituents and the chemical by-products generated during its polymerization process. This dual-component system comprises a base and a catalyst. The base predominantly contains bisphenol A diglycidyl ether (BADGE), an epoxy oligomer resin known for its adhesive qualities and potential antimicrobial activity. Upon mixing with the catalyst, which includes amines and bismuth subcarbonate, leading to the formation of a cross-linked polymer network. During this curing process, minimal amounts of formaldehyde may be released, which can interact with microbial proteins and nucleic acids, thereby exerting a bactericidal effect. Notably, the sealer's transient slight acidity during polymerization-pH values just below neutrality does not contribute measurably to its antimicrobial efficacy (22).

Based on current in vitro studies ADSEAL Plus has exhibited a moderate antifungal effect, demonstrating partial inhibitory capacity. However, its efficacy inferior when compared to other sealers, particularly in terms of sustained antimicrobial action. Although direct comparative studies between Adseal and MTA Fillapex are scarce, the prevailing evidence supports the superior antifungal performance of MTA-based sealers compare to epoxy resin based sealers as shown in study done by Türkyılmaz and

Erdemir (2020).

MTA Fillapex, a sealer formulated with Mineral Trioxide Aggregate, empirical evidence indicates that among the sealers evaluated, MTA Fillapex exhibits the moderate antibacterial efficacy against *E. Faecalis*, which is less than NeoSEALER® Flo, but more than ADSEAL Plus, as reflected by inhibition zones observed 1day in ADT, while in MDCT the antibacterial effect decreased with time after setting. This statement is supported by the result of Morgental et al. (2011) which is studied antibacterial effect of Adseal and MTA-Fillapex both at 24 and 48-hour intervals, its antimicrobial potency declines markedly post-setting, ultimately resulting in negligible antibacterial activity over time.

Torabinejad et al. (1995) investigated the antimicrobial properties of Mineral Trioxide Aggregate (MTA). Their findings indicated that MTA exhibits an initial alkalinity with a pH of 10.2, which escalates to approximately 12.5 within three hours. It is well-established that such elevated pH levels typically around 12.0 are capable of exerting inhibitory effects on a broad spectrum of microorganisms, including resilient strains such as *E faecalis* (12). MTA Fillapex, has demonstrated notable antifungal properties against *C. albicans* compared to other sealers in both ADT and MDCT. In a similar recent study, which is reported that MTA Fillapex produces significant zones of inhibition against *C. albicans*, with measurements up to 16.0 ± 0.20 mm, indicating strong antifungal efficacy. This activity is attributed to its high pH and the release of bioactive ions such as calcium and silicon, which can disrupt fungal cell structures and metabolic processes (26,27). Also due to their ability to maintain a high pH and continuously release antimicrobial ions. This makes them more effective in eliminating resistant bacteria commonly found in root canal systems. In a similar recent study done by Nunes Moreira et al. (2024), found that MTA-Fillapex demonstrated superior performance among the calcium silicate-based sealers, with stable inhibition levels observed for all tested fungal species over the 7-day period, and study performed by Türkyılmaz and Erdemir (2020), which is concluded that MTA-Fillapex inhibited bacterial and fungal growth in all freshly mixed and set form.

The present study demonstrated no ZOI in 1 hour and 1 week in ADT for all three tested sealers, The antibacterial effect of sealers in the direct technique was higher than the indirect technique, which might be attributed to the fact that in the indirect technique, the sealer need a longer time to exert its effect on microorganisms, because indirect technique demonstrates incubation period of antibacterial materials, while MDCT is a more reliable and reproducible method used to quantitatively assess the antibacterial effects of insoluble materials, specifically in evaluating the antimicrobial properties of root canal sealers (29, 30).

5. Conclusion

NeoSEALER® Flo demonstrates superior antibacterial properties against *E. faecalis* consistently across all time intervals compared to other tested sealers and decreased with increasing time interval, while MTA-Fillapex demonstrated superior antimicrobial activity against *C. albicans* and increased with increasing time interval, followed by NeoSEALER® Flo, in contrast to ADSEAL Plus decreased activity with increasing time.

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