Original Article

Evaluating the antimicrobial effectiveness of endodontic sealers against oral pathogens associated with failed root canal treatments

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Abstract

Overview: Residual pathogens lead to treatment failure. Microbes like *Enterococcus faecalis*, *Candida albicans*, and *Actinomyces israelii* are often isolated after failed root canal therapy. Therefore, the antimicrobial efficacy of sealers is critical for treatment outcomes.

Aim: To evaluate the antimicrobial efficacy of endodontic sealers against *E. faecalis* and *C. albicans*.

Methodology: The agar diffusion test was used to evaluate the antibacterial efficacy of four sealers: MTA Fillapex, Bio-C-Sealer, Sealapex, and Seal-Pex. Each microorganism's bacterial suspension was added to agar plates separately. The sterile discs were then placed on the plates; later, a freshly mixed sealer was coated on the sterile discs. After 24 h of incubation, inhibition zones were measured.

Results: The Kruskal–Wallis test was used to analyze the data, and the Mann–Whitney *U*-test was used for *post hoc* pairwise comparison. The results demonstrated that Seal-Pex has potent antibacterial activity against E. faecalis ($P < 0.05$). Against *C. albicans*, both MTA Fillapex and Seal-Pex were effective, with MTA Fillapex showing a statistically significant difference compared to Bio-C Sealer and Sealapex.

Conclusion: Seal-Pex, an epoxy resin-based sealer, exhibited a significant antimicrobial efficacy against *E. faecalis*. Conversely, MTA Fillapex demonstrated notable antifungal activity against *C. albicans*, surpassing other tested sealers. These findings highlight the differential antimicrobial and antifungal properties of the sealers.

Keywords: Antifungal agents; microbial sensitivity tests; root canal filling materials; root canal sealants

INTRODUCTION

Microbial infection is recognized as the primary causative factor of endodontic diseases.[1,2] Therefore, the elimination of pathogenic microorganisms is a primary goal in endodontic treatment.[3] This is achieved through a combination of mechanical instrumentation, chemical irrigation, and the application of antimicrobial medicaments to the root canals in between visits. Despite these measures, certain microorganisms are capable of surviving, posing a significant challenge to achieving complete disinfection.

Microorganisms that infect the dentine in the root canal may attach themselves to the dentine at the surface or can go deeper into the dentinal tubules.[4,5] Once established within the dentinal tubules, their removal becomes increasingly difficult.[6] Residual pathogens are a major

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cause of endodontic treatment failure, necessitating complete eradication of infection for long-term clinical success. It has been reported that microbes like *Actinomyces israelii* (3%–24%),[7] *Candida albicans* (9.3%–16%),[8] and *Enterococcus faecalis* (29%–28%)[9] are often isolated from teeth after root canal therapy has failed.[10]

Root canal sealers play a critical role in the success of endodontic treatment. Their primary function is to fill the interface between the root canal walls and gutta-percha cones, creating a void-free, cohesive obturation mass.[11] Furthermore, sealers with enhanced sealing properties and antibacterial qualities are clinically valuable, as they prevent bacterial recontamination of the canal and inactivate any residual microorganisms remaining after root canal obturation. This makes them an essential final component of the endodontic treatment regimen.

Given the persistent challenges posed by microbial infections, the development and evaluation of endodontic sealers with enhanced sealing capacity and antimicrobial efficacy are of significant clinical importance. In this *in vitro* study, we compare the antimicrobial effectiveness of four different endodontic sealers: Sealapex (calcium hydroxide-based), Seal-Pex (epoxy resin-based), Bio-C Sealer (calcium silicate-based), and MTA Fillapex (MTA-based). These sealers were selected due to their frequent usage and diverse chemical composition, which may influence their antimicrobial activity. The study specifically aims to assess the antimicrobial effectiveness of these sealers against two common pathogens, *C. albicans* and *E. faecalis*, which are frequently associated with failed root canal treatments.

METHODOLOGY

Testing samples

Four endodontic sealers were tested in this study:

- Calcium hydroxide-based sealer – Sealapex (Kerr, USA)
- Epoxy resin-based sealer – Seal-Pex (Waldent, India)
- Calcium silicate-based sealer – Bio-C sealer (Angelus, Brazil)
- MTA-based sealer – MTA Fillapex (Angelus, Brazil).

The manufacturer's instructions were followed while preparing the sealers. An agar disc diffusion test (ADT)[12] was performed with stringent aseptic measures to evaluate the sealer's antimicrobial effect.

Microorganism preparation and antimicrobial testing procedure

Antimicrobial properties were assessed concerning *E. faecalis* and *C. albicans* strains. The study includes clinical and American Type Culture Collection (ATCC) strains of *E. faecalis* (ATCC 29212) and *C. albicans* (ATCC 10321) from the Microbiology Laboratory at AIIMS Kalyani. The organism was inoculated in peptone water and incubated at 37°C for 2 h to reach the log phase of growth. The turbidity of the growth of the organism was matched to the 0.5 McFarland Standard by selecting 5–6 colonies for *E. faecalis* and 3–4 colonies for *C. albicans*, ensuring the appropriate inoculum density for preparing a lawn culture on the Mueller–Hinton agar plates.

The procedures outlined by Bauer *et al*.[12] were followed in the current study. The Kirby–Bauer method involves testing bacterial isolates for antibiotic resistance using antibiotic discs that are positioned on the surface of a Mueller–Hinton agar plate containing bacteria. This process is known as the disc diffusion susceptibility test.

An antimicrobial agent begins to diffuse and create a concentration gradient around the paper discs as soon as they are placed on the surface of a freshly inoculated plate. The highest concentration is found closest to the disc. After 16–18 h of incubation at 37°C, bacteria grow on the plate's surface, except in the zones where the antibiotic concentration within the gradient is sufficient to inhibit bacterial growth. Using an inhibition zone gauging scale, the diameter of the zone of inhibition surrounding each disc was measured following incubation.

Preparation of discs

With the aid of sterile forceps, sterile discs (6 mm in diameter, made from Whatman filter paper) were applied and gently pressed to ensure consistent interaction with the medium after the inoculum had dried. Using micropipettes, 100 μL (0.1 mL) of every sealer was put on the sterile disc after the sealers had been mixed following the manufacturer's instructions. After making a lawn culture with the test organism and application of the sealers on the inoculated plates, it was incubated at 37°C and the reading was taken after 24 h as this time frame is sufficient to observe the antimicrobial effects of endodontic sealers. Previous studies have shown that further incubation beyond 24 h does not significantly alter the inhibition zone. Therefore, we chose 24 h as it is a reliable time point for accurately assessing the antimicrobial efficacy of sealers.[13]

In our study, six plates were prepared as replicates for each pathogen tested to ensure statistical reliability and reproducibility of the results. The antimicrobial efficacy of four endodontic sealers was then evaluated by placing all four sealer discs on a single inoculated plate, allowing for an efficient and comparative analysis.

RESULTS

Table 1 presents the tabulated results of the antimicrobial efficacy of the four tested sealers. Figure 1 illustrates the

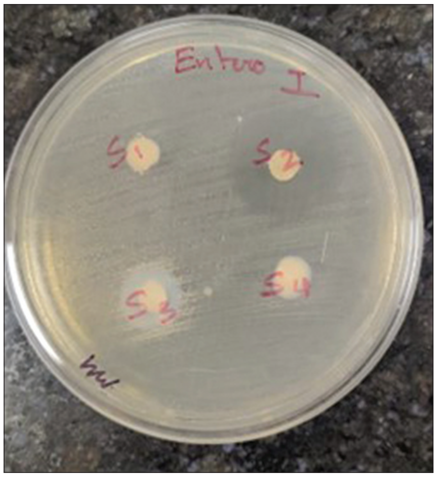

Figure 1: Sealers' zone of inhibition against *Enterococcus faecalis*

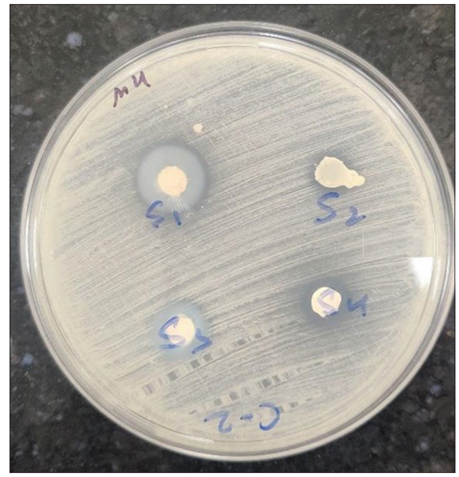

Figure 2: Sealers' zone of inhibition against *Candida albicans*

Table 1: Mean value of growth inhibition by diameter in mm for different groups of sealer at 24 h

Sealers	*E. faecalis*	*C. albicans*
Sealer 1 - Sealapex	7.5	6
Sealer 2 - Seal-Pex	24.5	8.6
Sealer 3 - Bio-C Sealer	6	6
Sealer 4 - MTA Fillapex	6	10.6

E. faecalis: Enterococcus faecalis, C. albicans: Candida albicans, MTA: Mineral trioxide aggregate

zone of inhibition for *E. faecalis*, and Figure 2 displays the zone of inhibition for *C. albicans*.

Statistical analysis was conducted using the Kruskal–Wallis test, which revealed a significant variation in the zone of inhibition across the four sealers at a 0.05 significance level.

The *post hoc* pairwise comparison (Mann–Whitney *U*-test) demonstrated a statistically significant variation in the zone of inhibition for *E. faecalis* between Seal-Pex and Sealapex. Similarly, Seal-Pex demonstrated a significant difference when contrasted to both Bio-C Sealer and MTA-Fillapex. The other pairs lacked statistically significant differences.

For *C. albicans*, a *post hoc* pairwise comparison demonstrated that the MTA Fillapex zone of inhibition is statistically significant with Sealapex and Bio-C Sealer. The other pairs did not show statistically significant differences.

DISCUSSION

Research indicates that the most common causes of endodontic failure are residual bacteria and debris in the root canal.[14] Therefore, the success of endodontic therapy depends on the complete elimination of pathogens within the root canal and achieving a three-dimensional seal using a sealer with antimicrobial properties to prevent reinfection. According to Grossman, sealers should exhibit bacteriostatic properties or, at the very least, should not support bacterial growth.[15]

E. faecalis was selected for this study due to its association with recurrent periapical infections and its challenging removal from root canals. As a facultative anaerobic Gram-positive bacterium, it can withstand a variety of adverse conditions including highly alkaline environments, contributing to its persistence in treated root canals. It utilizes an ATP-dependent potassium/proton antiport system to transport protons into its cells, helping to counteract internal alkalization.[16] Since this system relies on ATP for its function, it generates the necessary energy by fermenting carbohydrates and degrading proteins, such as gelatin. This indicates that *E. faecalis* may derive energy from nitrogen-rich compounds found in root canals, which fuels its ATP-linked proton transport system, allowing it to survive and thrive in alkaline environments. In addition, research indicates that *E. faecalis* produces a biofilm that protects against antibacterial agents and host defense mechanisms, further contributing to its persistence in root canal infections.[17] Yeasts have been found in 1%–17% of infected root canals and are most commonly associated with cases of persistent apical periodontitis. The genus Candida contains the majority of the significant oral yeasts. The species that is isolated most frequently is *C. albicans*. It can thrive in an environment deficient in nutrients, due to its secretion of enzymes that can break down dentinal collagen and aspartyl protease.[18]

As these two pathogens can withstand the challenging conditions found in the root canal, infection control is also impacted by the antifungal properties of root canal sealers. Therefore, this study compares the antimicrobial efficacy of four distinct endodontic sealers against *C. albicans* and *E. faecalis*.

E-test, agar disc diffusion, broth dilution, agar disc dilution, quantitative polymerase chain reaction, thin-layer chromatography-bioautography, spiral gradient test, and automated antimicrobial testing systems are some of the methods used to evaluate the effectiveness of antibiotics. Each method has innate benefits and drawbacks.[19,20] Conventionally, antimicrobial susceptibility has been determined using agar diffusion and agar dilution methods. For our investigation, the agar disc diffusion method, also known as the Kirby–Bauer method, was chosen over the agar dilution method. The latter has a drawback, as it may alter the characteristics of some test sealers. Furthermore, homogeneously dissolving certain sealers is a challenging and time-consuming process. As a result, the agar disc diffusion method was employed in this study. This approach allows for the evaluation of antimicrobial resistance by testing bacterial isolates against antimicrobial discs when preserving the chemical characteristics of the sealers. In addition, it is a straightforward method that is less technique dependent.[21]

In our study, the resin-based sealer Seal-Pex demonstrated significant antimicrobial activity against *E. faecalis*, as evidenced by the largest zone of inhibition (24.5 mm) compared to the other sealers tested, which can be attributed to the presence of titanium oxide (TiO_2) and methenamine in its composition. TiO_2, known for its high oxidative potential, facilitates the production of reactive oxygen species, which disrupt the bacterial outer cell membrane and lead to cell death.[22] In addition, methenamine hydrolyzes into formaldehyde and ammonia under aqueous or acidic conditions (pH <6), further contributing to its antimicrobial activity.[23] The superior performance of Seal-Pex is primarily due to these antimicrobial agents rather than its pH. Resin-based sealers typically exhibit a neutral to slightly acidic pH, which may not directly inhibit bacterial growth. Instead, the chemical composition of Seal-Pex ensures effective disruption of bacterial integrity.[24] As far as we are aware, this study is the first to specifically evaluate the antimicrobial efficacy of Seal-Pex. The significant zone of inhibition observed may be attributed to its epoxy resin-based composition, known for inherent antibacterial activity. While no prior studies on Seal-Pex exist, similar epoxy resin-based sealers like AH Plus have demonstrated significant antimicrobial properties in the literature.[25]

MTA Fillapex demonstrated enhanced antifungal activity against *C. albicans*, which can be attributed to MTA in its composition. During its setting process, the sealer achieves a pH of approximately 10–10.5, creating an environment lethal to many microorganisms, including fungi.[26] This alkaline pH contributes to the larger zone of inhibition observed for *C. albicans* (10.6 mm). In addition, it releases bioactive ions, such as calcium and silicon, which are known for their antimicrobial effects. These ions may disrupt the fungal cell structure or interfere with its metabolic processes, making *C. albicans* more susceptible. The superior antifungal activity of MTA Fillapex is further attributed to its sustained release of these ions over time, ensuring prolonged antifungal efficacy.[27,28] These results align with the findings of Türkyılmaz and Erdemir,[29] who reported that MTA Fillapex demonstrated the ability to inhibit bacterial and fungal growth in both its freshly mixed and set forms, outperforming other tested sealers. The calcium-based sealer Sealapex and the bioceramic sealer exhibited some zones of inhibition against *E. faecalis* and *C. albicans*. However, this activity was significantly lower compared to other sealers tested.

This study is being conducted to provide the practitioner with important knowledge about the antimicrobial characteristics of different endodontic sealers. Thus, *in vivo* testing is crucial to ascertain the actual efficacy of the sealer. In light of this, the results of this investigation demonstrate that the zones of inhibition of the different endodontic sealers reflect variations in their antimicrobial activity. Interestingly, despite the bioceramic sealer being biocompatible and widely regarded for its favorable properties, this study revealed that it exhibited lower antimicrobial efficacy compared to other sealers. The antimicrobial efficacy was assessed using the ADT, while other newer techniques may provide more detailed insights. Variations in results could arise due to the limitations of the method used.

CONCLUSION

Seal-Pex, an epoxy resin-based sealer, demonstrated the highest antimicrobial efficacy against *E. faecalis*, significantly outperforming other sealers. Sealapex, a calcium-based sealer, showed moderate activity against *E. faecalis*, while Bio-C sealer exhibited minimal antimicrobial efficacy against both *E. faecalis* and *C. albicans*. MTA Fillapex showed increased zone of inhibition against *C. albicans*, surpassing the antifungal performance of others. These results emphasize the distinct antimicrobial and antifungal properties of the tested sealers, with Seal-Pex excelling against bacteria and MTA Fillapex against fungi.

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Conflicts of interest
There are no conflicts of interest.

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