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Comparative *in vitro* analysis of the antifungal activity of different calcium silicate-based endodontic sealers

Luiz Felipe Nunes Moreira[1], Fernando Peña-Bengoa[2*], Sven Eric Niklander[3], Carlos Eduardo da Silveira Bueno[4], Alexandre Sigrist de Martin[4], Daniel Guimarães Pedro Rocha[4,5]

[1] Faculty of Dentistry, Vale do Rio Doce University, Governador Valadares, Minas Gerais, Brazil

[2] Departament of Endodontics, Dentistry Faculty, Andres Bello University, Viña del Mar, Chile.

[3] Unit of Oral Pathology and Medicine, Dentistry Faculty, Andres Bello University, Viña del Mar, Chile.

[4] Department of Endodontics, Faculty of Dentistry, São Leopoldo Mandic College of Campinas, Campinas, São Paulo, Brazil.

[5] Department of Endodontics, Faculty of Dentistry, Pontifical Catholic University of Campinas, Campinas, São Paulo, Brazil.

Corresponding author:
Fernando Peña-Bengoa
Department of Endodontics, Universidad Andres Bello.
Quillota 980, torre E, 2531015, Viña del Mar, Chile.
Phone number: 569 85481196
ORCID:0000-0001-9389-0468
E-mail: fernandopenab@gmail.com, fernando.pena.b@unab.cl

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Aim: This study aimed to perform an *in vitro* comparative analysis of the antifungal activity of different calcium silicate-based endodontic sealers against three fungal species. **Methods:** The antifungal properties of three calcium silicate-based sealers were tested: Bio-C Sealer, Cambiar a Sealer Plus BC, and MTA-Fillapex. Two commonly used sealers were used as controls: AH Plus and Endomethasone. An agar diffusion test was performed to analyze the antifungal activity of the sealers against *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and a mixed microbial culture medium. The results were analyzed using ANOVA ($p < 0.05$). **Results:** Endomethasone exhibited the highest inhibition against all strains examined, maintaining a consistent level of inhibition throughout 7 days. MTA-Fillapex demonstrated the best performance among the calcium silicate-based sealers for the three fungal species ($p < 0.05$), maintaining stable values over the 7 days, surpassing that of Endomethasone. Nevertheless, MTA-Fillapex only exhibited antimicrobial effect against the mixed culture for the first 24 hours, and no antimicrobial activity was observed at 48 hours, being surpassed by all tested sealers ($p < 0.05$). **Conclusion:** Of all silicate-based sealers tested, only MTA-Fillapex exhibited promising antifungal activity. Nevertheless, care must be taken when extrapolating these results, as MTA-Fillapex exhibited poor antimicrobial activity when tested in mixed microbial cultures.

Keywords: Endodontics. Bacteria. *Candida albicans*. Root canal filling materials.

Introduction

Microbiological factors are the main reason for endodontic failure[1,2]. The complexity of endodontic microbiota and their ability to colonize irregularities in the root canal system (RCS) pose challenges for clinicians, particularly in areas where instrumentation, irrigation, or intracanal medication may be difficult for anatomical reasons[3]. Although endodontic treatments have a high success rate, most failures are associated with persistent or secondary infections[3,4].

Bacteria are the main cause of endodontic infections; however, other microorganisms, such as fungi may be involved[3]. The proportion of *Candida spp.* in infected root canals ranges from 0.5% to 55%, with *C. albicans* being the most frequently involved species[5]. Other species, such as *C. tropicalis*, which is the most common non-albicans-candida species found in the oral cavity of healthy people[6], may also be involved in apical periodontitis[7]. Fungi in endodontic infections are commonly associated with secondary or persistent infections; however, they may also be present in primary infections[1,8]. Although fewer in number compared to bacteria, they are aggressive enough to maintain periapical disease because of their ability to adapt to the environment, adhere to surfaces, produce hydrological enzymes, undergo morphological transitions, form biofilms, and resist calcium hydroxide, the most commonly used intracanal medication[9].

Calcium silicate-based endodontic sealers have become popular in recent years because of their excellent properties and simplicity of use[10]. Their biocompatibility, high pH, and low cytotoxicity are just some of their favorable properties[11]. In addition, silicate-based endodontic sealers do not shrink after insertion and form hydroxyapatite, establishing a chemical bond with dentin[12-14]. This made it possible to simplify the filling stage by complying with all parameters of adequate root canal obturation. However, the use of these sealers in teeth treated for secondary or persistent endodontic infections, where fungi may be present, must be handled with care given that the setting of these materials depends on the local conditions of the RCS[15]. The persistence of fungi and their byproducts cause inflammation of the periradicular tissues and a reduction in the local pH, which can alter the properties of these sealers[16].

Considering the complexity of fungal infections and their resistance to endodontic procedures, it is essential to understand the antifungal properties of endodontic sealers. Calcium silicate-based sealers are frequently studied for their antibacterial properties; however, studies on their antifungal properties are limited. This study aimed to perform a comparative *in vitro* analysis of the antifungal activities of different calcium silicate-based sealers against three *Candida spp.* species.

Materials and Methods

This project was approved by the local ethics committee, which authorized its development under protocol number 2018/0997.

Tested Materials

Three calcium silicate-based endodontic sealers were tested: Bio-C Sealer (Angelus, Londrina, PR, Brazil), Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil), and MTA-Fillapex (Angelus, Londrina, PR, Brazil). Two of the most commonly used endodontic sealers, AH Plus (Dentsply Sirona, Ballaigues, Switzerland) and Endomethasone (Septodont, Saint-Maur-Des-Fossés, France), were used as controls.

Microbiological analysis

The methodology used in this study was adapted from Damasceno et al.[17] (2008), Gomes et al.[18] (2004), and Rahman et al.[19] (2017). An agar diffusion test was used to determine inhibitory effects against three fungal species: *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 90030), and *Candida tropicalis* (ATCC 750). The initially lyophilized fungi were reactivated, cultured, and maintained in trypticase soy broth (TSB) (Difco, Detroit, MI, USA). A 24-hour culture was used as the inoculum. To standardize the inoculum, TSB medium was prepared according to the 0.5 McFarland turbidity standard (1.5×10^8 colony-forming units/mL[20]).

Petri dishes containing TSA medium were inoculated with 0.1 mL culture of each fungal species using sterile swabs rubbed across the surface of the TSB medium. A mixed microbial culture collected from human saliva was used as a comparative parameter for antifungal tests. Saliva was diluted to a concentration of 1 mL in 4 mL of TSB. After the inoculation of fungal species and the mixed microbial culture on the TSB medium, three 6-mm-diameter and 5-mm-deep holes (dictated by the thickness of the agar layer) were made with a sterile metal hole punch. Endodontic sealers were manipulated simultaneously according to the manufacturer's instructions. Premixed sealers (Bio-C Sealer and Sealer Plus BC) were used directly from the syringes with dispensing tips, whereas MTA–Fillapex was prepared using the automix tip designed for the dual syringe of the sealer. For the preparation of AH Plus, equal volumes (1:1) of pastes A and B were dispensed on a glass slab and mixed using a metal spatula until a homogeneous consistency was obtained. Endomethasone was prepared by progressively pouring the powder into the liquid at a ratio of two spoons of powder to four drops of liquid until a homogeneous mixture was obtained. Sealers were introduced into the agar holes to be tested in triplicate. The plates were kept for 2 hours at room temperature to allow the diffusion of the sealers through the agar, and then they were transferred to a 37 °C aerobic incubation environment for 24 hours to allow microbial growth (Tanomaru-Filho et al.[21] (2007), Gomes et al.[18] (2004)). Growth inhibition zones were measured daily for 7 days using a manual caliper (Damasceno et al.[17] (2008), Rahman et al.[19] (2017)).

Results

When analyzing the performance of the sealers in terms of their ability to inhibit fungal growth over time, Endomethasone exhibited the highest inhibition against all strains examined, maintaining a consistent level of inhibition throughout the entire 7-day period. MTA-Fillapex demonstrated the best performance among the calcium silicate-based sealers for the three fungal species, maintaining stable values over 7 days, and was only surpassed by Endomethasone. Bio-C Sealer, Sealer Plus BC, and AH Plus exhibited similar levels of inhibition as MTA-Fillapex against *C. albicans* and *C. tropicalis* only after 24 hours, and at 48 hours, the antifungal activities of Sealer Plus BC and BIO-C Sealer decreased. At all the time points evaluated, AH Plus failed to exhibit any inhibitory effect against *C. glabrata* (Fig. 1).

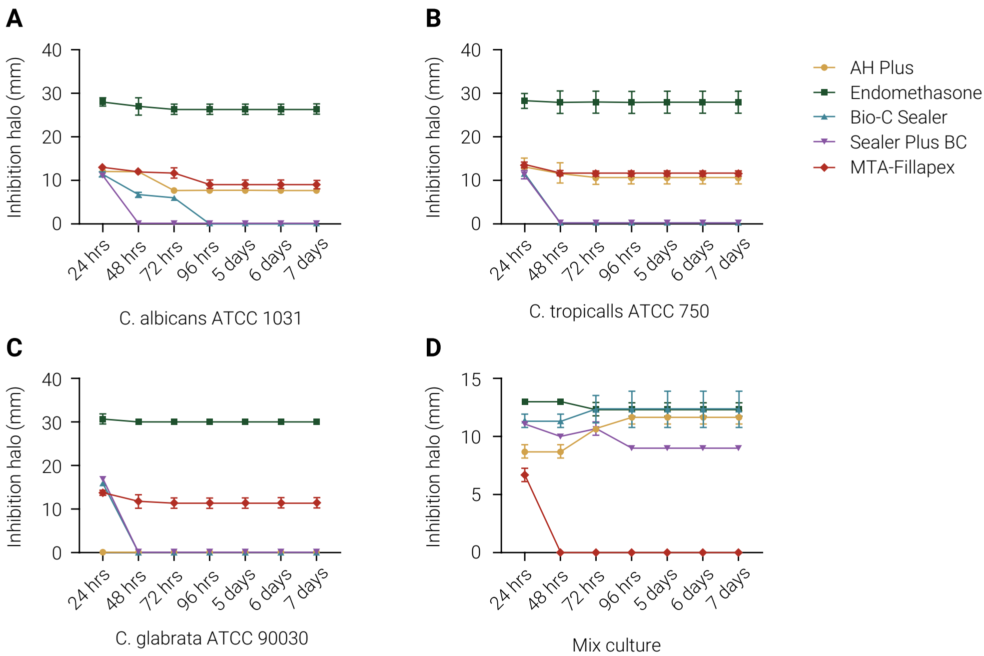
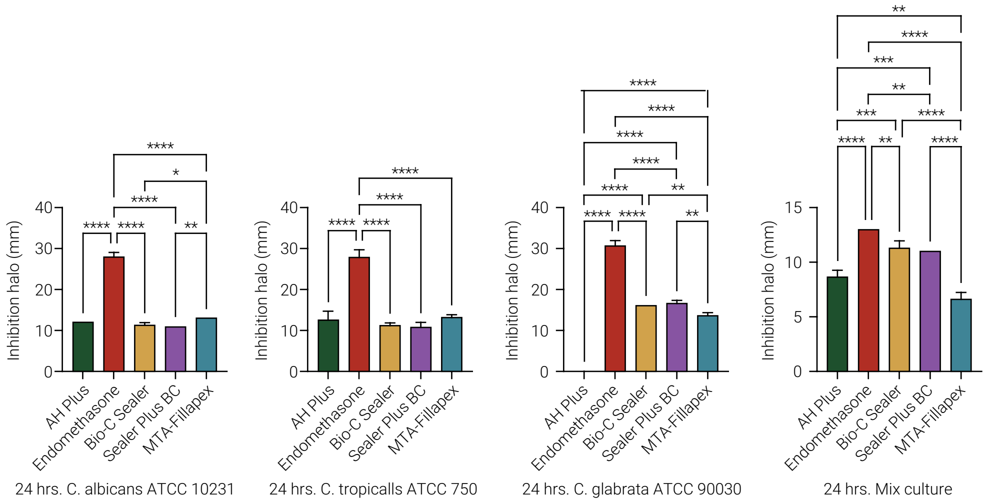


Figure 1. Variation over time of the inhibition halos produced by the sealers for the different species studied.

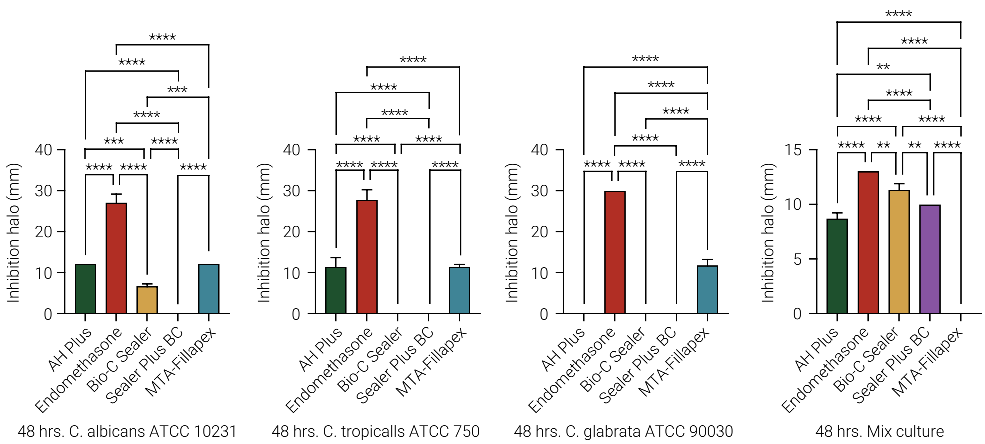
When analyzing the performance of calcium silicate-based sealers at 24 hours, MTA-Fillapex displayed the highest inhibition halo against *C. albicans*, exhibiting significant differences compared to Bio-C Sealer and Sealer Plus BC (p<0.008). However, for *C. tropicalis*, all three calcium silicate-based sealers demonstrated similar values. Concerning *C. glabrata*, the highest inhibition halo was achieved by Sealer Plus BC, showing significant differences compared to MTA-Fillapex (p<0.008) but not when compared to Bio-C Sealer (Fig. 2).



****<0.0001; ***<0.0008; **<0.008; *<0.05

Figure 2. Inhibition halos at 24 hours for the different sealers

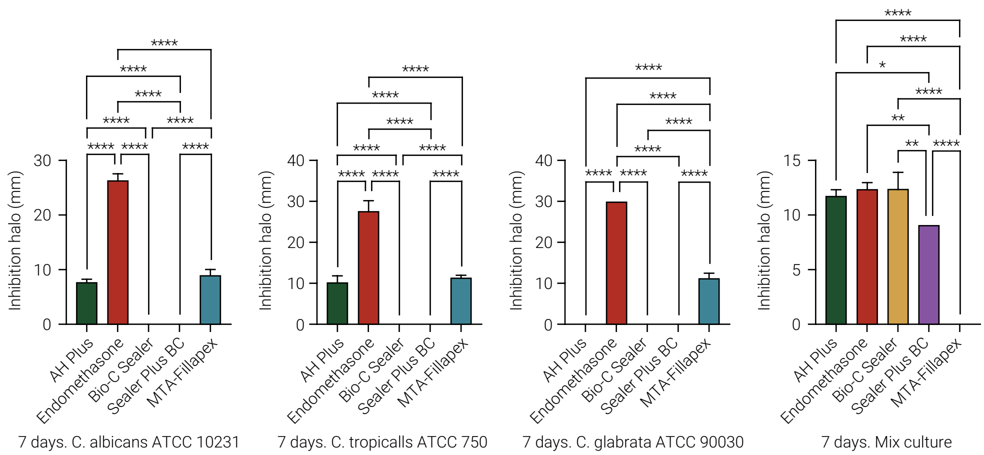
At 48 hours, MTA-Fillapex displayed larger inhibition halos against *C. albicans* than Bio-C Sealer (p<0.0008), whereas Sealer Plus BC failed to exhibit any inhibition. Only MTA-Fillapex produced inhibition halos for *C. tropicalis* and *C. glabrata*, an effect that was sustained only for fungal species, as no inhibition halos were observed for mixed bacterial cultures (Fig. 3).



****<0.0001; ***<0.0008; **<0.008

Figure 3. Inhibition halos at 48 hours for the different sealers

After 7 days, only MTA-Fillapex maintained its inhibitory effect against the three fungal species studied. In the mixed bacterial culture, Bio-C Sealer outperformed Sealer Plus BC (p<0.008), and the values remained stable from 72 to 96 hours, respectively (Fig. 4).



****<0.0001; **<0.008; *<0.05

Figure 4. Inhibition halos at 7 days for the different sealers

Discussion

Understanding the antifungal properties of sealers is crucial, given their direct contact with canal walls and the potential exposure to hard-to-reach areas that may be colonized by fungi or other species. These areas, such as isthmuses, lateral canals, and apical deltas, can serve as reservoirs for various microorganisms and contribute to the development of persistent apical periodontitis[3].

The simultaneous testing of the sealers and their measurement every 24 hours allowed the analysis of the antimicrobial behavior of each sealer over time. Periodic assessment of inhibition halos makes it possible to determine the time at which the sealers exhibit fluctuations, time–pointing when further attention is needed. In this study, we determined that the time points with the most significant fluctuations in antimicrobial activity were 24, 48, and 7 days.

Endomethasone exhibited the highest antimicrobial activity, as it was the only sealer that demonstrated inhibitory action against all fungal species and mixed microbial cultures over 7 days. This sustained and potent antimicrobial activity can be attributed to the combined effect of corticosteroids, formaldehyde, and zinc oxide eugenol, all of which are present in the sealer's composition[18]. AH Plus exhibited greater inhibition than Sealer Plus BC and Bio-C Sealer against *C. albicans* and *C. tropicalis,* with the highest inhibition observed at 48 hours. These findings can be explained by the release of formaldehyde during the setting reaction of AH Plus. Although AH Plus did not contain formaldehyde, it released small amounts of this compound during the setting reaction, reaching its maximum level after 48 hours[22]. Formaldehyde exhibits antimicrobial properties that alter the viability and growth of bacteria and fungi[22]. No significant differences were observed between the antifungal activity of AH Plus and MTA-Fillapex for *C. albicans* and *C. tropicalis.* These results can be attributed to the high salicylate resin content in Fillapex, which imparts antimicrobial properties to the sealer[23].

The analysis of the results of calcium silicate-based sealers during the first 24 hours showed that MTA-Fillapex, Bio-C Sealer, and Sealer Plus BC exhibited similar inhibitory effects on the three fungal strains studied. However, a sustained decrease in inhibition was observed between 48 hours and 96 hours for the latter two. These results demonstrate the resistance of fungi to pH changes, which could explain their recognized resistance to the action of calcium hydroxide when used as an intracanal medication[8]. Although calcium silicate-based sealers do not contain calcium hydroxide in their composition, they contain calcium oxide. The interaction of the latter with tissue fluids produces calcium hydroxide, which can act as a source of the calcium ions necessary for the growth and morphogenesis of *Candida*. It is important to consider that these results may have been influenced by the buffering ability of the culture broth, which can neutralize the pH of these materials. This effect was previously reported by Al Hezaimi et al.[24] (2006) and Tanomaru Filho et al.[21] (2007), who observed a similar behavior for MTA (mineral trioxide aggregate). In relation to the behavior of MTA-Fillapex, it presented a stable inhibitory action during the 7-day period for all the fungal species analyzed. This activity can be mainly attributed to the high percentage of salicylate resin present in its composition[19,25].

Regarding the performance of premixed calcium silicate-based sealers in mixed microbial cultures, stable inhibition halos were observed from 72 to 96 hours for Sealer Plus BC and Bio-C Sealer. This might be explained by the alkalization of the medium produced by these types of materials, which, from their hydration reaction, produces calcium silicate and calcium hydroxide, releasing hydroxyl ions[26]. Based on the obtained results, Bio C-Sealer produced greater inhibition halos than AH Plus during the 7-day testing period, showing an incremental inhibition that stabilized at 72 hours, at which time it showed inhibition halos similar to Endomethasone. According to Sfeir et al.[26] (2021), calcium silicate-based sealers have similar or even greater antibacterial properties than conventional sealers; however, the lack of standardized testing methologies makes it difficult to compare the results[27]. In contrast, MTA-Fillapex presented inhibitory halos only in the first 24 hours. The antimicrobial action of this sealer can be attributed to the resin and MTA present in its composition; however, this effect is lost with setting time[28]. The limited alkalization shown by MTA-Fillapex has been previously reported in other studies[25], which can be mainly attributed to the fact that it contains 13% of MTA in its composition[23]. This low percentage of calcium silicate differentiates it from other calcium silicate-based sealers that are characterized by high pH values over longer periods[26].

This study aimed to compare the antifungal properties of three calcium silicate-based sealers using an agar diffusion test. This test is commonly used to compare and analyze the antimicrobial activity of materials, facilitating direct comparisons between them[28]. However, this methodology is not exempt from limitations because it is incapable of distinguishing between bacteriostatic and bactericidal effects and can be influenced by the physical properties of the material. Materials with high diffusibility may exhibit larger inhibition halos independent of their antimicrobial ability[29].

The scarce literature on the antifungal properties of calcium silicate-based sealers makes it difficult to compare our results with those of other studies. Further studies are required to understand and support the clinical relevance of these findings.

In conclusion, of all the silicate-based sealers tested, only MTA-Fillapex exhibited promising antifungal activity. Nevertheless, care must be taken when extrapolating these results, as MTA-Fillapex exhibited poor antimicrobial activity when tested in mixed microbial cultures.

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None.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

Datasets related to this article will be available upon request to the corresponding author.

Author Contribution

Luiz Felipe Nunes Moreira: Conceptualization, Investigation, Methodology, Project administration

Fernando Peña-Bengoa: Investigation, Formal analysis, Methodology, Project administration, Writing - review & editing.

Sven Eric Niklander: Software, Formal analysis, Writing - review & editing.

Carlos Eduardo da Silveira Bueno: Conceptualization, Formal analysis, Methodology,

Alexandre Sigrist de Martin: Conceptualization, Formal analysis, Methodology.

Daniel Guimarães Pedro Rocha: Conceptualization, Formal analysis, Methodology

All authors approved the final version of the manuscript.

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