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Antifungal activity of several root canal sealers against *Candida albicans*

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Abstract

Objective. The purpose of this *in vitro* study was to evaluate the antifungal activity of several root canal sealers (iRoot SP, MTA Fillapex and GuttaFlow) against *Candida albicans* and compare them to that of AH Plus Jet. ***Materials and methods.*** A 10 μL fungi in suspension was allowed to directly contact the sealers, which were freshly mixed or allowed to set for 1 or 7 days. Fresh media was then added and survival of fungi was determined by using 10-fold serial dilution and inoculated onto agar plates. After incubation for 48 h, colony-forming units (CFU) were calculated and their $\log_{10}$ values converted. The data were analyzed using ANOVA and Tukey tests ($\alpha = 0.05$). ***Results.*** Freshly mixed AH Plus Jet totally inhibited the growth of fungi and showed the highest antifungal activity. GuttaFlow did not show any significant antifungal activity at all times. Freshly mixed iRoot SP and MTA Fillapex were found to be antifungal. Statistical differences were found between freshly mixed and set samples ($p < 0.05$) in favor of the former, except GuttaFlow. No statistically significant differences were found among the tested sealers at the 1 and 7-day samples ($p > 0.05$). ***Conclusions.*** Fresh AH Plus Jet had very potent antifungal activity. All sealers, except GuttaFlow, exhibited antifungal activity when freshly mixed.

Key Words: *AH Plus Jet, antifungal activity, GuttaFlow, iRoot SP, MTA Fillapex*

Introduction

Micro-organisms are the main etiologic factors of pulpal and periapical disease [1]. Numerous studies have revealed a possible pathogenic role of the occurrence of fungi in endodontic infections [2,3]. The incidence of fungi reported in infected root canals varies between 2–40% [4,5]. In addition, previous studies have reported that the presence of fungi could be associated with failed root canal therapies [2,6]. Waltimo et al. [7] showed that fungi can be found as either pure culture or along with bacteria in persisting endodontic infections. The most predominant and commonly detected fungi are *Candida albicans* isolates [7]. Rocas et al. [3] isolated *C. albicans* in 6% of endodontically-treated teeth with persisting apical periodontitis. Baumgartner et al. [8] detected *C. albicans* in 20.8% of root canal samples using a polymerase chain reaction assay.

It has been reported that *C. albicans* can invade the dentinal tubules [9] and use dentine as a hideaway [10]. *C. albicans* exhibit a variety of virulence factors that are capable of infecting the dentin-pulp complex [11]. It also is capable of adapting itself to a wide range of pH levels [12]. *C. albicans* has been found to be resistant to commonly used medicaments such as calcium hydroxide [13]. The presence of these organisms in infected root canals is one of the problems that must be solved in endodontic therapy and they must be eliminated for rehabilitation of periapical tissue in post-treatment apical periodontitis cases [14].

Elimination of micro-organisms from the infected root canals as well as complete sealing of the root canal system will enhance the success of endodontic therapy [15]. However, several studies have reported that micro-organisms remain in the infected root canals even after thorough cleaning, shaping, antimicrobial irrigation and intra-canal dressing due to the complicated anatomy of dentinal tubules, lateral canals and apical ramifications [16–18]. For this reason, the use of a sealer with substantial antimicrobial activity is believed to be beneficial in the further reduction of the number of remaining micro-organisms or eradication of these micro-organisms.

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Therefore, a number of studies have investigated antimicrobial activity of endodontic sealers [19–21].

Today numerous endodontic sealers are available and newer sealers are continually introduced to the market. AH Plus (Dentsply DeTrey, Konstanz, Germany), which is an epoxy resin-based root canal sealer, has been shown to be suitable for successful endodontic therapy [22,23] and is accepted to be the gold standard against which all new sealers are compared [22]. Furthermore, previous studies have indicated that AH Plus presented antimicrobial activity [24,25]. A relatively new sealer, GuttaFlow (Coltène Whaledent, Alstatten, Switzerland) is a silicone-based root canal filling material and consists of a mixture of gutta-percha powder, poly-dimethylsiloxane and silver particles. It is a cold flowable filling material and the manufacturer claims this material does not shrink during the setting. GuttaFlow has shown good homogeneity and adaptability to root canal walls [26]. Several studies have reported that GuttaFlow had poor antimicrobial properties [20,25]. iRoot SP (Innovative BioCeramix Inc., Vancouver, Canada) is an insoluble, radiopaque, aluminum-free, hydrophilic, ready-to-use, injectable, calcium silicate-based material that has recently been introduced for filling of root canal space [27]. The composition of material is similar to the white MTA and requires the presence of humidity to set and harden [28]. MTA Fillapex (Angelus, Londrina, PR, Brazil) is another recently developed calcium silicate-based root canal sealer. It is a paste–paste sealer and consists of resinous components. According to the manufacturer it has high radio-opacity, easy handling, adequate working and setting time and low solubility (http://www.angelus.ind.br/folders/mta_fillapex/). Studies evaluating the antimicrobial activity of GuttaFlow, iRoot SP and MTA Fillapex are limited and there is a lack of information about the antifungal activity of these sealers.

The aim of this study was to evaluate the antifungal activity of a poly-dimethylsiloxane-based sealer (GuttaFlow) and two calcium silicate-based sealers (iRoot SP and MTA Fillapex) against *C.albicans* by the direct contact test and compare them to that of a resin-based sealer (AH Plus Jet). The tested hypotheses were (i) there was no significant difference in antifungal activity among the sealers and (ii) antifungal activity of the sealers was not dependent on time.

Materials and methods

The root canal sealers evaluated in this study were prepared in accordance with the manufacturer's instructions.

Test micro-organism and media

The antifungal efficacy was evaluated using a standard strain of *C. albicans* (ATCC 10231). The lyophilized fungi were cultivated on tryptone soy agar (TSA, Merc, Germany) at 37°C after incubation in sterile saline solution. Inoculum for fungal strain was prepared by picking up three or four colonies and cultivating them into tubes containing 5 mL mL tryptone soy broth (TSB, Merc, Germany) at 37°C overnight. The cell suspension was adjusted to match the turbidity equivalent to 0.5 McFarland scale, corresponding to 1.5×10^8 CFU/mL.

Direct contact test

The methodology used to assess the antifungal activity of the root canal sealers was performed as described by Weiss et al. [29], with some modifications [21]. In brief, a 96-well microtiter plate was held vertically and a section of fixed area on the side wall of wells was coated with an equal amount of each freshly mixed sealer by using a cavity liner applicator. A 10 μL sample of the fungal suspension (~ 1.5×10^6 fungi) was placed on the surface of each test material, 20 min (designated as fresh samples), 1 and 7 days after mixing. Samples in the 1 and 7 day groups were allowed to set in a humid atmosphere at 37°C prior to testing. Wells containing only identical volumes of fungal suspension were used as a positive control. Sealers incubated without micro-organisms were used as negative controls. All plates were incubated at 37°C in a humid atmosphere for 1 h. Then, 240 μL of TSB was added to each of the wells and gently mixed with a pipette for 1 min. To determine the survival of fungi, 10-fold serial dilutions were prepared in TSB and cultured on TSA plates. After incubation at 37°C for 48 h, visible colonies were counted and converted to their $\log_{10}$ values. All experiments were made in triplicate.

Controls for carry-over effect

To assess the carry-over effect of the root canal sealers, procedures performed were adapted from Zhang et al. [21]. The same amount of sealers as for direct contact test was coated on the side wall of wells. Sterile distilled water (10 μL) was placed in direct contact with the sealers. After incubation at 37°C for 1 h, 240 μL of TSB was added to each well. After mixing gently with a pipette, 10 μL of the broth was transferred to a tube containing 970 μL of TSB. Then the suspension in the tube was completed to 1000 μL by adding 20 μL of the fungal inoculum. For controls, the same amount of sterile distilled water was placed on the side wall of wells in the absence of the test materials and processed as described above. Ten-fold serial dilutions were prepared and plated onto TSA plates to investigate the possibility of antifungal carry-over effect of the sealers. After incubation at 37°C for 48 h, survival of fungi was compared in the absence or presence of the sealers. The tests were made in triplicate.

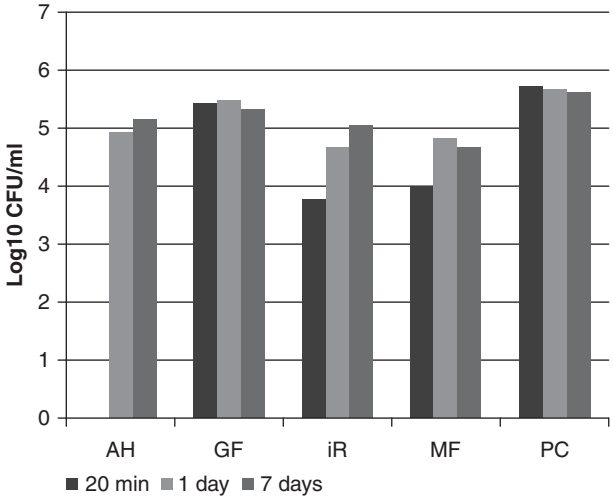

Figure 1. The fungal growth after direct contact with sealers used in this study (AH; AH Plus Jet, GF; GuttaFlow, iR; iRoot SP, MF; MTA Fillapex) for 20 min, 1 or 7 days after mixing.

Statistical analyses

The direct contact test results were analyzed by an ANOVA test using a factorial design with the root canal sealer and time. Multiple comparisons were performed using Tukey test to determine statistically significant differences at $\alpha = 0.05$.

Results

The results of the direct contact test for freshly mixed, 1 and 7-day old root canal sealers are shown in Figure 1. The positive controls exhibited fungal growth in all test periods; however, fungi was not recovered from the negative controls. Carry-over of the antifungal effect from the root canal sealers to the fungal cultures was not observed.

There was a significant difference among the fungal counts of the freshly mixed sealers ($p < 0.001$). Freshly mixed AH Plus presented the highest antifungal activity and showed complete inhibition of fungal growth, which was significantly different from the other sealers ($p < 0.05$). Freshly mixed iRoot SP and MTA Fillapex produced significant ($p < 0.05$) reduction in the fungal growth, with no statistically significant difference between them ($p > 0.05$) and with statistically significant difference from the freshly mixed GuttaFlow ($p < 0.05$). Further, no significant difference was found between GuttaFlow and positive control at all time intervals in fungal growth ($p > 0.05$). With samples set for 1 or 7-days, only slight or no antifungal efficacy was observed and there were no significant differences among the sealers tested and positive controls ($p > 0.05$).

Discussion

Residual micro-organisms resisting chemo-mechanical procedures may cause treatment failure in endodontic therapy. It has been reported that the presence of *C. albicans* in root canals may be associated with therapy-resistant periapical pathosis [3,7]. The use of a root canal sealer having good antimicrobial activity is essential for long-term success of endodontic therapy. The present study evaluated antifungal activity of several root canal sealers, including a poly-dimethylsiloxane-based sealer (GuttaFlow) and two calcium silicate-based sealers (iRoot SP and MTA Fillapex) against *C.albicans* and compare them to that of a resin-based sealer (AH Plus Jet). The outcomes of the present study showed that the freshly mixed epoxy resin-based sealer (AH Plus Jet) exerted a strong antifungal effect and freshly mixed calcium silicate-based sealers (iRoot SP and MTA Fillapex) exerted moderate antifungal effects on *C. albicans*, whereas the silicone-based sealer (GuttaFlow) did not show any antifungal effect. Furthermore, the results of the present study revealed that root canal sealers used in the present study lost their antimicrobial activity depending on time.

The most commonly used assays to evaluate antimicrobial activity of sealers and dental materials are the agar diffusion test and the direct contact test. The agar diffusion test is a relatively insensitive and semi-quantitative method; results highly depend on the solubility and diffusibility of test agent in the agar [30]. It is difficult to determine the true antimicrobial activity of water-insoluble materials using the agar diffusion test, because a less soluble and diffusible material could result in a smaller size of inhibition zone [20]. On the other hand, the direct contact test is a quantitative and reproducible assay which relies on direct contact of the test micro-organisms with the test material for a controlled period of time and independent of the diffusion and solubility properties of the material tested and media [29]. In addition, this method allows measurement of the exact number of surviving bacteria [21]. Therefore, in the present study, a direct contact test was used to evaluate the antifungal activity of the sealers.

Carry-over of the antimicrobial effect from the test materials to the culture media may inhibit the growth of micro-organisms surviving in spite of direct contact and lead to a false negative result [21]. In the present study, carry-over control experiments showed that there was no carry-over of antifungal effect from the sealer to the fungal culture.

AH Plus is an epoxy resin-based sealer. A lot of previous studies evaluated its antimicrobial activity and reported that AH Plus had very potent antifungal and antibacterial activity [24,30,31]. Miyagak et al. [24] showed that AH Plus had antifungal activity against *C.albicans*. Pizzo et al. [30] reported that freshly mixed AH Plus showed complete inhibition of microbial growth, whereas 1 and 7-days old material did not show any antimicrobial effect, corroborating the present findings. The antimicrobial activity of AH

Plus could be associated with bisphenol A diglycidyl ether, which was previously identified as a mutagenic component of the resin-based sealer [32].

The results of the present study showed that the silicone-based sealer, GuttaFlow, was ineffective against *C.albicans* and was not different from the positive control. This confirmed previous findings concerning the absence from an antimicrobial effect of GuttaFlow [20,25]. Willershausen et al. [33] also reported that the AH Plus Jet exerted a strong antimicrobial effect, but GuttaFlow had no antimicrobial effect. Nawal et al. [20] stated that the nanosilver component of GuttaFlow does not make a contribution to the antimicrobial efficacy.

The two calcium silicate-based sealers (iRoot SP and MTA Fillapex) were equally effective in inhibiting fungal growth, but they were less effective than AH Plus Jet, when freshly mixed. The present data also showed that the set sealers did not show antifungal activity. To date there is no evidence of antifungal activity of calcium silicate-based sealers. Zhang et al. [21] reported that fresh iRoot SP and AH Plus effectively eliminated *E. faecalis*. According to their report, antimicrobial activity of iRoot SP might derive from high pH of the sealer depending on calcium hydroxide releasing during the polymerization reaction. However, several studies addressed that Candida spp. were not susceptible to calcium hydroxide and high level of pH [12,13] and this could explain the difference. In a recent study, Morgental et al. [19] concluded that MTA Fillapex had an antimicrobial effect against *E. faecalis*, whereas it did not extend this effect to 7 days after mixing, in agreement with the present study.

Endodontic sealers with strong antimicrobial properties have been found to be cytotoxic and even mutagenic [34]. Cytotoxicity of AH Plus has been previously well documented [35]. Schwarze et al. [36] stated that the cytotoxic effect of AH Plus was observed immediately after mixing, which disappeared after 24 h of setting. Willershausen et al. [33] reported that the AH Plus Jet led to a significant decrease of cell proliferation and was significantly more toxic than GuttaFlow. Further studies also support these findings [37]. iRoot SP was found to be significantly less cytotoxic than AH Plus when tested as a fresh sample and it was not cytotoxic in a set situation after 24 h [28]. A recent report concluded that MTA Fillapex was a biocompatible material [38].

In conclusion, the sealers showed different antifungal effects depending on the types and time interval between mixing and testing; therefore, the two tested hypotheses were rejected. Freshly mixed AH Plus Jet completely inhibited fungal growth. GuttaFlow did not reveal any antifungal activity. Freshly mixed iRoot SP and MTA Fillapex were equally effective in reducing the number of viable *C.albicans*. None of the sealer maintained the antifungal activity 1 and 7 days after mixing.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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