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In vitro activity of zinc oxide-eugenol and glass ionomer cements on *Candida albicans*

Atividade in vitro *dos cimentos de óxido de zinco e eugenol e ionômero de vidro sobre* Candida albicans

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ABSTRACT: The aim of this study was to evaluate *in vitro* the antimicrobial activity of glass ionomer (GIC) and zinc oxide-eugenol (ZOE) cements against *Candida albicans*. Standardized GIC and ZOE specimens were maintained in contact with *C. albicans* suspension (1×10^6 cells/ml) at 37°C for 24 h, 48 h or 7 days. A control group without any testing cement was included. After the incubation period, aliquots of 0.1 ml were plated on Sabouraud's agar, and then the number of colonies was counted. The results were expressed as values of logarithms of colony-forming units per milliliter (log CFU/mL) and were analyzed statistically by Kruskal-Wallis ANOVA. After 48 h of incubation, the ZOE group presented no growth of *C. albicans*. GIC and control groups presented similar mean values at all tested periods. According to the results obtained, it could be concluded that, under the experimental conditions, ZOE cement was more effective *in vitro* against *C. albicans* than GIC.

DESCRIPTORS: *Candida*; Candidiasis, oral; Glass ionomer cements; Zinc oxide-eugenol cement; Antimicrobial activity.

RESUMO: O objetivo deste estudo foi avaliar *in vitro* a atividade antimicrobiana dos cimentos de ionômero de vidro (CIV) e óxido de zinco e eugenol (OZE) sobre *Candida albicans*. Corpos-de-prova padronizados de CIV e OZE foram mantidos em contato com suspensão (1×10^6 células/ml) de *C. albicans* a 37°C por 24 horas, 48 horas ou 7 dias. Um grupo controle sem nenhum cimento teste foi incluído. Após o período de incubação, alíquotas de 0,1 ml foram semeadas em ágar Sabouraud e o número de colônias foi contado. Os resultados foram expressos em logaritmos de valores de unidades formadoras de colônias por ml (log UFC/mL) e analisados estatisticamente pelo teste ANOVA Kruskal-Wallis. Após 48 horas de incubação, o grupo OZE não apresentou crescimento de *C. albicans*. Os grupos CIV e controle apresentaram médias similares em todos os períodos testados. De acordo com os resultados obtidos, pode ser concluído que, sob as condições experimentais testadas, o cimento OZE apresentou-se mais efetivo *in vitro* sobre *C. albicans* em relação ao CIV.

DESCRITORES: *Candida*; Candidíase bucal; Cimentos de ionômeros de vidro; Cimento de óxido de zinco e eugenol; Atividade antimicrobiana.

INTRODUCTION

Candida genus yeasts are usually isolated from the human oral cavity[21] and their prevalence among healthy individuals varies from 35-38% to 40-60%[3,12]. *C. albicans* may be isolated from the mouth of 5-7% of newborns few hours after birth, and from 14.2% of them one week later[17]. *C. albicans* is the most prevalent species in the mouth (60 to 70% of the isolates), followed by *C. tropicalis* and *C. glabrata*[21].

Under specific situations, *Candida* spp. may cause oral and systemic pathologies[6]. The transformation from the saprophytic to the parasitic form is related to microbial, environmental and individual factors[18]. The pathogenicity of *Candida*

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species is related to tissue invasion, enzyme production and to the adherence to the oral cavity's epithelium[2].

Systemic and local predisposing factors to candidosis, such as immunodepression, xerostomia, hormonal alterations, use of orthodontic devices or total dentures have been reported in the related literature[10,21,22]. Among immunocompromised patients, especially in AIDS cases, candidosis is related to increased rates of mortality and morbidity[22].

Oral environmental stabilization procedures are commonly employed in Dentistry. The aim of these procedures is the elimination of pathogenic microorganisms, preventing the progression of oral diseases and creating conditions for the improvement of oral health. Glass ionomer and zinc oxide-eugenol cements are the most frequently employed cements for this purpose[7,16].

The glass ionomer cement has a fluoride-releasing property that is desirable in the carious process control. Also, it presents good chemical adherence to the dental structure[19]. The zinc oxide-eugenol cement is also frequently used for provisional restorations due to its low cost and easy manipulation.

The studies of the antimicrobial activity of these materials have been generally performed against cariogenic microorganisms, in particular *Streptococcus mutans* and lactobacilli, and dental biofilm formation[1,7,8]. Chandler, Heling[4] (1995) reported that a zinc oxide-eugenol cement showed effective antimicrobial activity against *Enterococcus faecalis* after 24 hours.

Previous studies concerning the effect of endodontic sealers on *Candida* spp. growth showed contradictory results. Kaplan *et al.*[11] (1999) reported that fluoride-releasing materials did not show any activity against these yeasts. On the other hand, Palenik *et al.*[14] (1992) showed the antimicrobial effect of fluoride-releasing materials. A previous study[20] reported the relative effectiveness of an eugenol-containing endodontic sealer against *Candida*.

The information about the effect of dental materials on *Candida* spp. yeasts seems to be important for candidosis prevention purposes. Jacob *et al.*[9] (1998), studying candidosis among HIV-infected patients, observed that the elimination of carious dentine promotes the reduction of *Candida* spp. colonization sites, reducing the risk of infections caused by these microorganisms.

In our previous study[16], oral environmental stabilization procedures employing a zinc oxide-eugenol cement were more effective to reduce the levels of yeasts in saliva than those employing a glass ionomer cement. Based on these results and on the lack of conclusive studies, the aim of the present research was to evaluate *in vitro* the antimicrobial activity of glass ionomer and zinc oxide-eugenol cements on *C. albicans*.

MATERIAL AND METHODS

Candida albicans ATCC 18804 was plated on Sabouraud's dextrose agar (Difco, Detroit, USA) and incubated for 24 hours at 37°C. After this period, a suspension containing 1.0×10^6 viable cells per milliliter was prepared in sterile saline solution (0.85% NaCl) (Synth, São Paulo, Brazil), with the aid of a Neubauer's chamber (Inlab, São Paulo, Brazil), according to the exclusion method with 0.05% trypan blue (Merck, Darmstadt, Germany).

Standardized specimens (diameter = 0.5 mm and height = 0.3 mm) were obtained with the aid of a sterile aluminum matrix (Promontec Metais, São José dos Campos, Brazil) (Figure 1). Glass ionomer cement (GIC) (Vidrion, SS White Artigos Dentários Ltda., Rio de Janeiro, Brazil) and zinc oxide-eugenol cement (ZOE) (SS White Artigos Dentários Ltda., Rio de Janeiro, Brazil) specimens were prepared according to the manufacturers' instructions.

Immediately after the curing process that was standardized in 1 hour for ZOE and 5 minutes for GIC, according to the manufacturers' instructions, specimens were transferred to tubes (Corning, New York, USA) containing 3 mL of Sabouraud's dextrose broth (Difco, Detroit, USA). Then, 0.1 ml of

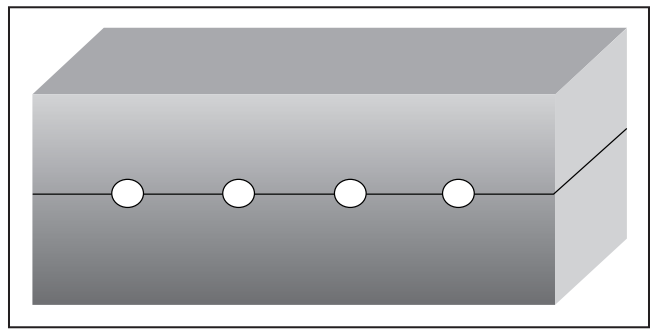

FIGURE 1 - Representative illustration of the aluminum matrix employed for obtaining standardized specimens (diameter = 0.5 mm and height = 0.3 mm).

the standardized *C. albicans* suspension was inoculated in each tube. Tubes were incubated at 37°C for 24 h, 48 h or 7 days according to the experimental group. Nine experimental groups were included in this study, as shown in Table 1. Three groups were composed of zinc oxide-eugenol (ZOE) specimens incubated for different periods of time. GIC groups included glass ionomer specimens incubated for 24 h, 48 h and 7 days. Control groups were incubated for different periods of time without any specimen of the tested materials.

After the incubation period, serial decimal dilutions (10^{-1} to 10^{-5}) were obtained from each initial suspension in sterile saline solution (0.85% NaCl). Then, aliquots of 0.1 ml were plated in duplicate on Sabouraud's dextrose agar (Difco, Detroit, USA) and incubated for 37°C for 48 h. After this period, the number of colony-forming units (expressed in values of logarithms of colony-forming units) per milliliter (log CFU/mL) was obtained. Values of mean, standard deviation and median of log CFU/mL were calculated for each experimental group.

Statistical analysis

The results obtained after 24 h of incubation were analyzed statistically by Kruskal-Wallis ANOVA and Dunn's test (5%). Considering that the counts obtained for zinc oxide-eugenol after 48 h and 72 h of incubation were zero, the results of the glass ionomer cement and control groups at these periods were analyzed by two-way ANOVA and Tukey's test (5%).

TABLE 1 - Experimental conditions included in the study.

Groups	n	Material tested	Period of incubation
ZOE 24 h	15	Zinc oxide-eugenol	24 h
ZOE 48 h	15	Zinc oxide-eugenol	48 h
ZOE 7 days	15	Zinc oxide-eugenol	7 days
GIC 24 h	15	Glass ionomer cement	24 h
GIC 48 h	15	Glass ionomer cement	48 h
GIC 7 days	15	Glass ionomer cement	7 days
C 24 h	15	-	24 h
C 48 h	15	-	48 h
C 7 days	15	-	7 days

ZOE – zinc oxide-eugenol cement; GIC – glass ionomer cement; C – control.

RESULTS

The values of mean, standard deviation and median obtained for the groups at each period are shown in Table 2.

Counts of *Candida albicans* after 24 h were lower and statistically different (p = 0.000) in the ZOE group in relation to GIC and control groups. No differences were observed between GIC and control groups at any experimental period.

After 48 h of incubation, the ZOE group presented no growth of *C. albicans*. GIC and control groups presented similar mean values (p > 0.05). The same results were observed after 7 days of incubation.

DISCUSSION

Preventive antifungal therapy has been adopted to avoid the occurrence of systemic candidosis. However, this practice may contribute to the appearance of resistant isolates. In fact, some authors[13,23] correlate the widespread use of azolic drugs in the prevention of systemic mycoses in patients with low immunity (i.e., transplant recipients or HIV-infected patients) with the selection of resistant isolates. Therefore, other concomitant preventive measures could be of great importance for patients under risk of systemic candidosis development.

TABLE 2 - Results of mean ± standard deviation and median of logarithms of colony-forming units per millilitre (log CFU/mL) of *Candida albicans* under the experimental conditions and after 24 h (ZOE 24, GIC 24 and C 24), 48 h (ZOE 48, GIC 48 and C 48) and 7 days (ZOE 7d, GIC 7d and C 7d) of incubation.

Groups	Mean ± SD	Median value
ZOE 24	3.62 ± 1.17*	3.73
GIC 24	7.15 ± 0.49	6.88
C 24	6.75 ± 0.27	6.69
ZOE 48	0	0
GIC 48	6.52 ± 0.57	6.64
C 48	6.77 ± 0.24	6.76
ZOE 7d	0	0
GIC 7d	5.61 ± 0.12	5.61
C 7d	5.67 ± 0.12	5.71

*Statistically different from GIC and control groups (p = 0.000); SD – standard deviation; ZOE – zinc oxide-eugenol; GIC – glass ionomer cement; C – control.

Oral *Candida* spp. level control might be an important preventive measure since the occurrence of oral candidosis may be considered a potential risk for the occurrence of systemic diseases among immunocompromised patients. However, few studies of this subject have been reported in the related literature.

Oral stabilization procedures may contribute to *Candida* spp. level control. A previous study[9] showed that the elimination of dentine affected by carious process reduced the colonization sites of *Candida* spp., reducing the risk of infection by these yeasts. Considering that zinc oxide-eugenol and glass ionomer cements are the most frequently employed materials in oral stabilization procedures, they were included in this study.

The findings of the present study showed that the zinc oxide-eugenol cement was more effective in reducing *Candida* spp. colony counts than the glass ionomer cement. These results may be related to the high antimicrobial activity demonstrated by eugenol-containing materials. Previous literature reports on eugenol-containing materials showed their significant antimicrobial effect on *Candida* spp. Siqueira Júnior *et al.*[20] (2000), testing the antimicrobial activity of root canal sealers, showed that an eugenol-containing sealer presented relative effectiveness against *Candida* spp. However, this activity was observed only after a long interval of time (40 days). Also, Kaplan *et al.*[11] (1999), evaluating the antimicrobial effect of endodontic sealers, showed that eugenol was effective against *Candida* spp.

The lack of activity of the glass ionomer cement against *Candida* spp. may be related to the fluoride concentration released by this material. These results are similar to those showed by Kaplan *et al.*[11] (1999). These authors, analysing fluoride-releasing endodontic sealers did not observe any inhibitory effect on *Candida* growth. These authors attributed this lack of activity to the low fluoride concentration in the studied materials.

Some methods have been suggested for testing the antimicrobial effect of dental materials. The most frequently employed methods are those based on direct contact test (DCT)[1,6,15]. However, no standardization among methods is found in the literature. The agar diffusion test was also performed in previous studies[5,19], although the differences between the diffusion velocities that are characteristic for each material seem to be a limitation for comparative purposes.

The methodology applied in this research was based on DCT[1,6,15] and focused on the standardization of the experimental conditions, in particular in relation to the specimens' dimensions and *C. albicans* suspension.

Our previous clinical study[16] showed that oral environmental stabilization procedures using zinc oxide-eugenol cement were effective to reduce the levels of *Candida* spp. in saliva. The present *in vitro* results corroborate these findings suggesting that the procedures employing zinc oxide-eugenol may be indicated for oral candidosis prevention purposes.

CONCLUSIONS

At all periods of time evaluated (24 h, 48 h and 7 days), the zinc oxide-eugenol cement showed effective antimicrobial activity on *Candida albicans*. The glass ionomer cement did not show any effect on the growth of *Candida albicans*.

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