SURFACE MICROHARDNESS OF DIFFERENT RESTORATIVE MATERIALS EXPOSED TO CANDIDA ALBICANS BIOFILM ISOLATED FROM THE ORAL CAVITY OF HIV-INFECTED CHILDREN

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Palavras- Chave: Candida Albicans. Biofilme. HIV.

RESUMO

Introdução: A *Candida albicans* é um dos microorganismos que mais frequentemente colonizam a cavidade bucal de crianças HIV+. Este fungo excreta ácidos, proporcionando uma diminuição do pH em um ambiente já altamente acidificado, como cavidade bucal dessas crianças devido sua dieta hipercalórica, uso de medicamentos açucarados e higiene oral deficiente. Considerando a elevada freqüência de restaurações dentárias em função da alta prevalência de cárie, todos esses fatores, incluindo o metabolismo da *C. albicans,* podem provocar alterações na superfície de materiais restauradores usados nesses pacientes. **Objetivo**: O objetivo do estudo foi avaliar, in vitro, a ação da *C. albicans,* isolada de uma criança HIV+, sobre a superfície de materiais restauradores utlizados na prática odontopediátrica. **Material e método**: Confeccionou-se 44 blocos de diferentes materiais (2 resinas, 1 compômero e 1 cimento ionomérico de Vidro) separados em 4 grupos (n=11) Todos os blocos foram submetidos a microdureza superfícial inicial (MDI). Posteriormente, foram expostos ao biofilme de *C. albicans* formado a partir de 1mL de uma suspensão padronizada contendo 10^5 cels/mL, durante 07 dias. Após, os blocos foram limpos e mantidos sob refrigeração (4ºC) e submetidos à mensuração da microdureza final (MDF). Foram utilizados o Teste de Mann-Whitney para comparação intra grupo entre os valores de MDI e MDF; os valores de perda percentual de microdureza (%PMD) foram comparados com o Teste de Kruskall-Wallis (95% IC). **Resultados**: Os valores de MDI variaram de 63,54±11,41 a 77,92±10,91, sem diferença entre os grupos (p=0,076). Após exposição ao biofilme, não foram observadas variações significativas na microdureza (MDI X MDF) exceto para o grupo 3 (compômero Vitremer™), cujo valor de MDF foi 40,45±7,57 (p=0,001) . O %PMD do compômero (grupo 3) foi significativamente maior (41,16%) que o dos outros materiais (5,35% grupo 1; 7,02% grupo 2; e 9,57% grupo 3) (p=0,036. **Conclusão:** Conclui-se que a *C. albicans* isolada do biofilme dental de criança HIV+ pode causar, in vitro, diminuição significante na microdureza superficial do compômero em comparação aos demais.

Keywords: Candida Albicans. Biofilm. HIV.

ABSTRACT

Introduction: *Candida albicans* is one of the microrganisms that most often colonizes the oral cavity of HIV-infected children. This fungus secretes organic acids, which decrease the pH of the oral cavity; an environment that is already particularly acidic in HIV-Infected children because of their hypercaloric diets, use of sugary medicines, and poor oral hygiene. Considering the large number of dental restorations and the high prevalence of caries in this population, these conditions, including the metabolism of *C. albicans*, can potentially cause problems in terms of the surface of restorative materials. **Objective**: Therefore, the aim of this study was to evaluate, *in vitro*, the potential of *C. albicans* isolated from the dental biofilm of HIV-infected children to cause surface demineralization of the restorative materials used in pediatric dentistry. **Material and method**: Forty-four blocks of four different materials (2 resins, 1 compomer, and 1 glass ionomer cement) were made and separated into four groups (n = 11). All blocks were submitted to initial surface microhardness (ISM) analysis. Subsequently, each block was exposed to *C. albicans* biofilm, formed from a 1 mL standard suspension containing 10^5 yeasts/mL, over seven days. The blocks were then cleaned and kept at 4 °C until being submitted for measurement of the final surface microhardness (FSM). The Mann-Whitney test was used for intragroup comparisons between ISM and FSM values. **Results**: The percentage of microhardness loss (% MHL) values between the four groups were compared using the Kruskall-Wallis test (95% CI). The ISM values ranged from 63.54 ± 11.41 to 77.92 ± 10.91, with no statistical differences being found (p = 0.76). After exposure to biofilm, no significant changes in surface microhardness were observed when comparing the values of ISM and FSM, except for group 3 (compomer Vitremer™), which had an FSM value of 40.45 ± 7.57 (p = 0.001). The % MHL of the compomer (group 3) was significantly higher (41.16%) than the other groups (5.35% group 1; 7.02% group 2; and 9.57% group 3) (p = 0.036). **Conclusion**: It can be concluded that, *in vitro*, *C. albicans* isolated from the dental biofilms of HIV-infected children can cause significant reduction in the surface microhardness of compomer compared with other materials.

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INTRODUCTION

Oral candidiasis (OC) is the most common oral lesion observed in HIV-infected children and may be the first clinically visible manifestation of the disease. It features three distinct clinical variations, each recognized as being associated with HIV infection: erythematous, pseudomembranous, and angular cheilitis.[1] The prevalence of OC varies from 6 to 45%, and has recently decreased because of the use of multiple anti-retrovirals in the treatment of HIV infection, such as highly active antiretroviral treatment (HAART).[2,3] The oral cavity colonization by *Candida albicans*, the main etiologic agent of OC, in Brazilian HIV-infected children treated with HAART is still very high, despite reports of a low prevalence (6.7%) of OC in these children.[4]

C. albicans excretes various organic acids, some of which are stronger than lactic acid, thus causing a decrease in the pH of the oral cavity,[5] an environment that is already particularly acidic in HIV-infected children because of their hypercaloric diets, use of sugary medicines, and poor oral hygiene.[6] Also, this fungus is a frequent constituent of dental biofilms in HIV patients, and the authors speculate that it is a supporting factor in the etiology and development of caries in such children.[7] Various studies have reported the ability of *C. albicas* biofilm to cause erosion and abrasion to dental surfaces, as a result of the loss of microhardness;[8,9] therefore, it is likely that restorative materials also suffer loss of microhardness because of the action of these microorganisms. According to Belduz et al. (2017),[10] dental restorative materials are a potential source of fungal infections and in general, *C. albicans* biofilms adhere firmly to composite and glass ionomer cements. However, fewer studies have been carried out on these materials and any little research into the effect of *Candida* biofilms on dental material surfaces is present in the literature.

Considering the high prevalence of *C. albicans* in HIV-infected children, its ability to produce a cariogenic environment associated with a high rate of caries, leading to the extensive use of restorative procedures in this population, the objective of this study was to evaluate changes to the surface microhardness of restorative materials after exposure to *Candida albicans* biofilm isolated from an HIV-infected patient.

MATERIALS AND METHODS

This *in vitro* study evaluated the potential of *C. albicans* biofilm, isolated from an HIV-infected patient, to demineralize the surface of restorative materials commonly used in pediatric dentistry. The present study was characterized as a descriptive, analytical, and laboratorial. Four restorative materials were used; two composites, one compomer, and one glass ionomer cement:

- Group 1 (composite): resin, low voltage nanofilled material, Filtek Z350 XT™, color A2D (3M Company, Minnesota, USA).
- Group 2 (composite): resin, low voltage, FiltekBulkFill™, color A2 (3M Company, Minnesota, USA).
- Group 3 (compomer): glass Ionomer resin, Vitremer™, color A3 (3M Company, Minnesota, USA).
- Group 4 (glass Ionomer cement): conventional glass ionomer, Ketac Molar Easymix® ™, color A3 (3M Company, Minnesota, USA).

Sample Preparation and Determination of Initial Surface Microhardness

A single operator made 60 blocks (15 blocks of each material) using a circular 3 x 5 mm radio device (Tabela 1). The blocks of restorative materials were prepared according to the manufacturers' recommendations. For the Z350™ and BulkFill™ composites, and the compomer Vitemer™, the same light was used for curing (DEMI, Kerr® number 910770). Later, the blocks were secured in a polypropylene device with sticky wax (Kota™ Indústria Com. Ltda., São Paulo, SP) and adapted in a metallographic polishing machine with 1200 grit sandpaper (Extec™, Connecticuti, USA), under refrigerated conditions, resulting in a glassy surface that allowed the measurement of the initial surface microhardness (ISM). At the end of the polishing stage, the specimens were immersed for in deionized water 10 minutes under the action of ultrasound (Ultrasonic Cleaner Mod USC 750™, Unique Ind. Com. Ltda Electronics, São Paulo, SP) to remove the grains produced by the polishing process.

Next, 44 blocks were chosen for further experiments and divided into 4 groups of 11 blocks each. The blocks were submitted for initial surface microhardness (ISM) analysis. Three indentations, positioned 100 µm from each other, were made at the center of the block using a diamond-tipped microdurometer Vickers under a static load of 50 g, applied for 15 seconds.[11] After this phase, the blocks were set in 24-well cell culture plates and subjected to sterilization using UV light.[12]

Exposure to *Candida albicans* Biofilm

One isolate of *C. albicans* from the dental biofilm of an HIV-infected child was randomly selected from the collection of isolates from the Paulo de Góes Microbiology Institute of the Universidade Federal do Rio de Janeiro (UFRJ), previously identified and stocked in Sabouraud medium at 4°C. Each well contained the restorative material blocks previously sterilized and 1 mL of inoculum standard cell suspension containing 10^5 yeasts/mL of *C. albicans*.[9] Prior to this, growth of the clinical isolates was induced in brain heart

infusion (BHI) liquid (BD Difco™®, Maryland, USA), while being mixed, for 48 hours at 37 °C, with the standardized cells and in BHI medium (BD Difco™®, Maryland, USA) supplemented with 20% sucrose. After the development of the biofilm, the blocks, which were already laid down in the wells, were kept for 7 days at 37 °C without agitation. During the seven days of the experiment, the medium was replaced every 48 hours, after the full seven days, the blocks were immediately cleaned with cotton and 10% formaldehyde and kept at approximately 4 °C until further evaluation.

Determination of Final Surface Microhardness

For each block of restorative material, the— same researcher who conducted the ISM readings also did the final FSM readings. Three spaced indentations were held 100 µm from the baseline.[13] The percentage of surface microhardness loss (% MHL) for all samples was calculated using the following equation:

% MHL = ISM - FSM / ISM x 100

Statistical Analyses

A database was created and data were analyzed using the statistical program SPSS, version 20.0. The Mann-Whitney test was used for comparing ISM and FSM values in each group (intragroup). The Kruskall-Wallis test was used to measure correlations between the groups by comparing % MHL. Data were considered statistically significant if analysis resulted in $p < 0.05$ at 95% CI.

RESULTS

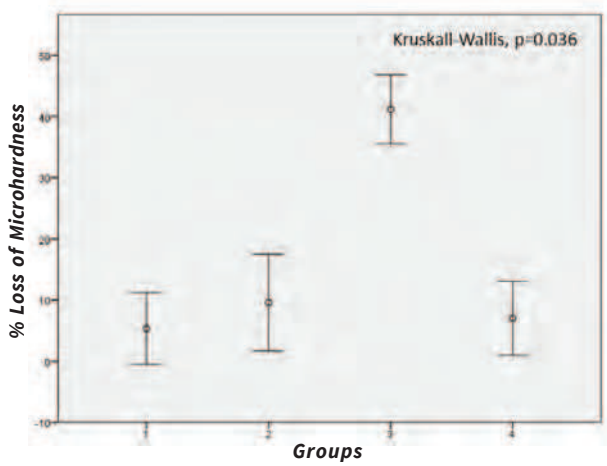
The ISM and FSM values in Table 1 show that the ISM values were similar between groups ($p = 0.76$). After exposure to the *C. albicans* biofilm, no significant intragroup variations were observed when comparing the ISM values with the FSM values, except for the compomer (Group 3, $p = 0.00$).

Considering only the samples that exhibited mircohardness loss after exposure to *C. albicans* biofilm, there was significantly greater mircohardness loss (41.16%) of the compomer Vitremer™ (Group 3, $p = 0.036$). The other materials also presented with mircohardness loss after exposure to the C. *albicans* biofilm. The % MHL values found for Groups 1 (Z250™), 2 (BulckFill™), and 4 (KetacMolar™) were 5.35%, 9.57%, and 7.02%, respectively (Figure 1).

***Table 1**: Average of microhardness of different restorative materials before exposure (ISM) and after (FSM) exposure to the biofilm of* Candida albicans *isolated from HIV +.*

Groups (N =11)	ISM(mean ± SD)	FSM(mean ± SD)	P value Mann-Whitney
Z350™	70.29 ± 17.42	70.98 ± 13.71	NS
BulkFill™	63.54 ± 11.41	60.16 ± 9.42	NS
Vitremer™	68.83 ± 9.33 *	40.45 ± 7.57 *	**0.01**
Ketac Molar™	77.92 ± 10.84	79.43 ± 18.36	NS
P valor(Kruskall Wallis)	0.76	**0.026**	—

Note: NS = not significant; ISM = initial surface microhardness; FSM = final surface microhardness.



Note: Groups: 1= Z250™; 2= Bulk Fill™; 3 Vitremer™; 4= Ketac Molar™

***Figure 1**: Surface hardness loss ratio (average percentage) between the different types of restorative materials after exposure to biofilm of Candida albicans isolated from HIV+.*

DISCUSSION

The literature shows that *C. albicans* is the most common species found in the oral cavity of HIV-infected patients with a prevalence of up to 65%, whereas other species of the genus *Candida* account for less than 35% of total isolates.[4,14] Considering the role of *C. albicans* in dental caries, Oliveira et al. (2016)[7] observed a significant positive correlation between the number of early caries lesions in enamel and the number of *C. albicans* colonies in the dental biofilm of HIV-infected children. This suggests that these fungi are not only associated with the development of oral candidiasis, but also with caries disease in HIV-infected children.

Some *in vitro* studies have verified that *C. albicans* has a high cariogenic potential that gives it the ability to

dissolve hydroxyapatite and challenges the enamel surface.[8,9] Studies in mice, conducted by Klinke et al. (2011)[15] showed that *C. albicans* is able to increase the incidence of caries when added to a mixed microbiota. Considering these results, we considered it important to investigate the action of the biofilm formed by isolates of *C. albicans* from the dental biofilm of HIV-infected children on the surface of the restorative materials most commonly used in patient procedures. Since the prevalence of caries and restorations required in HIV positive patients are high,[6] the number of dental restorations that can undergo this microorganism's destructive action is significant in these patients, which is the reason for this research.

Four different materials frequently used in pediatric dentistry were selected to observe challenges in microhardness after exposure to *C. albicans* biofilm. The majority of the studies in the literature regarding *Candida* and restorative materials focus on its ability to form biofilms[10] and/or antifungal activity,[16] making this study the first to evaluate the capacity of *C. albicans* isolated from oral cavity of an HIV infected children to cause alterations in the surface of restorative materials which are most used in children.

Our results showed that *C. albicans* is able to cause microhardness loss in all the restorative materials tested in this study. However, this loss was only significant for the compomer, with the resins and the glass ionomer cement (GIC) being the best at withstanding the effects of the fungi biofilm. The resins are load restorative dental materials, which gives them greater resistance,[17] and can probably explain the low variation in microhardness loss after the fungal challenge.

With regard to the GIC, it is important to note that this material undergoes syneresis within the first 24 h after preparation. Therefore, immediately after manipulation, its surface must be protected with liquid vaseline to ensure the physical properties of the material.[18] Also, although conventional GIC has been reported to have some negative features (low wear resistance, susceptibility to breakage, structure sensitivity to moisture contamination during hardening, e.t.c),[19] its performance in our study was very good, showing reduced values of microhardness loss. Unlike the resins, the GICs are not loaded materials but they do have antimicrobial properties, which may have been an advantage during the experiment. Cosgun et al. (2019)[20] observed in a recent study, using a different glass ionomer, that when cultured with $1x10^2$ cfu/mL of micoorganisms all the restorative materials inhibited bacterial and fungal growth. It is worth noting that the compomer material has neither the same resistance of a resin nor the antimicrobial activity of conventional GICs, which may have contributed to the results found in our study, showing lower values of final superficial microhardness after *Candida* biofilm exposure. Although one study carried out by Franciscone et al. (2008)[21] observed a decrease in the surface challenges of different restorative materials after being subjected to an erosive challenge, it is interesting to note that the same restorative compomer (resinous glass ionomer Vitremer™) had the worst performance compared to the other materials (Resin Z350™, Ketac Molar™, and Resin BulkFill™). These results corroborate with the Bonifácio et al. (2009)[22] observation that surface hardness is known to negatively correlate with wear on the surface of restorative materials, with lower hardness leading to higher wear.

Regarding the limitations of this study, it was an *in vitro* study, conducted with only one isolate of *C. albicans,* with a limited number of materials, and observing only one parameter, the microhardness. Therefore, their results should be evaluated with caution because they may not fully represent true conditions. Nevertheless, they are interesting results for some observations of clinical applicability regarding the restorative treatment of children infected with HIV.

As we have shown, restorative materials of the resin and GIC types can be susceptible to the action of *Candida* spp, which may increase their chance of wear and failure, although this was mainly observed with the compomer. We may also consider that because GICs potentially reduce microleakage by attaching to the tooth structure, they also inhibit the growth of oral microorganisms that result from caries and neutralize the acids produced by these microorganism through ion release.[23,24] Therefore, the use of this material could be the best recommendation for HIV-infected children because of its beneficial antimicrobial effects in cases where protection against caries is necessary.

We conclude and point out the importance of controlling the colonization of *C. albicans* in the oral cavity of HIV-infected children, not only for the prevention of oral candidiasis, but also to control the development of caries disease and reduce the need for invasive restorative procedures To achieve this, it is necessary for patients to maintain their oral hygiene, thereby minimizing the potential for colonization of these microorganisms in the oral cavity.

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