

Original Article

Evaluation of *Candida Albicans* Biofilm Formation on Various Dental Restorative Material Surfaces

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ABSTRACT

Aims: *Candida* adhesion to any oral substrata is the first and essential stage in forming a pathogenic fungal biofilm. In general, yeast cells have remarkable potential to adhere to host surfaces, such as teeth or mucosa, and to artificial, nonbiological surfaces, such as restorative dental materials. This study compared the susceptibility of six dental restorative materials to *Candida albicans* adhesion.

Materials and methods: Cylindrical samples of each material were made according to the manufacturers' instructions. The antifungal effect of the samples on *C. albicans* was determined with the disc-diffusion method. The samples were put in plates with sterile Mueller Hinton and Sabouraud dextrose agar previously seeded with *C. albicans*. After the incubation period, the inhibition zone around each sample was evaluated. To evaluate the biofilm formation, the XTT technique and scanning electron microscopy (SEM) were used. **Results:** No inhibition zone was observed around the samples. According to the XTT assays, the amalgam samples revealed the lowest quantity of biofilm formation ($P > 0.001$). The highest median XTT values, significantly higher than the other materials ($P < 0.001$), were found for the composite and the compomer samples. Within the SEM examination, the amount of candidal growth was significantly lower on the resin-modified glass ionomer and glass-ionomer cement samples. The compomer and the composite samples showed more candidal adhesion. **Conclusion:** This finding emphasizes the use of glass ionomer restorative cements and amalgam to reduce *C. albicans* adhesion to dental restorative materials especially in people with weakened immune systems, neutropenia, and cancer.

KEYWORDS: Adherence, biofilm, biofilm vitality, *Candida albicans*

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INTRODUCTION

Candida albicans is a commensal fungus normally found in the oral cavity of 20-40% of healthy individuals^[1] and the major pathogen in oral and systemic candidosis.^[2] *Candida* infections have increased in recent years. This is probably because of the increasing number of seriously ill patients, immunosuppressive therapies, as well as the increased use of antibiotics and more invasive therapeutic medical procedures.^[3]

Oral environmental stabilization procedures are commonly employed in dentistry. The aims of these procedures are elimination of pathogenic microorganisms, prevention of the progression of oral diseases, and creation of conditions for the improvement of oral health. However, with the increase in *C. albicans*

infections, resistance against commonly used antifungal agents has been observed.^[4,5] Targeted chemoprophylaxis with effective antifungal agents is the most effective prevention strategy against candidiasis.^[5] However, because of the development of antifungal resistance, its indications should be considered carefully. Alternative strategies would be advantageous in particular for long-term prevention of candidiasis.

Since adhesion is an essential prerequisite in colonization and infection, the role of adhesion in the pathogenesis of

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several diseases by *C. albicans* is widely acknowledged. Several studies have suggested that the initial stage of various microbial diseases involves microorganisms adhering to the target tissue.^[6,7] Thus, interest in using dental materials that might inhibit plaque formation and adhesion of oral microorganisms to restorative materials is increasing. Generally, it is important to obtain information on how biofilm may be influenced by various dental restorative materials, because microorganisms adhering to restorative materials can colonize other oral surfaces and eventually cause oral infections in predisposed individuals. Various kinds of supporting materials release diverse substances^[8], which exert an antibacterial action against oral microorganisms *in vitro*.^[9] Thus, studies concerning the adhesion of *C. albicans* to biomaterials have focused on the denture base and denture relining materials;^[10-12] however, fungi effectively adhere to all kinds of resin, glass, and even metal surfaces.^[13] Although dental restorative materials are known to be a potential source of fungal infections, fewer investigations have been carried out on these materials.^[12,14,15] In general, to ensure the clinical relevance of microbiological adhesion studies, materials should be used that are appropriate to clinical dentistry.^[11]

Against this background, we compared the susceptibility of six dental restorative materials (two compomers, two composites, two glass ionomer cements, three resin modified glass ionomer cements, one giomer, one amalgam, and one self-etching bonding agent) to *C. albicans* adhesion.

MATERIALS AND METHODS

Preparation of the Samples

In the present setup, six commercially available dental restorative materials (compomer, light curing glass ionomer cement, nanohybrid composite, amalgam, giomer, glass ionomer cement) were assessed. All materials, manufacturers, and material information are presented in Table 1. All materials were handled in strict compliance with their manufacturers' instructions. For each test material, 10 samples were prepared. Cylindrical samples (7 mm in diameter, 2 mm in height) were made, using a custom Teflon mold with calibrated circular holes. The materials were inserted into the mold, and their surfaces were covered with a transparent strip and pressed with two glass slides from the top and bottom. Light-activated materials were activated for 40 s of overlapping surface exposure using a polymerization light T-LED (Anthos, Italy, 1900 mW/cm²; 2 cm distance from the tip). The samples were then removed from the molds, mechanically polished sequentially with coarse, medium, fine, and superfine aluminum oxide abrasive discs (Sof-Lex, 3M, St Paul, MN, USA), and stored in distilled water before further processing.

Fungal growth conditions

The *C. albicans* clinical strain SC5314 was used. Cells were grown for 24 h at 37°C in the yeast nitrogen base (YNB; Sigma, St Louis, MO, USA) supplemented with 50 mM dextrose. After the incubation period, the cells were harvested, washed with phosphate-buffered saline (PBS; Cellgro, Media-tech, Herndon, VA, USA), and standardized to 1×10^7 cells/ml spectrophotometrically at 492 nm for the biofilm formation experiments.

Table 1: Details of the dental materials

Restorative Materials	Type	Composition	Manufacturer
1 Dyract Extra	Polyacid modified resin composite (compomer)	UDMA, TCB resin, TEGDMA, trimethacrylate resin, camphorquinone, ethyl-4-dimethylaminobenzoate, BHT, str-alumino-sodium-fluoro-phosphor-silicate glass, strontium fluoride, iron oxide and titanium dioxide pigments	Dentsply Detrey GmbH, Konstanz, Germany
2 Ionolux AC Capsule	Resin modified glass ionomer cement	Polyacrylic acid solution, 2-hydroxyethyl methacrylate, glycerin dimethacrylate, urethane dimethacrylate, tartaric acid, initiators	Voco GmbH, Cuxhaven, Germany
3 Grandio SO	Universal nanohybrid composite	BisGMA, BisEMA, TEGDMA, camphorquinone, butylated hydroxytoluene (BHT)	Voco GmbH, Cuxhaven, Germany
4 ANA 2000 Capsule	Amalgam	Ag 43.1%, Sn 30.8%, Cu 26.1%	Nordiska Dental, Angelholm, Sweden
5 Beautifil II	Giomer	Multifunctional glass filler and S-PRG filler based on fluoroboroaluminosilicate glass, BisGMA, TEGDMA resin	Shofu Inc., Kyoto, Japan
6 Ionofil Molar Capsule	Glass ionomer cement	Polyacrylic acid, tartaric acid, aluminofluorosilicate glass	Voco GmbH, Cuxhaven, Germany

Quantitative measurement of *C. albicans* biofilms

Metabolic activity of *C. albicans* biofilms was assessed using a colorimetric assay (XTT). To evaluate biofilm formation by *Candida* isolates, the samples were washed with PBS, placed in 24 well-culture plates with 2-ml standardized cell suspension (1×10^7 cells/ml), and incubated for 72 h at 37°C on a rocker. Biofilms were quantified using a tetrazolium XTT [2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide] assay as described previously.^[16]

Scanning electron microscopy

Three samples of each material were used for scanning electron microscopy (SEM) investigation. The samples with the adhering fungi were rinsed in PBS, fixed with ethanol, and air-dried. The test samples were then mounted on aluminum stubs and sputter-coated with gold. Samples were examined with a scanning electron microscope (magnification $\times 3000$ and $\times 5000$; EVO LS 10, Carl Zeiss Microscopy, LLC, NY / United States).

In vitro antifungal study

The antifungal effect of the restorative materials on *C. albicans* was determined with the disc diffusion method. Cylindrical samples of each material were put in plates with sterile Mueller Hinton agar (with 2% glucose) and Sabouraud dextrose agar previously seeded with *C. albicans*. The agar plates were incubated at 37°C for 24 h and then the inhibition zone was evaluated around each sample. The entire operation was carried out under aseptic conditions in duplicate.

Statistical analysis

A multiple significance test with the Duncan correction was used to compare the XTT and biofilm vitality values of *C. albicans* biofilm formation various restorative materials. Data are presented as the mean \pm standard deviation (SD), and the level of statistical significance was set at 5% for all analyses. The statistical analyses were performed using a computerized statistical software program SPSS 11.5 for Windows, (SPSS Inc., Chicago, IL, USA).

RESULTS

In general, the *C. albicans* biofilms adhered firmly to the restorative materials. No inhibition zone was observed around any sample; thus, none of the tested restorative materials reduced the amount of fungal growth. In addition, no differences were observed in the gross morphology and adhesion of biofilms formed by this pathogen on various types of restorative material [Figure 1].

During the scanning electron micrograph examination, a fine biofilm layer of *C. albicans* species was found on the surfaces of all examined materials. However, the quantity of adhering microorganisms varied among the materials. The amount of candidal growth was significantly lower on the surfaces of the resin-modified glass ionomer cement (Ionolux AC) and glass ionomer cement (Ionofil



Figure 1: The antifungal effect of the restorative materials on *C. albicans* strains 1, Dyract Extra; 2, Ionolux AC; 3, Grandio SO; 4, ANA 2000 Capsule; 5, Beautifil II; 6-Ionofil Molar.

Table 2: *C. albicans* biofilm XTT (OD492) and vitality values (%) on different restorative materials

Restorative Materials (n)	Type	OD value (mean \pm SD)	Vitality (%) (mean \pm SD)	F
Dyract Extra (10)	Compomer	0.1790 \pm 0.020a	55.0 \pm 6.07a	<i>P</i> < 0.001
Ionolux AC (10)	Resin modified Glass ionomer cement	0.1559 \pm 0.016b	48.4 \pm 4.59b	
Grandio SO (10)	Composite	0.1764 \pm 0.010a	54.1 \pm 2.98a	
ANA 2000 Capsule (10)	Amalgam	0.1205 \pm 0.010c	37.2 \pm 3.07c	
Beautifil II (10)	Giomer	0.1399 \pm 0.019d	43.1 \pm 5.83d	
Ionofil Molar (10)	Glass ionomer cement	0.1408 \pm 0.019d	43.3 \pm 5.85d	

F, Duncan test frequency; Means followed by the same letter are not significantly different at *P* = 0.05.

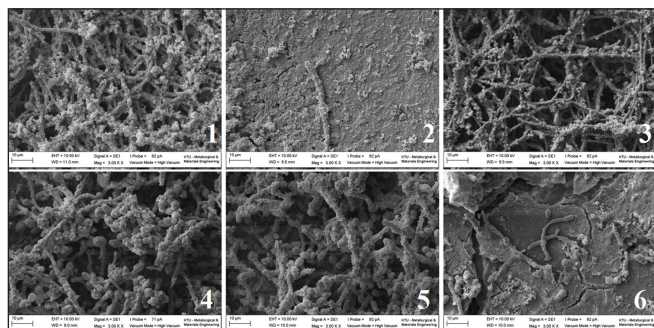


Figure 2: Scanning electron micrographs of *C. albicans* biofilm layers on the restorative materials

Note the heterogeneous nature of biofilm, showing a mixture of blastospore, pseudohyphae, and hyphae embedded in extracellular polymeric substance. 1, Dyract Extra; 2, Ionolux AC; 3, Grandio SO; 4, ANA 2000 Capsule; 5, Beautifil II; 6, Ionofil Molar.

molar) samples [Figure 2]. The compomer (Dyract Extra), the composite (Grandio SO), the amalgam (ANA 2000), and the giomer (Beautifil II) samples showed more candidal adhesion and on these restorative materials, dense oval colonies and round blastospore colonies dominated the fungal biofilm [Figure 2]. The biofilm formation appeared to be thicker on the surfaces of these restorative materials than on the glass ionomer cement samples.

When the adhering fungi were investigated, the amalgam (ANA 2000) samples revealed the lowest quantity of biofilms formed (median XTT value, 0.1205) of all the materials with statistically lower XTT values than the other materials ($P < 0.001$). The glass ionomer cement (Ionofil molar) and the giomer (Beautifil II) had low XTT values with similar values. The highest median XTT values, significantly higher than those of the other materials ($P < 0.001$), were for the composite (Grandio SO) and compomer (Dyract Extra) samples [Table 2]. Metabolic activity assays revealed that the *Candida* isolate tested formed significantly more vital biofilms on Dyract Extra than on the Grandio SO, Ionolux AC, Ionofil molar, Beautifil II, and ANA 2000 materials [Table 2]. Taken together, these results demonstrate that *Candida* can form biofilms on restorative materials and that this ability is influenced by the type of material.

DISCUSSION

This study compared the susceptibility of six dental restorative materials to *C. albicans* adhesion. The human mouth presents various surfaces to which microorganisms of the oral microbiota can adhere.^[14] Therefore, evaluating dental biofilms grown on typical dental restorative materials such as amalgam, resin composite, compomer, and glass-ionomer cement plays an important role in achieving long-term success of oral healing and

protection from oral diseases. Although other species of the genus *Candida* are known to be involved, *C. albicans* is the major microbiological factor in oral candidosis.^[2] Different fungal species have different adhesion potential for dental material surfaces.^[17] However, since we preferred to provide as simple a test model as possible, we used only one type of culture strain of *C. albicans*.

Studies concerning the adhesion properties of *C. albicans* have focused on the denture base and relining materials.^[10,12,17] To our knowledge, the current work is the first to compare differences in *C. albicans* adhesion to various dental restorative materials.

In the present study, we investigated the inhibition of fungal colonization around the restorative samples. However, *C. albicans* biofilms adhered firmly to the restorative materials, and no inhibition zone was observed around any sample. The tested materials did not reduce the amount of fungal growth, and none of the tested materials exhibited antifungal properties. In fact, this inhibition test revealed *C. albicans* adhered to the assessed material surfaces. In agreement with previous studies, our results [Figure 2] confirm that *C. albicans* can adhere directly to the surface of restorative materials.^[15]

Various material characteristics such as surface roughness, surface hydrophobicity, electrostatic forces, composition of the material, type of matrix, size of fillers, and configuration of fillers are relevant factors affecting attachment of organisms to the surface to form a biofilm.^[3,18-22] Surface roughness is well documented to have a crucial influence on microbial adhesion.^[23] This property affects the attachment of biofilm bacteria, because more or less “surface” is available for bacterial attachment and more or less protection is provided for colonizing bacteria.^[24] Higher numbers of *C. albicans* are found on rough surfaces than on polished, smooth surfaces.^[25] Theoretically, and as a consequence, dental materials should be polished *in situ*, so that a surface as plane as possible is provided. Therefore, in the present study, the tested materials were prepared and polished as close as possible for the minimal roughness surface. Moreover, the correlation between surface roughness and candidal adhesion was not evaluated in the present study. Therefore, the results cannot be related to specific surface characteristics.

The XTT values of the amalgam (ANA 2000) samples revealed the lowest quantity of biofilms formed of all materials. The glass ionomer cement (Ionofil molar) and the giomer (Beautifil II) showed low XTT values with similar values. The highest median XTT values were found for the composite (Grandio SO) and compomer

(Dyract Extra) samples. These important findings confirm the hypothesis that different types of material and their specific chemistry and composition (matrix and fillers) interfere considerably with *C. albicans* adherence.^[20,26,27] Heavy metals have antimicrobial properties on biofilm formation^[28], and amalgam liberates ions that kill bacteria in the adhering biofilms.^[28,29] Although the amalgam samples showed a very high percentage of surface coating within the scanning electron micrograph examination, low vitality values of the adhering *C. albicans* biofilm were found on the amalgam samples. This might be due to the metallic ions released from the surfaces. The low quantity of adhering *C. albicans* on glass ionomer cements in this investigation may be because glass ionomer cements are known to release significant quantities of fluoride, and fluoride components are associated with antimicrobial effects.^[30,31] However, it was not within the scope of the present investigation to analyze these diverse chemical entities (i.e., different metallic cations, fluoride ions, and organic molecules), so such conclusions must be regarded as rather speculative.

Bürgers *et al.*^[32] reported that the compomers Compoglass F and Dyract eXtra and the ormocer Admira revealed lower amounts of adhering fungi than the conventional hybrid composites and a novel silorane-based restorative. This finding is not in agreement with our findings. In our study, the highest XTT values were found for the composite (Grandio SO) and compomer (Dyract Extra) samples.

To prevent *C. albicans* biofilms from accumulating and to reduce adhesion, several promising inventions have been introduced.^[33-35] As the etiology of *Candida*-associated stomatitis is multifactorial with numerous influencing parameters, a better understanding of the essentials of fungal adhesion *in vitro* methods to study these adhesion processes is needed. In general, conclusions from this *in vitro* investigation and other related studies may not be transferred to the clinical situation without restriction of any kind, and results have to be interpreted carefully because only a limited number of parameters can be simulated outside the oral cavity. Considering the limitations of this study, the amalgam and glass ionomer cements revealed lower amounts of adhering fungi than the composites and compomers.

These findings emphasize the use of glass ionomer restorative cements and amalgam regarding reducing *C. albicans* adhesion to dental restorative materials especially in people with weakened immune systems, neutropenia, and cancer.

CONCLUSION

These findings emphasize the use of glass ionomer restorative cements and amalgam regarding reducing

C. albicans adhesion to dental restorative materials especially in people with weakened immune systems, neutropenia, and cancer.

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Nil

Conflict of interest

None

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