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Original research

ADHESION OF *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* TO DIFFERENT RESTORATIVE MATERIALS

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ABSTRACT

Objectives: The aim of this study is to compare the susceptibility of seven different restorative materials (three conventional composite resins, two bulk-fill composite resins, one giomer, and one high viscosity glass ionomer material) to adhere *Candida albicans* and *Candida parapsilosis.*

Materials and methods: In this study, thirty cylindrical specimens of each material were made according to instructions of the manufacturers. The surface roughness of the specimens was assessed using a profilometer. Thereafter, the specimens were incubated with a reference strain of *Candida albicans* (ATCC 64548) and *Candida parapsilosis* (ATCC 22019). The proliferated colonies counted as CFU/ml. One-way analysis of variance (ANOVA) was used to evaluate the surface roughness and the adhesion value of the materials. Tukey's post-hoc test was used for subsequent pairwise comparisons.

Results: There was a statistically significant difference between the groups in terms of the surface roughness of the materials ($p<0.05$). The high viscosity glass ionomer material exhibited significantly higher surface roughness values while X-trafil (a bulkfill composite resin) had the lowest surface roughness values. Also, there was a significant difference between *Candida* adhesion values of the materials ($p<0.05$).

Conclusions: There was no significant relationship between surface roughness and adhesion of *Candida albicans* and *Candida parapsilosis*. Involvement was seen more for *Candida albicans* compared to *Candida parapsilosis* in all restorative materials.

Keywords: Bulk fill composites, candida, dental materials, glass ionomer.

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INTRODUCTION

Dental caries is an infectious pathology of hard dental tissues which has a localized and transmissible destruction process.[1] Cariogenic microorganism eradication is one of the most important critical factors for preventing primary or residual caries lesions.[2] Previous studies show that *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, and *Lactobacillus rhamnosus* are the most frequent cariogenic microorganisms.[3,4] Moreover, it was reported that the existence of *Candida* spp. in the oral cavity is associated with the caries lesion progress.[5]

Candida albicans (C. albicans) is the most common fungus in the human oral cavity and *C. albicans* is normally present in the oral cavity in 20-40% of healthy individuals. *Candida parapsilosis (C. parapsilosis)* represents less than 10% of the *Candida* species in the oral cavity.[6-8] It is known that yeast cells have the adhesion potential to host surfaces such as mucosa and non-biological surfaces.[6] In dentistry, studies have focused on the attachment of dentures and the base materials due to adhesion of fungal cells to materials such as resin, glass or metal.[9-11] However, it is not known whether dental restorative materials are potential sources of fungal infections since very few studies have been performed on these materials.

Composite resins that were developed as an aesthetic alternative to amalgam in the 1960s are commonly used as direct restorative materials in modern dentistry. The manufacturers developed distinctive composites to improve the physical and mechanical properties of traditional composites of resin matrices and filler materials. A new restorative approach involves the use of high-viscosity bulk-fill composites. The use of bulk-fill composite resins for posterior restorations reduces the time and effort required for stratification and eliminates the possibility of voids between the layers by allowing 4 mm curing in one step without affecting the polymerization shrinkage and mechanical properties in the negative direction.[12,13]

In addition to composite resins, glass ionomer cements (GIC) have also been used in posterior restorations. Chemical bonding to tooth structures, decay-inhibiting effects due to fluoride release, remineralization, thermal expansion similar to dental structures, low toxicity and biocompatibility have made GIC a clinically preferred restorative material compared to resin composites.[14] Besides the many advantages of GIC, they have some disadvantages such as low wear resistance, low fracture toughness, and they are also greatly influenced by dehydration and initial moisture contamination. To reduce the moisture sensitivity of GIC in early stages of hardening, to increase their hardness and abrasion resistance, and to enable them to be used in areas exposed to chewing forces, bulk-fill glass hybrid restorative systems, which combine the main advantages of a highly viscous GIC with a nano-filled, light-curing varnish, were presented to the market.[14]

Giomer is a fluoride-releasing hybrid composite restorative material and it contains active glass ionomer particles (pre-reacted glass ionomer - PRG) formed as a result of the acid-base reaction in the aqueous medium between the fluoroaluminasilicate glass particles and the polyalkenoic acid.[15]

In the present study, it was aimed to compare the adhesion of the oral fungal pathogens *C. albicans* and *C. parapsilosis* to bulk-fill composite resin, a conventional composite resin, a giomer, and a high viscosity glass ionomer material. The null hypotheses of this study were that

1. the composition and chemistry of the materials would not affect *Candida* adhesion to the materials.

2. the surface roughness would not affect *Candida* adhesion to the materials.

MATERIALS AND METHODS

Preparation of the samples

This *in vitro* study was performed in accordance with the Helsinki Declaration. In this study, seven different restorative materials (three conventional hybrid composite resins, three bulk-fill composite resins, one giomer, and one high viscosity glass ionomer material) were used. The used materials, lot numbers, and their compositions are given in Table 1.

Table 1. Materials and compositions

MATERIALS	COMPOSITIONS
Arabesk N **VOCO GmbH, Cuxhaven, Germany** **Batch 1338579**	Bis-GMA, UDMA, TEGDMA, barium aluminium silicate glass, lithium aluminium silicate glass-ceramic, highly dispersed silicon dioxide
Clearfil Majesty Esthetic **Kuraray, Okuyama, Japan** **Batch 00051D**	Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, Silanated barium glass filler, prepolymerized organic filler
Beautifil-Bulk **Shofu, Kyoto, Japan** **Batch PN2034**	Bis-GMA, TEGDMA, inorganic glass filler, aluminuoxide, silica, pre-reacted glass ionomer filler, DL-camphorquinone
X-tra Fil **VOCO GmbH, Cuxhaven, Germany** **Batch 1612535**	Bis-GMA, UDMA, TEGDMA, Barium boron aluminum silicate glass
Filtek Bulk Fill Posterior Restorative **3M ESPE, St. Paul, MN, USA** **Batch N719528**	AUDMA, UDMA and DDMA, Zirconia / silica and ytterbium trifluoride filler.
Equia Forte Fil **GC, Tokyo, Japan** **Batch 160512A**	Powder: 95% strontium fluoroalumino-silicate glass, 5% polyacrylic acid. Liquid: 40% aqueous polyacrylic acid EQUIA Forte Coat: 40%-50% methyl methacrylate, 10%-15% colloidal silica, 0.09% camphorquinone, 30%-40% urethane methacrylate, 1%-5% phosphoric ester monomer
Filtek Ultimate **3M ESPE, St. Paul, MN, USA** **Batch N618541**	Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA, 20 nm silica particuls, 4 - 11 nm zirkonyum particuls

Bis-GMA; Bisphenol A-Glycidyl Methacrylate, UDMA; Urethane dimethacrylate, TEGDMA; Triethylene glycol dimethacrylate, PEGDMA; polyethyleneglycoldimethacrylate, Bis-EMA; ethoxylatedbisphenol-A dimethacrylate, DDMA; 1,12-dodecane dimethacrylate, AUDMA; Aromatic dimethacrylate.

All materials, Arabesk N (AN) (VOCO GmbH, Cuxhaven, Germany); Clearfil Majesty Esthetic (CME) (Kuraray, Okuyama, Japan); Beautifil-Bulk (BB) (Shofu, Kyoto, Japan); X-tra fil (XF) (VOCO GmbH, Cuxhaven, Germany); Filtek Bulk Fill Posterior Restorative (FBF) (3M ESPE, St. Paul, USA); Equia Forte Fil (EF) (GC, Tokyo, Japan); Filtek Ultimate (FU) (3M ESPE, St. Paul, USA) are applied in accordance with the manufacturer's instructions. For each test group, thirty disc-shaped samples (8 mm in diameter, 2 mm in height) (n=30) were prepared using a special teflon mold with calibrated circular holes. In the composite resin and giomer groups, the restorative materials were placed in molds on glass, their surfaces were covered with a mylar strip and another glass was pressed on top of the materials. All samples were polymerized on both sides for 40 s using a LED light source (VALO, Ultradent, UT, USA, 395-480 nm, 1000 mW/cm^2). In the EF group, glass ionomer material was placed in the mold on glass, its surface was covered with a mylar strip and another glass was pressed from top of the material. After the material hardened, Equia Forte Coat (GC) was applied to the surfaces of the samples and it was cured for 20 s. Thereafter, the specimens were removed from the molds and the excesses in the specimens were removed with Sof-Lex XT discs (3M ESPE, St Paul, USA), and the surfaces of the specimens were polished using aluminum oxide/diamond-abrasive-impregnated (Enhance/PoGo; Dentsply Caulk, Milford, USA). Specimens were stored in distilled water for further processing. The surface roughness of each disc-shaped specimens was measured with a profilometer (Mitutoyo SJ-301, Mitutoyo Corporation, Tokyo, Japan,). Roughness measurements were performed in three regions of each specimen and the arithmetic mean of the values was taken.

Adhesion testing for C. albicans and C. parapsilosis

C. parapsilosis (ATCC 22019) standard strain was used in the adhesion test. A suspension of the *C. parapsilosis* strain was prepared in 2.5 to 5x10^6 CFU / ml in sterile saline from a 24-hour culture on

a sabouraud dextrose agar (SDA) medium. From this suspension, 100µl of SDA-containing plaque medium was taken and composite discs were placed on top of it. Disc-shaped specimens were incubated at 37°C for 24 hours and then they were washed with 15 ml of SF. The washed specimens were taken in tubes of 1 ml SF and treated with an ultrasonic sonicator three times for 10 seconds at 30W. This suspension was spread on the supernatant by inoculating 100 µl of both 1:10, 1:100 and 1:1000 dilutions directly onto the SDA medium. These media were incubated at 37°C for 24 hours. The growth colonies on SDA were then counted as CFU / ml (R). The method of the application of *C. albicans* (ATCC 64548) to the samples was identical to the application of *C. parapsilosis* (ATCC 22019).

Scanning electron microscopy (SEM)

In order to observe the surface roughness of the specimens under a scanning electron microscope (SEM), one sample from each group was prepared. The samples were examined with a scanning electron microscope (magnification 3000x and 5000x, GeminiSEM 500, Zeiss, Germany).

Statistical analysis

The data were analyzed using statistical software (SPSS Statistics 22.0; SPSS Inc, IL, USA). The distribution of the data in this study was evaluated by the Shapiro Wilk test. One-way analysis of variance (ANOVA) was used to evaluate the surface roughness and the adhesion value of the materials. Tukey's post-hoc test was used for subsequent pairwise comparisons. The significance level was accepted as $p<0.05$.

RESULTS

Surface roughness

The surface roughness values of the groups are shown in Table 2.

Table 2. Surface roughness, *Candida albicans* and *Candida parapsilosis*, adhesion values of the materials (Mean ± SD (Standard Deviation))

Materials	Surface roughness Mean (± SD)	*Candida albicans* Mean (± SD)	*Candida parapsilosis* Mean (± SD)
Arabesk N	0.51 (± 0.13)^{a}	1.1×10^{3} (± 1.4×10^{3})^{a}	0.8×10^{3} (± 0.7×10^{3})^{a}
Clearfil Majesty Esthetic	0.56 (± 0.14)^{ab}	29×10^{3} (± 20×10^{3})^{b}	28×10^{3} (± 28×10^{3})^{b}
Beautifil-Bulk	0.66 (± 0.21)^{b}	16×10^{3} (± 11×10^{3})^{ab}	8.5×10^{3} (± 8×10^{3})^{a}
X-tra Fil	0.47 (± 0.12)^{a}	2.5×10^{3} (± 1.8×10^{3})^{a}	0.2×10^{3} (± 0.18×10^{3})^{a}
Filtek Bulk Fill	0.57 (± 0.16)^{ab}	58×10^{3} (± 36×10^{3})^{c}	11×10^{3} (± 8.3×10^{3})^{a}
Equia Forte Fil	1.13 (± 0.28)^{c}	63×10^{3} (± 32×10^{3})^{c}	8×10^{3} (± 6.7×10^{3})^{a}
Filtek Ultimate	0.63 (± 0.22)^{ab}	29×10^{3} (± 15×10^{3})^{b}	4.4×10^{3} (± 5×10^{3})^{a}

a,b,c shows statistical differences in the vertical column

The EF group exhibited significantly higher surface roughness values. A bulk-fill composite resin (XF) had the lowest surface roughness values and there was statistically significant difference between the XF group and BB group and, XF group and EF group ($p<0.05$). Also, there was a statistically significant difference between BB group and EF group ($p<0.05$). SEM images of the surface roughness of tested restorative materials are given in Figure 1.

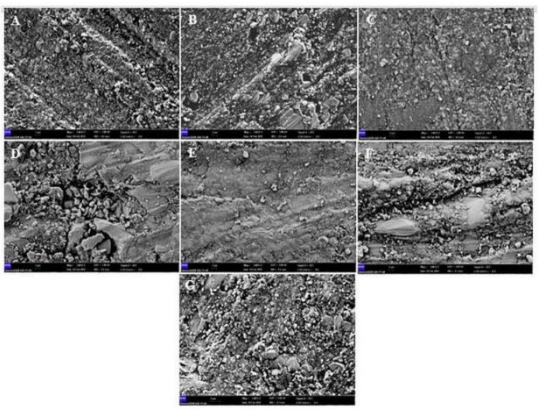

Figure 1. SEM images of the surface roughness of Arabesk N (A), Beautifil-Bulk (B), Clearfil Majesty Esthetic (C), Equia Forte Fil (D), Filtek Bulk Fill Posterior Restorative (E), Filtek Ultimate (F) and X-tra fil (G)

C. albicans adhesion values

Table 2 shows the comparative adhesion values of *C. albicans* in all test materials. Significant differences between the adhesion values of *C. albicans* were found according to the test results. Although the AN group showed the lowest *C. albicans* adhesion value among all tested materials, there was no statistically significant difference between the AN group and the XF and BB groups ($p=0.455$, $p=1.00$). The EF group showed the highest *C. albicans* adhesion value and there was no statistically significant difference between the EF and the FBF groups ($p=0.998$). Also, there was no statistically significant difference between the CME, BB and FU groups ($p>0.05$).

C. parapsilosis adhesion values

Table 2 shows the comparative adhesion values of *C. parapsilosis*. There were significant differences between the *C. parapsilosis* adhesion values according to the test results. The CME group showed significantly higher *C. parapsilosis* adhesion value ($p<0.05$). The XF group showed the lowest *C. parapsilosis* adhesion value among all tested materials and there was a statistically significant difference only between the XF and the CME groups ($p=0.001$), but not with XF and the other groups ($p>0.05$). The CME group showed significantly higher *C. parapsilosis* adhesion than other groups ($p<0.05$). Besides all these, *C. albicans* adhesion was significantly higher when compared to *C. parapsilosis* adhesion ($p<0.05$).

DISCUSSION

This study aimed to compare the adhesion of *C. albicans* and *C. parapsilosis* to traditional composite resins, bulk-fill composite resins, and restorative materials containing glass ionomer. While there was no significant relationship between surface roughness and *Candida* adhesion, the content of restorative materials was found to affect *Candida* adhesion. Therefore, the first hypothesis that the composition and chemistry of the materials would not affect *Candida* adhesion to the materials was rejected and the second hypothesis that the surface roughness would not affect *Candida* adhesion to the materials was accepted.

The human mouth provides different surfaces to which oral microorganisms can adhere.[9] *C. albicans* is the major microbiological factor in fungal infections. Besides, reports show that *C. parapsilosis* is often the second most commonly isolated *Candida* spp. from blood cultures.[16,17] Since we prefer to provide as simple a test pattern as possible, we used culture strains of *C. albicans* and *C. parapsilosis* in this study.

Studies on the adhesion properties of fungal species are commonly focused on denture base and relining material.[10,11,18] Despite their frequent use in clinical dentistry and the fact that they are potential sources for fungal infections, fewer studies have been conducted on composite resin and glass ionomer materials.[9,19] In order to ensure the clinical suitability of microbiological adhesion studies, appropriate materials for clinical dentistry should be used.[20] To the best of our knowledge, this is the first study to compare fungal adhesion to different bulk-fill composite resins.

The number of microorganisms adhering to the restorative material depends on different properties such as surface hydrophobicity, surface roughness, type of matrix, electrostatic forces, the composition of the material, filler size, and configuration of fillers.[6,16,21] It has been proven that the surface roughness has a very important effect for microbial adhesion.[22] The most commonly used parameter to define the roughness of a given surface is the arithmetic mean peak-valley value *Ra*.[23] Therefore, we compared the roughness values of restorative materials using *Ra* value.

Surfaces with *Ra* values below 0.2 μm are considered smooth.[21] Studies have shown that as the value of *Ra* increases, the *Candida* adhesion also increases.[20,22] In our study, the XF and AN groups have numerically the lowest surface roughness values and therefore show the lowest adhesion values. Although the EF group showed a high surface roughness, it showed a similar *Candida* adhesion with some composites. In this study, the low quantity of adhering *Candida* on glass ionomer cements which are known to release significant quantities of fluoride is associated with the antimicrobial effects of fluoride

components.[23,24] These findings support the alternate hypothesis that different types of materials and their composition and chemistry have a significant effect on *Candida* adhesion. However, a statistical correlation analysis between surface roughness and *Candida* adhesion showed no significant correlation in our study.

The capability of *Candida* to persist within the host and to cause infection has been attributed to its different virulence features which include the ability to adhere to host surface, the presence of hyphae, biofilm formation, and the secretion of hydrolytic enzymes.[17] Although *C. albicans* is the most prevalent fungus in humans[25], the incidence of *C. parapsilosis* has dramatically increased recently.[17] Besides, *C. parapsilosis* has low virulence properties compared to *C. albicans.*[26] The lower virulence of *C. parapsilosis* compared to *C. albicans* can also be attributed to the lack of formation of true hyphae (tube germ).[27] The ability of a microorganism to adhere to host surfaces is the critical first step for successful colonization and subsequent infection of host tissues by a potentially pathogenic *Candida* spp. *C. albicans* adhere to a greater extent to host surfaces than does *C. parapsilosis.*[28] Biofilms are surface-associated communities of microorganisms within the extracellular matrix and seem to contribute to cohesion.[29] Biofilm formation is a potent virulence factor for several *Candida* spp. but *C. parapsilosis* strains produce quantitatively and structurally less complex biofilm than *C. albicans.*[17] Secreted aspartic proteinases (Saps) facilitate the invasion and colonization of host surfaces. Compared to *C. albicans, C. parapsilosis* has less Saps activity.[17] Other enzymes that seem to play an important role in the pathogenesis of *Candida* species are phospholipase and lipases. These enzymes affect the adhesion and penetration of host cells. Phospholipase production is concentrated on the tips of hyphae, and the activity is greater when the hypha is in direct contact with the membrane.[29] The extra-cell phospholipases are relevant for *C. albicans*. In *C. albicans*, 10 lipase genes have been identified whereas, in *C. parapsilosis*, two lipase genes have been identified.[17,30] In our study, *C. albicans* may have shown greater adhesion to all tested composite resin surfaces than *C. parapsilosis* because of the above-mentioned virulence factors.

The limitation of this study was the use of standard species of *C. albicans* and *C. parapsilosis* rather than clinical isolates. Therefore, further studies with clinical isolates are needed.

CONCLUSIONS

Within the limitation of this study, X-trafil, which is a bulk-fill composite resin, showed similar surface roughness values and similar *Candida* adhesion with Arabesk N (a conventional composite resin). There was no relationship between surface roughness and *Candida* adhesion. Besides, the content of restorative materials was found to affect *Candida* adhesion. The involvement of *C. albicans* was seen more than that of *C. parapsilosis* in all tested restorative materials.

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None

CONFLICT OF INTEREST STATEMENT

None

Farklı Restoratif Materyallere Candida Albicans ve Candida Parapsilosis Adezyonu

ÖZ

Amaç: *Bu çalışmanın amacı, yedi farklı restoratif materyalin (üç adet geleneksel kompozit rezin, iki adet bulk-fill kompozit rezin, bir giomer ve bir yüksek viskoziteli cam iyonomer materyal) Candida albicans ve Candida parapsilosis adezyonuna olan duyarlılığını karşılaştırmaktır.* ***Gereç ve Yöntemler:*** *Bu çalışmada her materyalden otuz silindirik örnek üretici talimatlarına göre hazırlandı. Örneklerin yüzey pürüzlülüğü profilometre kullanılarak ölçüldü. Daha sonra örnekler, Candida albicans (ATCC 64548) ve Candida parapsilosis (ATCC 22019) referans suşu ile inkübe edildi. Materyallerin yüzey pürüzlülük ve adezyon değerlerini değerlendirmek için tek yönlü varyans analizi (ANOVA) kullanılmıştır. Grupların ikili olarak karşılaştırılması için Tukey post-hoc testi kullanıldı.* ***Bulgular:*** *Materyallerin yüzey pürüzlülüğü açısından gruplar arasında istatistiksel olarak anlamlı fark bulundu (p<0,05). Bir bulk-fill kompozit rezin olan X-trafil, en düşük yüzey pürüzlülüğü değerlerine sahipken, yüksek viskoziteli cam iyonomer material önemli ölçüde daha yüksek yüzey pürüzlülüğü değerleri gösterdi. Ayrıca materyallerin Candida adezyon*

değerleri arasında anlamlı fark bulundu (p<0,05). ***Sonuç:*** *Yüzey pürüzlülüğü ile Candida albicans ve Candida parapsilosis' in adezyonu arasında anlamlı bir ilişki bulunmazken, tüm restoratif materyallerde Candida albicans, Candida parapsilosis' ten daha fazla tutulum gösterdi.* ***Anahtar kelimeler:*** *Bulk-fill kompozit, candida adezyonu, restoratif material, yüzey pürüzlülüğü.*

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