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ORIGINAL ARTICLE

Candida albicans adhesion to composite resin materials

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Abstract The adhesion of *Candida albicans* to dental restorative materials in the human oral cavity may promote the occurrence of oral candidosis. This study aimed to compare the susceptibility of 14 commonly used composite resin materials (two compomers, one ormocer, one novel silorane, and ten conventional hybrid composites) to adhere *Candida albicans*. Differences in the amount of adhering fungi should be related to surface roughness, hydrophobicity, and the type of matrix. Cylindrical specimens of each material were made according to the manufacturers' instructions. Surface roughness R_a was assessed by perthometer measurements and the degree of hydrophobicity by computerized contact angle analysis. Specimens were incubated with a reference strain of *C. albicans* (DMSZ 1386), and adhering fungi were quantified by using a bioluminometric assay in combination with an automated plate reader. Statistical differences were analyzed by the Kruskal–Wallis test and Mann–Whitney *U* test. Spearman's rank correlation coefficients were calculated to assess correlations. Median R_a of the tested composite resin materials ranged between 0.04 and 0.23 μm, median contact angles between 69.2° and 86.9°. The two compomers and the ormocer showed lower luminescence intensities indicating less adhesion of fungi than all tested conventional hybrid composites. No conclusive correlation was found between surface roughness, hydrophobicity, and the amount of adhering *C. albicans*.

Keywords Adherence · *Candida albicans* · Composite · Hydrophobicity · Silorane · Surface roughness

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Introduction

Candida albicans is the most prevalent fungus in the human oral cavity and the major pathogen in both oral and systemic candidosis [1, 3]. Candidal adhesion to any oral substrata is the first and essential stage in the formation of a pathogenic fungal biofilm, which is in turn the prerequisite for the microorganism to ingress into the human host [7, 18, 34]. In general, yeast cells are known to have a remarkable potential to adhere to host surfaces, such as teeth or mucosa, and to artificial, non-biological surfaces, such as implanted dental devices [3]. The oral occurrence of *C. albicans* is strongly associated with denture-related stomatitis, also termed as *Candida*-associated denture stomatitis [4, 7, 34], whereas up to 67% of all denture wearers are affected by this fungal infection [1, 5, 43]. For this reason, studies concerning the adhesion of *C. albicans* to biomaterials have basically been focused on denture base and denture relining materials [24, 27, 28, 32–34, 44, 45], although it is well known that fungi effectively adhere to all kinds of resin, glass, and even metal surfaces [18, 24, 31]. Besides the typical older denture wearers with poor oral hygiene, oral candidosis and its sequelae are increasingly seen in patients without removable dentures. These are mainly medically compromised patients under immunossupression, such as those with AIDS and tumor patients being treated with chemotherapy or radiation [9, 17, 20, 29, 40]. Therefore, in addition to denture materials, direct restor-

ative materials should also be considered and assessed as a reservoir for (re)infection with *C. albicans* in the oral cavity.

Dental resin composites as the most popular and widely used direct restorative materials in modern dentistry have been developed as an aesthetic alternative to amalgam in the 1960/1970. To improve the mechanical and physical properties of conventional (hybrid) composites composed of a resin matrix and filler materials, dental research has innovated several capable matrix-modified composites. Compomers, also referred to as polyacid-modified resin composites, were marketed to provide the mechanical and esthetic benefits of composites and the fluoride-releasing advantages of glass ionomers [23]. They are mainly composed of silanized glass particles and a monomer matrix, made up of modified methacrylates (bisphenol-glycidyl-dimethacrylates, urethane dimethacrylate, etc.) and bifunctional monomers (acidic trichlorobenzene, dicarboxylic acid dimethacrylate, etc.) [23]. Ormocers (organically modified ceramics) are organic/inorganic copolymer systems based on multifunctional urethane- and thioether(meth) acrylate alkoxysilanes [21]. The latest innovation in matrix-modified composite restoratives is a silorane-based monomer, which is a combination of siloxane and oxirane moieties [14]. Siloranes were developed to reduce polymerization shrinkage and exhibit promising mechanical and biocompatibility characteristics [37, 46]. To our knowledge, only few studies have been carried out to investigate fungal adhesion on dental composites, and none of them aimed to compare a range of commonly used composite resin materials [13, 22, 36, 44]. In general, bacterial and fungal adhesion to biomaterials is known to be significantly influenced by substratum surface properties [11, 18, 19, 24–26, 30, 31, 33, 39, 42]. Surface roughness and hydrophobicity have been counted among the most significant factors to influence the adhesion of microorganisms. High surface roughness values have been found to promote bacterial adhesion on composite resins [2], but no further direct influence of surface roughness on the bacterial adhesion should be expected below 0.2 μm [2]. There are contrary reports regarding the influence of hydrophobicity and surface free energy on fungal adhesion [15, 18, 27, 31, 32, 34, 39, 45], but it becomes apparent that hydrophobic microorganisms preferentially adhere to hydrophobic surfaces, whereas hydrophilic bacteria or fungi preferentially adhere to hydrophilic surfaces [24, 45].

The purpose of the present study was to compare the adherence of oral fungal pathogen *C. albicans* to the surface of 14 widely used composite resin materials and reference material glass. Different adhesion potentials should be related to different material composition and specific surface properties (surface roughness and hydrophobicity). Scanning electron micrographs were made for validation.

Materials and methods

Preparation of specimens

Fourteen commercially available composite restorative materials (ten conventional resin-based hybrid composites, two compomers, one ormocer, and one novel silorane-based composite) were assessed in the present study. Glass was used as reference material. All materials, manufacturers, and material information are presented in Table 1.

Table 1 Manufacturer and material information, arithmetic surface roughness R_a, contact angles and relative luminescence intensities of all assessed materials; (medians and 25/75 percentiles)

Material	Manufacturer	Type	Surface roughness, R_a (μm)	Contact angles (degrees)	Relative luminescence (no units)
Admira	VOCO, Cuxhaven, Ger	Ormocer	0.04 (0.04/0.04)	74.0 (70.3/77.1)	43 (−44/208)
Arabesk Top	VOCO	Microhybrid	0.11 (0.08/0.11)	80.5 (80.4/81.3)	193 (133/217)
CeramX	Dentsply, Konstanz, Ger	Nanohybrid	0.04 (0.04/0.04)	86.9 (84.7/90.7)	311 (186/522)
Compoglass F	Ivoclar Vivadent, Schaan, FL	Compomer	0.04 (0.04/0.08)	77.5 (67.9/80.0)	19 (−32/32)
Dyract eXtra	Dentsply	Compomer	0.04 (0.04/0.04)	86.5 (70.9/90.7)	68 (58/116)
Enamel Plus HFO	GDF, Rosbach, Ger	Microhybrid	0.04 (0.04/0.04)	87.2 (79.4/90.0)	440 (236/485)
Filtek Silorane	3M ESPE, St. Paul, Minn, USA	Silorane	0.04 (0.04/0.04)	86.0 (82.3/87.3)	201 (180/332)
Filtek Supreme XT	3M ESPE	Nonohybrid	0.04 (0.04/0.08)	70.8 (60.9/75.6)	553 (469/600)
Filtek Z250	3M ESPE	Microhybrid	0.04 (0.04/0.04)	70.6 (69.2/74.5)	296 (274/399)
Grandio	VOCO	Nanohybrid	0.23 (0.11/0.23)	78.5 (75.3/81.8)	164 (140/212)
Heliomolar	Ivoclar Vivadent	Microhybrid	0.11 (0.08/0.11)	74.5 (73.8/85.3)	213 (141/278)
InTen-S	Ivoclar Vivadent	Microhybrid	0.08 (0.08/0.11)	72.9 (68.4/76.7)	249 (212/432)
Tetric EvoCeram	Ivoclar Vivadent	Nanohybrid	0.04 (0.04/0.08)	69.2 (68.4/71.9)	502 (294/597)
Venus	Heraeus Kulzer, Hanau, Ger	Microhybrid	0.08 (0.08/0.08)	83.9 (80.2/85.9)	376 (288/491)
Glass	Marienfeld, Koenigshofen, Ger	–	<0.01	65.2 (63.2/67.7)	103 (52/115)

All materials were handled in strict compliance with their manufacturers' instructions. Fifteen specimens were prepared for each test material. Cylindrical specimens (10 mm in diameter, 2 mm in height) were made, using a custom metal mold with calibrated circular holes. The materials were inserted into the mold and covered immediately with two glass slides (Alfred Becht GmbH, Offenburg, Germany) from the top and bottom. All specimens were light-polymerized for 1 min from both sides using polymerization light Heliolux DLX1 (Vivadent, Schaan, Liechtenstein, 100 W; 2 cm distance from the tip). Specimens were mechanically polished (Motopol 8; Buehler, Duesseldorf, Germany) with wet abrasive paper discs (Buehler, Lake Bluff, IL: grit 1000, 2000, and 4000), fixed into 48-well plates (Sarstedt, Newton, NC, USA) and stored in distilled water before further processing.

Surface roughness was determined with a stylus instrument (Perthometer S6P; Perthen, Goettingen, Germany). Roughness measurements were performed on three sites of five specimens of each material and values are expressed as the arithmetic average peak-to-valley value. Restorative surfaces with values below 0.2 μm were regarded as smooth.

Hydrophobicity of all test and reference materials was evaluated by measuring water contact angles. Hydrophobic surfaces resulted in high water contact angles. A computerized contact angle system (OCA 15 plus; Dataphysics Instruments, Filderstadt, Germany) in combination with SCA 20 software (Dataphysics Instruments) was used to analyze and calculate specific contact angles. Deionized water was used for all calculations. An image of the water drop on the surface of the specimen was taken exactly 20 s after the contact. Two calibrated droplets (2.0 μl) were assessed for each material with two measures (right and left contact angle) for each single droplet.

Adhesion testing

C. albicans human isolate (strain 1386; DSMZ, Braunschweig, Germany) was incubated overnight in yeast broth (3 g yeast extract/ Sigma-Aldrich, St. Louis, MO, USA; 3 g malt extract/ Sigma-Aldrich; 5 g peptone from Casein/Merck, Darmstadt, Germany; 10 g D(+)-glucose/Merck; 1,000 ml distilled water). Cells were harvested by centrifugation at 1,000×*g* for 5 min at 18°C. Pellet was washed using phosphate-buffered saline (PBS; Sigma-Aldrich) and adjusted to a suspension of 0.3 optical density at 540 nm. The suspension was incubated with the specimens for 2.5 h at 37°C. After washing three times with PBS [44], specimens were removed and placed into sterile polypropylene tubes at 4°C. Five hundred microliters perchloric acid (Merck) was added, cups were vortexed for 30 s, and 500 μl potassium bicarbonate (Sigma-Aldrich) was added for neutralization. Cups were then centrifuged at 7,300×*g* for 15 min at 4°C. One hundred microliters of each cup was pipetted into 96-well plates (Sarstedt), and 100 μl of bioluminescence adenosine triphosphate (ATP) detection kit VIA Light AMR-Plus buffer (Cabrex Bio Science, Rockland, ME, USA) was added to each well. Luminescence was recorded after 5 min by an automated multi-detection reader (Fluostar optima; BMG Labtech, Offenburg, Germany) at wavelengths of 590 nm emission. The relative luminescence intensities are calculated from the differences between the luminescence after adhesion testing and the background luminescence of the specimen itself. Eleven specimens for each material were used for luminescence quantification, two specimens served as dye controls (no bacterial solution), and two specimens served as bacteria control (no dye).

Scanning electron microscopy

Three specimens of each material were used for scanning electron microscopy (SEM) verification. The specimens with the adhering fungi were rinsed in PBS, fixed with methanol, and air-dried. The test specimens were then mounted on aluminum stubs and sputter-coated with 99.99% gold (Provac, Balzers, Liechtenstein). Specimens were examined with a scanning electron microscope (magnification ×800 and ×1,700; Stereoscan 240; Cambridge Instruments, Cambridge, UK).

Statistical analysis

Continuous data were summarized by using medians and interquartile ranges (25th to 75th percentile). Global between-group comparisons were done by the Kruskal–Wallis test. The detection of differences between the test materials ($n=14$) and reference material glass was performed by the pairwise Mann–Whitney U test in combination with the Bonferroni adjustment (two-sided $\alpha=0.003$). Spearman's rank correlation coefficients were calculated to assess correlations between the variables relative luminescence intensity, surface roughness, and hydrophobicity. Calculations were done using statistical software SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). For power calculation, relative effects for the 14 pairwise comparisons have been calculated with SAS (SAS Institute, Cany, NY, USA). The power calculation for the Wilcoxon (Mann–Whitney) rank-sum test was performed by using nQuery Advisor 7.0 (Statistical Solutions, Saugus, MA, USA).

Results

Surface roughness and hydrophobicity

All obtained surface roughness and contact angle values are displayed in Table 1. The Kruskal–Wallis test revealed

significant differences between the assessed materials ($p<0.001$ for both, surface roughness and contact angles). Median surface roughness values ranged between 0.04 and 0.23 μm. Except for Grandio, all roughness values were below 0.2 μm and therefore termed as smooth. Glass showed the significantly lowest surface roughness values ($p<0.001$ for all tests).

Contact angle measurements with deionized water ranged between 65.2 and 86.9°. Glass revealed significantly lower contact angles (meaning lower hydrophobicity) than all test materials ($p<0.001$), except for Filtek Supreme XT ($p=0.173$).

Quantification of adhering fungi

Figure 1 and Table 1 show the comparative adherence of *C. albicans* displayed as relative luminescence intensities of all test and reference materials. In general, statistically significant differences have been found after the Kruskal–Wallis rank analysis of variance ($p<0.001$).

Median luminescence intensities between 19 and 553 (no units) have been recorded. Compomer Compoglass F revealed the lowest quantity of luminescence (median luminescence value, 19) of all materials with statistically lower luminescence quantities than reference material glass ($p<0.001$). The second compomer Dyract eXtra (68) and the ormocer Admira (43) showed low luminescence intensities with no significant differences ($p=0.935/0.415$) to the reference material glass (103). The highest median luminescence intensity, significantly higher than reference glass ($p<0.001$), was found for Filtek Supreme XT (553). Tetric EvoCeram (502), Enamel plus HFO (440), Venus (376), CeramX (311), Filtek Z250 (296), InTen-S (249), Heliomolar (213), Filtek Silorane (201), Arabesk Top (193), and Grandio (164) revealed significantly higher luminescence quantities than glass ($p<0.002$ for all comparisons).

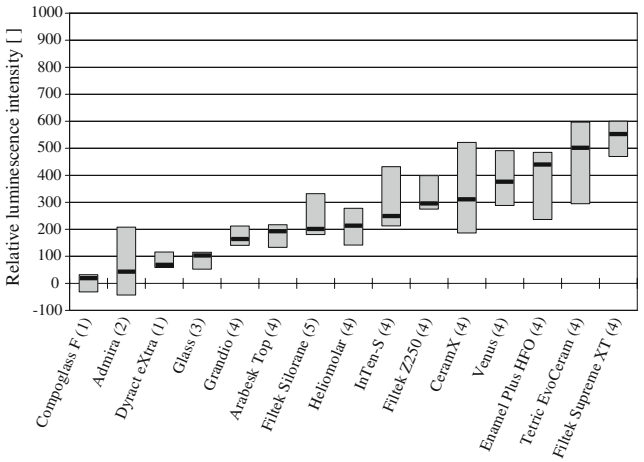

Fig. 1 Relative luminescence intensities (no unit) of two compomers (*1*), one ormocer (*2*), one reference materials (*3*), ten hybrid resin composites (*4*), and one novel silorane (*5*) (medians and 25/75 percentiles)

Spearman's rho coefficients (SR) were calculated to detect possible correlations between the assessed surface properties (surface roughness and hydrophobicity) and the quantity of bacterial adhesion. No significant correlations were found between luminescence and surface roughness (SR=0.064), between luminescence and contact angles (SR=0.064), and between surface roughness and contact angles (SR=0.105). Using 11 samples for each material and accepting a two-sided type I error of 5% for each comparison with the reference material, we would achieve 80% power to detect at least a relative effect of 0.862. Using an adjusted α-level of 0.003, relative effects of 0.988 could be detected with 80% power in our study.

Scanning electron microscopy

Within the scanning electron micrograph examination, a fine *C. albicans* mono-species monolayer was found on the surfaces of all examined materials. The number of adhering microorganisms varied on different locations on the same specimen and among the different materials. Dense colonies of oval and round blastospore colonies dominated the fungal biofilm. These blastospores appeared in different budding stages from 2 to 5 μm. (Fig. 2a, b).

Discussion

This study aimed to compare the susceptibility of 14 commonly used composite resin materials and reference material glass to adhere *Candida albicans*. Secondly, differences in the amount of adhering fungi should be related to surface roughness, hydrophobicity, and the type of matrix. Although other species are known to be involved, *C. albicans* is the major microbiological factor in oral candidosis [1, 3]. It was reported that different closely related fungal species and even different isolates have different adhesion potentials to acrylic surfaces [38]. As we preferred to provide a test model as simple as possible in which fungus-specific influences should be excluded, only one type culture strain of *C. albicans* was used.

The presence of an acquired pellicle is known to influence the complex process of fungal adhesion to substrata [27, 30, 36], but there is conflicting evidence if salivary coating reduces or enhances the adhesion of *C. albicans* [11, 13, 22, 31, 33]. Pellicle coatings in adhesion testing make interpretation of results even more complicating, and as we tried to cut the influences on fungal adhesion to material characteristics only, no acquired pellicle was

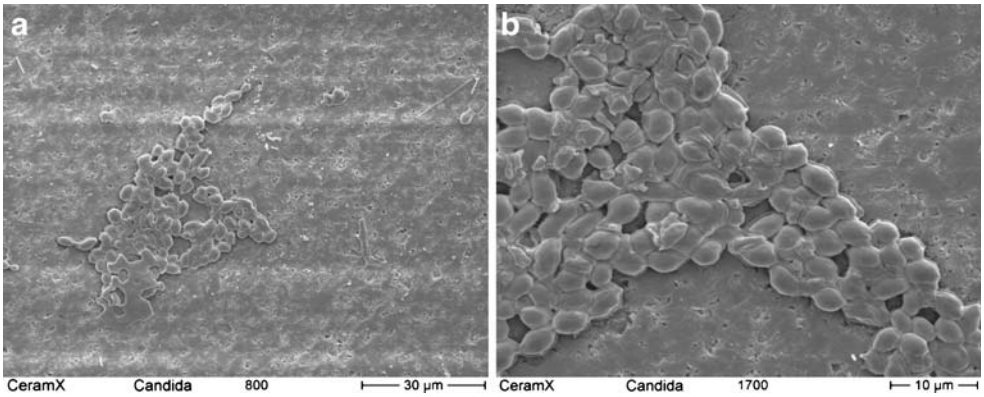

Fig. 2 Scanning electron micrographs of *C. albicans* monolayer to CeramX; magnification ×800 (**a**) and ×1,700 (**b**)

used in our assay. Additionally, it is not adequate to reduce the difference of the in vivo and the in vitro situation to the presence of saliva because innumerable other factors, like further co-adhering microorganisms, influence the fungal adhesion in the human oral cavity [12]. In the past, quantification of adherent fungi has been performed with a variety of *Candida* strains, different growth conditions, concentrations, and methods of analysis, and therefore, a comparison of the results is difficult [7, 39, 44, 45].

Studies concerning the adhesion properties of *C. albicans* have mainly been focused on denture base and relining materials [24, 27, 28, 32, 33, 44, 45]. Despite their increasing use in clinical dentistry and although they are known to be a potential source of fungal infections, fewer investigations have been carried out on composite resin materials [13, 22, 36, 44]. In general, to ensure the clinical relevance of microbiological adhesion studies, materials should be used that are appropriate to clinical dentistry [33]. To our knowledge, the current work is the first to compare differences in fungal adhesion to such a high number (n=14) of different composite resin materials.

In the present study, the Via Light kit for the quantification of viable fungal cells was used. This simple, reproducible, and precise in vitro bioluminometric test assay is based on the determination of cellular ATP, which is present in all metabolically active cells. The use of ATP luminescence as a measure of cell viability and quantity is accepted as reproducible and highly sensitive in literature [8, 10]. In agreement with previous studies, our results from the SEM investigations (see Fig. 2a and b) confirm that *C. albicans* can adhere directly to the surface of different composite resins [18, 22, 41]. As we intended to investigate the early steps of fungal colonization, which is the initial adhesion, a relative short incubation time of 2.5 h was chosen. At this point, a fungal monolayer, which is characteristic for the initial fungal adhesion could be observed on all specimens. A reduction of the incubation time would result in the presence of a few isolated, single fungal cells on the substratum, whereas longer incubation would lead to more developed multilayer biofilms.

The luminometric adhesion test revealed statistically significant amounts of adhering *C. albicans* on the assessed material surfaces. Except for the compomer Compoglass F, none of the tested composite materials exhibited antifungal properties compared to reference material glass. In fact, most test materials showed increased potentials to adhere *C. albicans*. There have been relatively high variances in measured luminescence (25/75 percentiles) on different specimens of the same material, which was also found by other authors who also reported large standard deviations [28, 39, 44, 45].

The amount of adhering microorganisms on composite resins is governed by various material characteristics like surface roughness, surface hydrophobicity, electrostatic forces, composition of the material, type of matrix, size of fillers, and configuration of fillers [11, 18, 19, 24, 25, 30, 31, 33, 39]. Surface roughness is well documented to have a crucial influence on microbial adhesion [2]. Higher numbers of *C. albicans* are found on rough surfaces than on polished, smooth surfaces [47]. The most widely used parameter to describe the roughness of a specific surface is the arithmetic average peak-to-valley value R_a [33, 34]. Below an R_a limit of 0.2 µm, no further influence on the quantity of adhesion should be expected [2]. All tested materials, except for Grandio (R_a=0.23 µm), ranged significantly below 0.2 µm, and therefore, roughness was not considered as a variable for these materials. Consequently, the statistical correlation analysis between surface roughness and relative luminescence (SR=0.105) did not prove a significant correlation in our study.

There are contrary reports regarding the role of the surface hydrophobicity on fungal adhesion [15, 18, 27, 31, 32, 34, 39, 45], but it becomes apparent that there is higher adherence of bacteria or fungi to substrata with a hydrophobicity close to that of the specific microorganism [24, 45]. *C. albicans* is reported to be rather hydrophilic with water contact angles between 23 and 51° [18, 24, 25]. Consequently, relative hydrophilic composite surfaces with lower contact angles should be more prone to *Candida* adhesion than hydrophobic composite resin materials. The

findings of the present studies are not consistent with this theory, as there is no significant correlation (SR=0.064) between hydrophobicity and the amount of adhering *C. albicans*. Although there have been statistically significant differences between the tested composite resin materials for contact angles, these variances were possibly too small to influence the quantity of adhering fungi.

In the current study, four different types of composite resin materials (one ormocer, two compomers, one novel silorane, and ten conventional hybrid composites) were compared according to their susceptibility to adhere *C. albicans*. Because of the hydrophobic nature of the siloxane backbone, Weinmann et al. [46] assumed a low susceptibility to adhere oral microorganisms. In the present study, the novel silorane-based composite material Filtek Silorane had comparable adhesion values to *C. albicans* than the low adhesion conventional hybrid materials. The two compomers Compoglass F and Dyract eXtra and the ormocer Grandio showed lower fungal adhesion quantities than reference material glass. These important findings confirm the hypothesis that different types of material and their specific chemistry and composition (matrix and fillers) considerably interfere with *C. albicans* adherence [6, 7, 37, 39]. The low quantity of adhering *C. albicans* on compomers in this investigation may be due to the fact that compomers are known to release significant quantities of fluoride, and fluoride components are associated with antimicrobial effects [16, 35]. As fluoride release was not measured in this investigation, such conclusions must be regarded as rather speculative.

The best strategy for preventing the accumulation of pathogenic biofilms and therewith infectious diseases like oral candidosis is to reduce initial microbial adhesion [13, 15]. Several promising inventions have been introduced, like increasing surface hydrophobicity, modifying surfaces with Teflon, salivary protein histatin or 2-methacryloyloxyethyl phosphorylcholine, copolymerizing of methacrylic acid, and adding disinfectant components [12, 15, 31, 46]. As the etiology of *Candida*-associated stomatitis is multifactorial with numerous influencing parameters, a better understanding of the essentials of fungal adhesion and true-to-life in vitro methods to study these adhesion processes are 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 there is a only a limited amount of parameters that can be simulated outside the oral cavity.

Considering the limitations of this study, a significant correlation between surface roughness, hydrophobicity, and the amount of adhering *C. albicans* was not found. In contrast to that, the adhesion potential of composite resin materials seems to differ considerably depending on the type of matrix of the composite. The compomers Compoglass F and Dyract eXtra and the ormocer Admira revealed lower amounts of adhering fungi than all assessed conventional hybrid composites and a novel silorane-based restorative. Applied to the clinical setting, compomers and ormocers might be superior in patients who are prone to oral candidosis, like denture wearers, medically compromised patients under immunosuppression, and tumor patients being treated with chemotherapy or radiation.

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