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Effects of Surface Characteristics of Conventionally Manufactured, CAD/CAM Milled, and 3D-Printed Interim Materials on Adherence of Streptococcus Mutans and Candida Albicans

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ABSTRACT

Objectives: The purpose of this *in vitro* study was to compare conventionally manufactured, CAD/CAM milled, and 3D-printed interim materials based on their susceptibility to adherence of *Streptococcus mutans* and *Candida albicans*, and examine the influence of surface roughness and hydrophobicity.

Materials and Methods: Eighty disc-shaped specimens fabricated from autopolymerized polymethyl methacrylate (A-PMMA), bis-acryl composite (Bis-acrylate), CAD/CAM PMMA-based polymer (Milled-PMMA), and 3D-printed resin (Printed) were subjected to 10,000 thermal cycles (5-55 °C) and divided into two groups (n=10) according to microbial suspension used: *Streptococcus mutans* and *Candida albicans*. Surface roughness (Ra) and hydrophobicity (WCA) of specimens were measured. An adhesion test was performed by incubating the specimens in *Streptococcus mutans* and *Candida albicans* suspensions at 37 °C for 24 hours, and the adherent cells were evaluated by counting colony-forming units (CFU/ml). Scanning electron microscopy (SEM) was performed to analyze the surfaces (n=2). Data were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Spearman's correlation analysis was used to determine correlation among the measurements (α=.05).

Results: Type of restorative material significantly influenced Ra and WCA. The highest adhesion of *Streptococcus mutans* was observed in Printed, followed by Bis-acrylate, A-PMMA, and Milled-PMMA (p=.001). The highest adhesion of *Candida albicans* was noted on A-PMMA, followed by Printed, Bis-acrylate, and Milled-PMMA (r=.001). The adhesion of *Streptococcus mutans* (r=.660) and *Candida albicans* (r=.413) showed a positive correlation with Ra. A negative correlation was found between WCA of the materials and *Streptococcus mutans* adhesion (r= −.373).

Conclusions: Surface roughness plays an important role in the adherence of microorganisms. CAD/CAM PMMA-based polymers may be a better choice to reduce microbial adhesion in long-term use.

Key words: Interim material, microbial adhesion, surface properties.

Konvansiyonel, CAD/CAM Frezeleme ve 3D Baskı Yöntemleriyle Üretilmiş Geçici Materyallerin Yüzey Özelliklerinin *Streptococcus Mutans* ve *Candida Albicans* Tutunumuna Etkileri

Süreç

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ÖZ

Amaç: Bu çalışmanın amacı konvansiyonel, CAD/CAM frezeleme ve 3D baskı yöntemleriyle üretilmiş geçici materyallerinin *Streptococcus mutans* ve *Candida albicans* tutunumu duyarlılıklarına göre karşılaştırılması ve yüzey pürüzlülüğü ve hidrofobikliğin buna etkisinin incelenmesidir.

Gereç ve Yöntemler: Otopolimerize polimetil metakrilat (A-PMMA), bis-akril kompozit (Bis-acrylate), CAD/CAM PMMA-bazlı polimer (Milled-PMMA) ve 3D baskı (Printed) rezinlerinden seksen adet disk şeklinde örnek üretildi. Örneklere 10,000 termal siklus (5-55°C) uygulandı ve kullanılan mikrobiyal süspansiyonlara göre örnekler iki gruba (n=10) ayrıldı: *Streptococcus mutans* ve *Candida albicans*. Örneklerin yüzey pürüzlülüğü (Ra) ve hidrofobikliği (WCA) ölçüldü. Örnekler Streptococcus mutans ve Candida albicans süspansiyonlarında 37°C'de 24 saat inkübe edilerek tutunum testi yapıldı ve yapışık hücreler koloni oluşturan birimler (CFU/ml) sayılarak değerlendirildi. Yüzeyleri analiz etmek için taramalı elektron mikroskobu (SEM) görüntüleri alındı (n=2). Veriler Kruskal-Wallis ve Mann-Whitney U testi ile analiz edildi. Ölçümler arasındaki korelasyonu belirlemek için Spearman korelasyon analizi kullanıldı (α=.05).

Bulgular: Restoratif materyalin türü, Ra ve WCA'yı önemli ölçüde etkiledi. En yüksek *Streptococcus mutans* adezyonu Printed'de gözlemlenirken, ardından Bis-akrilat, A-PMMA ve Milled-PMMA'da gözlemlenmiştir (p=.001). En yüksek *Candida albicans* adezyonu A-PMMA'da kaydedilirken, ardından Printed, Bis-acrylate ve Milled-PMMA'da (r=.001) kaydedilmiştir. *Streptococcus mutans* (r=.660) ve *Candida albicans* (r=.413) tutunumu ve Ra arasında pozitif korelasyon gözlemlenmiştir. Örneklerin WCA'sı ile *Streptococcus mutans* tutunumu arasında negatif bir korelasyon bulunmuştur (r=-.373).

Sonuçlar: Yüzey pürüzlülüğü mikroorganizmaların tutunumunda önemli rol oynar. CAD/CAM PMMA bazlı polimerler, uzun süreli kullanımda mikrobiyal yapışmayı azaltmak için daha iyi bir seçim olabilir.

Anahtar Kelimeler: Geçici diş restorasyonu, mikroorganizma tutunumu, yüzey özellikleri.

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Introduction

Interim fixed prostheses have an essential role in maintaining the patient's normal masticatory functions, in protecting the prepared teeth and periodontal tissues.[1] The interim restorations may be used for a longer duration to assess the course of treatment, particularly in multidisciplinary reconstructions. Therefore, the interim restorative materials should have certain mechanical and esthetic properties and biological behaviors.[1,2]

Accumulation of microorganisms on the surface of restorative materials contributes to the occurrence of caries, gingival inflammation, and denture stomatitis which is an important aspect related to the longevity of restorations.[2] Previous studies have reported *Streptococcus mutans (S. mutans)* as the primary etiological bacteria in the pathogenesis of caries.[2,3] *Candida albicans (C. albicans)* is the most prevalent fungus in the oral cavity and has been considered the major pathogenic agent related to denture stomatitis.[4] The factors that influence the quantity of microbial adhesion and biofilm formation on restorative materials include the chemical composition of the material, oral hygiene, salivary components, and dietary habits. Surface characteristics of materials such as surface free energy, surface roughness (Ra), and hydrophobicity were also reported to significantly influence the adhesion of microorganisms.[5-10] Surface irregularities can promote initial accumulation and provide niches in which microorganisms are protected against hydrodynamic shear forces.[11,12] Furthermore, manufacturing methods may affect the surface characteristics and biological behaviors of interim restorations. However, the relationship between these factors and microbial adhesion and subsequent biofilm formation still remains controversial.

Conventionally manufactured resin materials such as polymethyl methacrylate (PMMA) and bis-acryl composite resins are routinely used for fabrication of interim fixed prostheses because of their easy processability and relatively low cost. However, these materials have some disadvantages such that their fabrication is time consuming and their quality is dependent on hand skills.[13] Computer-aided design and computer-aided manufacturing (CAD/CAM) processes are increasingly used in dentistry to overcome some of the drawbacks of the conventionally manufactured materials.[14] Because CAD/CAM PMMA-based resin blocks are polymerized with a high degree of conversion and have a highly cross-linked structure, the surface properties and biocompatibility of milled interim restorations are better than those obtained with the conventional methods.[15,16]

The three-dimensional (3D) printing method produces restorations by building up a solid object from powdered or liquid-based material in layers and is a relatively low-cost alternative among digitally fabricating interim restorations.[17] The technology of stereolithography is commonly used in 3D-printing methods for dental applications and manufactured interim materials are largely based on photosensitive resin material in this technique.[18] Although acceptable mechanical and optical properties have been reported, investigations on the microbial accumulation on 3D-printed interim materials are limited.[17,19-21] Therefore, the purpose of this study was to investigate the effects of surface roughness and hydrophobicity of conventionally manufactured, milled, and printed interim materials on microbial adhesion of *S. mutans* and *C. albicans* in the presence of saliva after thermocycling. The first null hypothesis was that no difference would be found among the investigated interim materials by means of the quantity of *S. mutans* and *C. albicans* adhesion. The second null hypothesis was that the quantity of *S. mutans* and *C. albicans* adhesion would not be influenced by the surface roughness and hydrophobicity values of investigated interim materials.

Material and Methods

Four different interim materials, autopolymerized polymethyl methacrylate resin (A-PMMA), bis-acryl composite resin (Bis-acrylate), CAD/CAM PMMA-based polymer (Milled-PMMA), and 3D printed resin (Printed) were prepared to be assessed for their surface roughness, surface hydrophobicity, and susceptibility to adherence of the microorganisms after 10,000 thermal cycles (Table 1). Determination of sample size was performed by using a statistical power analysis program (G*Power 3.1.9.3; Heinrich-Heine-Universität Düsseldorf) which determined that 10 specimens per group provided a power of 0.8 at the significance level of 0.05.

Table 1. Materials used

Abbreviation	Product name	Manufacturer	Type	Interim material fabrication technique	Lot number
A-PMMA	Imident	Imicryl Dental	Conventional PMMA	Conventional; self-curing	20B976
Bis-acrylate	PreVISION® Temp	Kulzer GmbH	Bis-acrylate composite resin	Conventional; self-curing	72007838
Milled-PMMA	PMMA Disc Multi	Sagemax Bioceramics Inc	Polymethyl methacrylate-based polymer	CAD/CAM	YB357C
Printed	Temporis	DWS	Light curable nanocomposite	3D-Printing; SLA	1921741

A-PMMA: autopolymerized polymethyl methacrylate resin; CAD/CAM: computer-aided design and computer-aided manufacturing; 3D-Printing: Three Dimensional-Printing; SLA: stereolithography.

A-PMMA (Imident, Imicryl Dental, Konya, Turkiye) and Bis-acrylate (PreVISION® Temp, Kulzer GmbH, Hanau, Germany) Ø10×2-mm specimens were prepared by using stainless-steel mold. PMMA was mixed manually with a spatula and packed into the mold (n=20). Bis-acryl composite resin was mixed automatically and injected into the mold by using a cartridge system (n=20). Specimens then were polymerized according to the manufacturer's recommendations and removed from the mold after 15 minutes. Milled-PMMA specimens were designed as standard tessellation language files in the system software program (Nauta XFAB Edition, DWS, Thiene, Italy), 10 mm in diameter, and milled by using a CAD/CAM milling machine (Yenadent CAM 5.1, Yenadent Ltd, Istanbul, Turkiye) from CAD/CAM PMMA blocks (PMMA Disc Multi, Sagemax Bioceramics Inc, WA, USA). The specimens then were cut under water cooling to procure disk-shaped specimens 2 ±0.02 mm in thickness (n=20).

A virtual disk plate design (Ø10×2 mm) was performed by using the same software program and saved as a standard tessellation language file. The Printed specimens were fabricated by using an SLA-based 3D-printer machine (XFAB 2500PD, DWS, Thiene, Italy) with a galvanometer laser scanning technique from a composite resin material (Temporis, DWS, Thiene, Italy) according to the saved design (n=20). The thickness of the layer was 0.06 mm and the laser scanning speed was 5000 mm/sec. All printed specimens were cleaned with isopropyl alcohol for 1 minute and post polymerization procedures were completed in an ultraviolet curing unit (S2, DWS, Thiene, Italy) for 30 minutes.

After storage in 37 ºC distilled water for 24 hours, both sides of all specimens were trimmed and smoothed in an automatic polisher (LaboPol-20, Struers, Cleveland, OH, USA) with 500-, 800- 1000-, and 1200-grain sandpaper (SiC Foils, Struers, Cleveland, OH, USA), under water-cooling for 15 seconds for each. All specimens underwent additional polishing with a diamond solution on a felt disc (Struers, Cleveland, OH, USA) and were ultrasonically cleaned in distilled water for 5 minutes. Then all specimens were subjected to 10,000 thermocycles between 5 °C and 55 °C with a transport time for 15 seconds and a dwell time for 30 seconds by using a thermal cycler (MTE-101, Mod dental, Ankara, Turkiye) in distilled water to simulate 1-year clinical aging process.[22]

After thermocycling, the Ra values for each specimen were measured with a profilometer (MarSurf PS10, Mahr GmbH, Göttingen, Germany). The diamond stylus of profilometer (10 µm diameter) was moved across the surface under constant pressure and performed three measurements (cut-off: 0.8 mm; speed: 0.5 mm/sec) at different surface locations of each specimen, after which mean Ra values were calculated to obtain the general roughness profile of each specimen.

The surface hydrophobicity of all specimens was evaluated by using the static contact angle method with an automated contact angle measurement device (Attension Theta Flex, Biolin Scientific, Sweden) equipped with a digital camera and image analysis software (One Attension; 2.6 version). Specimens were cleaned with acetone and air dried. The water contact angle (WCA) values of all materials were obtained by dispensing a droplet (2 µL) of distilled water onto the specimens with 2 measurements for each droplet (right and left contact angle) at 20°C room temperature. Three WCA measurements were made from different areas of the surface for each specimen and then the average values were calculated.

To simulate the formation of an acquired salivary pellicle, unstimulated whole saliva was collected from two healthy volunteers after receiving their informed consent, as required by the Local Ethical Committee for Research (protocol 96/2021).[24] Volunteers refrained from oral hygiene for 24 hours, had no active dental disease, and did not have antibiotic therapy for at least three months before the experiments. All specimens were horizontally placed in presterilized 24-well plates, then were covered with 2 mL sterile human saliva prepared according to Baffone et al.[23] and incubated by shaking at 37 °C for 1 hour. Thereafter, the specimens were washed with 5 ml of saline and placed into the sterilized petri dishes.

Before biofilm adhesion test, all specimens were cleaned with an ultrasonic cleaner for 15 minutes and then disinfected with 70% alcohol, and ultrasonically cleaned with distilled sterile water for 15 minutes to remove any contaminants and residues from the surface, and then each material group was further divided into two subgroups based on the microbial suspensions used, with ten samples in each subgroup within the respective material group (n=10): *S. mutans* NCTC 10449 and *C. albicans* ATCC 10231 that were provided from a local laboratory (Near East University, Faculty of Medicine, Department of Medical Microbiology).

S. mutans obtained from stock were plated onto blood agar and incubated at 37°C in a 10% CO_2 atmosphere for 24 hours. Then transferred into tubes containing 5 ml of BHI and incubated at 37°C in a 10% CO_2 atmosphere for 18 hours. The tube contents were mixed using a centrifuge for 5 minutes. *S. mutans* suspensions were concentrated as $1.5x10^8$ bacteria/ml spectrophotometrically. The mixture of the *S. mutans* suspensions was applied to each specimen surface, and the *S. mutans* adhesion was provided for 15 minutes to the pellicle layer. BHI with 5% sucrose was added to each petri dish to cover all specimens, and dishes were placed into an incubator at 37°C in a 5% CO_2 atmosphere for 24 hours. The specimens were then placed into tubes containing 2 ml of PBS and mixed with a centrifuge for 30 seconds to separate the free *S. mutans*. After the incubation period, colony-forming units (CFU/ml) were determined and recorded for *S. mutans* for all groups.

C. albicans obtained from stock were plated onto SDA agar and incubated at 37°C in a 10% CO_2 atmosphere for 24-48 hours. Then transferred into tubes containing 5 ml of BHI and incubated at 37°C in a 10% CO_2 atmosphere for 24 hours. The tube contents were mixed using a centrifuge for 5 minutes. *C. albicans* suspensions were concentrated as $1.5x10^8$ yeast/ml spectrophotometrically. The mixture of the *C. albicans* suspensions was applied to each specimen surface, and the *C. albicans* adhesion was provided for 15 minutes to the pellicle layer. BHI with 5% sucrose was added to each petri dish to cover all specimens, and dishes were

placed into an incubator at 37°C in a 5% CO_2 atmosphere for 48 hours. The specimens were then placed into tubes containing 2 ml of PBS and mixed with a centrifuge for 30 seconds to separate the free *C. albicans*. After the incubation period, colony-forming units (CFU/ml) were determined and recorded for *C. albicans* for all groups.

For additional SEM evaluation, two specimens were randomly selected from each tested group (n=2), fixed for 1 h in 2.5% glutaraldehyde, dehydrated in increasing series of ethyl alcohol baths (10%, 25%, 50%, 75%, and 90% for 20 min and 100% for 1-h), and then dried overnight in a bacteriological incubator at 37°C. Then, specimens were coated with gold and evaluated under an SEM (Zeiss EVO 40, Carl Zeiss SMT, Cambridge, UK) at 20 kV, with magnifications of 1000× and 5000×. All images were examined by one observer.

Statistical analyses were performed by using a software program (Number Cruncher Statistical System 2007; NCSS Statistical Software). Descriptive statistical methods (mean, standard deviation [SD], median, minimum, maximum) and distribution of data were evaluated by the Shapiro-Wilk test. Because the data were non-normally distributed, the Kruskal-Wallis test was used to investigate the statistical difference between groups. The Mann-Whitney U test was then used to compare group pairs. Spearman's correlation analysis was used to determine correlation among the measurements (α=.05).

Results

The median, minimum, and maximum values of Ra and WCA for all tested interim materials are listed in Table 2. The Kruskal-Wallis test showed significant differences in the Ra and WCA results among groups (p<.05). The Ra values (0.21 to 0.83 µm) for all groups were higher than the plaque accumulation threshold (0.20 µm). The Mann-Whitney U test revealed the highest median Ra values for, Printed (0.61, p=.001) followed by A-PMMA (0.53, p=.001), Bis-acrylate (0.50, p=.001), and milled-PMMA (0.42, p=.001). The WCA values of all materials ranged between 71.15 and 101.65 degrees, so all had hydrophobic surfaces. The Mann-Whitney U test revealed the highest median WCA values for Milled-PMMA (93.38, p=.001), followed by A-PMMA (88.32, p=.001), Printed (84.94, p=.001), and Bis-acrylate (78.43, p=.001).

The median CFU/ml values for each group are presented in Figure 1. The Kruskal-Wallis test showed significant differences in the *S. mutans* adhesion results among groups (p<.05). The Mann-Whitney U test revealed the highest median CFU/ml values for Printed (220×10^6, p=.001), followed by Bis-acrylate (175×10^6, p=.001), A-PMMA (153×10^6, p=.001), and milled-PMMA (95×10^6, p=.001). The Kruskal-Wallis test showed significant differences in the *C. albicans* adhesion results among groups (p<.05). The Mann-Whitney U test revealed the highest median CFU/ml values for A-PMMA (275×10^6, p=.001), followed by Printed (200×10^6, p=.001), Bis-acrylate (150×10^6, p=.001), and Milled-PMMA (125×10^6, p=.001). As per the Mann-Whitney U test *S. mutans* adhesion was significantly higher than *C. albicans* adhesion in groups Bis-acrylate and Printed, whereas it was significantly less in groups A-PMMA and Milled-PMMA (p=.001). The representative SEM images of the S. mutans and C. albicans biofilm formation on the surface of the specimens are shown in Figure 2-3.

As per Spearman's correlation analysis, a statistically significant positive relationship was found between *S. mutans* adhesion and Ra values (r=.660, p=.000), and between *C. albicans* adhesion and Ra values (r=.413, p=.008). However, *S. mutans* adhesion showed a negative relationship with WCA values (r= −.373, p=.018), whereas the *C. albicans* adhesion did not show any significant relationship with the WCA values (r= −.133, p=.412).

Table 2. Median, minimum, and maximum surface roughness and water contact angle values for tested materials

	Surface roughness (µm)		Water contact angle (°)	
Groups	Median	Min/Max	Median	Min/Max
A-PMMA	0.53[a]	0.49/0.82	88.32[a]	79.6/99.69
Bis-acrylate	0.50[b]	0.43/0.58	78.43[b]	71.15/94.18
Milled-PMMA	0.42[c]	0.21/0.52	93.38[c]	79.31/101.65
Printed	0.61[d]	0.53/0.83	84.94[d]	75.35/94.08

A-PMMA: autopolymerized polymethyl methacrylate; Bis-acrylate: bis-acryl composite resin; Milled-PMMA: CAD-CAM PMMA resin; Printed: 3D-printed resin. Mean difference significant at p<.05. Means with same letters not statistically different.

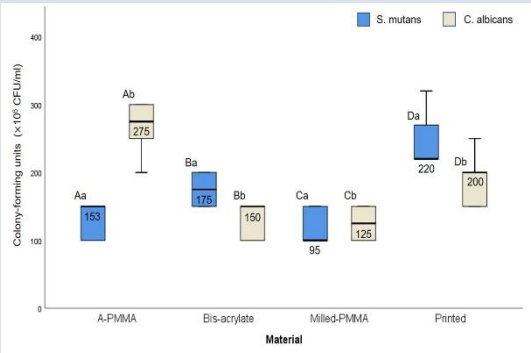

Figure 1. Box plot for colony-forming unit values of tested groups. Group codes as shown in

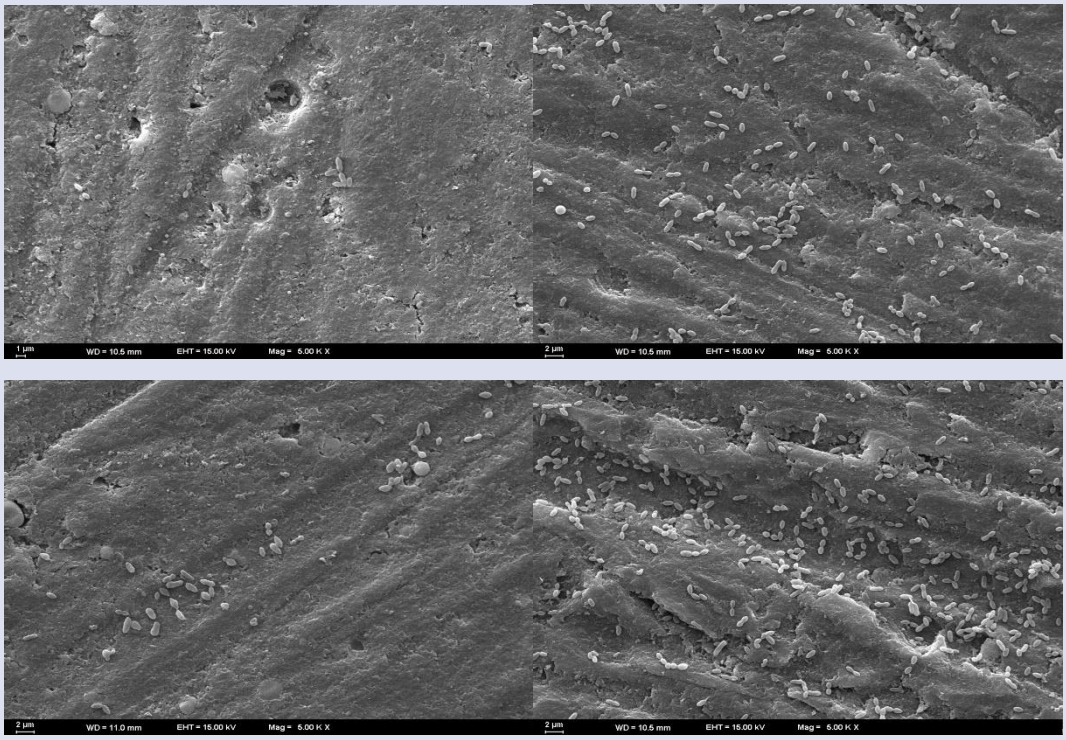

Figure 2. Representative scanning electron microscopy (SEM) images (5000×) of *S. mutans* adhesion on the specimens. A, A-PMMA. B, Bis-acrylate. C, Milled-PMMA. D, Printed

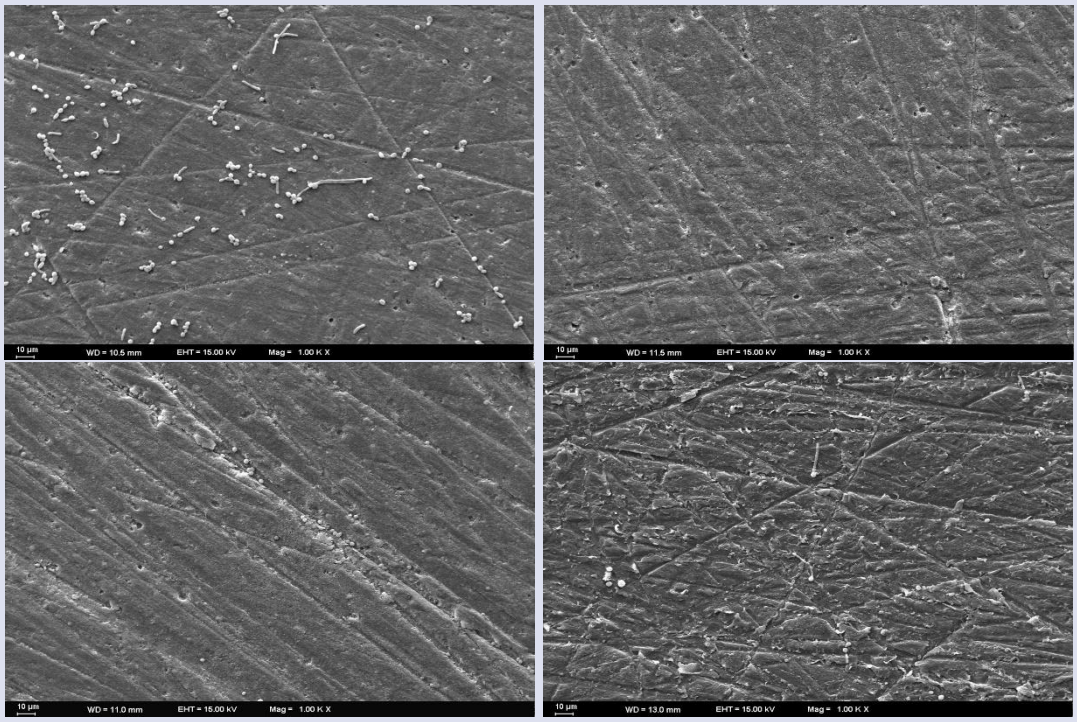

Figure 3. Representative scanning electron microscopy (SEM) images (1000×) of *C. albicans* adhesion on the specimens. A, A-PMMA. B, Bis-acrylate. C, Milled-PMMA. D, Printed.

Discussion

Based on the results of the current study, the first null hypothesis was rejected, as conventionally manufactured, CAD/CAM milled, and 3D-printed interim materials exhibited different susceptibility to adhere to *S. mutans* and *C. albicans*; the second null hypothesis was partially rejected, as correlations were observed among some of the tested parameters.

For restoration conditions in the oral environment to be simulated, all interim materials were submitted to thermocycling which mainly consists of water immersion and temperature change under standardized laboratory conditions, and after measurement of roughness, the hydrophobicity of all interim materials was evaluated.[22] The static contact angle method is an established method for obtaining hydrophobicity values of certain substrates.[6] In principle, the hydrophobic materials exhibit a higher water contact angle. Therefore, the sessile drop method, which involves measuring WCA, was used in the present study to evaluate the hydrophobicity of materials.[25] SEM observation is especially suited for the microscopic characterization of the surface topography and biofilm adhesion.[26] In the current study, scanning electron micrographs were used for supplementary verification of the results obtained from roughness and biofilm adhesion measurements.

Several researchers suggested that microbial adhesion is related to the components and compositions of materials.[11,27,28] Consistent with previous reports, [11,27,28] in present study, the quantity of adherent *S. mutans* and *C. albicans* cells varied depending on tested materials. Furthermore, higher amount of adherent *S. mutans* cells were observed in Bis-acrylate and Printed, which are composite-based materials, whereas higher amount of adherent *C. albicans* cells were observed in groups A-PMMA and Milled-PMMA, which are PMMA-based materials. In line with our study result, Ozel et al.[29] reported different susceptibilities of resin-based interim materials to the adherence of *S. mutans* and *C. albicans*. This may be related to the species-specific characteristics of different microorganisms, such as the cell surface protein antigen SpaP of *S. mutans* or the adhesion protein Hyphal wall protein 1 of *C. albicans*, which are associated with the initial adhesion of microorganisms to the surface, possibly resulting in the different sensitivities to materials.[30-33] However, a precise comparison concerning the interaction between bacterial adhesion and composition of the materials is not possible as manufacturers do not provide adequate information about the exact compositions of the materials.[17]

Surface roughness is generally regarded as an important surface property that affects microbial adhesion to restorative materials in the oral environment.[24,27,28,34,35] The current study found a significant, positive correlation between the Ra values and the quantity of adhesion *S. mutans* and *C. albicans* cells on the materials consistent with previous reports.[6,7,24,27,34,35] However, results of present study are in disagreement with other studies, where no correlation was observed between surface roughness of the resins and microbial adhesion.[36-38] In the present study, the lowest Ra values were observed on Milled-PMMA (0.42 µm). Additionally, the adhesion of *S. mutans* and *C. albicans* was found to be the least on Milled-PMMA, independent of the chemical composition and hydrophobicity of the materials. These findings supported that when Ra was above the threshold (Ra=0.2 µm), different susceptibilities to microbial adherence are primarily caused by variable Ra values. Corroborating the results of the current study, microscopic investigations reveal that the pits and fissures in substrata are responsible for initial adhesion of microorganisms.[12]

In the present study, Printed materials showed higher Ra than Bis-acrylate and Milled-CAD/CAM. These results are inconsistent with those of the previous study, which demonstrated that the Ra of bis-acryl and printed resin materials were similar and the Ra of 3D-printed resins were lower than that of conventionally fabricated and CAD/CAM milled interim materials.[39] These differences can also be related to the measurement of Ra at different aging periods. The layered nature of 3D-printing technology may initiate crack propagation between the layers due to the residual stress from temperature changes and result in increased Ra of the printed material.[17]

Hydrophobicity is another factor that has an influence on microbial adhesion and biofilm formation on material surfaces.[7] In the present study, Milled-PMMA showed a significantly higher WCA, and consequently higher hydrophobicity when compared to A-PMMA. This can be attributed to higher rates of residual components in A-PMMA which enhance its hydrophilic properties and to the reduced hydrolytic degradation processes in Milled-PMMA.[11,40] The lower WCA of Bis-acrylate and Printed groups in the present study confirmed that the composite-based resins are more hydrophilic and polar than PMMA-based resins. However, surface roughness may also affect the contact angle measurements, especially when Ra > 0.1 mm.[5,25] Therefore, it should be noted that dominant roughness values of the present *in vitro* study may obscure the effects of hydrophobicity on microbial adhesion.

The present findings showed a weak negative correlation between the hydrophobicity and *S. mutans* adhesion, which were in agreement with previous studies suggesting that the adhesion strength of *S. mutans* to hydrophobic surfaces was weaker than that to hydrophilic surfaces.[6,29] The published literature revealed different effects of the hydrophobicity on the adhesion of *C. albicans* to material surfaces. Several previous investigations[7-10] have indicated that increased hydrophobicity can lead to both higher and lower adhesion of *C albicans*; however, in the present study, surface hydrophobicity seemed to have no direct influence on the adhesion of *C albicans*, which is in agreement with other studies that found no correlation between hydrophobicity and Candida colonization.[38,41] Therefore, it is possible that the presence of an acquired

salivary pellicle has a homogenizing effect and masks originally distinct differences in material surface properties, which may explain the contradictory results in the literature.[41,42]

Limitations of the present study lie in its *in vitro* design, which did not fully imitate the complex and multifactorial process of the microbial adhesion mechanism. The biofilm formation is affected by the presence of other microorganisms in the dynamic environment of the oral cavity. It should also be noted that the design of the present *in vitro* study included the formation of monospecies *S. mutans* and *C. albicans* biofilm. Additionally, although saliva-coated specimens were evaluated in the present study, individual differences in saliva affect surface properties and microbial adhesion of the materials. Further clinical studies by using multispecies biofilm models on different brand materials in different incubation periods are needed.

Conclusions

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1. The type of restoration material significantly influenced the surface roughness, hydrophobicity, and susceptibility of the tested interim materials to adhere to *Streptococcus mutans* and *Candida albicans*.

2. A significant positive correlation was established between the surface roughness of tested materials and the quantity of adhesion of *Streptococcus mutans* and *Candida albicans* cells on the materials.

3. A negative correlation was found between water contact angle values of the tested materials and the quantity of adhesion of *Streptococcus mutans* cells, whereas no correlation was found between hydrophobicity of the tested materials and the quantity of adhesion of *Candida albicans.*

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Conflicts of Interest Statement

The authors do not have any financial interest in the companies whose materials are included in this article.

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