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Adhesion of *Candida albicans* and *Streptococcus mutans* to the Surface of a 3D-Printed Resin Used for the Fabrication of Prosthetic Bases

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Abstract

Background: Surface roughness and microbial adhesion are key determinants of the biological performance of prosthetic base resins. This study evaluated *Candida albicans* (ATCC 11225) and *Streptococcus mutans* (DSM 20523) adhesion to a conventional heat-polymerized acrylic resin (Probase Hot) and a 3D-printed resin (V-Print dentbase) fabricated at 0° and 90° build orientations. **Methods**: Fifteen disc-shaped specimens (10 mm × 2 mm) were polished using a standardized protocol and surface roughness was characterized by contact profilometry (Ra). After UV sterilization, specimens were incubated for 24 h with standardized suspensions of *C. albicans* or *S. mutans*. Microbial adhesion was assessed qualitatively by scanning electron microscopy (SEM). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test after confirmation of normality (Shapiro–Wilk) and homogeneity of variances (Levene). Statistical significance was set at $p < 0.05$, and Cohen's d was calculated to estimate effect size. **Results**: Surface roughness differed significantly among fabrication conditions ($p = 0.017$), with the conventional heat-polymerized resin showing the highest Ra values and the 3D-printed resin fabricated at 90° presenting the lowest roughness. SEM observations suggested a similar adhesion pattern for both *C. albicans* and *S. mutans*, with greater apparent microbial presence on 90°-printed specimens, followed by the conventional heat-polymerized resin, whereas 0°-printed specimens showed lower apparent adhesion in the analyzed fields. **Conclusions**: These findings suggest that printing orientation may influence surface-associated microbial adhesion patterns in 3D-printed prosthetic resins; however, further studies with larger sample sizes, orientation-specific surface characterization, and mono- and multispecies biofilm models are warranted to confirm these preliminary findings.

Keywords: *Candida albicans*; *Streptococcus mutans*; 3D; denture base resin; surface roughness; microbial adhesion; printing orientation; scanning electron microscopy; PMMA

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1. Introduction

Clinical performance of dental materials is strongly influenced by their intrinsic properties, which are determined by their composition and manufacturing processes [1,2]. Based on composition, polymerization method, and commercial formulation, different types of resins can be used for the fabrication of prosthetic bases [3]. Some studies have compared these materials after prolonged exposure to the oral environment, particularly regarding color stability, wear resistance, and susceptibility to microbial adhesion. Conventional heat-polymerized polymethyl methacrylate (PMMA) remains the reference material for denture base fabrication because of its well-established mechanical performance, hardness, dimensional stability, and long-term clinical behavior. Consequently, it is frequently used as the benchmark for evaluating the properties of digitally manufactured denture base resins [1,2].

In recent years, the integration of digital technologies into dentistry has significantly improved treatment planning and enhanced the predictability of clinical outcomes. Among these technologies, three-dimensional (3D) printing has emerged as a promising manufacturing approach. This technique enables the fabrication of prosthetic rehabilitations with complex geometries, allows the use of advanced biomaterials, and facilitates the production of customized structures while reducing the number of clinical appointments required. However, the manufacturing process itself may influence the final properties of printed restorations. In particular, printing orientation has emerged as an important processing variable because it determines the direction of layer deposition and may affect flexural strength, dimensional accuracy, surface roughness, degree of conversion, hardness, water sorption, and, potentially, microbial adhesion. Although several studies have investigated these effects, the optimal printing orientation remains inconsistent across different resin systems, printers, and post-processing workflows, suggesting that orientation-dependent behavior is material-specific rather than universal [4–6].

Porosity and surface roughness are critical properties that directly influence the aesthetic, physical, mechanical, and biological performance of dental materials [2]. Surface roughness is particularly relevant for additively manufactured prostheses because orientation-dependent layer deposition may generate distinct surface topographies even after standardized finishing procedures. These topographical differences are clinically important, as rougher denture surfaces have been associated with increased plaque accumulation and greater susceptibility to microbial colonization, particularly by *Candida albicans* [2,7–9]. In addition to fungal colonization, bacterial adhesion is also highly relevant for the biological performance of prosthetic base materials. Streptococcus mutans is one of the main early colonizers of oral surfaces and plays a central role in the development of cariogenic biofilms due to its ability to adhere to dental and biomaterial surfaces, produce extracellular polysaccharides, and tolerate acidic environments. Although *S. mutans* is classically associated with dental caries, its adhesion to prosthetic and restorative materials is clinically relevant because these surfaces may act as microbial reservoirs and contribute to biofilm accumulation in the oral cavity. Therefore, evaluating the adhesion of both *C. albicans* and *S. mutans* provides a broader assessment of the microbial susceptibility of denture base materials, including both fungal and bacterial components of oral biofilms [9–12].

Despite the increasing adoption of digital manufacturing technologies, the interaction between CAD/CAM 3D-printed resins and oral microorganisms remains insufficiently explored. In particular, the effect of printing orientation on surface topography and consequent microbial adhesion has received limited attention. Among the different build orientations reported for additively manufactured denture base resins, 0° and 90° represent the two extreme fabrication configurations and are the most frequently investigated in the literature. Printing orientation determines the direction of layer stacking during fabrication

and may result in distinct surface micro-irregularities depending on the angle used, potentially influencing fungal and bacterial adhesion behavior [6,13]. Therefore, further studies are required to better understand the biological performance of these emerging materials under different manufacturing conditions, particularly regarding their susceptibility to adhesion by clinically relevant oral microorganisms such as *C. albicans* and *S. mutans*.

2. Materials and Methods

This study is an in vitro experimental investigation in which the adhesion of *C. albicans* was evaluated by SEM on two different types of resins, both commonly used in the fabrication of denture bases: a heat-polymerized acrylic resin (Probase Hot, Ivoclar Vivadent, Schaan, Liechtenstein) and a 3D printing resin (V-Print dentbase, VOCO GmbH, Cuxhaven, Germany).

2.1. Specimens Preparation

Specimens (n = 5 per group) were fabricated using a mold designed in CAD software SOLIDWORKS® 2025 (Dassault Systèmes SOLIDWORKS Corp., Waltham, MA, USA) and produced via FDM 3D printing (Ultimaker 3) using polylactic acid (PLA). All specimens were disc-shaped (diameter: 10 mm; thickness: 2 mm) (Figure 1). Heat-polymerized acrylic resin was prepared according to the manufacturer's instructions, placed into the mold, and polymerized under pressure at 55 °C for 30 min, followed by cooling in water. 3D printed resin was exported as STL files, and fabricated using a DLP 3D printer (Asiga Max UV, ASIGA, Erfurt, Germany) at 0° and 90° orientations. After printing, specimens were ultrasonically cleaned in isopropyl alcohol (2 min × 2 cycles) and post-cured under UV light (280–580 nm). Subsequently, specimens were stored in sterile, sealed, and labeled packaging until further processing.

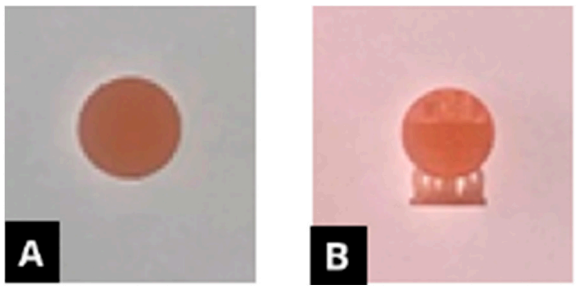

Figure 1. (**A**) Specimen printed at 0°; (**B**) specimen printed at 90°.

Surface finishing was performed using a standardized polishing protocol with sequential burs (green, gray, yellow, and final polishing bur with emulsion) to progressively reduce surface roughness and achieve a smooth, glossy surface. Polishing was carried out using a handpiece coupled to a micromotor (STRONG 206, Saeshin, Daegu, Republic of Korea) at 5000 rpm, with each bur applied for 30 s. All specimens were polished under identical conditions by the same operator to minimize variability.

2.2. Surface Roughness Assessment

Following completion of the standardized polishing procedure, three surface roughness (Ra) measurements were performed using a calibrated contact profilometer, under controlled and consistent measurement conditions in accordance with the manufacturer's guidelines. Surface roughness was quantified using the arithmetic mean roughness parameter (Ra, μm). Three measurements were obtained at different locations on each specimen surface, and the mean value was calculated to represent each specimen.

2.3. Surface Adhesion Study

Candida albicans ATCC 11225 and *Streptococcus mutans* DSM 20523 strains were used in the adhesion assays. *C. albicans* was stored at −80 °C in Sabouraud Dextrose Broth (SDB; Oxoid, Basingstoke, UK) supplemented with 20% (*v*/*v*) glycerol. *S. mutans* was stored under the same conditions in Trypticase Yeast Extract broth (ISP, Liofilchem, Roseto degli Abruzzi, Italy).

The fungus was subcultured on Sabouraud Dextrose Agar (SDA; Oxoid, UK) and prior to each assay, a single colony was inoculated into 5 mL of SDB and grown aerobically at 37 °C overnight at 170 rpm. *S. mutans* was subcultured in Trypticase Yeast Extract agar (ISP agar, Liofilchem, Roseto degli Abruzzi, Italy), and, prior to the experiments, one isolated colony was inoculated in 10 mL of Yeast Extract broth (ISP, Liofilchem, Roseto degli Abruzzi, Italy) and grown anaerobically at 37 °C overnight. An aliquot of each culture (300 µL) was transferred into a new fresh liquid medium and grown under the same growth conditions until the stationary growth phase was achieved.

Before each assay, all the resin specimens were exposed to UV light for 30 min to prevent contamination. This method was selected over autoclave sterilization to avoid potential surface alterations caused by heat and moisture exposure, which could confound roughness measurements and subsequent adhesion results. For the surface adhesion assays, overnight cultures of each microorganism, grown to thestationary phase, were diluted tenfold in the appropriate culture medium to obtain a final concentration of approximately 10^8 colony-forming units per millilitre (CFU/mL), corresponding to 0.5 McFarland. Then, 2 mL of each microorganism suspension was added to each well of a 24-well plate containing the resin specimens, followed by incubation at 37 °C for 24 h.

For scanning electron microscopy (SEM) analysis, samples were fixed in 2.5% (*v*/*v*) glutaraldehyde in phosphate-buffered saline (PBS) for 40 min at room temperature. Specimens were rinsed three times with PBS and dehydrated through a graded ethanol series (15%, 30%, 50%, 70%, 90%, 96%, and three times in 100%), each step for 15 min. The samples were then dried, mounted on aluminum stubs, sputter-coated with gold–palladium, and observed using a Field emission scanning electron microscope (FE-SEM; Hitachi S-4100, Hitachi Ltd., Tokyo, Japan) operating at 4.0 kV. For each microorganism and material condition, SEM observations were performed on one representative specimen, and three randomly selected fields were examined. To ensure reliable qualitative comparison among the experimental groups, all SEM images were acquired using identical imaging parameters, including accelerating voltage (4.0 kV), working distance, magnification, detector configuration, and acquisition settings.

Micrographs were used for qualitative and exploratory assessment of microbial adhesion. Because of the limited number of specimens analyzed by SEM, these observations were not used for inferential statistical analysis and should be interpreted as descriptive morphological evidence rather than as a quantitative biofilm assessment.

2.4. Statistical Analysis

Statistical analysis was performed using the average Ra values per specimen to maintain independence of observations. Statistical analysis was performed using the average Ra value of each specimen to maintain independence of observations. Prior to inferential testing, data distribution was assessed using the Shapiro–Wilk test because of the small sample size (n = 5 per group). Homogeneity of variances was evaluated using Levene's test (F = 2.25; p = 0.148).

Surface roughness was compared among the three fabrication groups (conventional heat-polymerized resin, 3D-printed resin at 90°, and 3D-printed resin at 0°) using one-way analysis of variance (ANOVA). When the ANOVA indicated statistically significant

differences, pairwise comparisons were performed using Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was established at $p < 0.05$.

Additionally, the effect size (Cohen's d) was calculated for the roughness comparison to evaluate the magnitude of the observed difference.

3. Results

3.1. Surface Roughness Measurement

The mean surface roughness values (Ra) for each specimen are presented in Table 1.

Table 1. Mean Surface Roughness (Ra) per specimen.

Specimen	Conventional Heat-Polymerized Resin	3D Printing (90°)	3D Printing (0°)
1	1.387	0.917	1.47
2	1.200	0.757	1.6
3	0.743	0.713	0.6
4	1.087	0.657	0.56
5	0.910	1.250	0.69

One-way ANOVA demonstrated statistically significant differences in surface roughness among the three evaluated fabrication conditions ($F(2,12) = 5.87$, $p = 0.017$).

Tukey's HSD post hoc analysis revealed that specimens fabricated using the conventional heat-polymerized resin exhibited significantly higher surface roughness than those produced by 3D printing at 90° ($p < 0.05$). No statistically significant differences were observed between the conventional resin and the 3D-printed 0° group, nor between the two printing orientations ($p > 0.05$). The conventional heat-polymerized resin exhibited the highest mean Ra values (1.06 ± 0.56 µm), whereas the 3D-printed resin fabricated at 90° showed the lowest roughness values (0.52 ± 0.03 µm). Specimens printed at 0° presented intermediate values (0.98 ± 0.51 µm) and greater variability. Tukey's HSD post hoc analysis revealed that the conventional heat-polymerized resin exhibited significantly higher surface roughness than the 3D-printed resin fabricated at 90° ($p = 0.013$). No statistically significant differences were observed between the conventional resin and the 3D-printed resin fabricated at 0° ($p = 0.236$), or between the two printing orientations ($p = 0.244$). Effect size analysis revealed a large effect magnitude ($\eta^2 = 0.49$), indicating that approximately 49% of the variability in surface roughness was associated with fabrication method and printing orientation. Pairwise comparisons demonstrated very large effect sizes between the conventional resin and the 90° printing orientation (Cohen's $d = 2.40$), as well as between the two printing orientations (Cohen's $d = 1.30$). Notably, both materials exhibited mean Ra values above the 0.2 µm threshold beyond which microbial adhesion is reported to increase significantly [6,8].

3.2. Candida albicans and Streptococcus mutans Adhesion by SEM

The adhesion of *C. albicans* and *S. mutans* to the surface of the resin specimens was evaluated by scanning electron microscopy (SEM) after 24 h of incubation with the microorganism. For comparative purposes, microbial adhesion was also assessed on a conventional heat-polymerized resin. Representative micrographs of each specimen are shown in Figures 2 and 3, for *C. albicans* and *S. mutans*, respectively.

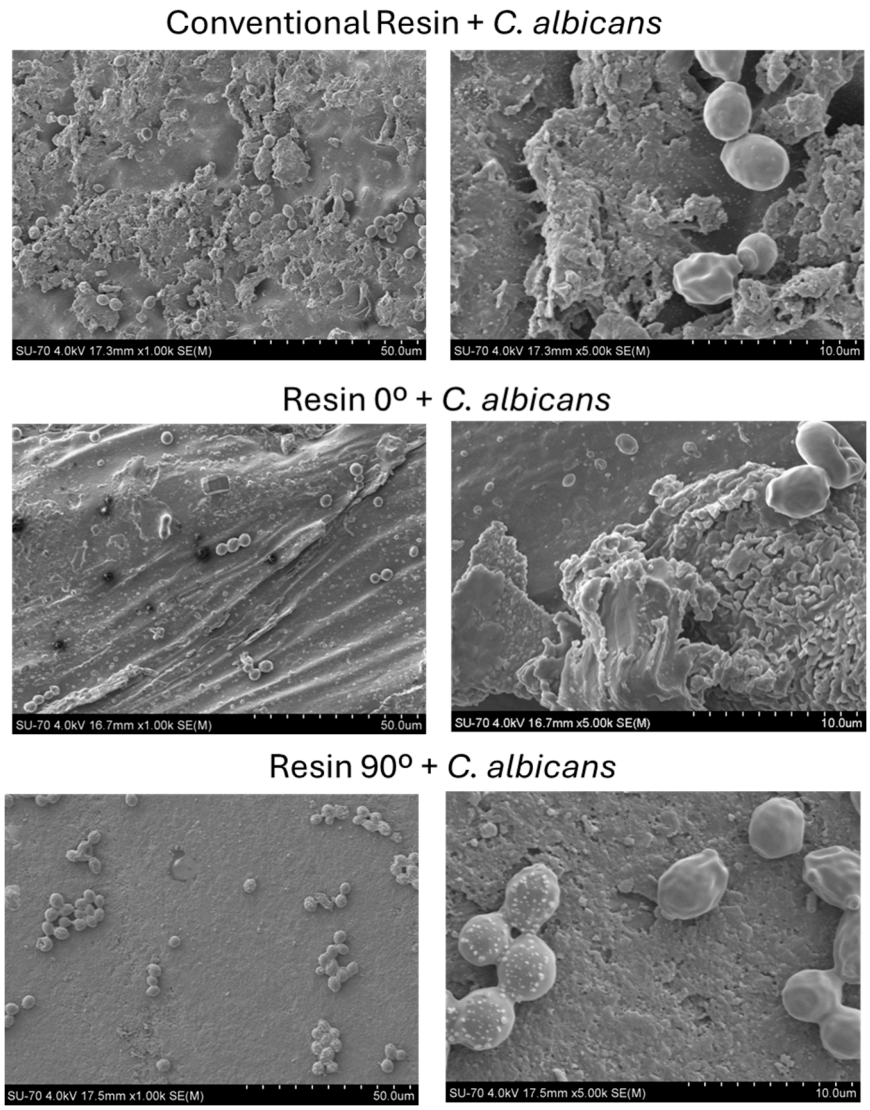

Figure 2. Representative SEM images of conventional heat-polymerized resin and 3D-printed denture base resins fabricated at 0° and 90° after 24 h incubation with *C. albicans*. Images are intended for qualitative exploratory assessment of microbial adhesion.

SEM analysis suggests differences in the extent of *C. albicans* adhesion according to the resin type and printing orientation.

Qualitative SEM observations suggested visible differences in *C. albicans* distribution among the tested surfaces (Figure 2). Representative images showed more evident fungal cell presence on the 90°-printed specimens, followed by the conventional heat-polymerized resin, whereas the 0°-printed specimens showed fewer visible adherent cells in the selected fields. adhesion. Qualitative SEM observations of *S. mutans* showed an apparent pattern similar to that observed for *C. albicans* (Figure 3). Representative images suggested more extensive bacterial presence on the 90°-printed specimens, intermediate colonization on the conventional heat-polymerized resin, and lower visible adhesion on the 0°-printed specimens. These observations are descriptive and require confirmation using quantitative biofilm assays.

Overall, the adhesion pattern observed for *S. mutans* appears to be consistent with that previously described for *C. albicans*, with apparent greater microbial colonization on the 90° 3D-printed resin specimens, intermediate adhesion on the conventional heat-polymerized resin, and lower adhesion on the 0° 3D-printed specimens. This suggests

that printing orientation may similarly influence the adhesion of both fungal and bacterial microorganisms to the surface of these resin materials.

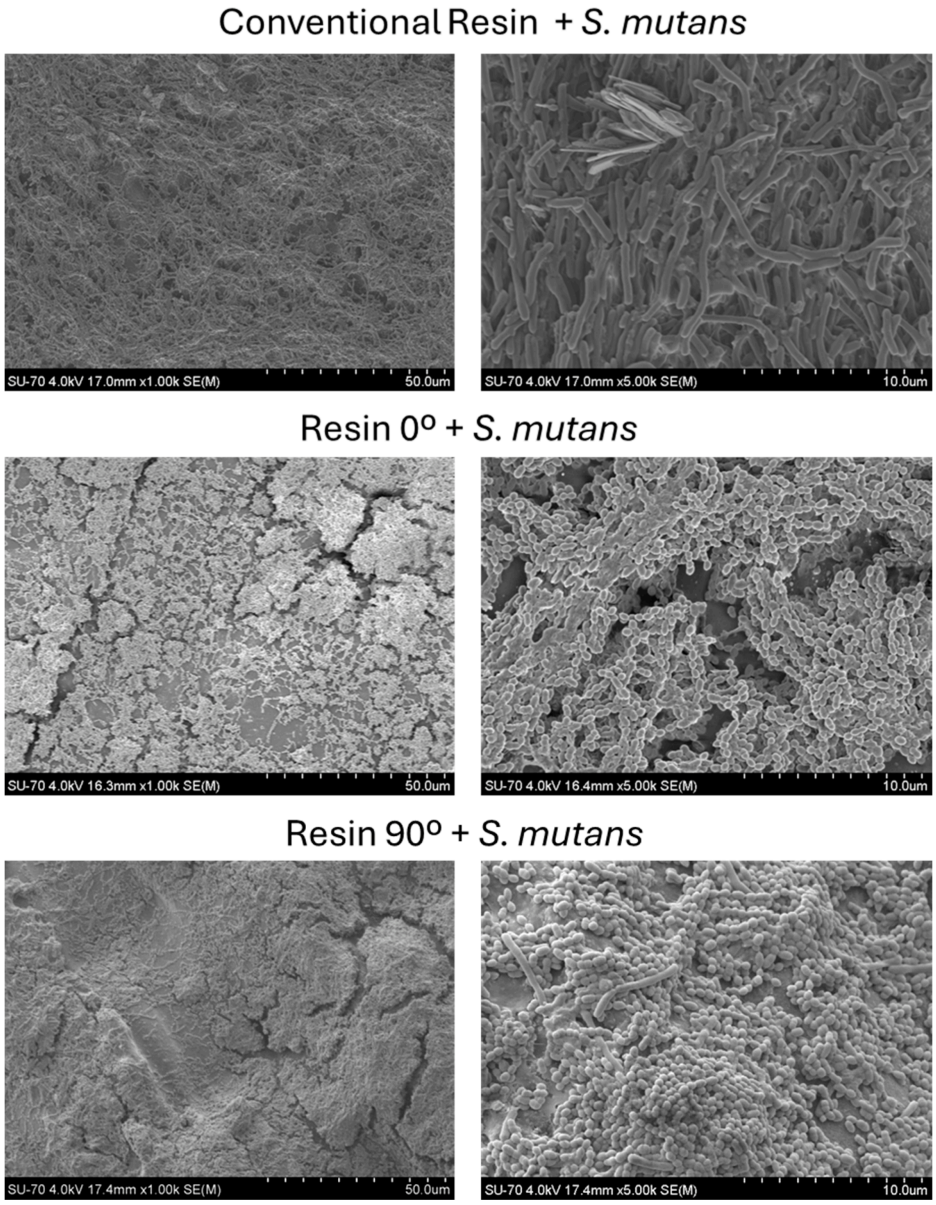

Figure 3. Representative SEM images of conventional heat-polymerized resin and 3D-printed denture base resins fabricated at 0° and 90° after 24 h incubation with *S. mutans*. Images are intended for qualitative exploratory assessment of microbial adhesion.

4. Discussion

The present study evaluated the adhesion of *C. albicans* and *S. mutans* to the surface of a 3D-printed resin (V-Print dentbase) and a conventional heat-polymerized acrylic resin (Probase Hot), using surface roughness characterization and scanning electron microscopy. The effect of printing orientation (0° vs. 90°) on fungal adhesion was also investigated.

The present findings indicate that printing orientation may influence the surface characteristics of additively manufactured denture base resins, as reflected by differences in Ra values among the tested groups. The 90° printing orientation produced smoother and more homogeneous surfaces, as reflected by the lower Ra values (0.52 ± 0.03 µm) and very large effect sizes when compared with both the conventional resin (Cohen's d = 2.40) and the 0° printing orientation (Cohen's d = 1.30). In contrast, the conventional

heat-polymerized resin exhibited the highest roughness values, while the 0° orientation showed intermediate roughness and greater variability (0.98 ± 0.51 µm). These findings suggest that printing orientation plays a substantial role in determining the final surface topography and manufacturing consistency of 3D-printed dental resins.

Despite the smoother surface profile observed in the 90°-printed specimens, SEM analysis revealed higher microbial adhesion in this group. This apparent discrepancy suggests that Ra alone may be insufficient to explain microbial adhesion behavior on additively manufactured resins. Microbial interactions with dental material surfaces are known to depend on multiple physicochemical and topographical parameters, including surface roughness, surface chemistry and wettability. However, these parameters were not directly assessed in the present study and should therefore be considered possible contributing factors rather than confirmed mechanisms. These results are consistent with previous studies reporting greater surface irregularity in conventionally processed PMMA resins compared with digitally manufactured materials, while additionally highlighting the importance of printing orientation as a determinant factor influencing both topographical behavior and microbial adhesion patterns in additively manufactured dental resins. Although no statistically significant differences were observed between the remaining pairwise comparisons, effect size analysis demonstrated a large effect magnitude (Cohen's $d > 0.8$), suggesting that fabrication method and printing orientation exerted a substantial influence on surface topography despite the limited sample size ($n = 5$ per group).

All evaluated groups exhibited mean Ra values above the 0.2 µm threshold commonly associated with increased microbial adhesion, suggesting that all tested surfaces may be favorable to microbial attachment under the experimental conditions [14,15]. These findings are consistent with previous studies reporting greater surface irregularity in conventionally processed PMMA resins when compared with digitally manufactured materials, while also highlighting the influence of printing orientation on the surface characteristics of additively manufactured resins [1,2].

With respect to *C. albicans* adhesion, SEM observations suggested greater apparent *C. albicans* presence on the 3D-printed resin fabricated at 90°, followed by the conventional resin and the 0°-printed specimens. Although the SEM observations are exploratory, the consistency of the apparent adhesion pattern across the analyzed fields supports the relevance of further investigating the effect of printing orientation on fungal adhesion.

The greater apparent microbial presence observed by SEM on the 90° specimens compared with the 0° specimens is a noteworthy observation. The printing orientation influences the direction of layer stacking and, consequently, the surface topography of the final product. From a manufacturing perspective, printing orientation may influence layer arrangement and local surface features, which could contribute to microbial attachment [4,16]. However, this interpretation remains hypothetical because detailed three-dimensional surface characterization was not performed. Therefore, no mechanistic explanation for the observed adhesion pattern can be established, and the present findings should be interpreted with caution. The lower apparent adhesion observed on 0°-printed specimens compared to the conventional resin may reflect the more homogeneous surface texture associated with that orientation, as well as compositional differences between photopolymerized resin and heat-polymerized PMMA, including differences in surface energy and hydrophobicity, both of which are known determinants of *C. albicans* adhesion [2,17].

Regarding *S. mutans*, SEM observations showed an apparent adhesion pattern similar to that observed for *C. albicans*, with greater visible bacterial presence on the 90°-printed specimens, intermediate presence on the conventional heat-polymerized resin and lower apparent adhesion on the 0°-printed specimens. This observation suggests that the possible influence of printing orientation on microbial adhesion patterns may not be restricted

to fungal cells but may also involve bacterial attachment. Since *S. mutans* is an early colonizer capable of initiating biofilm formation on oral surfaces, its adhesion to prosthetic base materials is clinically relevant, particularly because denture surfaces may serve as reservoirs for mixed microbial communities. The similar adhesion pattern observed for both microorganisms reinforces the hypothesis that orientation-dependent surface features, including layer arrangement and micro-irregularities, may modulate the initial attachment of different oral microorganisms [9,18].

The inclusion of *S. mutans* also strengthens the biological relevance of the present study, as oral biofilms are rarely composed of a single species. Interactions between *C. albicans* and oral streptococci, including *S. mutans*, may contribute to biofilm maturation, structural complexity, and increased microbial persistence on oral surfaces. Although the present study evaluated each microorganism independently, the comparable adhesion trend observed across both models suggests that surface characteristics associated with printing orientation may influence the early stages of both fungal and bacterial colonization. Future studies using dual-species or multispecies biofilm models would therefore be important to better approximate the clinical oral environment [19–21].

From a clinical perspective, these findings suggest that printing orientation should be considered not only as a manufacturing parameter influencing the mechanical performance of denture base resins but also as a factor that may affect their biological surface behavior. Although the present results are preliminary and based on qualitative SEM observations, the lower apparent microbial adhesion observed on the 0°-printed specimens indicates that optimization of printing parameters may contribute to reducing the susceptibility of prosthetic surfaces to early microbial colonization. Since denture biofilms are closely associated with the development of denture stomatitis and other biofilm-related complications, selecting appropriate manufacturing workflows, together with standardized finishing and polishing procedures, may improve the long-term biological performance of additively manufactured prostheses. Nevertheless, selecting appropriate manufacturing workflows, together with standardized finishing and polishing procedures, may improve the long-term biological performance of additively manufactured prostheses. Several limitations should be acknowledged. This was an in vitro study conducted under static incubation conditions, which do not replicate the dynamic oral environment, including salivary flow, pellicle formation, temperature fluctuations, and the complex oral microbiome. A further limitation is the absence of complementary quantitative biofilm assessment methods. The SEM analysis was primarily intended to provide morphological information regarding microbial adhesion to the tested surfaces, rather than to quantify total viable cells or global biofilm biomass. CFU counting would provide quantitative information on viable adherent microorganisms; however, in this model, it would require prior optimization of biofilm detachment, serial dilution ranges and recovery efficiency. Similarly, crystal violet staining would provide a global measurement of biomass retained on the entire disc, including microbial cells and extracellular matrix, without spatial or morphological resolution. Therefore, it would not allow direct assessment of the local adhesion patterns or surface-associated features observed by SEM. Also, a methodological limitation concerns the exclusive use of Ra as the surface roughness descriptor. Although Ra remains the most widely used parameter in dental research and was considered sufficient for predicting initial microbial adhesion to polished biomaterials in previous work [21], it only quantifies the arithmetic mean of vertical deviations from a mean line, and therefore provides no information on the shape, slope, or spatial distribution of surface asperities—all of which directly influence microbial adhesion and biofilm formation [22]. Ra is insensitive to the directionality of surface texture, which is particularly relevant for 3D-printed resins where build orientation creates anisotropic topographies. More informative alternatives include three-dimensional

areal parameters defined under ISO 25178 [23], such as Sa (areal arithmetic mean height), Sq (root mean square height), and Ssk (skewness), which characterize the surface rather than along a single profile line. The methodological framework for applying these parameters has previously been described by our research group; however, such analyses were beyond the scope of the present investigation, which was designed as a preliminary evaluation of surface roughness and microbial adhesion [8]. Contact profilometry, as used in the present study, is further constrained by its 2D nature and its inability to capture out-of-plane features. An additional limitation is that physicochemical surface properties potentially involved in microbial adhesion, including surface free energy, wettability, degree of conversion, and residual monomer content, were not evaluated. Consequently, the mechanisms underlying the observed discrepancy between surface roughness and microbial adhesion could not be established. Further investigation on 3D-printed prosthetic resins should consider complementing Ra with areal topographic analysis by confocal or white-light interferometry microscopy to obtain a more comprehensive characterization of surface texture and its relationship with fungal adhesion [8,23]. Future studies should also employ larger sample sizes, include three-dimensional surface characterization, and evaluate biofilm formation over extended time periods, including dual-species or multi-species biofilm models involving *C. albicans* and *S. mutans*, to better approximate clinical oral conditions and include optimized CFU counting, crystal violet staining, live/dead fluorescence assays and standardized image-based SEM quantification to validate and expand these SEM-based observations.

5. Conclusions

Within the limitations of this in vitro study, printing orientation influenced the surface characteristics of 3D-printed denture base resins and appeared to affect the adhesion pattern of both *C. albicans* and *S. mutans*. The 90° printing orientation exhibited greater apparent microbial adhesion despite presenting lower surface roughness, suggesting that Ra alone does not fully explain microbial attachment. These findings highlight the importance of considering printing orientation during the manufacturing of additively manufactured denture bases. Further studies incorporating quantitative biofilm assessment, three-dimensional surface characterization, and larger sample sizes are required to validate these preliminary observations.

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Abbreviations

The following abbreviations are used in this manuscript:

3D	Three-Dimensional
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
CAD	Computer-Aided Design
CAD/CAM	Computer-Aided Design/Computer-Aided Manufacturing
CFU	Colony-Forming Units
C. albicans	*Candida albicans*
DLP	Digital Light Processing
FDM	Fused Deposition Modeling
PBS	Phosphate-Buffered Saline
PLA	Polylactic Acid
PMMA	Polymethyl Methacrylate
Ra	Arithmetic Mean Surface Roughness
rpm	Revolutions Per Minute
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SEM	Scanning Electron Microscopy
STL	Standard Tessellation Language
UV	Ultraviolet
v/*v*	Volume per Volume
w/*v*	Weight per Volume

References

1. Alqutaibi, A.Y.; Baik, A.; Almuzaini, S.A.; Farghal, A.E.; Alnazzawi, A.A.; Borzangy, S.; Aboalrejal, A.N.; AbdElaziz, M.H.; Mahmoud, I.I.; Zafar, M.S. Polymeric Denture Base Materials: A Review. *Polymers* **2023**, *15*, 3258. [CrossRef]
2. Zafar, M.S. Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update. *Polymers* **2020**, *12*, 2299. [CrossRef] [PubMed]
3. Swaney, A.C.; Paffenbarger, G.C.; Caul, H.J.; Sweeney, W.T. American Dental Association Specification No. 12 for Denture Base Resin: Second Revision. *J. Am. Dent. Assoc.* **1953**, *46*, 54–66. [CrossRef] [PubMed]
4. Dimitrova, M.; Vlahova, A.; Kalachev, Y.; Zlatev, S.; Kazakova, R.; Capodiferro, S. Recent Advances in 3D Printing of Polymers for Application in Prosthodontics. *Polymers* **2023**, *15*, 4525. [CrossRef] [PubMed]
5. Manikandan, S.; Vinesh, E.; Selvi, D.T.; Kannan, R.K.; Jayakumar, A.; Dinakaran, J. Prevalence of Candida among Denture Wearers and Nondenture Wearers. *J. Pharm. Bioallied Sci.* **2022**, *14*, S702–S705. [CrossRef] [PubMed]
6. Bollenl, C.M.L.; Lambrechts, P.; Quirynen, M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. *Dent. Mater.* **1997**, *13*, 258–269. [CrossRef] [PubMed]
7. Schubert, A.; Wassmann, T.; Holtappels, M.; Kurbad, O.; Krohn, S.; Bürgers, R. Predictability of Microbial Adhesion to Dental Materials by Roughness Parameters. *Coatings* **2019**, *9*, 456. [CrossRef]
8. Quezada, M.M.; Fernandes, C.; Montero, J.; Correia, A.; Salgado, H.; Fonseca, P. A Different Approach to Analyzing the Surface Roughness of Prosthetic Dental Acrylic Resins. *Appl. Sci.* **2024**, *14*, 619. [CrossRef]
9. Manuschai, J.; Lomlim, L.; Ratti, P.; Kara, J.; Naorungroj, S. In vitro efficacy of synthetic lawsone derivative disinfectant solution on removing dual-species biofilms and effect on acrylic denture surface properties. *Sci. Rep.* **2023**, *13*, 14832. [CrossRef] [PubMed]
10. Salgado, H.; Gomes, A.T.P.C.; Duarte, A.S.; Ferreira, J.M.F.; Fernandes, C.; Figueiral, M.H.; Mesquita, P. Antimicrobial Activity of a 3D-Printed Polymethylmethacrylate Dental Resin Enhanced with Graphene. *Biomedicines* **2022**, *10*, 2607. [CrossRef] [PubMed]
11. Kozmos, M.; Virant, P.; Rojko, F.; Abram, A.; Rudolf, R.; Raspor, P.; Zore, A.; Bohinc, K. Bacterial Adhesion of Streptococcus mutans to Dental Material Surfaces. *Molecules* **2021**, *26*, 1152. [CrossRef] [PubMed]
12. Revilla-León, M.; Özcan, M. Additive Manufacturing Technologies Used for Processing Polymers: Current Status and Potential Application in Prosthetic Dentistry. *J. Prosthodont.* **2019**, *28*, 146–158. [CrossRef] [PubMed]
13. Shim, J.S.; Kim, J.E.; Jeong, S.H.; Choi, Y.J.; Ryu, J.J. Printing accuracy, mechanical properties, surface characteristics, and microbial adhesion of 3D-printed resins with various printing orientations. *J. Prosthet. Dent.* **2020**, *124*, 468–475. [CrossRef] [PubMed]

14. Alharbi, N.; Osman, R.; Wismeijer, D. Effects of build direction on the mechanical properties of 3D-printed complete coverage interim dental restorations. *J. Prosthet. Dent.* **2016**, *115*, 760–767. [CrossRef] [PubMed]
15. Assunção, W.G.; Gomes, É.A.; Barão, V.A.R.; Barbosa, D.B.; Delben, J.A.; Tabata, L.F. Effect of storage in artificial saliva and thermal cycling on Knoop hardness of resin denture teeth. *J. Prosthodont. Res.* **2010**, *54*, 123–127. [CrossRef] [PubMed]
16. Yacob, N.; Ahmad, N.A.; Safii, S.H.; Yunus, N.; Abdul Razak, F. Is microbial adhesion affected by the build orientation of a 3-dimensionally printed denture base resin? *J. Prosthet. Dent.* **2023**, *130*, 131.e1–131.e7. [CrossRef] [PubMed]
17. Quezada, M.M.; da Costa Gomes Fernandes, C.M.; Martín, J.M.; Correia, A.R.M.; da Fonseca, P.A.B. Influence of different processing techniques for prosthetic acrylic resins in the surface roughness parameters: A research article. *BMC Oral Health* **2024**, *24*, 641. [CrossRef] [PubMed]
18. Koo, H.; Andes, D.R.; Krysan, D.J. Candida–streptococcal interactions in biofilm-associated oral diseases. *PLoS Pathog.* **2018**, *14*, e1007342. [CrossRef] [PubMed]
19. Li, Y.; Huang, S.; Du, J.; Wu, M.; Huang, X. Current and prospective therapeutic strategies: Tackling Candida albicans and Streptococcus mutans cross-kingdom biofilm. *Front. Cell. Infect. Microbiol.* **2023**, *13*, 1106231. [CrossRef] [PubMed]
20. Salehi, B.; Kregiel, D.; Mahady, G.; Sharifi-Rad, J.; Martins, N.; Rodrigues, C.F. Management of Streptococcus mutans-Candida spp. Oral Biofilms' Infections: Paving the Way for Effective Clinical Interventions. *J. Clin. Med.* **2020**, *9*, 517. [CrossRef] [PubMed]
21. Pereira-Cenci, T.; Del Bel Cury, A.A.; Crielaard, W.; Ten Cate, J.M. Development of Candida-associated denture stomatitis: New insights. *J. Appl. Oral Sci.* **2008**, *16*, 86–94. [CrossRef] [PubMed]
22. Ramage, G.; Tomsett, K.; Wickes, B.L.; López-Ribot, J.L.; Redding, S.W. Denture stomatitis: A role for Candida biofilms. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2004**, *98*, 53–59. [CrossRef] [PubMed]
23. *ISO 25178-2:2012*; Geometrical Product Specifications (GPS)—Surface Texture: Areal—Part 2: Terms, Definitions and Surface Texture Parameters. International Organization for Standardization: Geneva, Switzerland, 2012.