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Evaluation of surface roughness, wettability and adhesion of multispecies biofilm on 3D-printed resins for the base and teeth of complete dentures*

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Abstract

Studies evaluating the roughness, wettability and microbial adhesion of 3D-printed resins for complete denture bases and teeth are scarce. Objective: This study evaluated the surface roughness, wettability and adhesion of multispecies biofilms (*Candida albicans*, *Staphylococcus aureus* and *Streptococcus mutans*) on 3D-printed resins for complete denture bases and teeth compared to conventional resins (heat-polymerized acrylic resin; artificial pre-fabricated teeth). Methodology: Circular specimens (n=39; 6.0 mm Ø × 2.0 mm) of each group were subjected to roughness (n=30), wettability (n=30) and biofilm adhesion (n=9) tests. Three roughness measurements were taken by laser confocal microscopy and a mean value was calculated. Wettability was evaluated by the contact angle of sessile drop method, considering the mean of the three evaluations per specimen. In parallel, microorganism adhesion to resin surfaces was evaluated using a multispecies biofilm model. Microbial load was evaluated by determining the number of Colony Forming Units (CFU/mL) and by scanning electron microscopy (SEM). Data were subjected to the Wald test in a generalized linear model with multiple comparisons and Bonferroni adjustment, as well as two-way ANOVA (α=5%). Results: The roughness of the conventional base resin (0.01±0.04) was lower than that of the conventional tooth (0.14±0.04) (p=0.023) and 3D-printed base (0.18±0.08) (p<0.001). For wettability, conventional resin (84.20±5.57) showed a higher contact angle than the 3D-printed resin (60.58±6.18) (p<0.001). Higher microbial loads of *S. mutans* (p=0.023) and *S. aureus* (p=0.010) were observed on the surface of the conventional resin (*S. mutans*: 5.48±1.55; *S. aureus*: 7.01±0.57) compared to the 3D-printed resin (*S. mutans*: 4.11±1.96; *S. aureus*: 6.42±0.78). The adhesion of *C. albicans* was not affected by surface characteristics. The conventional base resin showed less roughness than the conventional dental resin and the printed base resin. Conclusion: The 3D-printed resins for base and tooth showed less hydrophobicity and less adhesion of *S. mutans* and *S. aureus* than conventional resins.

Keywords: Computer-aided design. Three-dimensional printing. Acrylic resins. Candidiasis. Complete dentures.

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Introduction

Complete dentures are manufactured with polymethylmethacrylate (PMMA), which has advantages and disadvantages.[1] Alternatively, the CAD/CAM system has also been studied for the fabrication of complete dentures, as it can simplify the manufacturing process[2] and achieve good adaptation to the supporting tissues.[1] One of the methods used to fabricate CAD/CAM prostheses is additive manufacturing, which consists of depositing layer upon layer of light-curing resin until the desired geometry is obtained.[1,3] In this technique, prosthetic bases can be printed separately from the teeth and then joined together, or the base and teeth can be obtained in the same printing step, using the same resin for both, requiring subsequent characterization of the base.[2,4]

Compared to conventional resins, printed resins have a lower amount of filler to reduce the viscosity of the material and allow it to polymerize.[5] Furthermore, there are differences in the composition of base and tooth impression resins.[6] In general, the PMMA used in complete dentures can promote the accumulation of biofilm, which is composed mainly of *Candida* spp. and bacteria responsible for chronic atrophic candidiasis and halitosis.[1,7,8] 3D-printed resin has surface roughness and hydrophobicity characteristics different from those of conventional resins.[5] These properties can influence the adhesion of microorganisms and the formation of biofilm.[6,9-11]

The inner surface of upper dentures is an important reservoir of *C. albicans*, one of the main etiological agents of denture stomatitis.[6,12,13,14] A cohesive tendency between this fungus and *S. mutans* has been reported in the literature, as these bacteria secrete the enzyme mutanobactin A,[12] which acts as a retainer of hyphae, contributing to the adhesion of yeast to mucosal and resin surfaces.[12] This indicates a direct effect of *S. mutans* on the initiation and progression of denture stomatitis. *C. albicans* also interacts with *S. aureus*, increasing bacterial virulence and drug resistance.[12,15,16] Thus, evaluating the behavior of the multispecies biofilm of *C. albicans, S. mutans* and *S. aureus* is essential to prevent the development of inflammatory diseases and maintain individual health.[12]

Adhesion is the first step in microbial colonization, and understanding the interactions between microorganisms and surfaces is essential for biofilm control.[17] Research has been conducted in the medical field to find materials that are resistant to microbial adhesion.[18,19] Studies show that surface roughness tends to favor the adhesion of microorganisms by facilitating their retention in niches and protecting them from the shear forces that constantly occur on smooth surfaces of complete dentures.[8,13,20-23] Surfaces and microorganisms can have hydrophobic or hydrophilic properties, and the chemical composition of materials can influence their surface characteristics, which can favor microbial adhesion.[19,24-28]

Despite the importance of this area in dentistry, there are no studies to date that evaluate the roughness, wettability and microbial adhesion of multispecies biofilms on 3D-printed base and teeth resins for complete dentures. Since resin for bases and teeth are used in association, it is important to know the behavior of these materials in cases of microbial colonization and formation of multispecies biofilm.[2,10,23] This study investigated the surface roughness, wettability and adhesion of multispecies biofilms of *C. albicans*, *S. mutans* and *S. aureus* on 3D-printed resins for complete denture bases and teeth compared to conventional resins. The null hypothesis is that there are no significant differences in roughness, wettability, and adhesion of microorganisms between the materials.

Methodology

The sample size was determined using a pilot study. The difference between the means of the experimental and control groups was 0.064 with a standard deviation of 0.085, considering a power of 0.8 and a confidence interval of 95%. The test then indicated that n=29 per group was required to reject the null hypothesis. Therefore, n=30 was chosen for the roughness and wettability tests. The assessment of microbial adhesion was carried out in triplicate at three independent times with nine specimens of each group (n=9).[29] Figure 1 shows a flowchart of the experimental design of the study.

Preparation of the specimens

The specimens (6.0 mm Ø × 2.0 mm) of heat-polymerized acrylic resin for the base were prepared as described in the literature.[29] Artificial teeth model R17 color 2A Trilux (Vipi Produtos Odontologicos,

Pirassununga, SP, Brazil) were cut with a Maxicut bur to obtain specimens of the desired dimensions. The specimens were polished in a standardized way with sandpaper (nº 360, 600, 1200 and 2000 Norton Saint Gobain Accessories Ltda., Guarulhos, SP, Brazil) in a horizontal polisher (Panambra Industrial e Técnica AS, São Paulo, SP, Brazil) and with calcium carbonate (Branco Rio, Orlando Antônio Bussioli ME, Rio Claro, SP, Brazil) in a bench polisher (Nevoni, São Paulo, SP, Brazil).[29]

For the 3D-printed resin (experimental group), the specimens (6.0 mm Ø × 2.0 mm) were designed in Rhinoceros 6.0 software (Rhinoceros, Robert McNeel & Associates, Seattle, Washington, USA). The impression was fabricated using the Flashforge Hunter 3D Printer (Done 3D, Ribeirão Preto, SP, Brazil)[29] with a layer thickness of 0.05 mm, a layer time of 3.0 seconds for the base and 3.8 seconds for the tooth, 15 seconds of basecoat printing for both resins and a printing angle of 0°.

Medium pink acrylic resin for printing (Makertech Labs, São Cristóvão, Tatuí, SP, Brazil) was used for the base and PriZma 3D Bio Prov resin A2 color (Makertech Labs) for the tooth. These specimens were then washed with ethanol for 3 minutes, subjected to a post-cure process for 3 minutes (Curing Oven, Done 3D, Ribeirão Preto, SP, Brazil) and polished according to the methodology described in item 1.1.

The specimens were kept dry and stored at room temperature in the dark until the tests were carried out, in order to prevent sorption and solubility in water.[30]

Surface roughness assay

Measurements were made by Confocal Laser Microscopy (Olympus LEXT OLS4000®, Japan) at the center of the samples, with a 10 × objective and 216 × optical zoom, and a scanning area of 1280 × 1279 µm. Three images were obtained and a mean value (in µm) was calculated.[29]

Wettability assay

Wettability was analyzed in a goniometer (Goniometer Optical Contact Angle Measurements SCA20 - DataPhysics Instruments GmbH, Filderstadt, Baden-Württemberg, Germany) by the sessile drop

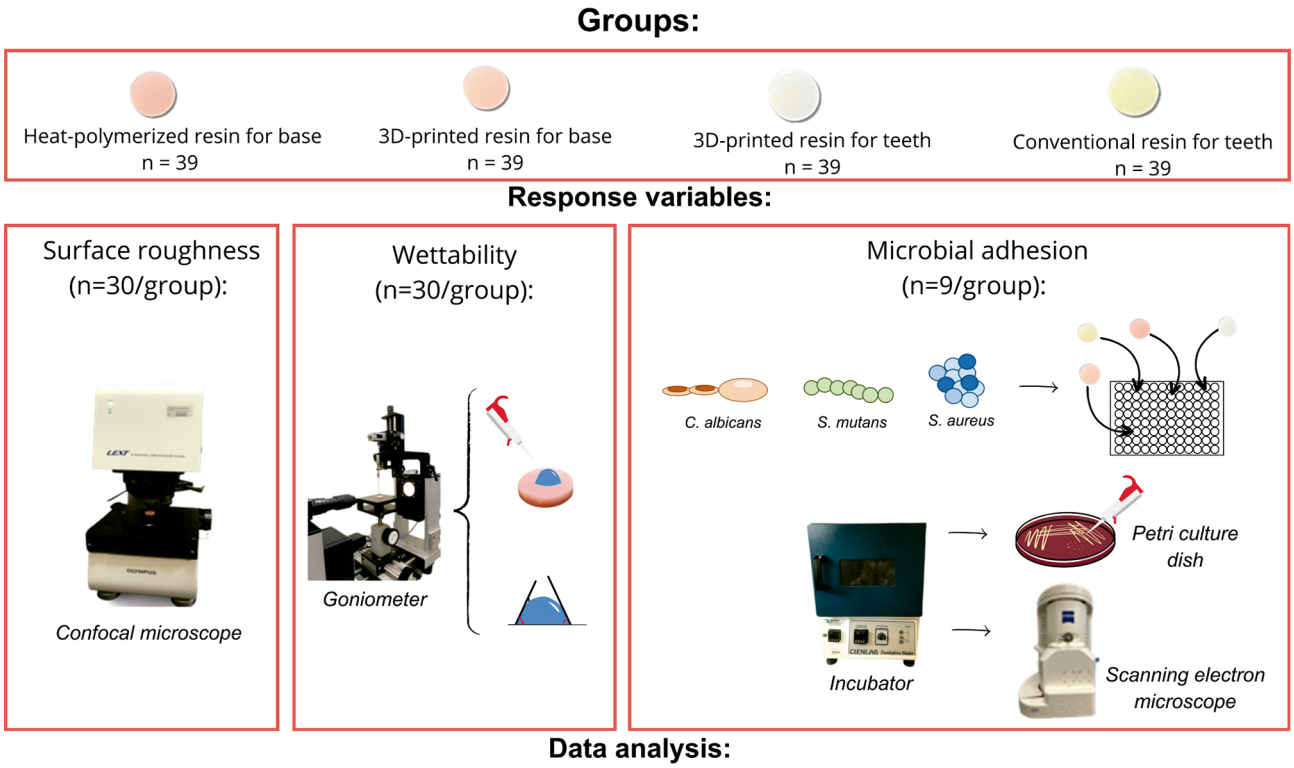

Figure 1- Experimental design of the study

method. Distilled water (15 µL) was applied to the surface of the specimens and images were captured using a Charge-Coupled Device (CCD) camera for contact angle calculation (OCA-20 Software, OneAttension, Biolin Scientific Inc., Manchester, North West, United Kingdom). The results were the arithmetic mean of the contact angle of 3 drops deposited on the surface, which was air dried between applications of each drop.[31]

Multispecies biofilm microbial load of *C. albicans*, *S. aureus* and *S. mutans*

Biofilm formation

The specimens were sterilized with hydrogen peroxide plasma (STERRAD® sterilizer, Advanced Sterilization Products, Irvina, CA, USA). The assay was performed in three technical replicates at three independent times (n=9). *Candida albicans* (ATCC 90028) frozen stock was thawed and cultured in Sabouraud Dextrose Broth (Kasvi). Similarly, *Streptococcus mutans* (ATCC 25175) and *Staphylococcus aureus* (ATCC 6538) were cultured in BHI. The species were incubated for 24 hours at 37ºC in a microbiological oven (De Leo – Equip. para Lab., Porto Alegre, RS, Brazil), and *S. mutans* was maintained in microaerophilic conditions. The culture suspensions were centrifuged (4200g; 5 min) and the cells were resuspended in phosphate-buffered saline (PBS). The inoculum concentration of 10^8 CFU/mL and 10^7 CFU/mL for bacteria and yeast, respectively, was verified according to the literature.[29]

The specimens were distributed in 48-well culture plates (Techno Plastic Products, Trasadingen, Switzerland). Each well received 400 µL of BHI inoculated with the three microorganisms (10^7 CFU/mL for bacteria and 10^6 CFU/mL for yeast) and incubated (Shaker Incubator, CE-320 Cienlab – Scientific Equipment, Campinas, SP, Brazil) at 37 ºC for 90 minutes at 75 rpm under microaerophilic conditions. The specimens and wells were washed twice with sterile PBS, filled with 600 µL of sterile culture medium and incubated under the same conditions for 48 hours. After 24 hours, half of the medium was replaced with fresh culture medium.[29]

Assessment of biofilm adhesion by microbial load

Each specimen was removed from the well, rinsed in PBS and immersed in 10 mL Letheen Broth (LB) (HiMedia Laboratories Pvt. Ltd. Mumbai, MH , India), sonicated at 40 KHz, 200 W (Altsonic, Clean 9CA, Ribeirão Preto, SP, Brazil) for 20 minutes.[29] The specimens were then vortexed (Phoenix, AP 56, Araraquara, São Paulo, Brazil) and 10-fold serial dilutions (10^0 to 10^{-3}) were seeded in Modified Sucrose Bacitracin Agar [SB-20 (15 g casitone; 5 g yeast extract; 0.2 g L-cysteine; 0.1 g sodium sulfite; 0.2 UI/mL bacitracin; 20 g sodium acetate; 200 g sucrose; 15 g agar-agar; 1,000 mL water)] supplemented with 200 UI/mL Nystatin for *S. mutans*; Mannitol Salt Agar (BD Difco, Sparks, MD, USA) supplemented with 200 UI/mL Nystatin for *S. aureus*; and Sabouraud Dextrose Agar (Kasvi) for *C. albicans*. Petri dishes were incubated at 37 °C for 48 hours in a microbiological oven. For *S. mutans*, incubation was performed under microaerophilic conditions. The number of colonies was registered and the CFU/mL was calculated taking into account the dilution and the volume, in milliliters, seeded on the agar surface. The values were expressed in $\log_{10}$ CFU/mL.[29]

Qualitative analysis of biofilm adhesion

One specimen of each material was analyzed using a scanning electron microscope (EVO MA10, CARL ZEISS, Jena, Thuringia, Germany). The samples were fixed with 1 mL of 2.5% glutaraldehyde (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), dehydrated in a graded ethanol series and immersed in 1 mL of Hexamethyldisilazane (Sigma-Aldrich, St. Louis, MO, United States) for 15 minutes.[31]

The surface of the specimens was metallized (Cressington Sputter Coater, TED PELLA, INC., Redding, CA, United States) with the machine operating for 60 seconds at 30 mA in an argon atmosphere.[30] The images were obtained at magnifications (Mag) of 3000 and 5000 ×, with a working distance (WD) of 9.0 mm and an acceleration voltage (EHT) of 20.00 Kv.

Data analysis

The roughness, wettability and microbial adhesion of *C. albicans*, *S. mutans* and *S. aureus* were considered independent variables. The factors of variation were resin (conventional or 3D-printed) and prosthetic application (base or tooth).

The data were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene tests, respectively. Roughness, wettability, and *S. aureus* and *S. mutans* microbial load data were analyzed with the Wald test in a generalized linear model with multiple comparisons and Bonferroni adjustment. The microbial load of *C. albicans*

was analyzed by two-way ANOVA, considering as variables the resin (conventional or 3D-printed) and the prosthetic application (base or tooth). Analyses were performed using SPSS, version 21.0 (SPSS for Windows; SPSS Inc, Chicago, IL, USA), with a significance level of 5%.

Results

Surface roughness

Surface roughness was influenced by the interaction between resin and application (p=0.003). The conventional base resin [median: 0.09] showed less roughness than the conventional tooth resin [median: 0.12] (p=0.023) and the printed base resin [median: 0.19] (p<0.001) (Table 1).

Surface wettability

Wettability was influenced by the type of resin (p<0.001). The conventional resin [median: 84.54] had a higher contact angle than the 3D-printed resin [median: 61.76] (Figure 2).

Biofilm adhesion

There was a significant difference between the conventional and 3D-printed resins for the adhesion of *S. mutans* (p=0.023) and *S. aureus* (p=0.010), with a higher microbial load on the surface of the conventional resin. A large variation was observed between the microbial load values of *S. mutans*, which is explained by the fact that three of the specimens (one of conventional resin and two of 3D-printed resin) had a count equal to zero.

Figure 3 shows the microbial load of *S. mutans* and *S. aureus* on the surface of the conventional [*S. mutans* - median*:* 5.60]; [*S. aureus* - median*:* 7.24] and 3D-printed resins for complete dentures [*S. mutans* – median: 4.53]; [*S. aureus* - median*:* 6.66].

Scanning Electron Microscopy (SEM)

The SEM images (Figure 4) show multispecies biofilm formation in layers with interaction between *C. albicans*, *S. mutans* and *S. aureus*, with probable co-adhesion and co-aggregation between the species.

Table 1- Comparison of surface roughness (Sa, µm) of conventional and 3D-printed resins for complete denture base and tooth, considering n=30 per group

	Conventional					3D-printed				
	Median	**Q1**	**Q3**	**Mean**	**SD**	**Median**	**Q1**	**Q3**	**Mean**	**SD**
Base	0.09^{Aa}	0.07	0.11	0.10	0.04	0.19^{Ab}	0.12	0.25	0.19	0.08
Teeth	0.12^{Ba}	0.11	0.15	0.14	0.45	0.16^{Aa}	0.11	0.22	0.16	0.59

*Capital letters: comparison between resins for the same application; lowercase letters: comparison between applications for the same resin. SD: standard deviation.

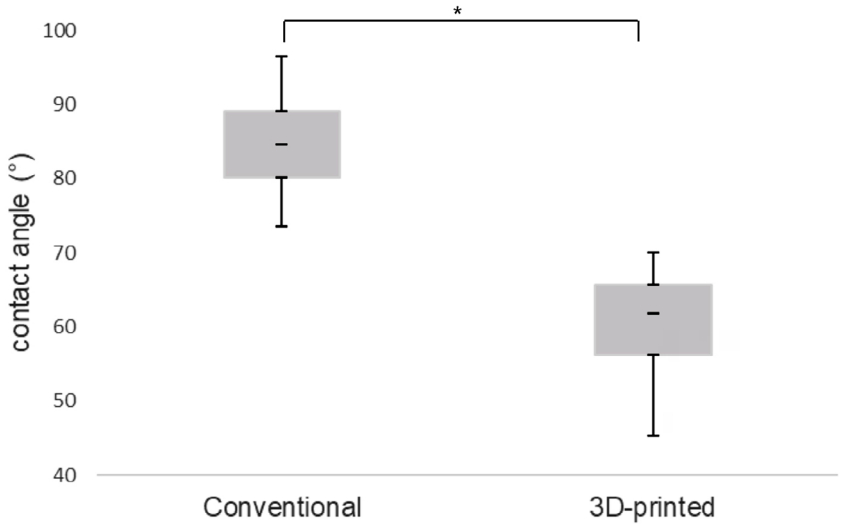

Figure 2- Comparison of the wettability of conventional and 3D-printed resins for complete dentures, represented by the contact angle (p<0.001). Signal (*) indicates a significant difference between the resins

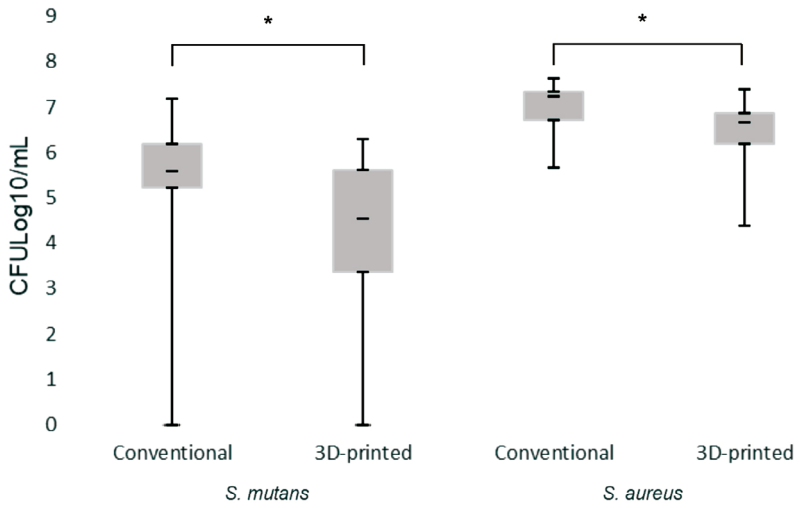

Figure 3- Microbial load (Log10CFU/mL) of *S. mutans* and *S. aureus* on conventional and 3D-printed resins for complete dentures (p=0.023). Signal (*) indicates a significant difference between the resins

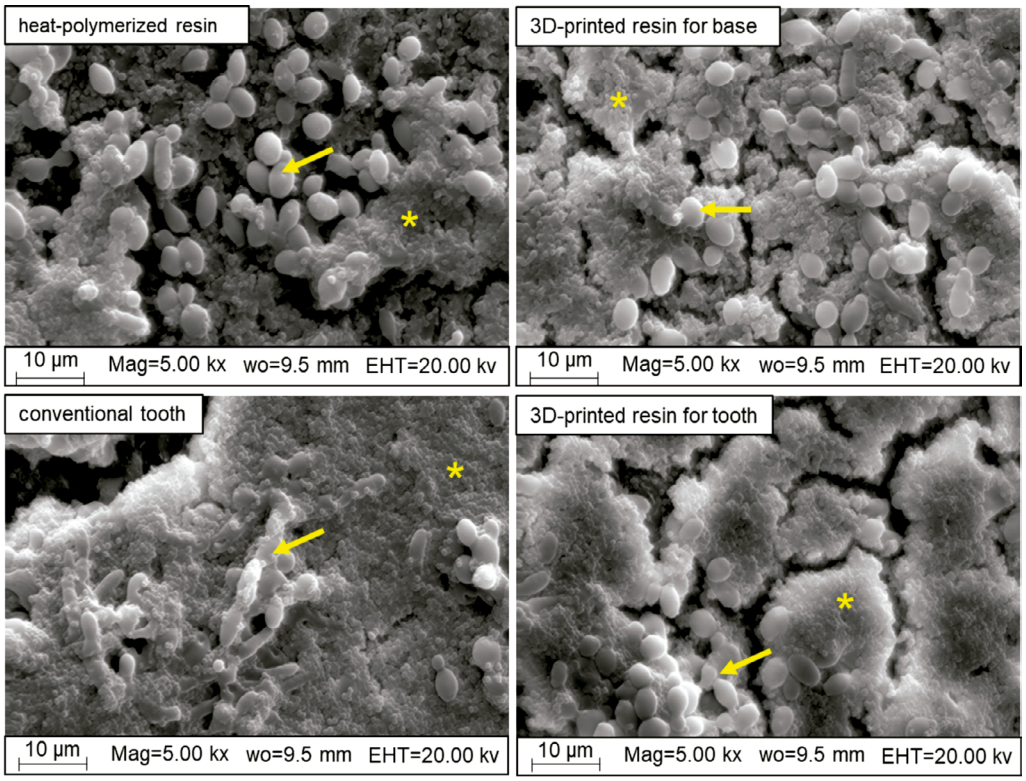

Figure 4- Multispecies biofilm on heat-polymerized resin for base, 3D-printed resin for base, conventional tooth and 3D-printed resin for tooth in 5000 x. The arrow indicates *C. albicans* and the symbol (*) indicates bacterial aggregates

Discussion

The null hypothesis was partially rejected as there was an interaction between resins and application for roughness and a significant difference between conventional and 3D-printed resins for wettability and adhesion of *S. mutans* and *S. aureus*. There was no significant difference between resins or application for *C. albicans* adhesion.

Microbial adhesion involves a thermodynamic model based on the interfacial free energies of liquids and interactive surfaces.[26] Therefore, it can be influenced by the surface properties of materials, such as roughness and wettability, as well as by the characteristics of microorganisms.[9,13,18,20,22,23,26]

In this study, the roughness parameter Sa was used instead of Ra. However, the literature shows a positive correlation between these two parameters, indicating redundancy and low discriminatory power between them.[19] The specimens were obtained with a printing angle of 0°, but the literature shows that different printing angles (0°, 45° and 90°) do not influence

microbial adhesion.[14] Furthermore, the specimens in this study were polished in a standardized way to achieve a roughness of approximately 0.2 µm.[9] The results of this study did not indicate significant differences in the adhesion of *C. albicans* to different surfaces, possibly because all materials were within the clinically acceptable roughness range. These results are consistent with the literature regardless of surface roughness standardization of ≤ 0.2 µm[22] or ≥ 0.2 µm[17]. For *S. mutans* and *S. aureus*, surface roughness also did not appear to have an effect on microbial counts, as there was greater adhesion to conventional resins compared to 3D-printed ones, even when roughness was standardized. It is possible that other factors, such as hydrophobicity, had a greater influence on bacterial adhesion.[21]

The wettability assay showed that the conventional resin had a higher contact angle (higher hydrophobicity) than the 3D-printed resin.[26] Adhesion of *C. albicans* to prosthetic surfaces *in vitro* has been associated with microorganism hydrophobicity.[9,28] However, different experimental and growth conditions can influence the hydrophobicity of yeast cells.[10] The influence of wettability did not prove to be an important factor for the adhesion of this microorganism, as there was no significant difference between adhesion to conventional (hydrophobic) and 3D-printed (hydrophilic) resin surfaces, which is in line with the literature.[13,24] In this study, the mixed model biofilm used included *S. mutans*, which forms the initial biofilm and influences subsequent biofilm formation by co-adhesion and co-aggregation with other species.[11] Thus, it is possible that the adhesion of bacteria to the substrates and the formation of an initial biofilm influenced the adhesion and retention of *C. albicans*, reducing the influence of surface properties on the adhesion of yeast cells to the biofilm.[12] In addition, the qualitative analysis of the biofilm in the SEM did not show significant differences between the biofilm formed on the different materials, suggesting that there was an interaction between the species.

It has been reported that adhesion forces are stronger on hydrophobic surfaces than on hydrophilic ones.[27] Since *S. mutans* shows adhesion to surfaces with hydrophobic properties, it is suggested that its adhesion to conventional surfaces is stronger than to 3D-printed surfaces.[25] This bacterium also tends to adhere strongly to PMMA surfaces via electrostatic interactions, which may have influenced its adhesion to the conventional resin.[21] It is known that 3D-printed resins have a lower filler content than conventional resins, which may also have influenced their adhesion to these surfaces.[5] Furthermore, two of the 3D-printed resin specimens and one of the conventional resins showed no growth of *S. mutans*, which may also be related to the adhesion of the microorganism. However, because the count was equivalent to zero in some of the specimens, the microbial load values varied widely between them.

The cell surface of *S. aureus* may have hydrophobic properties and tends to adhere strongly to hydrophobic materials.[16] This may explain the greater affinity of this microorganism for the surface of conventional resins compared to 3D-printed ones, corroborating a previous study that showed higher adhesion of *S. aureus* to hydrophobic surfaces than to hydrophilic ones.[15]

Furthermore, the resins for bases and teeth, both conventional and 3D-printed, showed no significant differences in wettability and bacterial adhesion. This may support the hypothesis that surface hydrophobicity is closely related to the adhesion of *S. mutans* and *S. aureus* to these materials. Thus, it can be suggested that the higher adhesion of bacteria to conventional resin is due to the higher hydrophobicity of the surface of this material compared to that of 3D-printed materials. However, it is important to highlight that conventional and 3D-printed resins for bases and teeth have different compositions, with specific initiators, additives and filler contents.[1-4,7] They also have different degrees of conversion to polymers[3]. This may also have influenced microbial adhesion and should be taken into account.[1-4,7]

Studying the different brands of 3D printers and commercially available resins would be difficult, and this is a methodological limitation of this study. Based on the literature referenced in this study, a master comparison table was created to report the results (Figure 5). Another limitation of this study is the fact that it did not evaluate microbial adhesion on resin samples aged by thermocycling or subjected to prolonged hygiene protocols.[7,29] In addition, in the oral environment, the mechanism of microbial adhesion is complex and multifactorial, influenced by the presence of salivary substrates and by the different microbial species present in the oral cavity.[6,13] These conditions are difficult to simulate in a laboratory environment, which is another limitation of this study.[13] Future research should investigate microbial adhesion on

Author	Title	Objectives	Result
Freitas, et al.[9] (2023)	Physical, mechanical, and anti-biofilm formation properties of CAD-CAM milled or 3D printeddenture base resins: *in vitro* analysis.	To evaluate roughness, wettability, flexural strenght and microbial adhesion of *C. albicans* biofilm on resin for complete denture bases.	The 3D-printed resin had the highest surface roughness and the lowest mini-flexural strength.
Foggi, et al.[24] (2016)	Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization	To evaluate effect of roughness on the hydrophobicity of a denture-base acrylic resin and the adhesion of *C. albicans*.	Roughnes increased hydrophobicity, but had no effect on the adhesion of *C. albicans*.
Liber-Kneć, et al.[26] (2021)	Testing of dental biomaterials-determination of contact angle and surface free energy.	To study the contact angle and surface free energy of denture acrylic resins, composite and PET-G dental retainer.	The contact angle can be used to show differences in surface properties of dental materials.
Osman, et al.[23] (2023)	Influence of fabrication technique on adhesion andbiofilm formation of *Candida albicans* to conventional, milled, and 3D-printed denture base resin materials:a Comparative *in vitro* study.	To evaluate *Candida albicans* adhesion and biofilm formation on conventional, milled and 3D-printed denture base resins.	3D printing results in greater candida adhesion compared to CAD/CAM.

Figure 5- Master comparison table of the results of the referenced studies

different resin brands subjected to thermocycling and simulation of hygiene protocols over long periods of time, and further clinical studies should evaluate microbial behavior on resins under clinical conditions.

Conclusion

The conventional base resin showed less roughness than the conventional tooth and the 3D-printed base. The 3D-printed resins for base and tooth showed less hydrophobicity and less adhesion of *S. mutans* and *S. aureus* than conventional resins. The microbial adhesion of *S. mutans* and *S. aureus* was higher on the 3D-printed resin than on the conventional resin. The adhesion of *C. albicans* was not influenced by the surface properties of the resins.

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Lastly, they thank Lourivaldo dos Santos Pereira for his support in the wettability assay.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

All data generated and analyzed during this study are included in this published article.

Authors' contributions

Poker, Beatriz de Camargo: Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Writing – original draft (Equal); Writing – review & editing (Equal). **Oliveira, Viviane de Cassia:** Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Writing – original draft (Equal). **Macedo, Ana Paula:** Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Writing – original draft (Equal). **Gonçalves, Mariane:** Investigation (Equal); Methodology (Equal); Writing – original draft (Equal). **Ramos, Ana Paula:** Methodology (Equal); Writing – original draft (Equal). **Silva, Cláudia Lovato:** Conceptualization (Lead); Data curation (Lead); Formal analysis (Lead); Funding acquisition (Lead); Investigation (Lead); Methodology (Lead); Project administration (Lead); Resources (Lead); Software (Lead); Supervision (Lead); Validation (Lead); Visualization (Lead); Writing – original draft (Lead); Writing – review & editing (Lead).

References

1- Tasaka A, Matsunaga S, Odaka K, Ishizaki K, Ueda T, Abe S, et al. Accuracy and retention of denture base fabricated by heat curing and additive manufacturing. J Prosthodont Res. 2019;63(1):85-9. doi: 10.1016/j.jpor.2018.08.007

2- Baba NZ, Goodacre BJ, Goodacre CJ, Müller F, Wagner S. CAD/CAM complete denture systems and physical properties: a review of the literature. J Prosthodont. 2021;30(S2):113-24. doi: 10.1111/jopr.13243

3- Kessler A, Reymus M, Hickel R, Kunzelmann KH. Three-body wear of 3D printed temporary materials. Dent Mater. 2019;35(12):1805-12.
4- Fekri LK, Abdelaziz MS. Digital duplication of maxillary complete denture: a dental technique. J Esthet Restor Dent. 2023;35(7):1139-43.
5- Gruber S, Kamnoedboon P, Özcan M, Srinivasan M. CAD/CAM complete denture resins: an *in vitro* evaluation of color stability. J Prosthodont. 2021;30(5):430-9. doi: 10.1111/jopr.13246
6- Schubert A, Bürgers R, Baum F, Kurbad O, Wassmann T. Influence of the manufacturing method on the adhesion of *candida albicans* and *streptococcus mutans* to oral splint resins. Polymers (Basel). 2021;11;13(10):1534. doi: 10.3390/polym13101534
7- Alfouzan AF, Alotiabi HM, Labban N, Al-Otaibi HN, Al Taweel SM, AlShehri HA. Color stability of 3D-printed denture resins: effect of aging, mechanical brushing and immersion in staining medium. J Adv Prosthodont. 2021;13(3):160-71. doi: 10.4047/jap.2021.13.3.160
8- Atalay S, Çakmak G, Fonseca M, Schimmel M, Yilmaz B. Effect of different disinfection protocols on the surface properties of CAD-CAM denture base materials. J Prosthet Dent 2022;8:S0022-3913(21)00692-2. doi: 10.1016/j.prosdent.2021.12.007
9- Freitas RF, Duarte S, Feitosa S, Dutra V, Lin WS, Panariello BH, et al. Physical, mechanical, and anti-biofilm formation properties of CAD-CAM Milled or 3D printed denture base resins: *in vitro* analysis. J Prosthodont. 2023;32(S1):38-44. doi: 10.1111/jopr.13554
10- Goswami RR, Pohare SD, Raut JS, Karuppayil SM. Cell surface hydrophobicity as a virulence factor in *candida albicans*. Biosci Biotech Res Asia. 2017;14(4). doi: http://dx.doi.org/10.13005/bbra/2598
11- Hotta M, Morikawa T, Tamura D, Kusakabe S. Adherence of *Streptococcus sanguinis* and *Streptococcus mutans* to saliva-coated S-PRG resin blocks. Dent Mater J. 2014;33(2):261-7.
12- Sampaio C, Pessan JP, Nunes GP, Magno MB, Maia LC, Exterkate R, et al. Are the counts of *Streptococcus mutans* and *Staphylococcus aureus* changed in complete denture wearers carrying denture stomatitis? A systematic review with meta-analyses. J Prosthet Dent. 2023;18:S0022-3913(23)00180-4. doi: 10.1016/j.prosdent.2023.03.015
13- Murat S, Alp G, Alatalı C, Uzun M. *In vitro* evaluation of adhesion of *candida albicans* on CAD/CAM PMMA-based polymers. J Prosthodont. 2019;28(2):e873-e879. doi: 10.1111/jopr.12942
14- Li P, Fernandez PK, Spintzyk S, Schmidt F, Yassine J, Beuer F, et al. Effects of layer thickness and build angle on the microbial adhesion of denture base polymers manufactured by digital light processing. J Prosthodont Res. 2023;67(4):562-7.
15- Pagedar A, Singh J, Batish VK. Surface hydrophobicity, nutritional contents affect *Staphylococcus aureus* biofilms and temperature influences its survival in preformed biofilms. J Basic Microbiol. 2010;50(Suppl 1):S98-106. doi: 10.1002/jobm.201000034
16- Kouidhi B, Zmantar T, Hentati H, Bakhrouf A. Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in *Staphylococcus aureus* associated to dental caries. Microb Pathog. 2010;49(1-2):14-22.
17- Song F, Koo H, Ren D. Effects of material properties on bacterial adhesion and biofilm formation. J Dent Res. 2015;94(8):1027-34. doi: 10.1177/0022034515587690
18- Taghizadeh A, Taghizadeh M, Yazdi MK, Zarrintaj P, Ramsey JD, Seidi F, et al. Mussel-inspired biomaterials: from chemistry to clinic. Bioeng Transl Med. 2022;7(3):e10385. doi: 10.1002/btm2.10385
19- 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(7):456.
20- Quirynen M, Marechal M, Van Steenberghe D, Busscher HJ, Van Der Mei HC. The bacterial colonization of intra-oral hard surfaces in vivo: Influence of surface free energy and surface roughness. Biofouling. 1991;4(1-3):187-98. doi: https://doi.org/10.1080/08927019109378209
21- Yuan C, Wang X, Gao X, Chen F, Liang X, Li D. Effects of surface properties of polymer-based restorative materials on early adhesion of *Streptococcus mutans in vitro*. J Dent. 2016;54:33-40. doi: 10.1016/j.jdent.2016.07.010
22- Ozel GS, Guneser MB, Inan O, Eldeniz AU. Evaluation of *C. Albicans* and *S. Mutans* adherence on different provisional crown materials. J Adv Prosthodont. 2017;9(5):335-40. doi: 10.4047/jap.2017.9.5.335
23- Osman RB, Khoder G, Fayed B, Kedia RA, Elkareimi Y, Alharbi N. Influence of fabrication technique on adhesion and biofilm formation of *candida albicans* to conventional, milled, and 3d-printed denture base resin materials: a comparative *in vitro* study. Polymers (Basel). 2023;15(8):1836. doi: 10.3390/polym15081836
24- Foggi CC, Machado AL, Zamperini CA, Fernandes D, Wady AF, Vergani CE. Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization. J Investig Clin Dent. 2016;7(2):141-8. doi: 10.1111/jicd.12125
25- Viitaniemi L, Abdulmajeed A, Sulaiman T, Söderling E, Närhi T. Adhesion and early colonization of *s. mutans* on lithium disilicate reinforced glass-ceramics, monolithic zirconia and dual cure resin cement. Eur J Prosthodont Restor Dent. 2017;25(4):228-34.
26- Liber-Kneć A, Łagan S. Surface testing of dental biomaterials-determination of contact angle and surface free energy. Materials (Basel). 2021;21;14(11):2716. doi: 10.3390/ma14112716
27- Wang C, van der Mei HC, Busscher HJ, Ren Y. *Streptococcus mutans* adhesion force sensing in multi-species oral biofilms. NPJ Biofilms Microbiomes. 2020;24;6(1):25. doi: https://doi.org/10.1038/s41522-020-0135-0
28- Gad MM, Abualsaud R, Khan SQ. Hydrophobicity of denture base resins: a systematic review and meta-analysis. J Int Soc Prev Community Dent. 2022;8;12(2):139-59. doi: 10.4103/jispcd.JISPCD_213_21
29- Ribeiro AB, Tinelli BM, Clemente LM, Poker BC, Oliveira VC, Watanabe E, et al. Effect of hygiene protocols on the mechanical and physical properties of two 3d-printed denture resins characterized by extrinsic pigmentation as well as the mixed biofilm formed on the surface. Antibiotics. 2023;12(11):1630. doi: https://doi.org/10.3390/antibiotics12111630
30- Zidan S, Silikas N, Haider J, Yates J. Long-term sorption and solubility of zirconia-impregnated pmma nanocomposite in water and artificial saliva. Materials (Basel). 2020;13(17):3732. doi: 10.3390/ma13173732
31- Ururahy MS, Curylofo-Zotti FA, Galo R, Nogueira LF, Ramos AP, et al. Wettability and surface morphology of eroded dentin treated with chitosan. Arch Oral Biol. 2017;75:68-73. doi: 10.1016/j.archoralbio.2016.11.017