Research

Evaluation of optical, surface, and microbiological properties of computer-aided design/computer-aided manufacturing-milled and three-dimensional-printed denture bases after aging by thermocycling: An *in vitro* study

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Abstract

Aim: The purpose of this study was to evaluate the surface properties, optical stability, and *Candida albicans* adhesion to resins used in the base of conventional, milled, and three-dimensional (3D)-printed dentures after aging by thermocycling.

Settings and Design: *In vitro* study.

Materials and Methods: A total of 96 resin samples were manufactured, divided into 3 resin groups ($n = 32$) (conventional thermally activated, milled and 3D-printed), and subdivided into 4 aging times by thermocycling at temperatures 5 ± 1°C and 55 ± 1°C in 24h, 5.000, 10.000, 20.000 cycles ($n = 8$), evaluating the adhesion of *C. albicans*, optical stability, surface roughness, hydrophilicity, and energy surface.

Statistical Analysis Used: Data were evaluated by two-way ANOVA and Tukey's test for pairwise comparisons ($P < .05$).

Results: Conventional resin exhibited lower C. albicans colonization, with significant reductions at T1 (1.05±1.38) ($P = .003$) and T3 (3.67 ±1.86) ($P = .023$) compared to other resins. The conventional resin had significantly the lowest ΔE00 value at T0 (0.98 ± 0.41) ($P < .05$). The milled resin had significantly lower roughness value at T0 (0.031 ± 0.00) ($P < .05$). The conventional resin had significantly higher surface energy values compared to the other resins ($P < .05$). The conventional resin exhibited a significantly lower water contact angle than the other resins ($P < .001$).

Conclusion: The conventional thermally activated resin showed less adhesion of *C. albicans* than the resins manufactured by CAD/CAM. Among CAD/CAM resins, milled resin showed better properties than 3D-printed resin over time.

Keywords: *Candida albicans*, computer-aided design/computer-aided manufacturing, denture stomatitis, dentures

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INTRODUCTION

The incidence of total edentulism is rising alongside increasing life expectancy, prompting a surge in research aimed at enhancing materials and techniques for denture fabrication.[1-3] Polymethylmethacrylate (PMMA) remains the material of choice owing to its affordability, accessibility, and user-friendliness. It can be processed using either conventional methods or digital approaches, including computer-aided design/computer-aided manufacturing (CAD/CAM), through milling and three-dimensional (3D) printed.[3,4]

Conventional PMMA exhibits increased polymerization contraction and a higher quantity of residual monomers, resulting in a porous surface and, consequently, a heightened susceptibility to bacterial plaque accumulation.[3,4] In contrast, CAD/CAM milling, a subtractive technique, utilizes blocks of acrylic resin condensed under high heat and pressure, conditions to minimize porosity. Meanwhile, 3D printed, an additive approach, employs liquid resin to construct dentures through the layered deposition of resin, with simultaneous photopolymerization by ultraviolet light, leading to reduced material waste.[5-7]

The microorganism *Candida albicans* is prevalent in the oral cavity's commensal flora, capable of adhering to and proliferating on the oral mucosa and denture surfaces.[8] The attachment and colonization of *C. albicans* are predisposing factors for oral candidiasis, leading to denture stomatitis, which can result in denture loss in cases of super-resistance of the microorganism, causing relapses.[9,10] Since *C. albicans* infection is closely associated with its adhesion to material surfaces, properties such as roughness, surface energy, and hydrophilicity play significant roles in fungal colonization. Rough or irregular surfaces and hydrophobic materials facilitate noncovalent interactions with hydrophobic proteins in the *C. albicans* cell wall, influencing denture longevity.[11,12] The success of a denture depends on its physical properties and also on its esthetic characteristics; color changes can affect the quality of the prosthesis and patient satisfaction.[13]

Over the past decade, there has been a significant increase in studies comparing the surface properties of milled and 3D-printed resins with those of conventional resins.[14-17] While CAD/CAM-fabricated dentures are promising, further research is necessary, particularly for long-term follow-up, as there is a paucity of literature on the performance of these resins under aging conditions, mainly related to 3D-printed resins and the adhesion of microorganisms.[13,17] Consequently, this *in vitro* study aimed to evaluate the surface properties, optical stability, and *C. albicans* adhesion to resins used in the base of conventional, milled, and 3D-printed dentures after aging by thermocycling. The null hypothesis posits that no significant differences will exist between the types of resins and aging periods.

MATERIALS AND METHODS

Specimen preparation

The sample size was calculated using G*Power v. 3.1.9.4 software (Heinrich-Heine-Universität Düsseldorf). The effect size was 1.66, $\alpha = 0.05$, and power = 80%, according to a previous study.[18-23] A minimum of 7 specimens per group were required. The sample size was adjusted to 8 specimens per group [Figure 1]. A total of 96 resin samples measuring $10 \times 3.0 \pm 0.03$ mm in diameter were manufactured in accord to ISO 20795-1:2013 standards,[18] divided into three groups of denture resin ($n = 32$) (G1: conventional thermally activated, G2: milled, and G3: 3D printed), according to the manufacturing method [Table 1] and subdivided into four aging times ($n = 8$) in T0: 24 h (baseline) and cycles (T1: 5.000, T2: 10.000, and T3: 20.000), evaluating the adhesion of *C. albicans*, optical stability, roughness, hydrophilicity, and surface energy [Figure 2].

Specimen fabrication

The G1 (conventional thermally activated) used acrylic

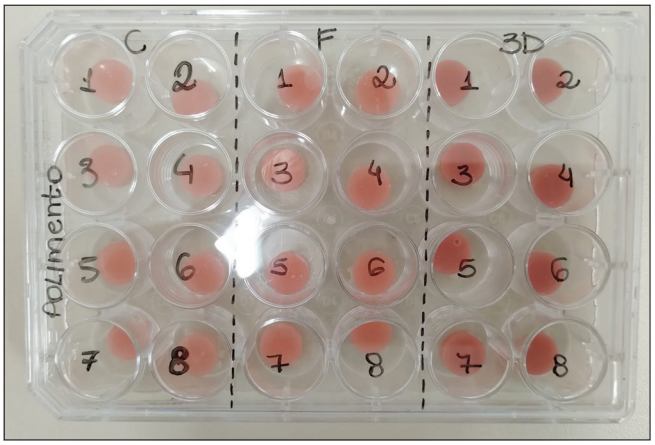


Figure 1: Specimens are divided into three groups of denture resin

Table 1: Acrylic resins for denture bases used in the study

Groups	Material	Brand	Proportion	Making method	Composition
G1	Thermally activated resin	Clássico	14 g powder for 6.5 ml monomer	60' water bath in 100°C	Powder: Polymethylmethacrylate, benzoyl peroxide, and biocompatible pigments Liquid: Methyl methacrylate monomer and inhibitor
G2	Resin pressed into disc	BlueDent	Pressed disc	Milled	Polymethylmethacrylate, EDMA, Fluorescent, organic additives, and biocompatible pigments
G3	Liquid resin	SmartDent	Liquid resin	3D-printed	Monomers, oligomers, photoinitiators, pigments, and stabilizers

3D: Three-dimensional, EDMA: Etilenoglicol dimetacrilato

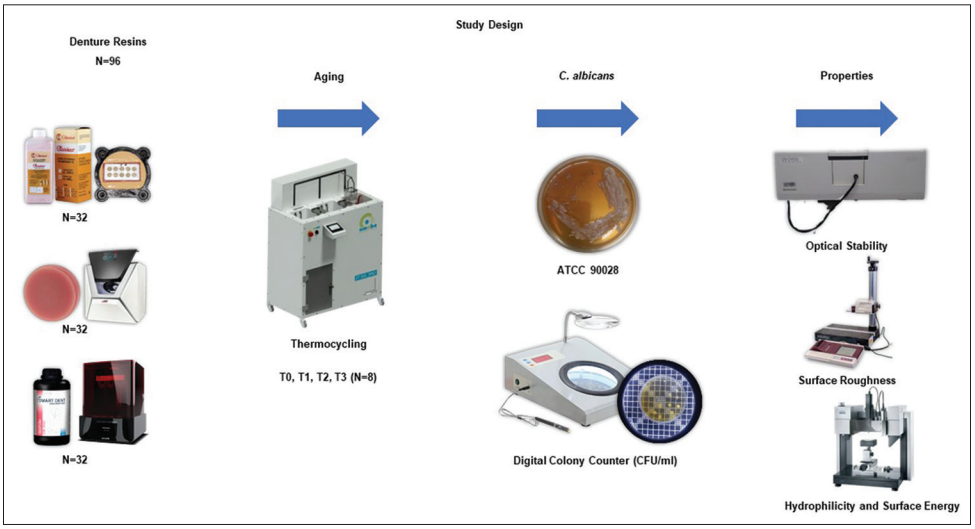


Figure 2: Diagram representing the study design

resin (Clássico®) placed on metal molds in a plastic muffle (Vipi–STG Ltd.), receiving 14.71 kN for 5 min in a hydraulic press (Maxx 1; Essence Dental). The polymerization was realized in a boiling water bath (100°) for 1 h. The G2 (milled) used discs resin (BlueDent Ltd.), CAD software (Exocad; Exocad Gmbh) for determination of the sample's dimensions, and CAM software (Exocad; Exocad Gmbh) for milled in a 5-axis milling machine (SilaMill 5R; SILADENT). The G3 (3D-printed) used liquid resin (SmartDent Ltd.), CAD software (Exocad; Exocad Gmbh) for determination of the sample's dimensions, and stereolithographic printer (MoonRay Model S; VertySystem), configured in 50 μm layer thickness and 45° impression. The postcuring was 30 min in 90% intensity (Dymax ECE 5000). All samples were subjected to a standardized polishing: 200 μm, 600 μm, and 1000 μm (Carbimet; Buehler) and 800 μm and 1200 μm (Microcut; Buehler) in an automatic polishing machine (AutoMet 250; Buehler) for 30 s on each face.[18-20] All samples were fabricated and finished by a single operator (V.A.A.B.).

Thermocycling

All samples were randomized according to aging time, with T0 being considered samples stored in distilled water in an oven (Equipamentos Cientéticos; Cienlab) at 37°C ± 2°C for 24 h.[18,21] Others were subjected to thermocycling, using a thermocycler (Model MSCT-3, Convel) with distilled water in temperatures of 5°C ± 1°C and 55°C ± 1°C (70 s per cycle; residence time: 30 s; transfer time: 5 s). The cycles were determined in 5.000 (T1), 10.000 (T2), and 20.000 (T3), in which 5.000 represents a 6-month clinical aging.[22,23]

Candida albicans

All samples were randomized and sterilized using ethylene oxide gas (Oximed®) according to the ISO9001:2015 standard protocols for microbiological tests.[9] *C. albicans* ATCC#90028 was streaked on an agar plate on Sabouraud dextrose agar (SDA) and transferred to tubes containing 10 mL brain heart infusion, and both were incubated at 37°C for 24 h. The colonies (C. albicans) were transferred to a Petri dish with SDA and incubated in an oven at 37°C with 5% CO2 for 24 h. The C. albicans were transferred to yeast malt broth (YMB) for absorbance mensuration in a spectrophotometer at a wavelength of 625 nm until reaching an optical density of the 350 ABS. The YMB were distributed 2 mL for each sample, which was kept in an oven at 37°C with 5% CO2 for 48 h. The samples were transferred to test tubes with 5 mL of 0.9% NaCl, and serial dilutions were made 1:9 μL (from 10^{-1} to 10^{-6}). Three drops of 25 μL were placed in each quadrant in a plate with SDA and incubated in an oven at 37°C with 5% CO_2 for 24 h. The counting (10 CFU/mL) was performed

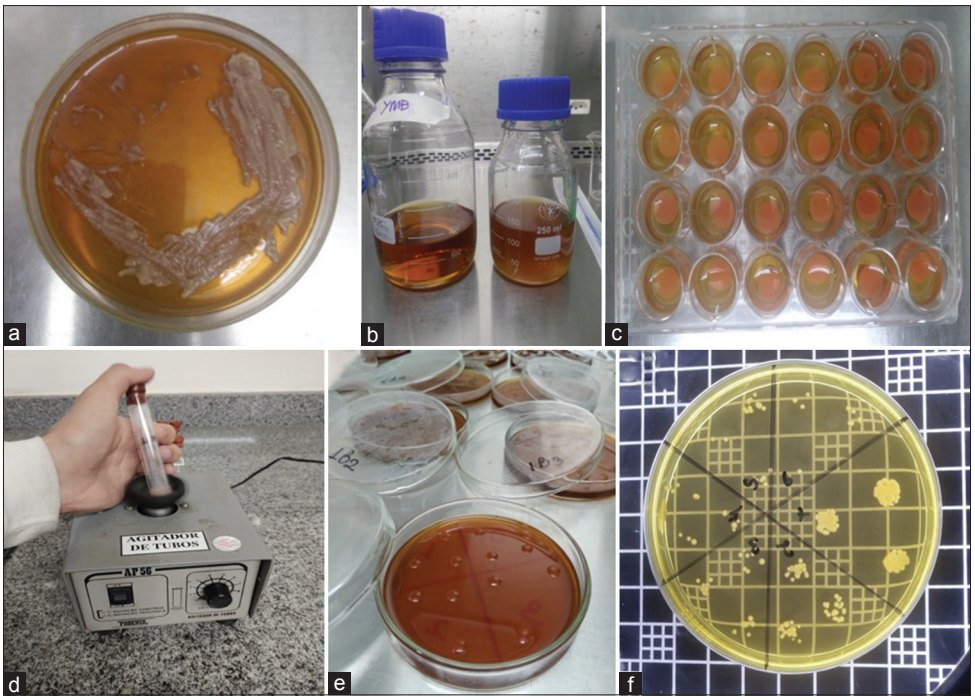


Figure 3: *Candida albicans* colonization: (a) *C. albicans* ATCC#90028 isolate on an agar plate on Sabouraud dextrose agar (SDA); (b) The colonies formed on yeast malt broth until cloudy; (c) 2 mL of broth was distributed in each well for each sample; (d) Samples in test tubes with 5 mL of 0.9% NaCl in vortex processing for 1 min; (e) A plate with SDA divided into six quadrants and three drops of 25 μL in each quadrant; (f) Courting *C. albicans* colonies (10 CFU/mL) using a digital colony counter

using a digital colony counter, choosing the quadrant with 3–30 colonies per drop [Figure 3].[9,19,20]

Optical stability analysis

The optical stability analysis was evaluated by a spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan) with mensuration the CIE L*a*b* color scale (Commission Internationale de I'Eclairage). The spectrophotometer was configured in a wavelength range of 380–780 nm and an opening of 10 mm in diameter. With the data collected before and after thermocycling and *C. albicans*. The chromatic difference was calculated using the CIEDE 2000 system (ΔE00). The higher the ΔE00 value, the greater the color change of the material, resulting in lower optical stability.[18]

Surface roughness analysis

The average roughness (Ra) was analyzed by contact profilometry (SJ-401 Mitutoyo) featuring a diamond with a diameter of 2 mm (Knoop) set at λ = 0.08 mm cutoff wavelength and 0.25 mm transverse length at a speed of 0.05 mm/s. Three measurements were performed on each sample by a single operator (V.A.A.B.).[18-20]

Surface energy and hydrophilicity analysis

Surface energy and hydrophilicity were analyzed by Drop Shape Analysis System DSA100E automatic dispenser digital goniometer (Krüss GmbH), calculating the average of five measurements of the contact angle (ø) of a 20 μL drop of distilled and diiodomethane. The Owens–Wendt method was defined in the Drop Shape Analysis software 1.51.[5,19]

Statistical analysis

Continuous measures with mean and standard deviation values were computed using SigmaPlot 15.0 statistical software (SYSTAT Software, San Jose, California, USA). Statistical differences were evaluated by a two-way analysis of variance (ANOVA) and Tukey's test for pairwise comparisons ($P < 0.05$).

RESULTS

Candida albicans

ANOVA (two-way) revealed that both resin ($F = 295.793$; $P < 0.001$) and aging ($F = 295.793$; $P < 0.001$) significantly influenced *C. albicans* colonization (105 CFU/mL). Tukey's test for pairwise comparisons indicated that conventional resin exhibited lower *C. albicans* colonization in all aging times, with significant reductions at T1 (1.05 ± 1.38) ($P = 0.003$) and T3 (3.67 ± 1.86) ($P = 0.023$). The conventional resin's colonization levels did not differ significantly over time ($P > 0.05$). In contrast, milled resin showed a significant increase at T3 (7.33 ± 1.60) compared to T0 (2.68 ± 1.74) ($P = 0.027$), and 3D-printed resin had a significant rise at T3 (7.50 ± 1.96) relative to both T0 (3.37 ± 2.68) and T1 (3.67 ± 2.57) ($P < 0.05$) [Figure 4].

Optical stability

ANOVA (two-way) revealed that both resin ($F = 153.499$; $P < 0.001$) and aging ($F = 566.196$; $P < 0.001$) significantly affected optical stability. Tukey's test for pairwise comparisons indicated that the conventional resin had the significantly lowest ΔE00 value at T0 (0.98 ± 0.41) ($P < 0.05$). In contrast, the milled resin exhibited significantly lower ΔE00 values at T2 (3.11 ± 3.31) ($P < 0.05$) and T3 (3.57 ± 0.25) ($P < 0.001$) compared to other resins. Over time, ΔE00 values increased significantly in all resins ($P < 0.05$), with the 3D-printed resin showing significantly higher values than the others at T3 (5.53 ± 0.35) ($P < 0.001$) [Figure 5].

Surface roughness

ANOVA (two-way) revealed that both resin ($F = 18.127$; $P < 0.001$) and aging ($F = 19.987$; $P < 0.001$) significantly affected surface roughness. Tukey's test for pairwise comparisons indicated that the milled resin had a significantly lower roughness value at T0 (0.031 ± 0.00) ($P < 0.05$). However, over time, there were no significant differences in roughness values among the resins ($P > 0.05$). All resins exhibited a significant increase in roughness at T3 compared to T0 and T1 ($P < 0.05$), with the higher value being 3D-printed resin (0.065 ± 0.00) [Figure 6].

Surface energy

ANOVA (two-way) revealed that both resin ($F = 40.064$; $P < 0.001$) and aging ($F = 136.388$; $P < 0.001$) significantly influenced surface energy. Tukey's test for pairwise comparisons indicated that the conventional resin had significantly higher surface energy values compared to the other resins ($P < 0.05$), with the exception of time T1 (49.98 ± 1.16) ($P > 0.05$). A significant reduction in surface energy was observed for all resins at time T3 compared to other time points ($P < 0.001$), with the lower values presented by milled (36.67 ± 3.79) and 3D printed (33.80 ± 2.03) [Figure 7].

Hydrophilicity

ANOVA (two-way) revealed that both resin ($F = 84.335$; $P < 0.001$) and aging ($F = 55.071$; $P < 0.001$) significantly influenced hydrophilicity. Tukey's test for pairwise comparisons indicated that the conventional resin exhibited a significantly lower water contact angle than the other resins in all aging times ($P < 0.001$), being the lowest at T0 (66.15 ± 2.02) and the highest at T3 (76.33 ± 1.43). Over time, all resins demonstrated an increase in water contact angle, with T3 showing a significantly greater duration compared to T0 across all

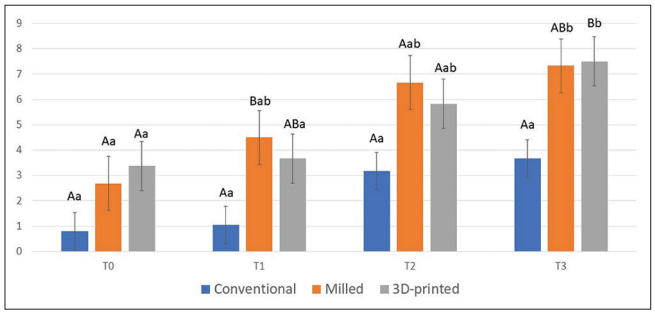


Figure 4: Result of *Candida albicans* colonization (105 CFU/mL). Different uppercase letters at the same time and different lowercase letters in the same resin show a significant difference ($P < 0.05$). T0: 24 h, T1: 6 months, T2: 12 months, T3: 24 months, 3D: Three-dimensional

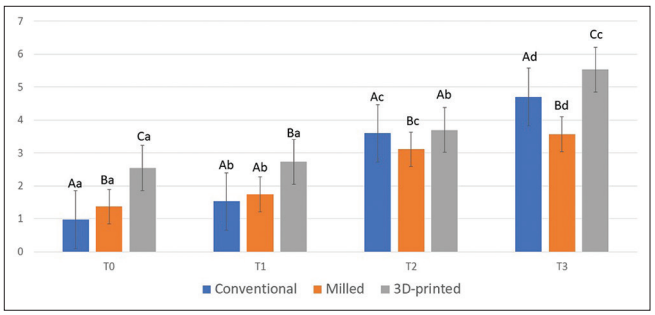


Figure 5: Result of optical stability. Different uppercase letters at the same time and different lowercase letters in the same resin show a significant difference ($P < 0.05$). T0: 24 h, T1: 6 months, T2: 12 months, T3: 24 months, 3D: Three-dimensional

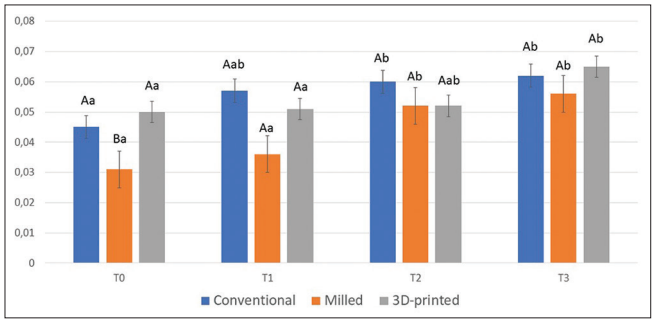


Figure 6: Result of surface roughness. Different uppercase letters at the same time and different lowercase letters in the same resin show a significant difference ($P < 0.05$). T0: 24 h, T1: 6 months, T2: 12 months, T3: 24 months, 3D: Three-dimensional

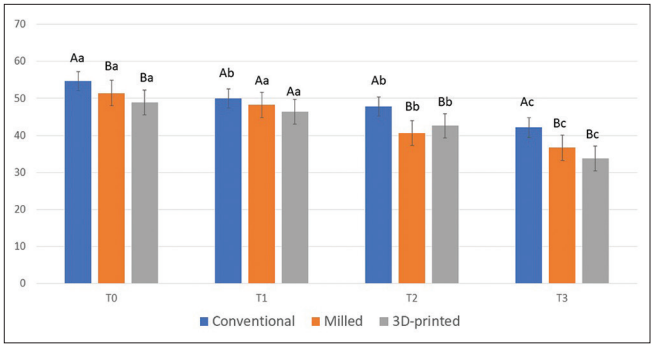


Figure 7: Result of surface-free energy. Different uppercase letters at the same time and different lowercase letters in the same resin show a significant difference ($P < 0.05$). T0: 24 h, T1: 6 months, T2: 12 months, T3: 24 months, 3D: Three-dimensional

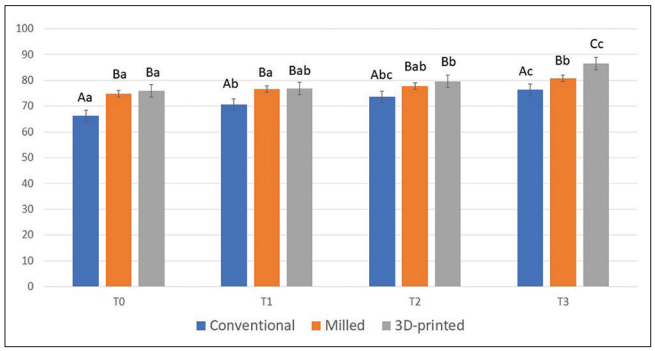


Figure 8: Result of hydrophilicity. Different uppercase letters at the same time and different lowercase letters in the same resin show a significant difference ($P < 0.05$). T0: 24 h, T1: 6 months, T2: 12 months, T3: 24 months, 3D: Three-dimensional

resins ($P < 0.05$), with the higher value being 3D-printed resin (86.58 ± 1.29) [Figure 8].

DISCUSSION

The two-way ANOVA results presented in this study reject the null hypothesis, indicating that both the resin type and aging significantly influenced the evaluated properties. The findings demonstrate that the conventional resin exhibited superior performance regarding *C. albicans* adhesion, surface energy, and hydrophilicity. Conversely, the milled resin showed the best long-term results in optical stability and surface roughness.

In this study, the conventional resin exhibited the highest surface energy values and the lowest water contact angle measurements over time, indicating increased hydrophilicity compared to the other resins. Additionally, the CAD/CAM resins demonstrated surface energy values below the critical threshold (41 mJ/m^2) after 24 months of aging (milled: 36.67 ± 3.79; 3D printed: 33.80 ± 2.03), leading to enhanced adhesion of *C. albicans*.[24] This outcome may be attributed to the denture manufacturing process, where the control of heat and temperature during the polymerization of conventional resin reduces the presence of surface residual monomers.[5] A higher surface energy and hydrophilicity result in improved wettability of the material, which plays a crucial role in hygiene and the retention of microorganisms on the denture. Hydrophobic prostheses, conversely, increase the adhesion of *C. albicans* hyphae, thereby facilitating denture stomatitis.[24,25] Consequently, denture base materials with greater wettability should be the preferred choice for clinical application.[24] In this context, conventional resin remains the superior option for dental professionals. However, if a prosthesis is fabricated using the CAD/CAM system, milled resin should be given precedence.

Several studies have indicated that the adhesion of *C. albicans* to denture resins produced by CAD/CAM is comparable to that of conventional resins, suggesting that CAD/CAM dentures may be less prone to pathogenic biofilm formation due to their more hydrophilic surfaces.[10,26] Conversely, other research has shown increased microbial adhesion to conventional resins, attributed to air entrapment during mixing, inadequate polymer and monomer blending, and improper muffle compression, all of which can influence microbial attachment to the denture surface. In the current study, no significant difference in adhesion between the resins was observed within the first 24 h ($P > 0.05$).[9,27] However, with aging–particularly after simulating 24 months of denture use, the conventional resin exhibited significantly lower adhesion compared to the other resins (3.67 ± 1.86; $P = 0.023$), a finding not previously reported in the literature. Notably, the studies reporting poorer performance of conventional resins employed a long polymerization cycle at low temperature (9 h at 74°C), whereas the present study utilized the Australian cycle (1 h at 100°C). Studies have shown that higher temperatures can reduce resin porosity and enhance material properties.[28,29]

Köroğlu *et al*.[30] demonstrated that the chromatic difference threshold for denture resins is ΔE00 = 1.30 for clinical perceptibility and ΔE00 = 2.25 for clinical acceptability. In this study, the 3D-printed resin did not exhibit clinical acceptability after 24 h (ΔE00 = 2.54), with values increasing over time. Conversely, both conventional and milled resins remained within acceptable limits up to 6 months of aging (1.53 ± 0.25 and 1.75 ± 0.29, respectively), with the milled resin maintaining significantly lower values over time ($P < 0.001$). The pronounced change in color stability of the 3D-printed resin may be attributed to bubble formation and structural irregularities during the printing process, as well as the low double bond conversion of these resins, which facilitates pigment entrapment and molecular interactions.[31,32]

In vitro studies have established a surface roughness (Ra) of 0.20 μm as the threshold for clinical acceptance of denture resins.[3,18-20] In the current study, all resins remained below this value at every aging interval, with no significant differences observed between them ($P > 0.05$). This indicates their suitability for long-term use.[26] These roughness findings diverge from those related to *C. albicans* adhesion, corroborating the conclusions of Gad *et al*.[7] and Silva *et al*.[33] that surface roughness is not the sole or most critical factor in *C. albicans* colonization. Instead, colonization is more closely associated with surface energy and hydrophilicity.[29]

Thermocycling is a widely used *in vitro* method for simulating the physiological aging of dental materials in the oral environment. This process involves subjecting specimens to several cycles of immersion in water at high and low temperatures.[22,23] In the current study, the established number of cycles was intended to simulate 6, 12, and 24 months of clinical use of dentures. This simulation directly influenced the surface energy, hydrophilicity, and consequently, the adhesion of *C. albicans*, an important factor in the development of denture stomatitis. These results are justified by the sudden temperature changes that cause successive contractions and volumetric expansions on the material's surface, directly affecting its surface characteristics.[22,28]

Overall, conventional resin exhibited less microbial adhesion compared to CAD/CAM resins, and the 3D-printed resin demonstrated properties that were unsuitable for long-term use. The limitation of this study was its *in vitro* design (sample size, different brands of CAD-CAM resins, blinding of samples is not possible), which could not precisely replicate clinical conditions. Consequently, clinical studies are necessary to yield more reliable results and conclusions. This *in vitro* study provides important insights into the microbiological behavior of resins used for CAD/CAM denture bases, particularly concerning the adhesion of *C. albicans*, the primary etiological agent in the development of denture stomatitis, the most common oral pathology among denture users. Thus, these findings could inform future *in vivo* research and ultimately guide the safe use of these resins in fabricating prosthesis bases, ensuring the well-being of patients.

CONCLUSION

The results of this *in vitro* study led to the following conclusions:

1. The conventional thermally activated resin showed less adhesion of *C. albicans* than the resins manufactured by CAD/CAM
2. Among CAD/CAM resins, milled resin showed better properties than 3D-printed resin over time.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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