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Original article

Comparison of the flexural and surface properties of milled, 3D-printed, and heat polymerized PMMA resins for denture bases: An *in vitro* study

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Abstract

Purpose: To compare the flexural properties and the adhesion of Lactobacillus salivarius (LS), Streptococcus mutans (SM), and Candida albicans (CA) on heat-polymerized (CV), CAD-CAM milled (CAD), or 3D-printed (3D) Poly (methylmethacrylate) (PMMA).

Methods: Ultimate Flexural Strength (UFS), Flexural Strain (FS) (%) at Flexural Strength, and Flexural Modulus (FM) of specimens (65.0×10.0×3.3 mm) from each PMMA group (n=6) were calculated by using the 3-point bending test. The surface roughness profiles (R) were measured before and after polishing with a contact profilometer. LS, SM, and CA adhesion on PMMA specimens (n=18) (10 mm in diameter, 3 mm in height) was assessed after 90 minutes and 16 hours by using scanning electron microscopy. The Kruskal-Wallis test with post hoc analysis was performed to compare the groups (alpha=0.05).

Results: Mean UFS values were 80.79±7.64 MPa for CV, 110.23±5.03 MPa for CAD, and 87.34±6.39 MPa for 3D. Mean FS values were 4.37±1.04% for CV, 4.71±0.62% for CAD, and 6.19±0.13 % for 3D. Mean FM values were 2542±301 MPa for CV, 3435±346 MPa for CAD, and 2371±197 MPa for 3D. CAD had the lowest average R value (0.29±0.16 µm) before polishing, and bacterial adhesion after 90 minutes of incubation. R value and microbial adhesion were not different amongst groups after polishing and 16 hours of incubation, respectively.

Conclusion: The CAD group displayed the best flexural properties, except for FS, the lowest roughness before polishing and bacterial adhesion after 90 minutes of incubation. All tested PMMAs had similar surface roughness after polishing, and microbial adhesion after 16 hours of incubation.

Keywords: CAD/CAM, 3D-printer, PMMA, Complete denture, Overdenture

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1. Introduction

Poly (methylmethacrylate) (PMMA) resin is the main material to fabricate complete dentures and implant-supported dentures due to its low cost, good physicochemical properties, and acceptable esthetics.[1-4] Dimensional changes, roughness, susceptibility to fracture, and wettability have been reported as the main drawbacks of PMMA. [5] Issues with its mechanical properties due to manufacturing process, polishing techniques, and dental hygiene of patients may increase denture-associated infections, [6] plaque accumulation, [7] and adhesion of candida albicans. [8,9]

Removable dentures are commonly fabricated conventionally pouring a fluid resin and mold filling techniques (compression and injection molding). [10-12] In recent years, computer-aided design and computer-aided manufacturing (CAD-CAM) technologies have allowed the fabrication of removable dentures, record bases, and implant-supported overdentures with subtractive (milling) or additive (3D printing) procedures. [13] Compared with the traditional workflow, the digital workflow reduces time, cost, processing steps,

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and increases accuracy. [14,15] However, few studies assessed the mechanical characteristics of PMMA processed by using CAD-CAM milling and 3D printing technologies. When compared with 3D-printed complete dentures, the intaglio surface of CAD-CAM milled complete dentures had favorable trueness and mechanical properties. [16,17] In addition, industrially prefabricated PMMA block for CAD-CAM milling presented a general improvement in material properties compared with heat-polymerized PMMA due to the strict control over pressure and temperature conditions during polymerization. [18] Previous studies demonstrated improved surface properties with PMMA CAD-CAM blocks compared with heat-polymerized PMMA. [19] However, the surface roughness values of all tested denture base materials [17,20] were below 0.2 µm, which was reported as clinically acceptable. [20] Comparison of surface roughness also with 3D-printed PMMA may be beneficial for clinicians to understand the differences amongst PMMAs fabricated by using varying techniques.

Knowledge of the surface roughness, mechanical characteristics, and biological behavior of microorganisms on denture base materials is important to achieve clinically successful dentures with PMMA bases. Therefore, the purpose of this in vitro study was to assess the effect of manufacturing method (CAD-CAM milling, 3D printing, or heat-polymerized) on flexural properties and surface roughness of PMMA. In addition, the adhesion of Streptococcus mutans, Lactobacillus salivarius, and Candida albicans was aimed to be evaluated. The null hypothesis was that the manufacturing process would not affect the flexural properties, surface roughness, and microbial adhesion of PMMAs.

2. Material and Methods

Mechanical and biological properties of heat-polymerized (CV), CAD-CAM milled (CAD), or 3D-printed (3D) PMMA were investigated. Rectangular prism-shaped specimens (65.0×10.0×3.3 mm (±0.2 mm)) were fabricated for mechanical tests (ISO 20795-1)[21](n=6), and cylindrical specimens (10 mm in diameter, 3 mm in height) were fabricated for the microbiological investigation by using each manufacturing method (n=18) (**Fig. 1 and 2**).

2.1. Specimens

For heat-polymerized specimens (Aesthetic Blue Clear; Candulor), the powder and the liquid were mixed according to the manufacturers' recommendations and packed into two custom silicone molds (Eurosil A & B; CHT Germany GmbH); one with the dimensions of 65.2×10.2×3.5 mm and the other with a cylinder-like shape (10 mm in diameter and 3 mm in height). The molds were then placed in a pressure pot (Palamat practice EL T; Kulzer GmbH) for 15 minutes at 45°C water temperature and 0.2 MPa pressure.

A CAD software (Exocad; Exocad Gmbh) was used to virtually design milling and printing groups according to the abovementioned dimensions. The CAD standard tessellation language files were sent to the CAM software of the milling machine and the 3D-printer. A PMMA block (Ruthinium Disc; Dental Manufacturing Spa) was milled to obtain the specimens using a 5-axis milling machine (SilaMill 5R; SILADENT). The specimens were 3D-printed using a stereolitography printer with digital light processing technology (MoonRay Model S; VertySystem) and liquid PMMA resin (Next Dent Denture Base). After printing, all specimens were cleaned using a detergent (VertySplash; VertySystem) applied in the cleaner machine (MoonWash; VertySystem) for 5 minutes according to the manufacturer's indications.

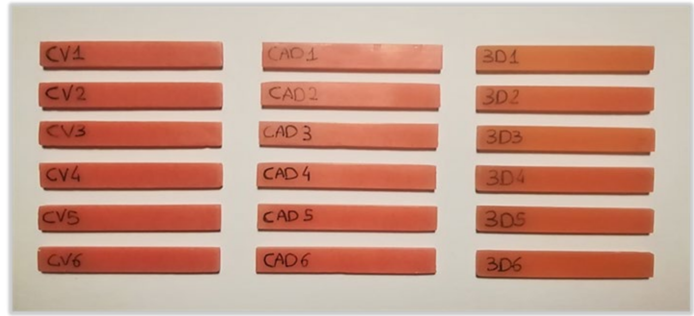

Fig. 1. Specimens from all groups (CV, CAD, and 3D) used for mechanical investigations according to ISO 20795-1.

Fig. 2. The cylinder-shaped specimens from groups (CV, CAD, and 3D) used for biological investigations.

Then, the specimens were polymerized for 20 minutes using a light-box (Moonlight; VertySystem).

According to ISO 20795-1, [21] all specimens were then ground to their final dimensions by using silicon carbide papers (Paper SiC P1200; Struers GmbH). The specimens were measured by using digital calipers with a 0.01-mm resolution (ABSOLUTE Digimatic Caliper Series 551 Mitutoyo Europe GmbH) ensuring that the specimens conformed to the dimensional tolerances of the ISO 20795-1. [21] After measurements, all specimens were disinfected by using 70% ethanol for 5 minutes and were stored in 37°C water for 50 hours according to ISO 20795-1. [21]

2.2. Flexural properties and surface roughness assessments

The 3-point bending test was performed using a universal testing machine (Acumen 3; MTS Systems Corp) with a crosshead speed of 5 mm/min according to ISO 20795-1. [21] Ultimate Flexural Strength (MPa), Flexural Strain (%) at Ultimate Flexural Strength and Flexural Modulus (MPa) were calculated. [21-22]

The surface roughness profile (R-profile) for each specimen was measured before and after polishing using a contact profilometer (Form Talysurf i-Series; Ametek Taylor Hobson). The polishing procedure consisted of the use of a silicon rubber polishing bur (141LMF2; Identoflex) and pumice powder (Polyglass Ultra ponce, Kaladent) with a polishing buff without water. The Ra was calculated using Gaussian filters with the tracing length set to 5.6 mm, the cut-off

Table 1. Mean values and standard deviations (SD) of flexural properties and surface roughness of heat-polymerized (CV), milled (CAD), and 3D-printed (3D) PMMA resins.

	Ultimate Flexural Strength (MPa)	Flexural Strain (%)	Flexural Modulus (MPa)	Ra (µm) before polishing	Ra (µm) after polishing	Rt (µm) before polishing	Rt (µm) after polishing
CV	80.79 (7.64)	4.37 (1.04)	2542.47 (301.55)	0.59 (0.3)	0.24 (0.08)	5.44 (2.97)	2.72 (1.70)
CAD	110.23 (5.03)	4.71 (0.2)	3435.07 (346.34)	0.29 (0.16)	0.22 (0.04)	2.09 (1.01)	2.15 (0.59)
3D	87.34 (6.39)	6.19 (0.13)	2371.37 (197.30)	0.38 (0.08)	0.29 (0.05)	3.91 (1.55)	3.04 (1.65)
P-value	*P<0.001*	*P<0.001* (CAD vs CV *P=0.25*)	*P<0.001* (CV vs 3D *P=0.13*)	*P<0.001*	*P=0.22*	*P<0.001*	*P=0.28*

length to 0.8 mm, and the stylus speed to 0.25 mm/s, according to ISO 16610-21. [23] Six measurements were performed for each specimen. The average roughness (Ra) and maximum roughness (Rt) were analyzed. Ra (µm) is the arithmetic mean value of all heights (peaks and valleys) in the given roughness profile. Rt (µm) is the maximum of all roughness depths (distance between the deepest valley and the highest peak). A plaque accumulation threshold of Ra=0.2 mm was used for comparisons. [20]

2.3. *Microbial assessment*

For the microbiological investigation, Lactobacillus salivarius (ATCC 33592) (L. salivarius), Streptococcus mutans (ATCC 25175) (S. mutans), and Candida albicans (ATCC 18804) (C. albicans) were purchased from ATCC (LGC Standards; Milan, Italy). L. salivarius and S. mutans were grown in brain heart infusion (BHI) broth or agar at 37°C. C. albicans was cultured in yeast malt broth or agar at 30°C. At the time of the experiment, microbial cultures were diluted in growth medium and cell density was adjusted to 1×106 colony-forming unit (CFU)/ml. For adherence assay, microbial preparations were added to the previously sterilized materials. Samples were placed into a 24-well tissue culture plate (Corning, Milan, Italy) and incubated for 90 minutes or 16 hours at 37°C or 30°C, as indicated above. At the end of incubation, the tested materials were washed in sterile PBS to remove non-adherent microbes. Samples were incubated in 1 mL of fresh cultured media for 30 minutes with shaking at 80 rpm to favor detachment of adherent microbes. Cell suspensions were then recovered, properly diluted, and plated on agar media. The CFU were enumerated 24 hours later. Experiments were performed in three independent replicates. Results are reported as CFU/ml of microbial species that adhere to the tested materials. All the specimens were analyzed using scanning electron microscopy (JSM 6490; Jeol). Before the measurements, all specimens were incubated into 3% glutaraldehyde solution for 2 hours to fix the bacteria and then in an ethylic alcohol solution at a concentration of 50, 70, 90, 100% for 10 minutes in each incubation.

2.4. *Statistical analysis*

Ultimate Flexural strength (MPa), Flexural Strain (%) at Ultimate Flexural Strength, Flexural Modulus (MPa), Ra (µm), and Rt (µm) were considered as the statistical unit. Descriptive and comparative statistics were analyzed, and the results were submitted to the Shapiro-Wilk test, where p=0.001 was considered not normally distributed. The Kruskal-Wallis test with a post hoc analysis using Dunn's test was used to compare the groups. The level of statistical significance was set as α = 0.05 and statistical power of 80% (SPSS v16.0; SPSS Inc, Chicago, IL).

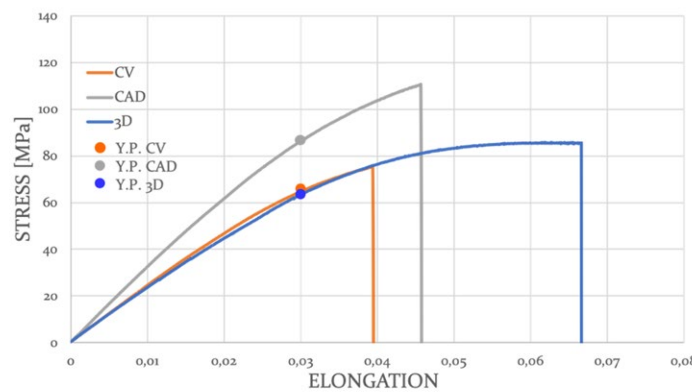

Fig. 3. Stress-Stain curve of groups with the respective Yield point.

3. Results

Table 1 presents the average and standard deviations of Ultimate Flexural Strength (MPa), Flexural Strain (%), Flexural Modulus (MPa), Ra (µm), and Rt (µm) for each of the heat-polymerized, milled, and 3D-printed PMMAs. All investigated mechanical properties were significantly affected by the manufacturing process (P<0.001). The CAD group presented higher flexural strength and modulus compared to CV (P<0.001) and 3D groups (P<0.001). No statistically significant difference for the Flexural Modulus (P = 0.13) was found between the CV and 3D groups and for the Flexural Strain (%) between the CAD and CV (P = 0.25). However, the Flexural Strain together with stress/strain diagrams (an example in **Fig. 3**) highlighted the wider plastic deformation with 3D printed PMMA compared with heat-polymerized or milled PMMA. The analysis of the surface roughness (Ra and Rt) revealed statically significant differences among the 3 groups before polishing (P<0.001). The average Ra of CAD group was lower compared with the average Ra of CV and 3D groups (**Table 1**, **Fig. 4**). After polishing, the average Ra (P = 0.22) and Rt (P= 0.28) of groups had no significant difference, and all average values were within the plaque accumulation threshold of 0.2 µm. (**Fig. 4**)

For microbial adhesion, the CAD group had the lower average microbial adhesion value after 90 minutes of incubation (**Table 2**). After 16 hours of incubation, the microbial proliferation progressed regularly and gradually on all surfaces, but no difference was found among the average microbial adhesion values of groups (**Table 2**). The SEM images before and after 90 minutes of incubation for all groups are reported in **Figure 5**.

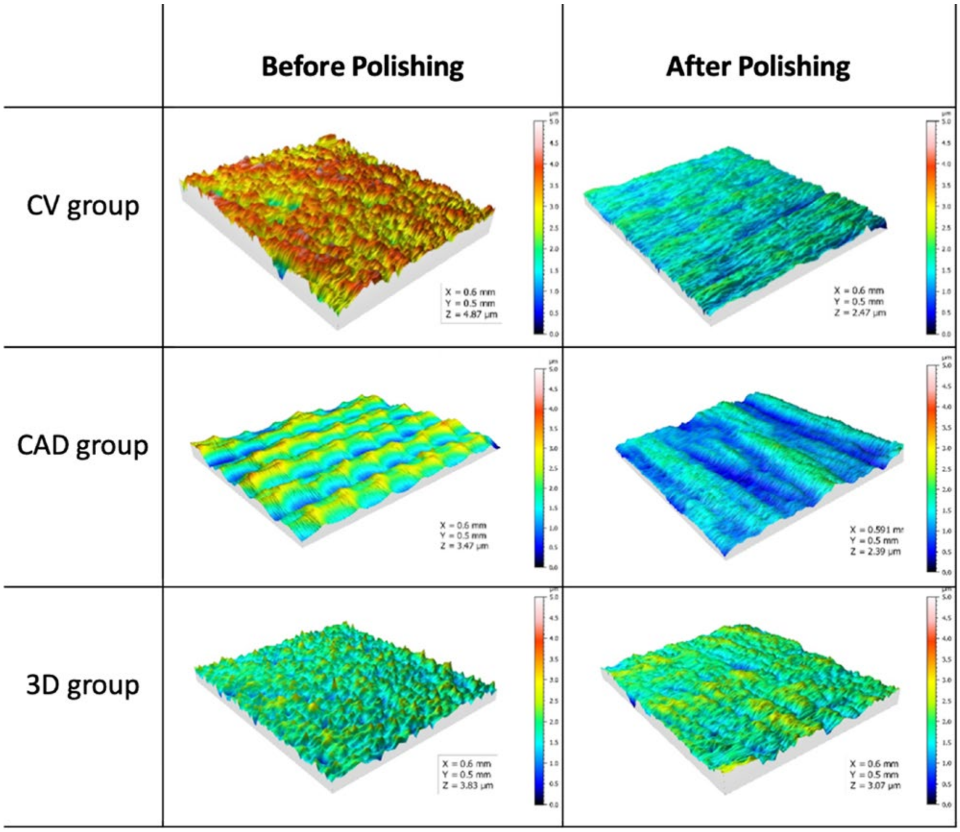

Fig. 4. Profilometer scan images of the groups before and after polishing.

Table 2. Mean values and standard deviations (SD) of microbial species that adhere to the tested materials after 90 minutes and 16 hours.

	Streptococcus mutans [CFU/ml]		Lactobacillus salivarius [CFU/ml]		Candida albicans [CFU/ml]	
	90 minutes	16 hours	90 minutes	16 hours	90 minutes	16 hours
CV	3.03E+03 (7.02E+02)	3.25E+06 (4.64E+05)	3.57E+03 (4.51E+02)	3.88E+06 (1.56E+05)	3.13E+03 (2.31E+02)	3.07E+06 (1.65E+05)
CAD	1.97E+03 (3.51E+02)	3.32E+06 (4.69E+05)	2.27E+03 (2.52E+02)	3.92E+06 (9.71E+04)	1.97E+03 (1.53E+02)	2.78E+06 (2.20E+05)
3D	2.97E+03 (3.21E+02)	3.15E+06 (1.12E+06)	2.97E+03 (3.06E+02)	3.27E+06 (6.05E+05)	1.83E+03 (2.52E+02)	2.93E+06 (1.31E+05)
P-Value	P<0.05	P>0.05	P<0.05 (CV vs CAD P= 0.001)	P>0.05	P<0.05 (CV vs 3D P= 0.001) (CV vs CAD P = 0.001)	P>0.05

4. Discussion

The heat-polymerized, milled, and 3D-printed denture base PMMAs were tested for their mechanical properties, surface roughness, and bacterial adhesion. The milled PMMA presented lower surface roughness before polishing, favorable flexural properties, and less microbial adhesion at 90 minutes of incubation compared with the heat-polymerized and 3D-printed PMMAs. Therefore, the null hypothesis that manufacturing process would not affect the mechanical properties and microbial adhesion of PMMA was rejected. Nevertheless, the average flexural strength values of all groups were above the required value of 65.0 MPa according to ISO 20795-1, [21] which may be considered clinically acceptable. In addition, surface roughness after polishing and microbial adhesion values after 16 hours of incubation were similar for all groups.

Conventional denture base fabrication method has been used for many years, however, porosity, roughness, volumetric and linear shrinkages were frequently encountered with the conventional dentures. Manual skills of the operator and manufacturing processes contributed to these flaws. [24,25] The CAD/CAM technology has standardized the workflow, reduced the fabrication time, [14] minimized the flaws of the conventional process, [18] and improved the trueness. [16,17] However, a difference has also been observed be-

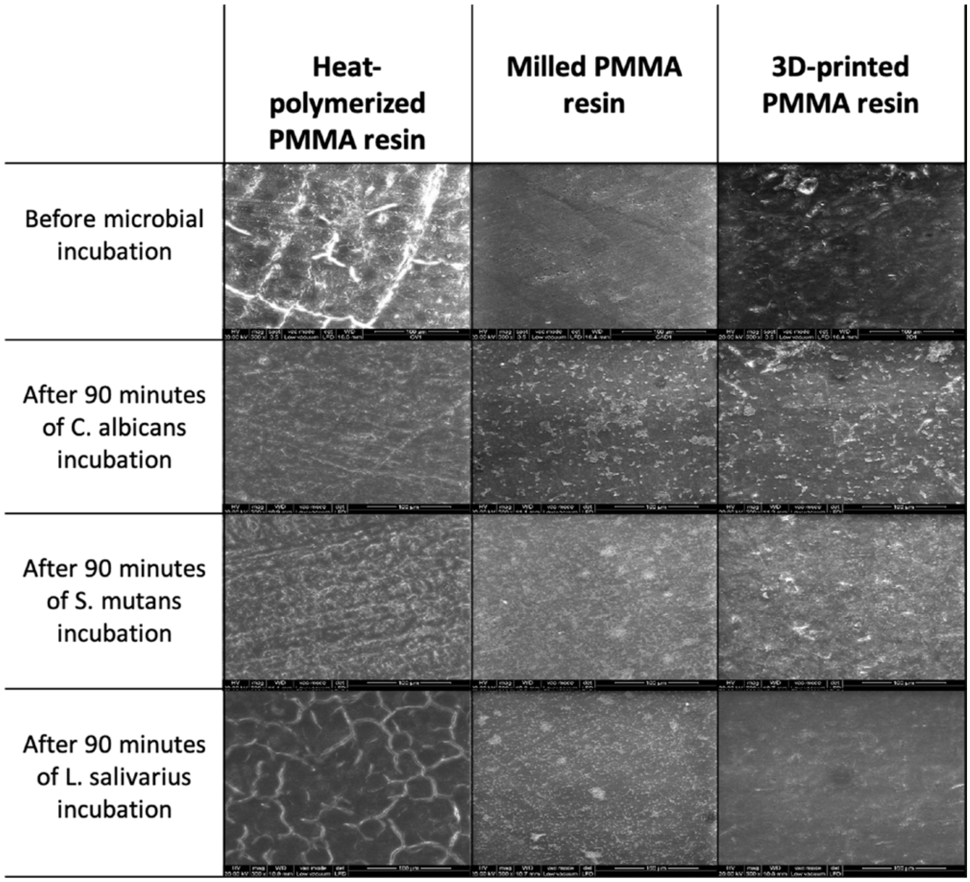

Fig. 5. SEM images (500X magnification) of the groups before and after microbial incubations.

tween the additive and subtractive processes. Srinivasan et al. [16] and Kalberer et al. [17] tested the trueness of the intaglio surface of milled or 3D-printed complete dentures. Both authors reported better trueness for milled prostheses. [16,17]

To the authors' knowledge, there are no published studies, which investigated the mechanical behavior, surface properties, and microbial adhesion with conventional heat-polymerized, milled, and printed PMMA resins. Al-Dwairi et al. [19] demonstrated significant superiority in surface wettability, surface roughness, and surface hardness of milled PMMA than the conventional heat-polymerized PMMA. Similar results regarding the mechanical properties were reported by Srinivasan et al. [18] The authors reported better mechanical performance with the milled PMMA compared with the conventional-heat polymerized PMMA, although both resins showed similar flexural elastic modulus (2.7 ±0.1 GPa for milled PMMA resin, 2.7 ±0.2 GPa for heat-polymerized PMMA resin). Similarly, in the present study, milled PMMA presented the highest Ultimate Flexural Strength and Flexural modulus compared with heat-polymerized and 3D-printed PMMAs. Clinically, the higher mean flexural modulus value (3435.07±346.34 MPa) may allow the fabrication of removable dentures with thinner bases without compromising the mechanical properties. Therefore, the patients might improve their natural speech and comfort due to less additional volume of the removable denture. It also has to be considered, that the base of an overdenture might be realized without metal or fiber reinforcement in the areas where the volume is not enough for the attachment system, reinforcement, and denture base. Moreover, the realization of thinner bases with high mechanical properties may improve the quality of the maxillo-facial obturator. However, clinical trials are needed to know long-term clinical performance.

Improved mechanical properties of the milled PMMA may be attributed to the industrial fabrication of the PMMA block. The blocks are pre-polymerized and manufactured under high pressure and temperatures. These procedures control the volumetric and shrinkage distortions. [26]

Heat-polymerized and 3D-printed PMMAs showed similar mechanical characteristics in the present study. However, different elongation values were recorded; 3D-printed PMMA presented a wider elongation of plastic type (**Fig. 3**). Heat-polymerized PMMA and milled PMMA presented less elongation due to the rigid plastic behavior of the materials, in fact, heat-polymerized PMMA showed only elastic elongation. The manufacturing process might be the reason for the difference in behavior whether being plastic or elastic. In heat-polymerizing technique, the resin was filled in a silicon mold and completely polymerized in one step. However, multiple polymerizations during the deposition of several PMMA layers through a 3D-printer may have contributed to the plastic behavior of the printed PMMA. [27,28]

For surface roughness before polishing, a difference was observed among the groups in the present study. Milled PMMA presented a lower average Ra value (0.29±0.16 μm) than the other PMMAs before polishing. The surfaces of the 3D printed PMMA resin (0.38±0.08 μm) were smoother than that of the conventional heat-polymerized PMMA (0.59±0.3 μm). However, the data showed a high variability in Ra value (high standard deviations) for the milled PMMA and conventional heat-polymerized PMMAs. The 3D printed PMMA resin presented homogeneous specimen surfaces. The variation in Ra for the heat-polymerized PMMA may be due to a potential difference in how the resin was manually handled by the operator during the fabrication of specimens. For the milled PMMA, the reason for variation in Ra may be attributed to the dynamic interaction of the milling tool with the PMMA block's surface during the milling operation. After polishing, all groups showed similar average Ra values within the plaque accumulation threshold of 0.2 μm. [29] Similar results have been presented in previous studies when the milled PMMA was compared with the conventional heat-polymerized PMMA. [1,18,19] The enhanced surface characteristics with milled PMMA might be attributed to the manufacturing process of the PMMA blocks; reduced levels of residual monomers, and the indirect polymerization method. [30] Although the milled PMMA presented an average Ra value similar to the threshold value before polishing, the appropriate polishing procedure after manufacturing, regardless of the PMMA type, should be applied to prevent high surface roughness and to obtain less microbial adherence. The applied polishing procedure enabled low surface roughness for all PMMA groups. Even though not statistically significant, milled PMMA had smaller roughness values than the other groups after polishing.

Al-Fouzan et al. [8] and Murat at al. [9] found lower average Candida adhesion values on milled PMMA than on the conventional heat-polymerized PMMA after 90 minutes [8] and 2 hours of incubation. [9] Therefore, the authors concluded that the milling procedure might decrease the incidence of denture stomatitis. [8,9] In the present study, in addition to Candida albicans, the adhesion of Lactobacillus Salivarius and Streptococcus mutans after 90 minutes and 16 hours of incubations were investigated. The milled PMMA showed a lower mean adhesion value than the other groups after 90 minutes. However, no difference emerged among the groups after 16 hours of incubation. Increased incubation time might favor the formation of bacterial biofilm and candida adhesion independent of the surface roughness. Therefore, daily hygiene maintenance is recommended to decrease the plaque accumulation on PMMA. According to the SEM images, the CV group appeared with deep scratches, grooves, and more porous surface than the other groups. The CAD group presented a smooth surface with less scratches. However, the 3D group showed multiple dots and surface irregularities that were probably caused by the polymerization process during printing. The difference in surfaces may have influenced the microbial adhesion in the initial evaluation (90 minutes), but, after 16 hours of incubation, the mean microbial adhesion value was similar among the groups.

Based on the findings of the present study, milled PMMA presented better flexural properties, and lower surface roughness and bacterial adhesion. However, future experiments, particularly on 3D printing of denture base resins are needed to confirm their clinical performance in the long term. Moreover, long-term evaluation of milled PMMA needs to be done to completely understand its clinical performance. Thermocycling was not performed in the present study and should be included in testing to see the effect of aging on tested parameters in future studies.

5. Conclusion

Based on the findings of the present in vitro study, the following conclusions were drawn:

1. The milled PMMA displayed improved flexural properties, surface roughness before polishing, and lower bacterial adhesion after 90 minutes of incubation.
2. The 3D printed PMMA showed a wider plastic deformation than the milled and the heat-polymerized PMMA.
3. All tested PMMAs had similar surface roughness after polishing.
4. All tested PMMAs had similar microbial adhesion after 16 hours of incubation.

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Conflicts of interest

The authors have no commercial or financial relationship with tested product manufacturers that may pose a conflict of interest or potential conflict of interest.

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