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Influence of Surface Properties on Candida albicans Adhesion to Denture Base Materials Fabricated by Different Techniques

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Research Article

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ABSTRACT

Objectives: This in vitro study evaluated and compared the surface roughness, wettability (contact angle), and *Candida albicans (C.albicans)* adhesion of denture base materials fabricated by different methods.

Materials and Methods: Six materials were tested: conventional heat-polymerized polymethyl methacrylate (PMMA) resin (Meliodent; Kulzer GmbH, Germany), CAD/CAM PMMA-based polymer (KeyMill; Keystone Industries, USA), CAD/CAM polyetheretherketone (PEEK) block (Audental; Shenzhen, China), thermoplastic polyamide-based resin (Nuxen; SRL, Argentina), titanium alloy block (Grade V, Ti-6Al-4V) (Audental; Shenzhen, China), and cobalt-chromium (Co-Cr) alloy (Wironit; BEGO GmbH, Germany). Surface roughness (Ra) and contact angle (°) were measured, and *C. albicans* adhesion was quantified as colony-forming units per milliliter (CFU/mL). Data were analyzed using Kruskal–Wallis and Mann–Whitney U tests with Bonferroni correction; correlations were examined with Spearman's coefficient.

Results: Significant differences were found among materials for surface roughness, contact angle, and *C. albicans* adhesion ($p < 0.05$). Conventional PMMA, polyamide, and PEEK showed the highest roughness; Co-Cr and titanium had the lowest. Co-Cr displayed the highest contact angle, titanium the lowest. *C. albicans* adhesion was greatest on polyamide and PEEK, and lowest on Co-Cr. Surface roughness correlated positively with fungal adhesion ($\rho = 0.676$, $p < 0.001$), while contact angle showed no significant correlation ($\rho = 0.006$, $p = 0.964$).

Conclusions: Surface roughness was the main factor influencing *C. albicans* adhesion; smoother surfaces reduced fungal colonization. Choosing denture base materials with low roughness may help limit biofilm formation and prosthesis-related infections.

Keywords: Candida albicans, denture bases, surface properties, wettability.

Farklı Tekniklerle Üretilen Protez Taban Malzemelerine Candida albicans Yapışmasının Yüzey Özelliklerine Etkisi

Araştırma Makalesi

Süreç

Geliş: 18/07/2025
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ÖZ

Amaç: Bu in vitro çalışma, farklı yöntemlerle üretilmiş hareketli protez kaide materyallerinin yüzey pürüzlülüğü, ıslanabilirliği (temas açısı) ve *Candida albicans* adezyonunu değerlendirmek ve karşılaştırmak amacıyla yapılmıştır.

Gereç ve Yöntemler: Altı farklı materyal test edilmiştir: konvansiyonel polimetil metakrilat (PMMA) rezin (Meliodent; Kulzer GmbH, Almanya), CAD/CAM PMMA (KeyMill; Keystone Industries, ABD), CAD/CAM polietereterketon (PEEK) blok (Audental; Shenzhen, Çin), termoplastik poliamid bazlı rezin (Nuxen; SRL, Arjantin), titanyum alaşımı blok (Grade V, Ti-6Al-4V) (Audental; Shenzhen, Çin) ve kobalt-krom (Co-Cr) alaşımı (Wironit; BEGO GmbH, Almanya). Yüzey pürüzlülüğü (Ra) ve temas açısı (°) ölçülmüş, *C. albicans* adezyonu ise mililitre başına koloni oluşturan birim (CFU/mL) olarak kantitatif değerlendirilmiştir. Veriler Kruskal–Wallis ve Mann–Whitney U testleri ile (Bonferroni düzeltmesi uygulanarak) analiz edilmiş, korelasyonlar Spearman katsayısı ile incelenmiştir.

Bulgular: Yüzey pürüzlülüğü, temas açısı ve *C. albicans* adezyonu açısından materyaller arasında anlamlı farklılıklar bulunmuştur ($p < 0.05$). En yüksek yüzey pürüzlülüğü geleneksel PMMA, poliamid ve PEEK'te gözlenirken; en düşük değerler Co-Cr ve titanyumda saptanmıştır. Co-Cr en yüksek temas açısını, titanyum ise en düşük temas açısını göstermiştir. *C. albicans* adezyonu en fazla poliamid ve PEEK'te, en az Co-Cr'da kaydedilmiştir. Yüzey pürüzlülüğü ile fungal adezyon arasında pozitif korelasyon bulunmuştur ($\rho = 0.676$, $p < 0.001$), ancak temas açısı ile anlamlı bir ilişki saptanmamıştır ($\rho = 0.006$, $p = 0.964$).

Sonuç: *Candida albicans* adezyonunu etkileyen temel faktör yüzey pürüzlülüğüdür; daha düzgün yüzeyler fungal kolonizasyonu azaltmıştır. Daha düşük yüzey pürüzlülüğüne sahip protez kaide materyallerinin tercih edilmesi, biyofilm oluşumunun ve protezle ilişkili enfeksiyonların sınırlanmasına katkı sağlayabilir.

Anahtar kelimeler: Candida adezyonu, ıslanabilirlik, kaide materyalleri, yüzey pürüzlülüğü.

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Introduction

Polymethyl methacrylate (PMMA) has historically maintained its position as the most commonly preferred material for denture bases, primarily owing to its ease of fabrication, satisfactory esthetics, and adequate mechanical properties. However, conventional heat-polymerized PMMA also has notable limitations, such as the presence of residual monomers, internal porosity, and a tendency to support microbial attachment—most notably by *Candida albicans(C. Albicans)*, an opportunistic microorganism frequently associated with prosthesis-related oral infections, mainly in the form of denture stomatitis.[1]

The integration of digital workflows into prosthodontic practice has facilitated the introduction of novel manufacturing approaches, including CAD/CAM (Computer aided design-computer aided manufacturing) systems, which aim to overcome the limitations of conventional techniques. PMMA blocks fabricated via CAD/CAM technology undergo polymerization under elevated temperature and pressure conditions, leading to enhanced polymer network crosslinking, minimized residual monomer levels, and improved structural homogeneity with lower porosity. These properties lead to improved mechanical stability, increased longevity, and enhanced biocompatibility.[2]

Beyond PMMA-based options, a number of alternative materials have gained interest for denture base fabrication. Polyetheretherketone (PEEK) is a thermoplastic material that exhibits superior mechanical performance low plaque affinity, and biocompatibility.[3,4] Polyamide resins, commonly used in flexible denture applications, have been introduced as metal-free alternatives for removable prostheses and are often preferred for their elasticity and patient comfort.[5] However, their higher water absorption and surface irregularities may raise concerns regarding microbial adhesion.[6,7]

Titanium has also emerged as a promising denture base material, particularly in digitally fabricated metal bases. Its high corrosion resistance, superior biocompatibility, and low bacterial affinity make it a valuable alternative to traditional cobalt-chromium (Co-Cr) alloys, which can release metal ions and potentially trigger mucosal irritation or allergic reactions in susceptible individuals.[8,9,10]

Microbial adhesion, especially by *Candida albicans* is influenced by various surface properties of the prosthetic material. Among these, surface roughness and wettability are considered critical factors.[8] Irregular or rough surfaces facilitate mechanical adhesion of microorganisms, while more hydrophobic surfaces (exhibiting higher contact angles) may enhance fungal adhesion by promoting hydrophobic interactions between the microbial cell wall and the material surface.[11,12] It is well established that roughness values above 0.2 µm tend to increase microbial retention.[13-16] Similarly, high surface energy and hydrophobicity can promote biofilm formation, although the exact nature of these interactions remains complex.[17]

Although the interaction between *Candida albicans* and conventional PMMA has been the subject of numerous investigations, comparative studies focusing on CAD/CAM PMMA, PEEK, polyamide, titanium, and Co-Cr alloys remain relatively scarce. [18,19]

Although various denture base materials are now in use, the specific roles of surface irregularities and hydrophilicity in shaping microbial adhesion patterns remain insufficiently investigated. Accordingly, this laboratory-based investigation was designed to examine differences in surface texture, wettability and the degree of *Candida albicans* colonization among six distinct denture base materials: conventional heat-cured PMMA, CAD/CAM-fabricated PMMA, PEEK, titanium, cobalt–chromium alloy, and polyamide.

This study was based on a single null hypothesis stating that there would be no statistically significant differences among the tested materials in terms of Candida albicans adhesion, surface roughness, and contact angle, and that no correlation would exist between surface characteristics and fungal colonization.

Materials and Methods

Materials and Specimen Preparation

The required sample size was determined through a power analysis based on 95% confidence level and 80% statistical power an effect size (d) 0.86 and 10 specimens were prepared for each group accordingly. The sample size was calculated with G*Power software(version 3.1.9.6, Franz Faul, University of Düsseldorf, Germany).

In this investigation, six denture base materials were selected and analyzed: a CAD/CAM PMMA-based polymer (KeyMill, Keystone Industries, Gibbstown, NJ, USA), a CAD/CAM PEEK block (Audental, Shenzhen, Guangdong, China), a titanium alloy block (Grade V, Ti-6Al-4V) (Audental; Shenzhen, China), a conventional heat-polymerized PMMA resin (Meliodent, Kulzer GmbH, Hanau, Germany), a cobalt-chromium alloy (Wironit, BEGO GmbH & Co. KG, Bremen, Germany), and a polyamide-based resin (Nuxen, SRL; Ayacucho 1053 3-A, Buenos Aires, Argentina). For each material, ten disc-shaped samples were fabricated.

The heat-polymerized PMMA specimens were prepared following the manufacturer's instructions using conventional flasking and processing techniques. CAD/CAM materials (PMMA, PEEK, and titanium) were milled from prefabricated blocks (10 mm diameter × 2 mm thickness). Specimen modeling was carried out using dental CAD software (ExoCad), and files were converted into STL format for production purposes. A 5-axis milling machine (VHF Devcon, China) was used to produce the specimens.

To mimic the tissue-facing side of a denture, specimens were left unmodified after fabrication. No additional surface treatments such as polishing or grinding were applied. The samples were incubated in distilled water at 37 °C, and the water was refreshed daily throughout the 6-day storage period.

Surface Roughness Measurement

To determine surface roughness (Ra, µm) contact profilometer (Perthometer M2; Mahr GmbH, Göttingen, Germany) was used with an area of 100 ∗ 100 µm^2. Three linear measurements were recorded per specimen—one at the center and two at peripheral regions—and the average of these three values was calculated to obtain a single measurement per sample. Subsequently, data were analyzed using non-parametric tests and results were expressed as median (min–max). Measurements were conducted on ten specimens per material group (n = 10).

Contact Angle Measurement

Contact angle data were obtained to evaluate the wettability of each material, utilizing a drop analysis system (Easy Drop, Krüss GmbH, Hamburg, Germany). A droplet of distilled water, measuring 15 picoliters in volume, was carefully placed on each sample's surface using a micropipette under standardized illumination. The contact angle was determined via image analysis software (DSA2, Krüss GmbH, Hamburg, Germany). For each group (n = 10), measurements were conducted in triplicate, and the resulting data were averaged for analysis.

Candida Albicans Adherence Assay

The ATCC 10231-1 strain of *C. albicans* was reactivated by culturing on SDA medium (Merck, Darmstadt, Germany) under standard incubation conditions (24 h, 37 °C). Subsequent identification was performed using MALDI-TOF MS, a technique based on matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). One isolated *Candida albicans* colony was transferred into Sabouraud Dextrose Broth (SDB; Merck, Darmstadt, Germany) and cultured at 37 °C for 18 hours with constant shaking. Centrifugation of the *Candida albicans* suspension was carried out at 3000 × g for 10 minutes in order to collect the cellular pellet. The pellet was rinsed in two steps using PBS (pH adjusted to 7.0) prepared with materials sourced from Thermo Fisher Scientific, Waltham, MA, USA. Cells were then diluted in Sabouraud Dextrose Broth until the concentration reached approximately 1×10^7 cells per milliliter. Before conducting the *Candida albicans* adhesion procedure, each specimen underwent surface decontamination by immersion in 70% ethanol, followed by sonication for 30 minutes. To remove residual ethanol, the specimens underwent an additional 10-minute wash in distilled water, using the same conditions as before. Sterile cylindrical specimens (n = 10 per material) were allocated into separate sterile tubes, each tube containing 2 mL of *Candida albicans* suspension prepared in advance. Following preparation, the test specimens underwent incubation at 37 °C for 90 minutes with gentle agitation (75 rpm) to facilitate fungal attachment. To eliminate loosely bound cells, each specimen underwent triple washing using phosphate-buffered saline (PBS).

Following this, the samples were transferred into fresh sterile containers filled with PBS and mixed via vortex agitation for 5 minutes to detach firmly adhered cells.

Aliquots of 0.1 mL and 0.01 mL from each suspension were cultured on SDA plates. Incubation was carried out at 37 °C over a 24-hour period. Once completed, the number of colonies on each plate was recorded as CFU per milliliter.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software (version 22.0; IBM Corp., Armonk, NY, USA). The distribution of data was assessed with the Shapiro–Wilk test, and normality assumptions were not met ($p < 0.05$). Therefore, differences in surface roughness (Ra, µm), contact angle (°), and *Candida albicans* adhesion (CFU/mL) among the six material groups were evaluated using the non-parametric Kruskal–Wallis H test. When significant differences were detected, pairwise comparisons were conducted using the Dunn–Bonferroni post-hoc test. The relationships between continuous variables (surface roughness, contact angle, and *Candida albicans* adhesion) were analyzed using Spearman's rank correlation coefficient (ρ). All tests were performed at a 95% confidence level, and a p-value of < 0.05 was considered statistically significant.

Results

The measured values of surface roughness (Ra), contact angle (°), and *C. albicans* adhesion (CFU/mL) for each tested denture base material summarized in Table 1.

Surface Roughness

Statistical analysis using the Kruskal–Wallis test demonstrated statistically significant differences in surface roughness values among the tested material groups (H = 44.09, $p < 0.001$). The highest median roughness values were observed in the polymer-based materials—conventional PMMA (2.61), PEEK (2.57), and polyamide (2.53). In contrast, the lowest values were recorded for the alloy-based materials—titanium (0.99) and Co-Cr (0.92)—with CAD-CAM PMMA (0.96) also exhibiting a similarly low roughness value (Figure 1, Table 1). Pairwise comparisons revealed that Co-Cr exhibited significantly lower roughness than polyamide ($p = 0.001$), PEEK ($p = 0.001$), and conventional PMMA ($p < 0.001$). Similarly, titanium showed significantly lower roughness than polyamide ($p = 0.004$), PEEK ($p = 0.002$), and conventional PMMA ($p = 0.001$). CAD-CAM PMMA also had significantly lower roughness than polyamide ($p = 0.017$), PEEK ($p = 0.009$), and conventional PMMA ($p = 0.004$). No statistically significant difference was found between Co-Cr and titanium ($p > 0.05$).

Contact Angle

Statistical analysis using the Kruskal–Wallis test revealed significant differences in contact angle measurements among the tested materials (H = 40.97, $p < 0.001$). The highest median contact angle was recorded for the Co-Cr group (89.55), followed by polyamid (73.9), conventional PMMA (73.3), PEEK (69.65), CAD-CAM PMMA (61.2), and titanium (38.7) (Table 1, Figure 2). Pairwise comparisons showed that titanium had significantly lower contact angles than PEEK ($p = 0.021$), polyamide ($p < 0.001$), conventional PMMA ($p < 0.001$), and Co-Cr ($p < 0.001$). Additionally, CAD-CAM PMMA demonstrated a significantly lower contact angle compared with Co-Cr ($p = 0.001$). No statistically significant differences were found between Co-Cr and conventional PMMA, polyamide, or PEEK ($p > 0.05$), between conventional PMMA and polyamide ($p > 0.05$), between PEEK and CAD-CAM PMMA ($p > 0.05$), or between CAD-CAM PMMA and titanium ($p > 0.05$).

Table 1. Mean (± SD) values of Candida albicans adhesion (CFU/mL), surface roughness (Ra, µm), and wettability (contact angle, °) of different denture base materials. Different superscript letters within the same row indicate statistically significant differences between groups (p < 0.05, Bonferroni-adjusted Mann–Whitney U test)

Material	*C. albicans* Adhesion (CFU/mL) (Mean ± SD) Median (Min - Max)	Surface Roughness (Ra, µm) (Mean ± SD) Median (Min - Max)	Contact Angle (°) (Mean ± SD) Median (Min - Max)
Conventional PMMA	44.0 ± 33.07 [a] 45 (10 - 90)	2.92 ± 1.18 [b] 2.61 (2.09 - 6.07)	79.06 ± 17.37 [a] 73.30 (47.4 - 102.7)
CAD-CAM PMMA	10.0 ± 11.0 [b] 10 (0 - 30)	0.98 ± 0.31 [a] 0.96 (0.50 - 1.45)	63.38 ± 8.73 [b] 61.2 (56.8 - 86.8)
PEEK	76.0 ± 36.27 [a] 80 (20 - 130)	2.58 ± 0.71 [b] 2.57 (1.64 - 3.61)	71.65 ± 7.06 [ab] 69.65 (60 - 83.4)
Polyamide	184.0 ± 121.0 [a] 185 (50 - 420)	2.65 ± 1.15 [b] 2.53 (1.09 - 5.32)	77.97 ± 10.39 [a] 73.9 (65.3 - 93.6)
Co-Cr	3.0 ± 7.0 [b] 0 (0 - 20)	0.85 ± 0.15 [a] 0.92 (0.57 - 1.01)	89.42 ± 2.92 [a] 89.55 (83.7 - 94.9)
Titanium	14.0 ± 5.16 [b] 10 (10 - 20)	0.74 ± 0.38 [a] 0.99 (0.27 - 1.11)	39.62 ± 7.04 [c] 38.7 (26.9 - 49.7)

Different superscript letters indicate statistically significant differences within each parameter (p < 0.05). Post-hoc pairwise p-values (Bonferroni correction): Candida adhesion:Polyamide vs Co-Cr (p < 0.001), Polyamide vs CAD-CAM PMMA (p < 0.001), Polyamide vs Titanium (p = 0.004), PEEK vs Co-Cr (p < 0.001), PEEK vs CAD-CAM PMMA (p = 0.008), Conventional PMMA vs Co-Cr (p = 0.031). Surface roughness:Co-Cr vs Polyamide (p = 0.001), Co-Cr vs PEEK (p = 0.001), Co-Cr vs Conventional PMMA (p < 0.001), Titanium vs Polyamide (p = 0.004), Titanium vs PEEK (p = 0.002), Titanium vs Conventional PMMA (p = 0.001), CAD-CAM PMMA vs Polyamide (p = 0.017), CAD-CAM PMMA vs PEEK (p = 0.009), CAD-CAM PMMA vs Conventional PMMA (p = 0.004). Contact angle: Titanium vs PEEK (p = 0.021), Titanium vs Polyamide (p < 0.001), Titanium vs Conventional PMMA (p < 0.001), Titanium vs Co-Cr (p < 0.001), CAD-CAM PMMA vs Co-Cr (p = 0.001).

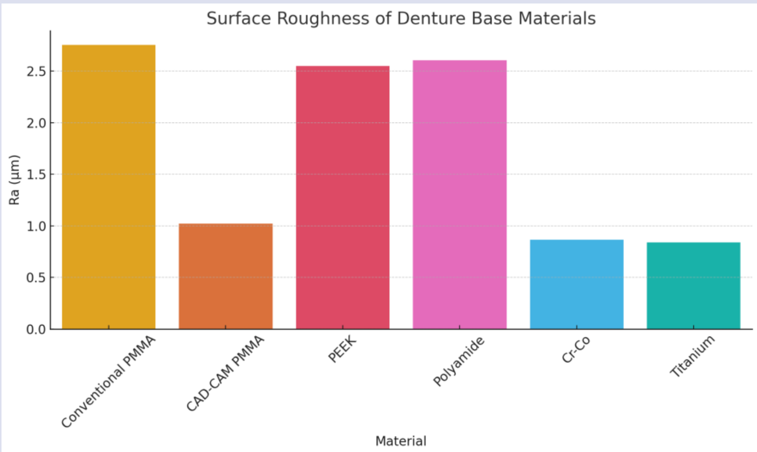

Figure 1. Mean surface roughness values of denture base materials.

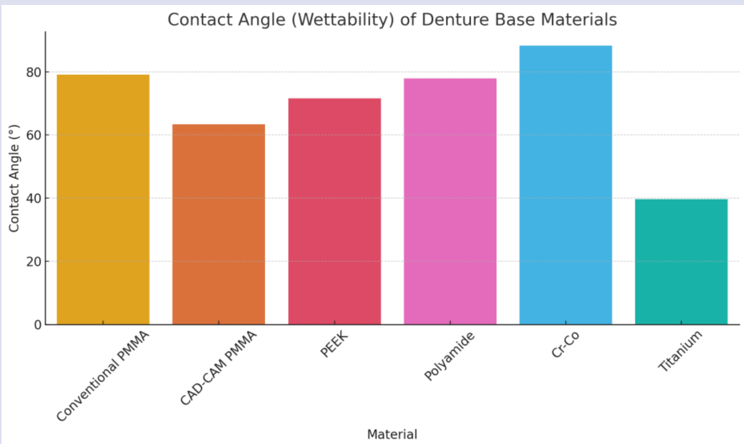

Figure 2. Contact angle measurements of denture base materials.

C. albicans Adherence

Significant differences in *Candida albicans* adhesion (CFU/mL) were observed among the tested materials ($H = 43.14$, $p < 0.001$). The highest median for fungal adhesion was recorded for polyamide (185 CFU/mL) and PEEK (80 CFU/mL), followed by conventional PMMA (45 CFU/mL), titanium (10 CFU/mL), CAD-CAM PMMA (10 CFU/mL), and Co-Cr (0 CFU/mL) (Table 1, Figure 3). Pairwise comparisons revealed that polyamide exhibited significantly higher adhesion than Co-Cr ($p < 0.001$), CAD-CAM PMMA ($p < 0.001$), and titanium ($p = 0.004$). PEEK demonstrated significantly higher adhesion than Co-Cr ($p < 0.001$) and CAD-CAM PMMA ($p = 0.008$). Conventional PMMA showed significantly higher adhesion than Co-Cr ($p = 0.031$). No statistically significant differences were observed between polyamide and PEEK, or between titanium and CAD-CAM PMMA or Co-Cr ($p > 0.05$).

Spearman's rank correlation analysis demonstrated a significant positive correlation between surface roughness and *C. albicans* adhesion ($\rho = 0.676$, $p < 0.001$), indicating that rougher surfaces were more susceptible to fungal colonization. In contrast, no significant correlation was found between contact angle and fungal adhesion when all materials were evaluated together ($\rho = 0.006$, $p = 0.964$).

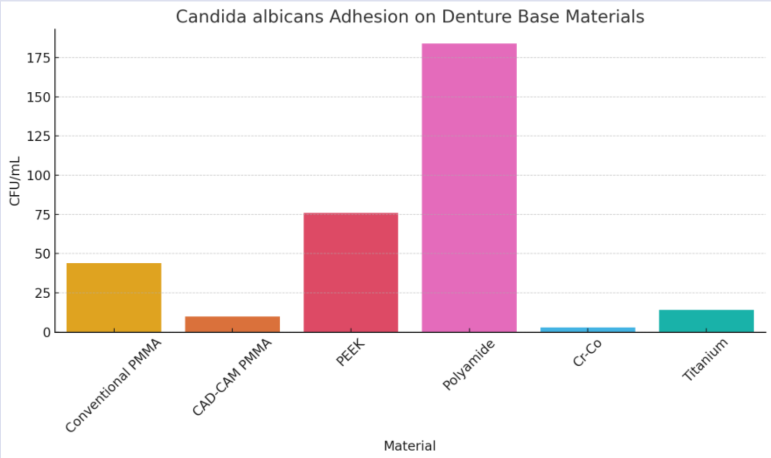

Figure 3. Candida albicans adhesion on denture base materials.

Discussion

Based on the findings of the present study, the null hypothesis was rejected. Significant differences were observed among the denture base materials in terms of *Candida albicans* adhesion, surface roughness, and contact angle values ($p < 0.05$). Polymer-based materials—particularly conventional PMMA, polyamide, and PEEK—exhibited significantly higher surface roughness compared with alloy-based materials (Co-Cr and titanium) and CAD/CAM PMMA. This increased roughness was positively correlated with *C. albicans* adhesion ($\rho = 0.676$, $p < 0.001$), indicating that rougher surfaces were more prone to fungal colonization. In contrast, contact angle did not show a statistically significant correlation with fungal adhesion when all materials were evaluated together ($\rho = 0.006$, $p = 0.964$), suggesting that wettability alone may not be a primary determinant of microbial attachment under the conditions of this study.

These findings align with previous reports indicating that surface roughness above the 0.2 µm threshold can significantly enhance microbial retention on denture base materials.[20.21] Similar to our results, recent studies have also highlighted that while hydrophobicity can influence microbial adhesion, its effect may be overshadowed by surface topography and material-specific chemical properties.[22,23]

Moreover, the statistically significant correlation between surface roughness and *Candida albicans* adhesion further supported the rejection of the null hypothesis. A moderate to strong positive relationship was observed between surface roughness and *C. albicans* adherence, as shown by Spearman correlation analysis ($\rho = 0.676$, $p < 0.001$)[20,21]. In contrast, no statistically significant correlation was found between contact angle and fungal adhesion when all materials were evaluated together ($\rho = 0.006$, $p = 0.964$). Overall, these findings confirm that material selection and surface topography—rather than wettability alone—play a primary role in influencing microbial adhesion.[22] In general, smoother, less retentive surfaces may be preferable in clinical prosthodontic applications to reduce the risk of biofilm formation and prosthesis-related mucosal infections.[23]

This suggests that surface characteristics, while important, are not the sole determinants of microbial colonization, and other material-related or biological factors may also contribute. In contrast, CAD/CAM PMMA, titanium, and cobalt-chromium (Co-Cr) alloy demonstrated smoother surfaces, which were associated with reduced *Candida* colonization. These findings are consistent with recent studies reporting lower *C. albicans* adhesion on CAD/CAM PMMA compared to conventional PMMA, primarily attributed to the reduced surface roughness and higher manufacturing precision achieved with industrial polymerization processes.[20,21] In line with our results, previous investigations have demonstrated a significant positive association between surface roughness and *Candida* adhesion (ρ = 0.676, p < 0.001), supporting the notion that increased surface irregularities facilitate microbial retention.[22,23] This is also consistent with reports identifying roughness thresholds (Ra > 0.2 µm), beyond which microbial accumulation significantly increases.[21]

Surface roughness plays a crucial role in *C. albicans* adhesion, as microtopographical features such as pits and grooves on the material surface can enhance fungal retention by providing mechanical anchorage and protection from shear forces.[21] In the present study, conventional PMMA (2.92 ± 1.18 µm) and polyamide (2.65 ± 1.15 µm) exhibited surface roughness values well above the commonly cited threshold of 0.2 µm, which may explain their higher CFU counts. In contrast, CAD/CAM PMMA (0.98 ± 0.31 µm) and titanium (0.74 ± 0.38 µm) demonstrated Ra values below this threshold, corresponding to significantly reduced fungal adhesion. Recent research has also confirmed that CAD/CAM PMMA generally exhibits lower roughness and reduced microbial retention compared to conventionally processed PMMA, likely due to the precision of industrial polymerization and milling techniques.[20,22] This variability highlights the importance of standardized testing protocols when evaluating surface characteristics and their microbiological implications.[23]

The role of surface hydrophobicity in fungal adhesion remains complex. In the present study, no statistically significant correlation was found between contact angle and *Candida albicans* adhesion when all materials were evaluated together (ρ = 0.006, p = 0.964). This finding aligns with recent reports indicating that wettability alone may not be a reliable predictor of microbial colonization on denture base materials.[21,22] Notably, cobalt–chromium, despite exhibiting the highest contact angle among the tested materials, demonstrated the lowest *Candida albicans* adhesion. This exception suggests that surface chemistry and material composition may exert a greater influence on microbial colonization than wettability alone. The metal's passive oxide layer and low surface free energy might contribute to its antifungal properties, a phenomenon supported by studies reporting consistently reduced fungal colonization on Co-Cr frameworks.[23,24] These findings indicate that material-specific properties such as surface chemistry and alloy microstructure play crucial roles in microbial colonization dynamics, overshadowing the isolated effect of hydrophobicity.

To replicate the clinical state of the intaglio surface immediately after denture delivery, specimens in this study were tested without any surface coating or polishing. This methodological choice was intentional, aiming to reflect the natural surface condition encountered intraorally upon prosthesis insertion. Previous investigations have adopted similar models, recognizing that the unmodified denture surface more accurately represents the initial phase of *Candida albicans* adhesion and early biofilm formation.[20,22] Such an approach avoids introducing polishing-related surface alterations, which can significantly influence roughness and wettability measurements, thereby ensuring that the findings are directly applicable to the clinical scenario.[23] From a clinical standpoint, the early adhesion stage is critical because microorganisms that successfully attach during this period can serve as the foundation for mature biofilm development, making subsequent decontamination more challenging and increasing the risk of persistent prosthesis-related infections.

The absence of a salivary pellicle in the present in vitro model may have influenced the results, as pellicles can modify surface free energy, alter protein–surface interactions, and thereby affect microbial adhesion. Some studies have suggested that pellicle formation can reduce fungal retention by creating a predominantly hydrophilic barrier that limits direct microorganism–surface contact, whereas others have reported that certain pellicle proteins may act as specific receptors facilitating fungal attachment.[25,26] Recent investigations indicate that the overall effect of the salivary pellicle on *Candida albicans* adhesion may depend on the pellicle's protein composition, the surface chemistry of the substrate, and the duration of pellicle maturation.[27,28] Therefore, the presence or absence of a salivary pellicle has the potential to alter microbial adhesion outcomes, which should be taken into account when interpreting and comparing in vitro and in vivo findings.

Although polyamide offers greater flexibility and enhanced patient comfort, it exhibited considerably higher median values of surface roughness and *Candida albicans* adhesion compared to conventional PMMA. However, despite these numerical differences, the variations did not reach statistical significance (p > 0.05), likely due to the high variability within the polyamide group, as reflected by its elevated standard deviation values**.** This high variability can be attributed to the manufacturing process of polyamide, which involves multiple manual and operator-dependent steps during injection molding, resulting in inconsistencies in surface topography and subsequent microbial adhesion potential. Most studies report that polyamide exhibits higher *Candida* adherence than conventional PMMA; however, the statistical significance of this difference often depends on factors such as surface roughness variability, polishing methods, and aging conditions. In our dataset, although mean *Candida* counts were notably higher in polyamide, the difference did not reach statistical significance, aligning with findings from similar in vitro investigations where high intra-group variance masked differences in the average values. Its higher water absorption capacity and less rigid structure may further contribute to increased microbial retention, consistent with previous reports.[20,29-31]

In contrast, CAD/CAM PMMA demonstrated reduced porosity and fungal adhesion, likely due to industrial polymerization under high pressure and temperature, as previously reported.[2,20] Several studies corroborate these findings: Murat et al. (2018) reported that CAD/CAM-milled PMMA-based polymers exhibited significantly lower *C. albicans* adhesion, along with reduced surface roughness and contact angle values, compared to conventional heat-polymerized PMMA ($p < 0.05$).[16] Similarly, Osman et al. (2023) observed that denture base specimens fabricated by CAD/CAM milling had the lowest *Candida* biofilm formation *in vitro*, attributing this to their highly cross-linked, less porous structure.[32] From a clinical perspective, these improvements may translate into reduced incidence of denture-related stomatitis and longer prosthesis lifespan, particularly in patients with a history of recurrent fungal infections, as also suggested by Al-Fouzan et al. (2017) and Goodacre et al. (2018).[33,34] These results reinforce the concept that CAD/CAM fabrication techniques enhance material properties in ways that mitigate fungal colonization.

Titanium and Co-Cr alloys also demonstrated minimal fungal colonization, supporting earlier reports that these materials possess stable oxide layers which reduce microbial affinity.[35] Titanium, in particular, is suitable for patients with acrylic or metal sensitivities.[36] However, the use of titanium as a denture base material presents certain limitations. Its high production cost, the need for specialized equipment, and the limited number of dental laboratories capable of processing titanium restrict its widespread clinical application. Furthermore, the marginal reduction in Candida adhesion observed in comparison to more accessible materials, such as CAD-CAM PMMA, may not justify the increased financial burden in routine prosthodontic practice.

This study has certain limitations, such as being conducted in vitro, relying solely on a single Candida species, and omitting simulation of intraoral conditions like thermal cycling or salivary pellicle formation.

Additionally, employing only one *Candida* strain may not reflect the diverse adhesion behaviors observed clinically. The oral environment harbors a complex microbiome where bacterial–fungal interactions can modulate *Candida* colonization.[36,37] Future studies should investigate these interactions and incorporate in vivo conditions to enhance clinical relevance.

Overall, this study reinforces that surface roughness is a key factor influencing Candida adhesion, whereas wettability, within the limitations of this study, did not show a significant effect. Selecting materials with smoother and more inert surfaces, such as CAD/CAM PMMA and titanium, may help reduce the risk of prosthesis-related fungal infections. However, clinicians should also consider other factors such as patient-specific risk profiles, prosthesis design, and maintenance protocols, as material choice alone may not fully prevent microbial colonization. Combining appropriate material selection with meticulous oral hygiene instruction and regular professional maintenance is likely to offer the greatest protection against prosthesis-associated Candida infections.

Conclusions

Based on these findings, the following clinical implications can be drawn:

Denture base materials exhibit significant differences in surface roughness, wettability, and *Candida albicans* adhesion.

Conventional PMMA, polyamide, and PEEK demonstrated higher surface roughness and greater fungal adhesion.

CAD/CAM PMMA, titanium, and Co-Cr presented smoother surfaces with reduced fungal colonization.

Surface roughness showed a significant positive correlation with fungal adhesion, whereas no significant correlation was found between wettability (contact angle) and fungal adhesion.

Within the limitations of this in vitro study selecting materials with smoother surfaces may help minimize fungal biofilm formation and lower the risk of prosthesis-related infections. Additional in vivo studies are required to confirm these findings and enhance clinical applicability.

Conflicts of Interest Statement

No conflicts of interest.

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