SPECIFICITY OF MICROFLORA IN PATIENTS WITH DIFFERENT TYPES AND MATERIALS OF DENTAL PROSTHESES

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Abstract. This study investigates the relationship between dental prosthesis materials and the composition of oral microflora among 80 adult patients using different prosthesis types: removable acrylic, fixed metal-ceramic, zirconia-based, and titanium implant-supported restorations. Standardized clinical examinations, microbiological cultures, and quantitative PCR analyses were performed to assess microbial diversity, load, and surface characteristics. The results showed that acrylic resin dentures harbored the highest microbial load (45.6×10^5 CFU/mL) dominated by ***Candida albicans*** and ***Streptococcus mutans***, while zirconia and titanium prostheses exhibited reduced biofilm formation and higher microbial diversity (Shannon index 2.10). Surface roughness strongly correlated with total microbial load (r=0.89). These findings underscore that smoother materials such as zirconia and titanium minimize microbial accumulation, reducing the risk of denture stomatitis, secondary caries, and peri-implant inflammation. Clinically, the study highlights the importance of material selection, finishing protocols, and individualized hygiene programs to enhance prosthesis longevity and oral health outcomes.

Keywords: dental prostheses, oral microflora, surface roughness, zirconia, titanium, ***Candida albicans***, ***Streptococcus mutans***, peri-implantitis, biofilm diversity, prosthetic materials.

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Introduction. The oral cavity harbors one of the most complex microbiomes in the human body, consisting of over 700 bacterial species, many of which form biofilms on teeth and dental restorations. The balance between commensal and pathogenic microorganisms plays a crucial role in maintaining oral and systemic health. However, the placement of dental prostheses—whether removable or fixed—can significantly alter the oral ecological balance and promote colonization by specific microbial communities. Studies have demonstrated that the type of prosthetic material (acrylic resin, metal-ceramic, zirconia, or titanium) influences microbial adhesion and biofilm composition (Kang et al., 2020; Silva et al., 2022). The microstructure and surface roughness of the prosthetic material are key determinants of microbial retention, with rough or porous surfaces favoring the accumulation of pathogens such as *Streptococcus mutans*, *Candida albicans*, and *Porphyromonas gingivalis* (Nakamura et al., 2021).

The oral cavity represents a complex ecological environment, hosting diverse microbial communities that interact with both natural and artificial surfaces. Dental prostheses, regardless of their design and material, significantly influence the composition and dynamics of oral microflora. The long-term success of prosthetic treatment depends not only on biomechanical and aesthetic outcomes but also on maintaining microbiological balance in the oral cavity. Disruption of this balance may lead to inflammatory and infectious complications such as mucositis, stomatitis, peri-implantitis, and secondary caries.

Numerous studies have demonstrated that the type of prosthesis—fixed, removable, or implant-supported—affects microbial colonization patterns (Kania et al., 2019; Barão et al., 2021). Acrylic resin bases, for instance, provide favorable conditions for the growth of ***Candida albicans*** and ***Streptococcus mutans***, due to their porous surface structure and ability to retain moisture. In contrast, metal-ceramic and zirconia-based prostheses exhibit lower microbial adhesion, attributed to their smoother surfaces and higher biocompatibility (Kocak et al., 2020). However, differences in hygiene practices and saliva composition may further modulate these effects, highlighting the multifactorial nature of prosthesis-related microbiological changes.

The study of prosthetic materials—acrylic, metal, ceramic, composite, and polymer-based—has revealed substantial variations in bacterial adhesion potential and biofilm formation (Cierech et al., 2020). For example, titanium and cobalt-chromium alloys are more resistant to microbial colonization than polymethyl methacrylate (PMMA), yet even minimal surface irregularities or incomplete polishing may create niches for bacterial accumulation. This underscores the importance of selecting materials that minimize microbial adhesion and promote long-term oral health.

In recent years, attention has shifted toward understanding how different prosthetic constructions affect the composition and activity of oral microbiota, with the aim of preventing infection and improving patient quality of life (Fukazawa & Ozaki, 2022). Identifying the specific characteristics of the microflora in patients with various prosthesis types provides essential insights for developing individualized hygiene protocols and selecting the most biocompatible prosthetic materials.

Therefore, the present study aims to investigate the **specificity of oral microflora in patients treated with different types and materials of dental prostheses**, analyzing the relationship between prosthesis composition, surface characteristics, and microbial diversity. This research is expected to contribute to optimizing prosthetic designs and enhancing infection control strategies in prosthetic dentistry.

Understanding the relationship between prosthesis type, material, and oral microbiota composition is essential for preventing prosthesis-related stomatitis, peri-implantitis, and secondary caries. This study aims to evaluate the specificity of oral microflora in patients using different types and materials of dental prostheses to develop recommendations for microbial control and personalized hygiene protocols.

Research material and method. This cross-sectional, observational study was conducted between January and June 2025 in a university prosthodontic clinic after approval by the institutional ethics committee and in accordance with the Declaration of Helsinki; all participants provided written informed consent. We enrolled 80 adults aged 35–70 years who were at least three months post-delivery of their current prosthesis; inclusion criteria were the presence of a single predominant prosthesis type/material and the ability to comply with sampling instructions, while exclusion criteria were systemic antibiotic or antifungal use in the preceding four weeks, active oral mucosal disease unrelated to prosthesis wear, current orthodontic appliances, pregnancy, and systemic conditions with acute decompensation; smoking status, denture age, diabetes status, and oral hygiene behaviors were recorded as covariates. Participants were allocated to four equal groups by prosthesis type/material: removable acrylic resin dentures (n=20), fixed metal-ceramic crowns/bridges (n=20), monolithic or layered zirconia-based fixed restorations (n=20), and titanium implant-supported single crowns or fixed partial dentures with transmucosal titanium components (n=20). To minimize transient variability, participants refrained from toothbrushing, denture cleansing, eating, and drinking (water permitted) for 12 hours prior to sampling, and none had undergone professional prophylaxis within the prior three months; clinical examinations were performed by two calibrated clinicians (intra- and inter-examiner ICC for plaque indices ≥ 0.86).

Plaque and biofilm sampling was standardized and executed in a single visit in the late morning (09:00–11:30). For fixed restorations, supragingival plaque was collected from the prosthetic surface margins on the index restoration using sterile Gracey curettes, while subgingival plaque was obtained from the mesio-buccal sulcus with sterile endodontic paper points inserted to the base of the sulcus for 10 seconds; for removable acrylic dentures, biofilm was gently scraped from a 1 cm^2 standardized palatal fitting-surface template and from the maxillary molar denture tooth cervical area; for implant-supported prostheses, peri-implant sulcus samples were obtained with paper points, and supramucosal biofilm from the transmucosal titanium abutment surface was collected using a Teflon-coated scaler to avoid metal contamination. All samples were placed into pre-reduced transport fluid and transported on ice to the microbiology laboratory within two hours. Surface roughness (Ra, µm) of representative prosthetic surfaces was measured in situ using a portable contact profilometer (cut-off 0.8 mm, evaluation length 4 mm) with three passes in orthogonal directions at five locations per subject; the mean Ra per subject was used for analysis.

Microbiological processing comprised serial ten-fold dilutions and plating on selective and differential media as follows: Mitis-Salivarius-bacitracin agar for *Streptococcus mutans*, Rogosa SL agar for *Lactobacillus* spp., Sabouraud dextrose agar with chloramphenicol for yeasts (*Candida* spp.), and Wilkins–Chalgren anaerobe agar supplemented with hemin and vitamin K for obligate anaerobes; aerobic plates were incubated at 37 °C for 48 hours and anaerobic plates at 37 °C for 72 hours in an anaerobic workstation (85% N_2, 10% H_2, 5% CO_2). Colonies were quantified as CFU/mL and presumptively identified by morphology and biochemical tests (catalase, API Rapid ID systems) with confirmation of representative isolates using MALDI-TOF MS. Species-specific quantitative PCR (qPCR) targeting *gtfB* (*S. mutans*), 16S rRNA (*Porphyromonas gingivalis*, *Prevotella intermedia*), *ltxA* (*Aggregatibacter actinomycetemcomitans*), and ITS regions (*Candida albicans*) was performed on DNA extracted from primary samples to verify culture results and to detect low-abundance taxa below culture thresholds. Community diversity metrics (Shannon and Simpson indices) were computed from merged culture and qPCR presence–absence matrices at the species level.

Clinical parameters recorded included the Oral Hygiene Index–Simplified (OHI-S, Green–Vermillion), the Gingival Index (Löe–Silness) at index sites, bleeding on probing (BOP) at subgingival and peri-implant sites, probing depths for natural teeth and implants, and for removable dentures, the Newton classification of denture stomatitis; a denture plaque index was scored on a four-point scale for the acrylic group. A pilot power analysis (α=0.05, two-sided) indicated that 20 subjects per group would provide ≥80% power to detect a between-group difference of 0.5 in the Shannon index (SD 0.6) or a 0.35 log10 difference in total CFU/mL (SD 0.4).

Statistical analysis was performed using R (v4.3). Distributions were inspected visually and with Shapiro–Wilk tests; CFU/mL were log10-transformed when appropriate. Group comparisons used one-way ANOVA or Kruskal–Wallis tests with

Tukey HSD or Dunn's post-hoc procedures, respectively. Linear models and ANCOVA adjusted for age, sex, smoking, OHI-S, and denture/implant age evaluated associations between material type and microbial outcomes; logistic regression modeled odds of high *Candida* carriage ($\geq 10^3$ CFU/mL). Correlations between surface roughness and microbial load used Pearson's or Spearman's coefficients as appropriate; multiple comparisons were controlled using the Benjamini–Hochberg false discovery rate with $q<0.10$ considered exploratory while primary hypotheses used $p<0.05$ as the significance threshold.

Research results. A total of 96 patients were screened, 12 were excluded (recent antibiotics n=6, active mucosal lesions n=3, mixed prosthesis materials n=3), and four declined participation; thus, 80 participants were enrolled and completed all procedures with no adverse events. Groups were comparable at baseline for age (mean 54.8±8.9 years), sex distribution (56% female overall), diabetes status (12% overall), smoking (21% overall), and OHI-S (overall mean 1.78±0.42) with no significant between-group differences (all $p>0.10$). Median denture/implant age was 2.1 years (IQR 1.3–3.4). The sampling workflow adhered to protocol in 100% of cases with duplicate laboratory measures (10% random subset) demonstrating high technical reproducibility (CV ≤7%).

Culture and qPCR revealed distinct material-specific microbial signatures. Acrylic resin dentures showed the greatest carriage of *Candida albicans* and cariogenic streptococci, while titanium implant-supported restorations exhibited higher proportions of obligate anaerobes typical of peri-implant niches; zirconia-based fixed prostheses consistently demonstrated the lowest total biofilm burden and the most even microbial communities. Below, we present detailed summaries with explanatory notes for each table.

Table 1. Distribution of dominant microorganisms in different prosthesis groups

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Microorganism	**Acrylic**	**Metal-ceramic**	**Zirconia**	**Titanium**
S. mutans	++++	++	+	+
C. albicans	++++	+	+	–
P. gingivalis	++	+++	++	++++
A. actinomycetemcomitans	++	++	+	+++
Lactobacillus spp.	+++	++	+	+

Notes to Table 1: The semi-quantitative scale maps to detection frequency: − <20%, + 20–39%, ++ 40–59%, +++ 60–79%, ++++ 80–100% of subjects within each group. Applying this mapping, *S. mutans* was detected in approximately 18/20 acrylic wearers (90%), 9/20 metal-ceramic (45%), 6/20 zirconia (30%), and 6/20 titanium (30%). *C. albicans* carriage was ~17/20 (85%) in acrylic, 6/20 (30%) in metal-ceramic, 6/20 (30%) in zirconia, and <4/20 in titanium (below the 20% threshold, consistent with qPCR low-level signals but culture negativity). Conversely, *P. gingivalis* positivity rose from ~8/20 (40%) in acrylic to ~16/20 (80%) in titanium, aligning with the anaerobic conditions of peri-implant sulci. These patterns were corroborated by species-specific qPCR with strong concordance (Cohen's κ=0.82 for *S. mutans*, 0.79 for *P. gingivalis*).

Table 2. Average microbial load (CFU/mL) by prosthesis type

Group	Mean total CFU/mL (×10^5)	SD	95% CI	Pairwise post-hoc vs Acrylic
Acrylic	45.6	6.2	43.1–48.2	reference

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Metal-ceramic	32.4	5.1	30.2–34.6	−12.9 (p<0.001)
Zirconia	22.7	4.8	20.7–24.8	∑22.9 (p<0.001)
Titanium	18.9	3.9	17.1–20.7	∑26.7 (p<0.001)

Notes to Table 2: One-way ANOVA on log10-transformed CFU showed a strong material effect ($F(3,76)=54.3$, $p<0.0001$, $\eta^2=0.68$) with a monotonic decrease in total biofilm from acrylic to titanium. Adjusted ANCOVA controlling for age, sex, smoking, OHI-S, and prosthesis age preserved significance ($p<0.001$ for material term) and explained 72% of variance (adjusted $R^2=0.72$). The largest mean difference was between acrylic and titanium (−26.7×10^5 CFU/mL, 95% CI −30.4 to −23.0), whereas metal-ceramic vs zirconia also differed significantly (−9.7×10^5, p=0.002), highlighting a biologically meaningful reduction associated with smoother ceramics.

Table 3. Distribution of aerobic and anaerobic species (%)

Prosthesis Type	Aerobes	Anaerobes
Acrylic	68	32
Metal-ceramic	55	45
Zirconia	48	52
Titanium	41	59

Notes to Table 3: The aerobic-to-anaerobic shift across materials was significant (Cuzick trend test p<0.001), paralleling increasing subgingival/peri-implant probing depths (median 3.1 mm acrylic-associated teeth vs 3.7 mm around titanium abutments, p=0.03) and higher BOP near implant sites (38% vs 24% at tooth sites, p=0.04). Implants demonstrated a 1.56-fold higher odds of anaerobe predominance than tooth-borne restorations after adjustment (OR 1.56, 95% CI 1.11–2.22, p=0.01), supporting the ecological impact of transmucosal geometry and oxygen tension.

Table 4. Species diversity indices by prosthesis type

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Group	Shannon Index	Simpson Index
Acrylic	1.45	0.65
Metal-ceramic	1.78	0.72
Zirconia	1.95	0.79
Titanium	2.10	0.83

Notes to Table 4: Diversity increased progressively from acrylic to titanium (ANOVA $p<0.001$ for Shannon; $p=0.002$ for Simpson), with post-hoc differences significant for acrylic vs zirconia (+0.50, $p=0.001$) and acrylic vs titanium (+0.65, $p<0.001$). Higher diversity correlated with lower total CFU ($r=-0.62$, $p<0.001$) and lower OHI-S ($r=-0.41$, $p<0.001$), suggesting that smoother surfaces promote more balanced, less biomass-dense communities rather than overgrowth by a few dominant taxa.

Table 5. Correlation between prosthesis material roughness and microbial load

Material Type	Surface Roughness (Ra, µm)	Total CFU/mL (×10^5)	Correlation (r)
Acrylic	2.5	45.6	0.89
Metal-ceramic	1.8	32.4	0.76
Zirconia	0.9	22.7	0.64
Titanium	0.6	18.9	0.58

Notes to Table 5: At the subject level, surface roughness demonstrated a strong positive correlation with total CFU (Pearson $r=0.71$, $p<0.001$) that remained robust after adjustment for OHI-S, smoking, and prosthesis age ($\beta=0.38$ log10 CFU per 0.5 µm Ra increase, $p<0.001$). Within the acrylic group, each 0.2 µm increment in Ra was associated with 12% higher odds of high *Candida* carriage (OR 1.12, 95% CI 1.04–1.22, $p=0.004$). Sensitivity analyses excluding smokers (n=17) and patients with diabetes (n=10) produced similar estimates (<10% attenuation), indicating limited confounding by these factors.

Additional findings of clinical relevance included a higher prevalence of Newton type II–III denture stomatitis among acrylic wearers with high *Candida* loads (12/20, 60%) compared with those with low *Candida* loads (2/20, 10%; χ^2 p=0.002), and lower microbial loads among participants reporting daily denture cleaning with mechanical brushing plus effervescent cleanser versus mechanical brushing alone (difference −6.1×10^5 CFU/mL, p=0.04). For implant-supported prostheses, *P. gingivalis* qPCR copy numbers were positively associated with peri-implant probing depth (ρ=0.46, p=0.03) and BOP presence (point-biserial r=0.42, p=0.04), underscoring peri-implant health monitoring needs.

Discussion. The present analysis demonstrates that the ecological profile of oral biofilms in prosthesis wearers is shaped jointly by prosthesis type and, critically, by material-linked surface characteristics that govern initial pellicle formation, microbial adhesion forces, and oxygen diffusion, yielding predictable community structures that carry distinct clinical risks, and in our cohort acrylic resin dentures, by virtue of higher roughness and porosity, preferentially supported dense, low-diversity biofilms dominated by *Candida albicans*, *Streptococcus mutans*, and *Lactobacillus* spp., thereby predisposing to denture stomatitis and secondary caries, whereas smoother, chemically inert ceramic and titanium surfaces harbored less biomass with greater evenness and, in the peri-implant context, an anaerobe-enriched community featuring *P. gingivalis* and *A. actinomycetemcomitans* that aligns with peri-implant mucosal inflammation; mechanistically, the roughness–biomass gradient we observed (β=0.38 log10 CFU per 0.5 μm Ra) is consistent with the concept that micro-irregularities shield pioneer colonizers from shear forces, accelerate maturation, and bias nutrient gradients, while the anaerobic shift around implants likely reflects the transmucosal design and deeper sulcular microenvironments that reduce redox potential; clinically, these findings argue for material-informed prevention strategies, including prioritizing highly polished zirconia or well-finished titanium wherever feasible, implementing rigorous post-delivery finishing and glazing protocols for ceramics and meticulous chairside polishing for acrylics, and tailoring hygiene regimens by risk—such as

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recommending daily mechanical cleansing plus effervescent oxidizing cleansers for acrylic denture wearers and professional maintenance intervals of three to four months for high-risk groups, alongside adjunctive antifungal therapy when high *Candida* carriage is documented, while for implant-borne restorations, early identification and suppression of anaerobe-dominant communities through mechanical debridement, chlorhexidine or essential-oil rinses as appropriate, and strict control of modifiable cofactors like plaque accumulation and smoking may reduce progression to peri-implantitis; beyond biomedical outcomes, the economic implications are substantial because prosthesis-associated infections drive unplanned chair time, medication costs, and premature remakes or revisions, and prevention via optimal material selection and finishing is typically less costly than treating established disease, while socially, preserving comfortable function with low-biomass, balanced microbiota supports nutrition, speech, self-esteem, and work productivity, particularly in older adults who depend on removable prostheses; our results should be interpreted considering limitations including the cross-sectional design that precludes causal inference, culture-based detection that may underrepresent fastidious taxa despite qPCR supplementation, and a single-center sample that may limit generalizability, though the consistency of trends across multiple metrics, robustness to covariate adjustment and sensitivity analyses, and biological plausibility strengthen confidence in the conclusions and provide a practical framework for integrating microbiological risk into prosthesis material selection, finishing standards, and personalized maintenance protocols.

Conclusion. In an 80-patient cross-sectional cohort, prosthesis material and associated surface roughness emerged as dominant determinants of oral biofilm burden and composition: acrylic resin supported the highest biomass with frequent *Candida* and cariogenic streptococci, titanium and zirconia exhibited lower biomass and higher community evenness with a peri-implant shift toward anaerobes, and roughness correlated strongly and independently with total CFU; these findings translate into actionable recommendations—select smoother materials when clinically possible, apply stringent finishing/polishing protocols to minimize Ra, institute risk-stratified hygiene (daily mechanical cleansing plus effervescent cleanser for acrylic wearers; peri-implant biofilm control and close monitoring for titanium restorations), and incorporate routine microbiological surveillance for high-risk patients—to reduce stomatitis, secondary caries, and peri-implant disease, improve long-term prosthetic success, and lower economic and social costs by preserving comfortable function, nutrition, and quality of life.

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