

Original Article



Comparison of Oral Biofilm Growth on Removable Partial Dentures Fabricated From Conventional Heat-Cured PMMA and Polyamide Denture Base Materials

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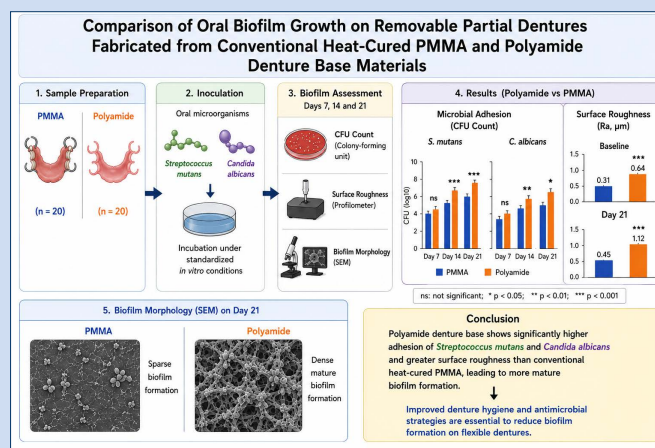
Abstract

Introduction: Surface characteristics of denture base materials influence microbial adhesion and oral biofilm formation. Heat-cured polymethyl methacrylate (PMMA) and polyamide are widely used; however, comparative evidence regarding their susceptibility to microbial colonization under standardized in vitro conditions remains limited. The present study compared oral biofilm formation on removable partial dentures fabricated from heat-cured PMMA and polyamide denture base materials under standardized in vitro conditions.

Methods: This in vitro experimental study evaluated 20 heat-cured PMMA and 20 polyamide removable partial denture specimens. The specimens were inoculated with standardized cultures of *Streptococcus mutans* and *Candida albicans*. Biofilm formation was assessed by colony-forming unit analysis on days 7, 14, and 21. Surface roughness was measured at baseline and on day 21 using a contact profilometer, and biofilm morphology was qualitatively evaluated by scanning electron microscopy. Statistical significance was set at $P < 0.05$.

Results: No significant difference in *Streptococcus mutans* adhesion was observed on day 7 ($P = 0.340$). However, polyamide showed significantly higher *S. mutans* counts on days 14 and 21 ($P < 0.001$). *C. albicans* adhesion was also significantly greater on polyamide on days 14 ($P = 0.006$) and 21 ($P = 0.027$), with no difference from day 7. Polyamide exhibited greater surface roughness at baseline and day 21, while SEM confirmed mature biofilm formation on both denture materials.

Conclusion: Polyamide demonstrated higher microbial adhesion and surface roughness than PMMA. Improved denture hygiene and antimicrobial approaches may help reduce biofilm formation on flexible dentures.



Introduction

Our oral cavity harbors many microorganisms, primarily bacteria, viruses, fungi, and protozoa, which exist either as planktonic cells or as structured biofilms. Colony

formation of some pathogenic microbes on denture bases often leads to stomatitis or even deterioration of existing teeth by contributing to periodontal diseases and caries. It may even contribute to systemic conditions

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such as diabetes.^{1,2} Once established, microorganisms within a biofilm become more resistant to antimicrobial agents and host defense mechanisms, making their elimination considerably more difficult than that of their planktonic counterparts.³

Microbial adhesion to denture base materials is the initial step in biofilm formation and is influenced by several factors, including surface roughness, surface energy, hydrophobicity, and chemical composition.^{4,5} Previous studies have proposed a critical threshold for the surface roughness of dental materials in the oral cavity, defined as $R_a = 0.2 \mu\text{m}$, above which plaque accumulation increases significantly.⁵⁻⁷

Gram-positive bacteria such as *Streptococci* and *Lactobacilli* form supragingival plaque, whereas Gram-negative anaerobic bacteria, such as *Fusobacterium* and *Porphyromonas gingivalis*, form subgingival plaque.^{8,9} Subgingival plaque in individuals with gingival and periodontal diseases also harbors *Candida albicans*, whose adhesion to the denture base is a critical factor behind denture stomatitis.¹⁰⁻¹³ The synergistic interactions between *C. albicans* and *S. mutans* enhance biofilm maturation and contribute to persistent microbial colonization of prosthetic surfaces.

Polymethylmethacrylate (PMMA) is an acrylic resin widely used as a denture base material for both complete and removable partial denture fabrication. Dr. Wright developed it in the early 1930s, and dentistry has adopted it for its numerous advantages, including biocompatibility in the complex oral environment, ease of processing, low cost, stability due to its mechanical and physical properties, and, most importantly, acceptable esthetic value. It also has disadvantages, such as brittleness due to low fracture toughness, impact strength, flexural strength, fatigue strength, dimensional inaccuracy, and allergic reactions to its monomer.^{14,15} Because of these properties, researchers have developed alternative denture base materials with improved mechanical and esthetic properties.

Polyamide-based flexible denture base materials have gained considerable popularity as an alternative to PMMA denture bases. Their flexibility, lightweight nature, superior esthetics, lack of visible metal clasps, and ability to engage soft tissue undercuts have contributed to improved patient acceptance.^{16,17} Post-insertion maintenance is also very “user friendly” with these materials.¹⁸⁻²² However, concerns remain regarding their surface characteristics and potential susceptibility to microbial colonization. Because of greater water sorption and surface irregularities, flexible denture bases may provide a more favorable substrate for biofilm formation, increasing the risk of denture-related infections.

Various authors have shown that roughened surfaces of PMMA-fabricated dental prostheses facilitate adhesion of *Candida* species and *Streptococcus oralis*, thereby exacerbating dental biofilm formation and contributing to oral health issues such as denture stomatitis, gingival inflammation, and root decay on abutment teeth.²³ However, most investigations have been conducted in vivo,

where patient-related variables cannot be standardized. Furthermore, few studies have simultaneously evaluated bacterial and fungal adhesion and surface roughness using complementary microbiological and microscopic techniques under controlled laboratory conditions.

Therefore, this in vitro study aimed to evaluate microbial colonization of oral biofilms formed on the intaglio surfaces of two different polymeric denture base materials in patients wearing removable partial dentures under standardized experimental conditions. The denture base resin materials investigated were heat-cured PMMA and a commercially available flexible denture base material (polyamide). The null hypothesis was that biofilm formation would not differ between the two denture base materials.

Methods

Study Design and Sample Selection

This in vitro study was conducted in the Department of Prosthodontics, Institute of Dental Sciences, Siksha ‘O’ Anusandhan, Bhubaneswar, Odisha, India. Ethical approval was obtained from the Institutional Ethics Committee before commencement of the study, and written informed consent was obtained from all participants. Twenty partially edentulous patients aged 30–60 years who required removable partial dentures were recruited, irrespective of sex. Unstimulated saliva samples were collected from each participant under aseptic conditions for microbiological isolation. Two removable partial dentures (RPDs) were fabricated for each participant using different denture base materials:

Group I: polyamide denture base material (n = 20)

Group II: heat-cured PMMA denture base material (n = 20)

The dentures were fabricated following the manufacturers’ recommended processing protocols and polished using a standardized finishing procedure to minimize variability in surface characteristics.

Biofilm Formation

The fabricated dentures were transferred to the Central Research Laboratory at the Institute of Dental Sciences for standardized biofilm formation experiments. Each denture specimen was immersed separately in modified nutrient broth inoculated with standardized cultures of *S. mutans* and *C. albicans*. The inoculum density for both microorganisms was standardized using a 0.5 McFarland standard before inoculation. Fresh culture medium was replaced every 48 hours throughout the 21-day incubation period to maintain microbial viability and standardized experimental conditions. Microbial adhesion was evaluated after 7, 14, and 21 days of incubation.²³

Microbiological Identification

Following incubation, microbial isolates recovered from the denture surfaces were identified using conventional microbiological techniques. *S. mutans* was identified based on Gram staining, colony morphology on blood

agar, catalase reaction, and carbohydrate fermentation tests (mannitol, sorbitol, melibiose, raffinose, and inulin). *C. albicans* was identified by colony morphology on Sabouraud dextrose agar (SDA), Gram staining, and confirmation by the germ tube test.²³

Colony-Forming Unit Analysis

At each observation period (days 7, 14, and 21), adherent microorganisms were recovered from the denture surfaces, and colony-forming units (CFUs) were determined using standard microbiological culture techniques. The colonies were counted using a digital colony counter, and the mean CFU count for each specimen was recorded. Separate analyses were performed for *S. mutans* and *C. albicans*.²³

Surface Roughness Evaluation

Surface roughness (Ra) of all denture specimens was measured using a contact profilometer before microbial inoculation (baseline) and after 21 days of incubation. Three measurements were obtained from standardized locations on each specimen, and the mean Ra value was calculated for statistical analysis. The profilometer was calibrated before each measurement session according to the manufacturer's recommendations.²⁴

Scanning Electron Microscopic Evaluation

Representative denture specimens from each group were examined using scanning electron microscopy (SEM) at the Central Instrumentation Facility, Odisha University of Agriculture and Technology, Bhubaneswar, India. Before examination, the specimens were sputter-coated with platinum to render the surfaces electrically conductive. SEM images were obtained at standardized magnifications of 4.38 and 20.00 KX. The micrographs were used to qualitatively evaluate the morphology, distribution, and surface characteristics of microbial biofilms formed on the denture base materials. Representative images were selected to illustrate differences in biofilm architecture between polyamide and PMMA specimens.²³

Statistical Analysis

Data were entered into Microsoft Excel 2016 and analyzed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean $\pm$ standard deviation (SD). The normality of the data was assessed before analysis, and Levene's test was used to evaluate homogeneity of variance. Comparisons between polyamide and PMMA denture base materials at each observation period were performed using the independent-samples t-test. A two-tailed P-value of <0.05 was considered statistically significant.

Results

Identification of Microorganisms

The isolates recovered from the denture specimens were identified as *S. mutans* and *C. albicans* based on colony morphology, Gram staining, and biochemical characteristics. *S. mutans* appeared as Gram-positive cocci arranged in chains, producing small, granular α -hemolytic colonies on blood agar. The isolates were catalase-negative and fermented the tested carbohydrates. *C. albicans* was identified as Gram-positive budding yeast cells producing creamy white colonies on SDA and a positive germ tube test. Table 1 presents the microbiological characteristics of both organisms.

Biofilm Formation

The number of *S. mutans* colonies increased progressively with time on both denture base materials. On day 7, the mean CFU count was similar in the polyamide and PMMA groups, with no statistically significant difference between them ($P=0.340$). By day 14, the polyamide group demonstrated a significantly higher mean CFU count than the PMMA group (10.55 ± 3.10 vs. 6.30 ± 2.00 ; $P<0.001$). A similar trend was observed on day 21, with polyamide exhibiting significantly higher CFU counts than PMMA (15.65 ± 4.43 vs. 9.90 ± 2.04 ; $P<0.001$) (Table 2, Figure 1). Similarly, an increase in *C. albicans* colony counts was observed in both groups throughout the study period (Table 2, Figure 2). No statistically significant difference was observed between the polyamide and PMMA groups on day 7 ($P=0.158$). However, by day 14, the polyamide group demonstrated significantly higher CFU counts than the PMMA group (10.95 ± 2.68 vs. 8.70 ± 2.20 ; $P=0.006$). This difference remained significant on day 21, with higher fungal colonization observed on polyamide than on PMMA (15.65 ± 4.55 vs. 13.10 ± 1.94 ; $P=0.027$).

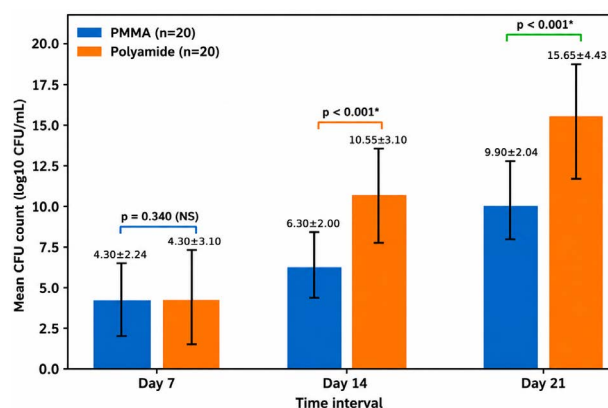


Figure 1. Comparison of mean *S. mutans* colony-forming unit (CFU) counts on polyamide and PMMA removable partial denture (RPD) base materials on days 7, 14, and 21. Data are presented as mean $\pm$ SD

Table 1. Identification of microorganisms isolated from oral biofilm

Microorganism	Gram staining	Colony morphology	Biochemical characteristics	Confirmatory test
<i>Streptococcus mutans</i>	Gram-positive cocci in chains	Small, granular, α -haemolytic colonies on blood agar	Catalase negative; ferments mannitol, sorbitol, melibiose, raffinose and inulin	Carbohydrate fermentation
<i>Candida albicans</i>	Gram-positive budding yeast cells	Creamy white colonies on Sabouraud dextrose agar	Germ tube formation	Germ tube positive

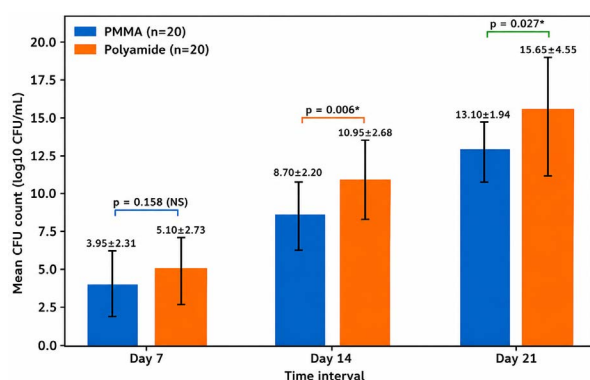
Table 2. Comparison of microbial colony-forming units (CFU) between Polyamide and PMMA denture base materials

Microorganism	Time	Polyamide (Mean $\pm$ SD)	PMMA (Mean $\pm$ SD)	Mean Difference	P value
<i>Streptococcus mutans</i>	Day 7	4.30 $\pm$ 3.10	4.30 $\pm$ 2.24	0.00	0.340
	Day 14	10.55 $\pm$ 3.10	6.30 $\pm$ 2.00	4.25	<0.001*
	Day 21	15.65 $\pm$ 4.43	9.90 $\pm$ 2.04	5.75	<0.001*
<i>Candida albicans</i>	Day 7	5.10 $\pm$ 2.73	3.95 $\pm$ 2.31	1.15	0.158
	Day 14	10.95 $\pm$ 2.68	8.70 $\pm$ 2.20	2.25	0.006*
	Day 21	15.65 $\pm$ 4.55	13.10 $\pm$ 1.94	2.55	0.027*

Table 3. Surface roughness (Ra) comparison between Polyamide and PMMA denture base materials

Time point	Polyamide (Mean $\pm$ SD) (μ m)	PMMA (Mean $\pm$ SD) (μ m)	Mean Difference	P value
Baseline	2.11 $\pm$ 0.28	1.47 $\pm$ 0.29	0.64	<0.001*
Day 21	2.52 $\pm$ 0.20	2.29 $\pm$ 0.36	0.23	0.014*

Values are expressed as Mean $\pm$ SD. Independent samples t-test. *Statistically significant ($P < 0.05$).

**Figure 2.** Comparison of mean *C. albicans* colony-forming unit (CFU) counts on polyamide and PMMA removable partial denture (RPD) base materials on days 7, 14, and 21. Data are presented as mean $\pm$ SD

Surface Roughness Analysis

Before microbial inoculation (baseline), the polyamide specimens demonstrated significantly greater surface roughness than the PMMA specimens ($2.11 \pm 0.28 \mu\text{m}$ vs. $1.47 \pm 0.29 \mu\text{m}$; $P < 0.001$). After 21 days of incubation, surface roughness increased in both materials. However, the polyamide group continued to exhibit significantly greater surface roughness than the PMMA group ($2.52 \pm 0.20 \mu\text{m}$ vs. $2.29 \pm 0.36 \mu\text{m}$; $P = 0.014$). Table 3 summarizes the surface roughness values.

Scanning Electron Microscopic Observations

SEM demonstrated microbial adhesion on the denture base surfaces following incubation. At low magnification (4.38 KX), the biofilm covered extensive areas of the denture surface, whereas high-magnification images (20.00 KX) revealed closely packed microbial cells embedded within an extracellular polymeric substance (EPS) matrix, indicating mature biofilm formation (Figure 3). The qualitative SEM observations supported the microbiological findings, showing greater biofilm accumulation on polyamide surfaces than on PMMA surfaces.

Discussion

The present study evaluated two commonly used removable partial denture base materials—heat-cured PMMA and polyamide—and compared their susceptibility

to microbial colonization under standardized in vitro conditions. In the study, polyamide denture bases showed significantly higher CFU counts than PMMA denture bases, leading to the rejection of the null hypothesis. These findings highlight the importance of denture base surface characteristics in microbial adhesion and biofilm formation.

Microbial adhesion to denture surfaces is a vital mechanism in the biofilm formation lifecycle. Following adhesion, the microbial colonies produce EPS, which promotes irreversible attachment and biofilm stability. Biofilm stability is heightened with increased surface roughness.^{14,25-28} A rough prosthetic surface can lead to discoloration, patient discomfort, and increased microbial adhesion and biofilm formation.^{5,29} Therefore, it is important to assess the surface roughness of materials intended for dental prostheses before patient use. Colonization of both *S. mutans* and *C. albicans* increased progressively on both denture base materials throughout the study duration. No significant changes were observed between the two groups up to day 7. On days 14 and 21, CFU counts increased significantly in the polyamide group compared with the PMMA group. This demonstrates that polyamide surfaces have a more conducive environment for microbial proliferation and subsequent biofilm formation than PMMA surfaces.

The roughness and topographic features of the denture base surface play a crucial role in regulating microbes' ability to form biofilm and in increasing biofilm thickness by shielding them from shear forces, thereby enhancing retention on the denture base surface.³⁰ A study in Germany reported that both *S. oralis* and *Actinomyces viscosus* adhered to dentures with increased surface roughness.³¹ Likewise, a Belgian study reported that the surface roughness of acrylic resin varies with the polishing grit used. Their research revealed that microorganisms such as *S. sanguis*, *Bacteroides gingivalis*, and *C. albicans* attach to roughened acrylic resin surfaces at significantly higher numbers than to smooth acrylic resin surfaces.⁶ Similarly, a British study investigated the influence of denture acrylic resin type and surface roughness on biofilm accumulation. The study also tested the susceptibility of

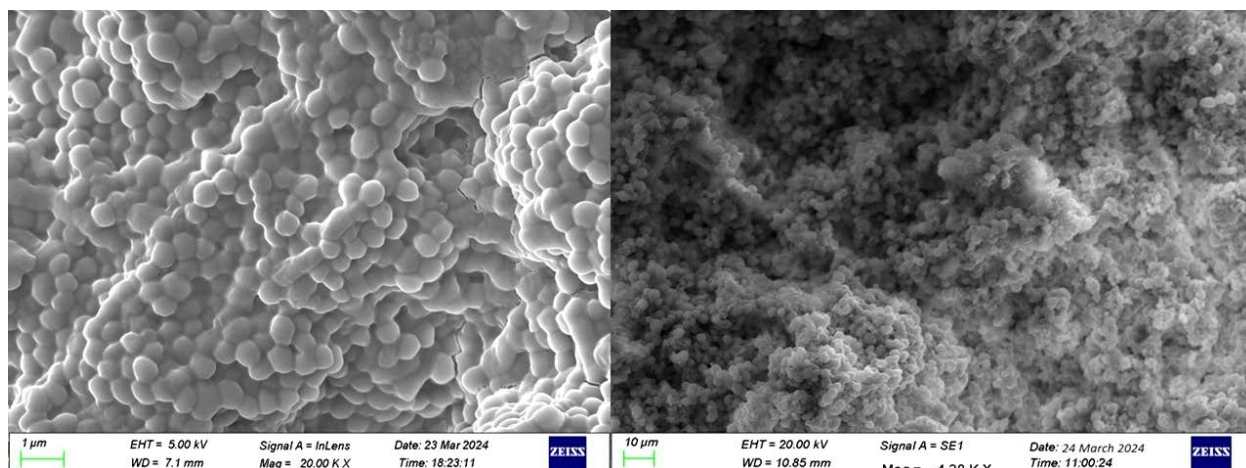


Figure 3. Representative scanning electron micrographs of mature microbial biofilm formed on the surface of a removable partial denture base. (A) High-magnification image (20.00 KX). (B) Low-magnification image (4.38 KX)

heat-polymerized and auto-polymerized acrylic resins to *S. oralis*. Surface roughness significantly affected *S. oralis* adhesion to the denture base materials.³²

SEM provided qualitative evidence of microbial colonization. Previous SEM-based studies demonstrated bacterial cells embedded within fibrous or amorphous EPS, validating the representative structural organization of mature biofilms.³³ The SEM observations of the present study support the quantitative colony counts and indicate the course of microorganisms from initial microbial adhesion to organized biofilm development.

The physicochemical properties of the two materials may have contributed to the differences in microbial colonization. Polyamide materials are relatively hydrophilic in nature. Consequently, they tend to retain more moisture and provide a suitable environment for microbial growth.^{34,35} However, PMMA offers a dimensionally stable, smooth denture surface, thereby preventing easy colonization by microbial species. These characteristics were not directly evaluated in the present investigation.^{14,34,35}

Limitations

The present study was conducted under standardized in vitro conditions which do not replicate the dynamic environment of the oral cavity. Several clinically relevant factors, including variations in salivary composition, diet, oral hygiene practices, masticatory forces, and temperature changes, were not simulated, thereby limiting the generalizability of the findings to clinical settings. Additionally, the study did not evaluate essential material characteristics that affect microbial adhesion to denture base materials (surface energy, chemical composition, and hydrophobicity).^{36,37} A study from the USA demonstrated the importance of evaluating multiple surface parameters to provide a more comprehensive understanding of microbial adhesion and colonization.³⁸

Future Research

Future research should explore incorporating antimicrobial agents into polyamide denture base

materials and examine their effectiveness in preventing biofilm formation without compromising the material's mechanical properties. Simultaneous evaluation of multiple surface parameters, including roughness, surface free energy, hydrophobicity, water sorption, wettability, and chemical composition, is warranted to provide a more comprehensive knowledge regarding the mechanisms governing microbial adhesion. Well-designed in vivo studies and long-term clinical trials are essential to validate the present study's findings, examine biofilm development over time, and verify the clinical relevance of these results under the dynamic conditions of the oral cavity. As denture base materials continue to advance with emerging technologies, future research will be essential to enhance biological performance, clinical durability, and treatment outcomes.

Conclusion

Within the limitations of this study, the polyamide denture base material exhibited greater surface roughness with significantly greater biofilm accumulation than the PMMA denture base material. Despite offering superior esthetics, polyamide dentures show increased susceptibility to colonization by *S. mutans* and *C. albicans*, which may concern patients predisposed to denture-associated infections. In contrast, PMMA exhibited comparatively lower microbial adhesion, suggesting reduced tendency for biofilm accumulation and maturation. Incorporating antimicrobial agents into polyamide may minimize microbial colonization while preserving its properties. Regardless of the material used, meticulous denture hygiene and regular follow-up are imperative to prevent biofilm accumulation and maintain oral health.

AI Declaration

AI-assisted tools were used only for language editing and improving manuscript readability. The authors generated and verified the scientific content, data analysis, interpretation, figures, and conclusions.

Authors' Contribution

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Competing Interests

None declared.

Ethical Approval

This study was conducted with approval from the institutional Ethics Committee vide letter no IEC-IDS/IDS/SOA/2023/I-13 dated 10th October 2023.

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