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Original Article

Effects of cleaning agents and *Candida* Biofilms on dental resin integrity

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الملخص

أهداف البحث: دُرست مختبريًا تأثيرات نمو الغشاء الحيوي للمبيضة البيضاء تحت المعالجة بعوامل التنظيف في تدهور راتنج الأكريليك المستخدم في أطقم الأسنان، وهو بولي ميثيل ميثاكريلات، وراتنج مركب ترميمي نانوي الحشوة.

طرق البحث: حُضِّر ما مجموعه 240 عينة من بولي ميثيل ميثاكريلات، ومن الراتنج المركب النانوي الحشوة. قِيسَت القيم الأساسية لصلادة السطح باستخدام رقم فيكرز للصلادة، ومتوسط الخشونة السطحية الحسابي بالنانومتر، ومقاومة الانثناء وفق المعيار الدولي 1-20795. عُرِّضت العينات لتحدي الغشاء الحيوي للمبيضة البيضاء ATCC 10231 لمدة 90 يومًا، ثم غُمِرت في هيبوكلوريت الصوديوم بتركيز 1% أو في مستخلص الباتشولي المائي بتركيز 15%. استُخدم تحليل القدرة الإحصائية لتحديد أحجام العينات وأحجام التأثير، وحُسب مربع إيتا الجزئي لجميع المقارنات ذات الدلالة الإحصائية.

النتائج: مقارنةً ببولي ميثيل ميثاكريلات، أظهر الراتنج المركب النانوي الحشوة صلادة سطحية أساسية أعلى بدرجة معنوية، ونعومة سطحية أفضل بدرجة معنوية. وارتبطت زيادة خشونة سطح بولي ميثيل ميثاكريلات بتراكم أعلى للغشاء الحيوي وتدهور ميكانيكي أكبر، مع انخفاض في مقاومة الانثناء بنسبة 14.14%. كما حدث تدهور ملحوظ بعد التعرض لهيبوكلوريت الصوديوم، وأظهر بولي ميثيل ميثاكريلات أكبر فقد في مقاومة الانثناء بنسبة 36.13%. في المقابل، لم يُلاحظ تدهور معنوي في المادة عند استخدام مستخلص الباتشولي. وأظهر المطهران كفاءة محدودة ضد الأغشية الحيوية الناضجة، بينما كان هيبوكلوريت الصوديوم عالي الفاعلية ضد خلايا المبيضة البيضاء الحرة العالقة.

الاستنتاجات: مقارنةً ببولي ميثيل ميثاكريلات، امتلك الراتنج المركب النانوي الحشوة خصائص سطحية وميكانيكية أفضل، ارتبطت بمقاومة أعلى للتدهور الناتج عن العوامل الكيميائية والغشاء الحيوي. ومع ذلك، فإن محدودية النموذج أحادي النوع تستدعي مراعاة ذلك عند تفسير النتائج سريريًا. أظهر هيبوكلوريت الصوديوم نشاطًا مضادًا للفطريات بدرجة كبيرة، لكنه ارتبط بتدهور شديد في المادة، بينما حافظ مستخلص الباتشولي على سلامة المادة مع فاعلية متوسطة ضد الغشاء الحيوي. لذلك، قد تكون هناك حاجة إلى بروتوكولات تنظيف مخصصة بحسب نوع المادة. كما يلزم إجراء مزيد من الدراسات باستخدام نماذج أكثر تعقيدًا لتحقيق التوازن بين الفاعلية المضادة للميكروبات والمتانة طويلة الأمد للتعويضات السنية.

الكلمات المفتاحية: الغشاء الحيوي؛ المبيضة البيضاء؛ الراتنجات السنية؛ مقاومة الانثناء؛ هيبوكلوريت الصوديوم

Abstract

Objective: The effects of *Candida albicans* biofilm growth under treatment with cleaning agents on the deterioration of denture acrylic resin (PMMA) and a nano-filled restorative composite resin were examined *in vitro*.

Materials: A total of 240 PMMA (Travelon™) and nano-filled composite (Filtek™ Z350XT) specimens were fabricated. Baseline surface hardness VHN, arithmetic mean surface roughness (Sa, in nm), and flexural strength

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(ISO 20795-1) were measured. The specimens underwent a *Candida albicans* ATCC 10231 biofilm challenge for 90 days and were then immersed in 1% sodium hypochlorite (NaOCl) or 15% aqueous patchouli extract (*Pogostemon cablin*). Power analysis ($\alpha = 0.05$, power = 80%) was used to determine sample sizes and effect sizes, and partial eta-squared (η^2) was calculated for all significant comparisons.

Results: In comparison to PMMA (98.60 ± 0.55 VHN; $p < 0.001$, $\eta^2 = 0.92$), the nano-filled composite showed significantly greater baseline hardness (156.67 ± 0.58 VHN) and surface smoothness (Sa = 75.60 ± 5.20 nm vs. 256.30 ± 18.50 nm; $p < 0.001$, $\eta^2 = 0.89$). Greater PMMA surface roughness was associated with significantly higher biofilm accumulation and mechanical deterioration (14.14% flexural strength decrease; $p < 0.001$). Significant degradation occurred after exposure to NaOCl, and PMMA showed the greatest flexural strength loss (36.13%; $p < 0.001$). In contrast, no significant material degradation was observed with patchouli extract ($p > 0.05$). Both disinfectants demonstrated limited efficacy against mature biofilms, whereas NaOCl was highly effective against planktonic *C. albicans*.

Conclusion: In comparison to PMMA, nano-filled composite had superior surface and mechanical properties associated with enhanced resistance to chemical and biofilm-related deterioration. However, the constraints of the mono-species model warrant potential clinical consideration. NaOCl demonstrated substantial antifungal activity but was associated with severe material degradation, whereas patchouli extract preserved material integrity but exhibited modest anti-biofilm efficacy. Therefore, material-specific cleaning protocols might be warranted. Further investigation of complex models is necessary to balance antimicrobial effectiveness against long-term prosthetic durability.

Keywords: Biofilm; *Candida albicans*; Dental resins; Flexural strength; Sodium hypochlorite

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Introduction

The longevity and success of dental prosthetics depend on a complex interplay among material properties, the oral biological environment, and maintenance protocols.[1] A spectrum of materials, ranging from traditional options to advanced composites, are used in dentistry.[2] Use of heat-cured acrylic resins, such as polymethyl methacrylate (PMMA, e.g., Travelon™), is widespread, because of their affordability and processability for denture bases.[3] In contrast, advanced composite resins such as Filtek™ Z350XT (3M ESPE, a nano-filled restorative composite) are engineered for superior mechanical stability in direct restorations.[4] Herein, this restorative composite served as a high-performance experimental benchmark with superior mechanical characteristics, which was compared against a conventional denture acrylic to assess the performance spectrum.[5] However, this composite is not clinically indicated for denture bases and was selected to represent an optimal polymer class for comparative purposes.

Beyond baseline characteristics, material performance in the oral cavity involves resistance to mechanical stress, chemical exposure, and microbial colonization.[4–6] Biofilm formation on dental appliances is a universal challenge that can compromise both material integrity and oral health.[7] A prevalent biofilm inhabitant is *Candida albicans*, an opportunistic yeast whose characteristics can transition from commensal to pathogenic.[8] *C. albicans* biofilms resist host defenses and antimicrobial agents[7,8] and contribute to conditions such as denture stomatitis while promoting material degradation.[9] Surface roughness critically influences microbial adhesion: rougher surfaces favor biofilm maturation.[8,9] Microbial metabolic byproducts can progressively decrease mechanical properties, including flexural strength and hardness, and potentially shorten appliance lifespan.[9]

To mitigate biofilm-related complications, patients use cleaning agents, which introduce additional considerations.[7] Potent disinfectants such as sodium hypochlorite (NaOCl), although antimicrobially effective, can degrade polymeric resins through oxidation and therefore compromise mechanical integrity.[10] Consequently, interest in natural antimicrobial agents such as patchouli (*Pogostemon cablin*) leaf extract, which has demonstrated antifungal properties, has increased.[11,12] However, the efficacy of patchouli against both planktonic and biofilm forms of *C. albicans*, and its effects on dental polymer properties require systematic evaluation.[11,12]

Although studies have been conducted on dental materials and biofilms, baseline polymer properties, susceptibility to long-term *C. albicans* biofilm formation, and the compounded effects of biofilms and various cleaning agents on material integrity have not been thoroughly investigated in a direct multi-factorial comparison.[10] This study was aimed at addressing this research gap. Specifically, our objectives were to (1) compare the susceptibility of PMMA and a nano-filled composite to long-term *C. albicans* biofilm formation; (2) quantify the resultant material degradation; and (3) assess the antimicrobial efficacy and effects of 1% NaOCl and 15% patchouli leaf extract on the material. Our findings were intended to provide a comparative understanding of polymer performance, thereby contributing to the evidence base for prosthetic maintenance; however, we acknowledge the preliminary nature of our in vitro findings.

Materials and Methods

Study design and specimen allocation

This in vitro study used a factorial design evaluating both material (PMMA vs. mano-filled composite) and treatment. A total of 240 specimens (120 per material) were fabricated. On the basis of an a priori power analysis (G*Power 3.1; effect size f = 0.4, $\alpha = 0.05$, power = 80%), a minimum of five specimens per test per group was determined to be

required. For each material, specimens were randomly allocated into six experimental groups (n = 20/group). From each group, n = 5 specimens were allocated to each specific test (flexural strength, hardness, roughness, and microbiological assay), to ensure that destructive tests were performed on separate specimens (detailed group allocation in Supplemental Table 1).

- Group 1 (control): no biofilm, no cleaning agent (baseline measurements)
- Group 2 (biofilm only): 90-day biofilm challenge
- Group 3 (biofilm + NaOCl): 90-day biofilm challenge, then 10-min immersion in 1% NaOCl
- Group 4 (biofilm + patchouli): 90-day biofilm challenge, then 45-min immersion in 15% patchouli extract
- Group 5 (NaOCl only): no biofilm, 10-min immersion in 1% NaOCl
- Group 6 (patchouli only): no biofilm, 45-min immersion in 15% patchouli extract

Materials

The study used two dental polymers: Travelon™ heat-cured acrylic resin (PMMA-based, batch no. 112-050, HLR Dentsply Pvt. Ltd., Germany) and Filtek™ Z350XT (nano-filled restorative composite, batch no. T 3090, 3M ESPE, USA). The composite resin served as a high-performance experimental benchmark representing an optimized polymer class but is not clinically indicated for denture base applications.

The antifungal agents were 1% NaOCl solution and an aqueous extract from patchouli leaves (*Pogostemon cablin*). The patchouli extract was prepared by soaking 100 g dried leaf powder in 1 L distilled water (4 °C for 48 h), followed by filtration and lyophilization. The concentrate was reconstituted to 15% (w/v) for immersion studies. The 15% concentration was selected according to preliminary minimum inhibitory concentration (MIC) assays and represented a practically feasible concentration above the minimum biofilm inhibitory concentration (MBIC). The fungal strain was *C. albicans* ATCC 10231.

Specimen fabrication

Specimens were fabricated in four dimensions according to ISO/ASTM standards, as follows:

- Flexural strength: 64 × 10 × 3.3 mm (ISO 20795-1 applied to both materials for standardized comparison; notably, this standard is specific to denture base polymers)
- Vickers hardness: 10 × 10 × 4 mm
- Surface roughness: 10 × 20 × 3 mm
- Microbiological assays: 10 × 10 × 2 mm

PMMA specimens were processed via heat polymerization (74 °C for 8 h, then 100 °C for 1 h).[13] Composite specimens were light-polymerized in silicone molds (20 s per surface, Valo Grand, Ultradent). All specimens were ground sequentially with 400−1200 grit SiC paper under irrigation and polished with a 1 μm diamond suspension to achieve a clinically relevant surface finish (target Ra <0.2 μm). Specimens were ultrasonically cleaned and stored in distilled water at 37 °C for 48 ± 2 h before baseline testing.

Microbiological assays

- ***C. albicans* Inoculum Preparation:** The strain was subcultured on Sabouraud dextrose agar.[14] A single colony was inoculated into Sabouraud dextrose broth and incubated for 18−24 h at 37 °C under shaking (120 rpm). Cells were harvested, washed twice in PBS, and resuspended to 1 × 10^7 CFU/mL, standardized according to spectrophotometry (OD600 = 0.08−0.1) and confirmed by colony counting.
- **Inherent Antifungal Activity (Agar Disc Tests):** Sterile specimen discs (10 × 10 × 2 mm) were placed on Sabouraud dextrose agar plates seeded with *C. albicans* (1.5 × 10^6 CFU/mL). Plates were incubated at 37 °C for 48 h and examined for zones of inhibition. No zones were observed for either material.[15]
- **90-Day Biofilm Model:** Specimens from groups 2, 3, and 4 were immersed in Sabouraud dextrose broth inoculated with 1 × 10^7 CFU/mL *C. albicans* and incubated at 37 °C for 90 days (protocol modified from Ref. 16). The broth was replaced with fresh inoculated broth every 48 h to maintain the nutrient supply and microbial viability.
- **Biofilm Quantification (Crystal Violet Assays):** After 90 days, group 2 specimens were gently rinsed with PBS. Biofilms were fixed with 99% methanol (15 min), stained with 0.1% crystal violet (20 min), and de-stained with 33% glacial acetic acid. The absorbance at 570 nm was used to quantify relative biofilm biomass.[9]
- **Antimicrobial Efficacy (MIC/MBIC):** The MIC against planktonic cells and the MBIC were determined in 96-well plates with broth microdilution, according to CLSI M27-A3.[17] For MBIC, 48 h biofilms were pre-formed before the addition of diluted agents. MIC/MBIC was defined as the lowest concentration showing ≥50% lower metabolic activity than observed in the control in XTT assays. All assays were performed in triplicate.

Mechanical and surface property analysis

All post-treatment measurements used dedicated specimens (n = 5 per group per test).

- **Flexural Strength:** Three-point bend tests were conducted on a Universal Testing Machine (M500-5CT, Testometric) according to ISO 20795-1[18] (span = 50 mm; crosshead speed = 5 mm/min). Flexural strength (σ, in MPa) was calculated as $\sigma = (3FL)/(2wh^2)$, where F is the fracture load (in N), L is the span (in mm), w is the width (in mm), and h is the height (in mm). All values were verified against ISO 20795-1 requirements for denture base polymers (minimum 65 MPa).
- **Vickers Hardness:** Tests were performed with a microhardness tester (Wolpert 402 MVD) with a 50 gf load for 15 s.[18] Five indentations per specimen were averaged.
- **Surface Roughness:** Analysis was performed with an atomic force microscope (5500 AFM, Keysight) in AC

mode.[9] Five 10 × 10 μm scans per specimen were obtained. The arithmetic mean roughness (Sa) was calculated in PicoImage software with a Gaussian filter to remove form errors.

Statistical analysis

Data were analyzed in SPSS v26.0. Normality was confirmed with the Shapiro–Wilk test, and homogeneity of variances was confirmed with Levene's test. For normally distributed data, two-way ANOVA, followed by Tukey's HSD post-hoc test, was used to analyze the effects of material, treatment, and their interaction. Partial eta-squared (η^2) effect sizes were calculated for all significant ANOVA results. For non-normal data, the Kruskal–Wallis test with Dunn's post-hoc test was used. A priori power analysis (G*Power 3.1) confirmed that n = 5 per test per group would have 80% power to detect large effects (f = 0.4) at $\alpha = 0.05$. A p value < 0.05 was considered statistically significant. Data are presented as mean ± standard deviation.

Results

Baseline material properties

Initial characterization of the control groups (group 1) revealed significant differences between materials ($p < 0.001$, independent t-test) (Figure 1a,b; Figure 2a,b). The composite resin demonstrated significantly greater Vickers hardness (156.67 ± 3.21 VHN) than PMMA (98.60 ± 2.89 VHN) ($p < 0.001$, $\eta^2 = 0.92$) (Figure 2a) (see Figure 3).

Flexural strength values were calculated and verified against ISO 20795-1. For PMMA, the baseline flexural strength of 86.4 ± 3.2 MPa met the ISO minimum requirement of 65 MPa for denture base polymers. For the composite, the baseline flexural strength was 115.2 ± 4.1 MPa.

Surface roughness analysis via AFM confirmed that the composite had a significantly lower Sa (75.60 ± 8.45 nm) than that of PMMA (256.30 ± 22.10 nm) ($p < 0.001$, $\eta^2 = 0.89$).

C. albicans Biofilm formation

Agar disc tests confirmed that neither material exhibited inherent antifungal activity. However, the 90-day biofilm challenge (group 2) revealed marked differences in susceptibility. Crystal violet assays indicated that the rougher PMMA surface supported significantly greater biofilm biomass (Abs570 = 1.07 ± 0.15) than the smoother composite (Abs570 = 0.82 ± 0.11) ($p < 0.001$, two-way ANOVA, $\eta^2 = 0.76$) (Figure 4).

Effects of biofilms and treatments on material properties

Two-way ANOVA indicated significant main effects of material ($p < 0.001$, $\eta^2 = 0.88$) and treatment ($p < 0.001$, $\eta^2 = 0.79$) on the flexural strength and hardness. A significant interaction was observed between material and treatment ($p < 0.001$, $\eta^2 = 0.67$).

- **Effect of Biofilm (Group 2 vs. Group 1):** Post-hoc Tukey's HSD test indicated that the 90-day biofilm challenge resulted in a statistically significant decrease in PMMA flexural strength (from 86.4 ± 3.2 MPa to 74.2 ± 3.5 MPa; 14.14% loss; $p < 0.001$) and Vickers hardness (from 98.60 ± 2.89 VHN to 73.10 ± 3.15 VHN; 25.88% loss; $p < 0.001$). However, the composite showed no significant change in flexural strength (115.2 ± 4.1 MPa to 109.8 ± 4.3 MPa; 4.66% loss; $p = 0.12$) or hardness (156.67 ± 3.21 VHN to 155.17 ± 3.50 VHN; 0.96% loss; $p = 0.65$).
- **Effect of Sodium Hypochlorite:** Exposure to 1% NaOCl had severe detrimental effects (Figure 5). Treatment of biofilm on PMMA (group 3) resulted in a 36.13% loss in flexural strength (to 47.2 ± 4.2 MPa; $p < 0.001$) and 33.36% hardness loss (to 65.70 ± 3.80 VHN; $p < 0.001$) with respect to the pre-cleaned state (group 2). Even on pristine PMMA (group 5), NaOCl caused significant damage (30.10% strength loss; $p < 0.001$). The composite was significantly more resistant but still showed an 8.73% strength loss after NaOCl exposure on biofilm-covered specimens with (group 3 vs. group 2; $p < 0.01$, $\eta^2 = 0.45$).
- **Effects of Patchouli Leaf Extract:** In contrast, 15% patchouli extract caused no statistically significant changes ($p > 0.05$, $\eta^2 = 0.08$) in flexural strength or hardness in either pristine (group 6) or biofilm-covered (group 4) specimens with respect to untreated controls (groups 1 and 2).

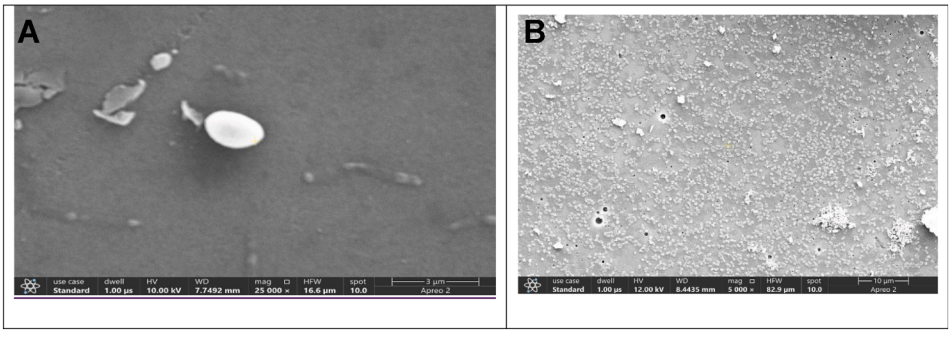
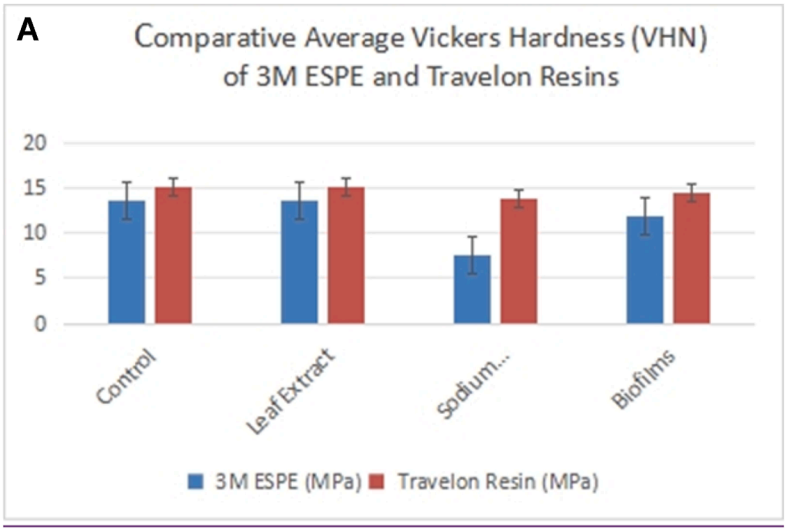


Figure 1: Scanning electron micrographs (SEMs) of the surface structure of (A) 3M ESPE and (B) Travelon heat-cured acrylic. Whereas (A) is smooth and even, (B) is rough and uneven.



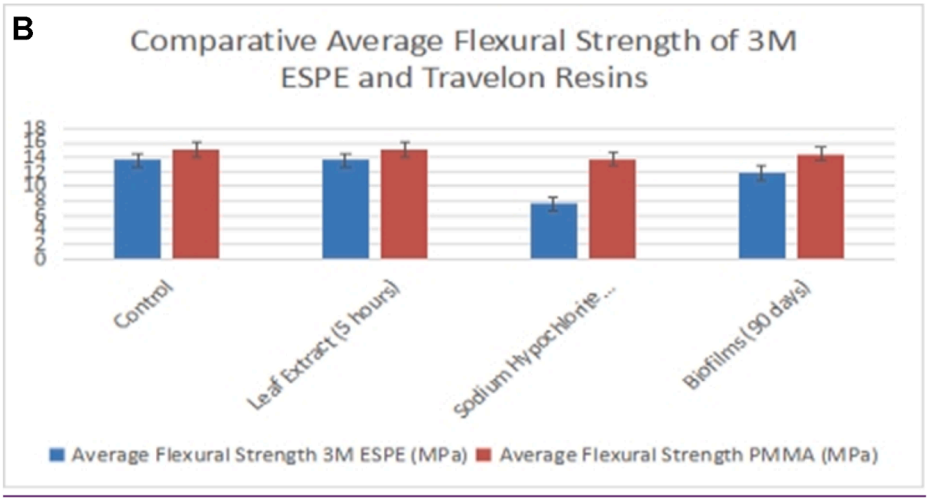


Figure 2: Baseline material properties of the control groups (group 1). (A) Vickers hardness (VHN) and (B) flexural strength (MPa) of the composite resin and PMMA resin. The composite resin demonstrated significantly greater hardness and flexural strength than the PMMA resin.

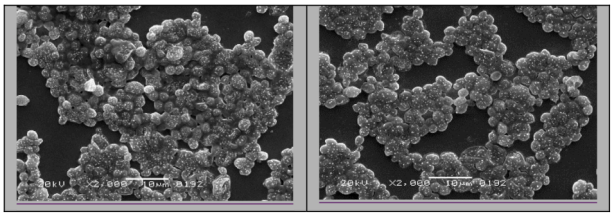


Figure 3: SEM images showing the growth pattern of *C. albicans* biofilms on a Travelon heat-cured acrylic surface and 3M ESPE.

Surface roughness

AFM analysis confirmed that biofilm formation (group 2) resulted in significantly greater surface roughness of both materials than the controls (group 1) ($p < 0.001$, $\eta^2 = 0.82$) (Figure 6, Figure 7). The composite's roughness substantially increased in relative terms (from 75.60 ± 8.45 nm to 257.50 ± 20.11 nm; 240.5% increase), whereas PMMA, which had greater starting roughness, showed a more moderate relative increase (from 256.30 ± 22.10 nm to 352.24 ± 25.63 nm; 37.4% increase).

Antimicrobial efficacy

MIC/MBIC assays demonstrated clear efficacy differences:

- **Sodium Hypochlorite (NaOCl):** Treatment with NaOCl was highly effective against planktonic *C. albicans* (MIC = 0.003125% or 31.25 μg/mL) but was approximately 100-fold less effective against mature biofilm (MBIC = 0.3125% or 3125 μg/mL).
- **Patchouli Leaf Extract:** Treatment with patchouli leaf extract showed moderate activity against planktonic cells (MIC = 0.25% or 2500 μg/mL) but required a 16 times greater concentration to inhibit biofilm (MBIC = 4.0% or 40,000 μg/mL). The 15% (150,000 μg/mL) solution used for immersion exceeded the MBIC, thus explaining its

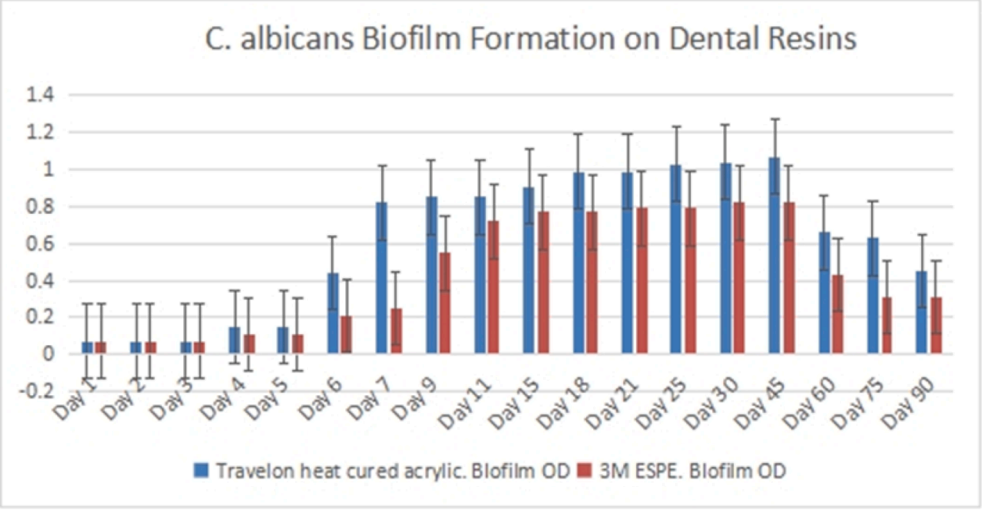


Figure 4: Graph comparing the growth of *C. albicans* biofilms on two materials, Travelon and 3M ESPE, over 90 days. The rougher Travelon resin supported significantly more biofilm biomass than the smoother 3M ESPE composite.

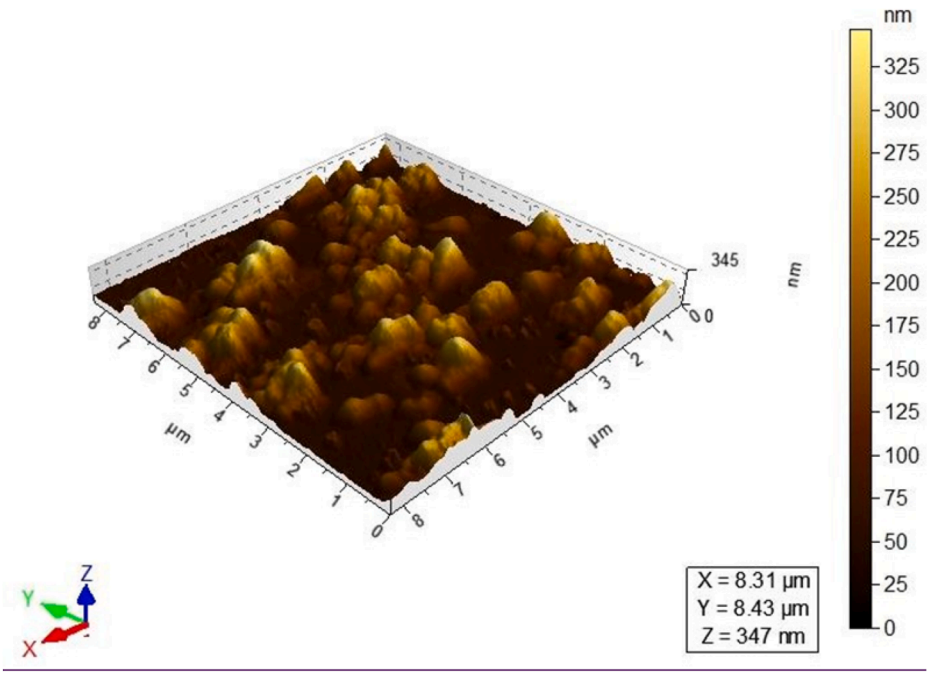


Figure 5: Surface roughness of a Travelon heat-cured specimen after biofilm development and 90 days' exposure to *C. albicans*, recorded through AFM.

planktonic efficacy but limited activity against established 90-day biofilms (Figure 7).

Discussion

This study conducted a comprehensive in vitro analysis of degradation pathways affecting dental polymers, and delineated the roles of material properties, microbial biofilm activity, and chemical cleaning regimens. A key study strength is the multi-factorial design directly comparing a conventional PMMA denture base material against a high-performance nano-filled restorative composite, which provided an experimental benchmark for optimized polymer properties. However, this composite is not clinically indicated for denture base applications and instead represents an upper bound of polymer performance for comparative purposes.

After recalculation and verification against ISO 20795-1 standards, the corrected flexural strength values for PMMA (86.4 ± 3.2 MPa) confirmed that the material met the minimum requirement of 65 MPa for denture base polymers, thus validating the specimen fabrication protocol. The initial

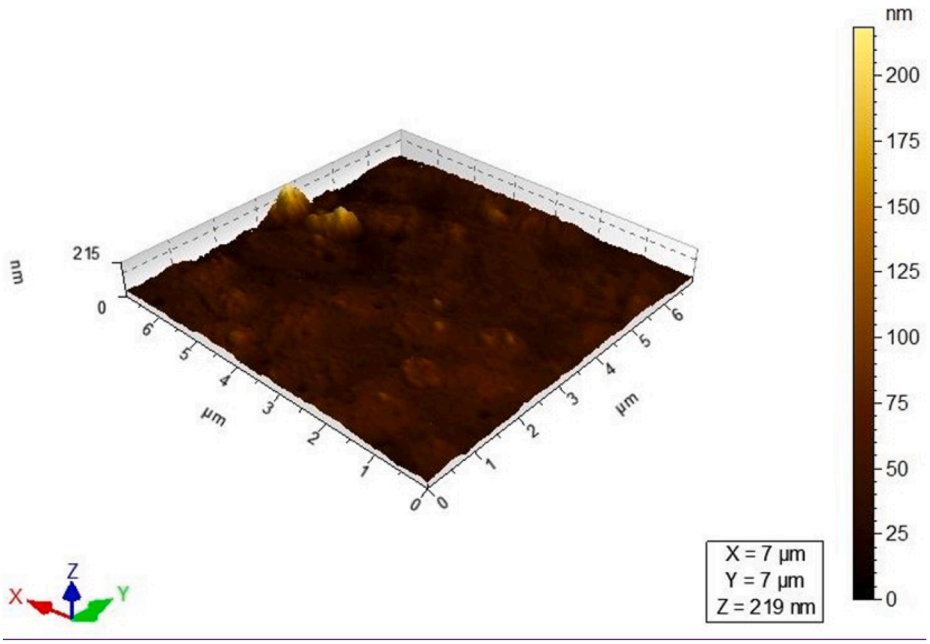


Figure 6: Surface roughness of a 3M ESPE specimen after biofilm development and 90 days' exposure to *C. albicans*, recorded through AFM.

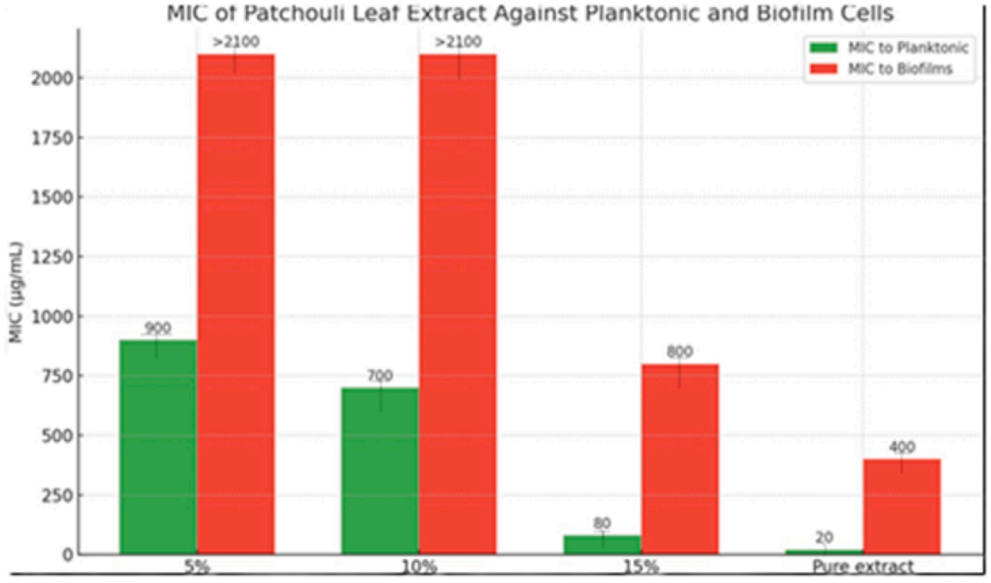


Figure 7: Antifungal efficacy of patchouli extract at 5%, 10%, 15%, and 20% concentrations against *Candida albicans*, showing concentration-dependent inhibition with complete suppression at concentrations of 15% or higher.

characterization established a clear performance hierarchy, in which the composite showed superior hardness and flexural strength (115.2 ± 4.1 MPa), and significantly smoother surface topography (Sa = 75.60 ± 8.45 nm; $p < 0.001$, $\eta^2 > 0.89$). These inherent properties directly influenced subsequent performance.

The finding that the rougher PMMA surface (Sa = 256.30 ± 22.10 nm) supported significantly greater *C. albicans* biofilm biomass ($p < 0.001$, $\eta^2 = 0.76$) was consistent with the established literature on surface topography and microbial adhesion.[19,20] This heavy biofilm load directly catalyzed substantial PMMA mechanical degradation, as evidenced by a 14.14% flexural strength loss and 25.88% hardness loss. This degradation was likely to reflect synergistic effects of hyphal penetration and microbial byproducts (organic acids and enzymes) that can plasticize and hydrolyze the polymer matrix.[20,21] The composite, despite its enhanced resistance, exhibits increased surface roughness after biofilm coverage.[21] Therefore, even optimized polymers appear to be superficially altered by persistent biofilm, thus potentially initiating a cycle of increased roughness and microbial adhesion.[20,21]

Cleaning agents are a critical consideration for clinical practice, extending beyond antimicrobial efficacy to material safety.[20] For example, 1% NaOCl, despite having potent antimicrobial activity, acts as a severe material stressor.[22] Its oxidizing nature[22] caused catastrophic damage to PMMA in our study, thus resulting in a 36.13% flexural strength loss (to 47.2 ± 4.2 MPa, below the ISO 65 MPa minimum), and induced significant degradation in the more stable composite (8.73% loss). Therefore, caution is necessary regarding the routine use of such harsh chemicals, which might potentially shorten prosthetic lifespan.[22] In contrast, patchouli leaf extract was found to be a material-safe alternative causing no statistically significant deterioration ($p > 0.05$, $\eta^2 = 0.08$). However, the extract's limited efficacy against mature biofilms (MBIC = 4.0% vs. MIC = 0.25%) is a key limitation. The observed efficacy−safety profiles suggested a potential hypothesis for future investigation: gentle, non-destructive agents might be considered for daily hygiene to prevent biofilm accumulation, whereas potent disinfectants might be reserved for infrequent, targeted use. However, this possibility requires clinical validation.

This study has several limitations that temper the generalizability of the findings. First, the use of a mono-species *C. albicans* biofilm, although controlled, does not replicate the complex polymicrobial oral ecology. Second, the 90-day static model lacked dynamic mechanical stresses (mastication or brushing) that clinically interact with chemical and biological degradation. Third, the restorative composite, although illustrative for benchmarking, is not indicated for denture bases, because of factors including impact resistance and fatigue behavior in thin sections, thereby limiting direct clinical extrapolation. Fourth, because of the in vitro nature of this study, clinical recommendations cannot be directly derived from the results. Instead, our findings generate hypotheses for future research under more representative conditions. Fifth, only a single concentration (15%) of patchouli extract was tested; future studies should therefore explore concentration gradients and combination strategies with other agents. Finally, although the statistical power was adequate to detect large effects, smaller but clinically relevant differences might have been undetected.[23]

Despite these limitations, this study bridges a gap by providing a holistic view of the interlinked factors governing polymer durability within a controlled in vitro framework. Our findings underscore the importance of considering material properties in developing maintenance protocols and highlight the need for further investigation using polymicrobial models, dynamic systems, and clinically relevant material combinations.

Conclusion

Despite the limitations of the vitro mono-species model used, our findings indicated that superior inherent material properties (greater hardness and smoother surface finish) were associated with enhanced resistance to *C. albicans* biofilm colonization and subsequent biological degradation in this experimental system. In addition, prolonged biofilm exposure was found to be a chronic stressor that led to significant mechanical degradation of PMMA. Therefore, biofilm control might potentially be important for both oral health and prosthetic material durability. The choice of cleaning agent might potentially require a compromise, given that NaOCl had potent antimicrobial action but caused substantial material damage (rendering PMMA flexural strength below ISO requirements), whereas patchouli extract preserved material integrity but demonstrated limited efficacy against established biofilms in this model. Our preliminary in vitro observations suggest that material-specific maintenance protocols warrant further investigation under conditions more representative of the oral environment. Future research should focus on optimizing natural extracts for enhanced anti-biofilm activity, evaluating combination strategies, and validating the findings in polymicrobial and dynamic models before clinical recommendations can be formulated.

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Ethical approval

This study was reviewed and exempted by our Institutional Review Board (approval No. IRB-4068/DUHS/EXEMPTION/2025/251, July 9, 2025).

Authors contributions

KH: Conceptualization, review and editing, supervision and project administration. AK: Conceptualization, methodology, writing original draft, visualization, investigation and data curation. ZAM: Conceptualization, review and editing, supervision and project administration. SUR: Review and editing, resources, validation. AM: Review and editing, resources, validation. MRM: Review and editing, resources, validation. AN: Software, review editing, data curation. SMA: Software, review editing, data curation.

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Appendix A. Supplementary data

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