THE ASEAN
JOURNAL OF MILITARY
AND PREVENTIVE MEDICINE

ISSN (e): 3031-870X
Vol. 2 No.1 (2025)
https://journal.ajmpm.net/

DOI: https://doi.org/10.47353/ajmpm.v2i1.25

Effect of Filler and Slip Casting Methods with Nano TiO2 Anastase as Anti-*Candida Albicans* on Extraoral Maxillofacial Prostheses: A Laboratory Experiment

Teguh Tri Widodo[1], Silvia Vera Indrawati[1]

[1] Department of Dentistry, Faculty of Dentistry, Sultan Agung University, Semarang,
Central Java, Indonesia

Email: teguh2sbyng22@yahoo.com

Abstract

Background: Polyurethane is widely used for extraoral maxillofacial prostheses because of its favorable elasticity, tear resistance, and tissue-like surface characteristics. However, polyurethane surfaces may support Candida albicans adhesion and biofilm formation, which may compromise prosthesis hygiene and durability. This study aimed to evaluate the antifungal effect of anatase titanium dioxide (TiO_2) nanoparticles applied to polyurethane plates using filler and slip-casting coating methods.

Methods: This in vitro laboratory experimental study used 54 polyurethane plates divided into nine groups: one control group, four filler groups with 1%, 2%, 3%, and 4% TiO_2, and four slip-casting coating groups with 1%, 2%, 3%, and 4% TiO_2. Treated samples were irradiated with ultraviolet light at 366 nm for 1 hour, exposed to Candida albicans suspension, incubated for 48 hours at 37°C, cultured on Sabouraud Dextrose Agar, and counted as CFU/mL. Data were analyzed using the Kolmogorov-Smirnov test, Levene's test, Welch-ANOVA, and Games-Howell post hoc test.

Results: The 4% TiO_2 slip-casting coating group showed the lowest mean colony count (128.83 ± 7.96 CFU/mL), while the control group showed the highest count (272.00 ± 47.44 CFU/mL). Welch-ANOVA showed significant differences among groups ($F = 37.664$; $p < 0.001$).

Conclusion: Surface coating with 4% anatase TiO_2 using slip casting produced the greatest inhibitory effect against Candida albicans on polyurethane plates in this laboratory model.

Keywords: *Candida albicans, laboratory experiment, maxillofacial prosthesis, polyurethane, TiO_2 anatase*

Background

Maxillofacial defects caused by trauma, congenital abnormalities, or surgical removal of neoplastic tissue may result in considerable aesthetic, functional, and psychological impairment. When surgical reconstruction is not feasible or cannot fully restore the defect, extraoral maxillofacial prostheses play an important role in restoring facial contour, improving speech and mastication, supporting tissue rehabilitation, and improving quality of life.[1–4]

Materials for extraoral maxillofacial prostheses should resemble human tissue, remain biocompatible, maintain color and mechanical stability, and tolerate routine handling and hygiene procedures. Polyurethane elastomers are used because of their favorable elasticity, tear resistance, edge strength, and tissue-like surface texture.[5] Nevertheless, polyurethane has limitations, including moisture sensitivity, reduced color stability, and susceptibility to microbial and fungal colonization. Candida albicans is clinically relevant because it can adhere to prosthetic materials and form biofilms, which may contribute to odor, discoloration, local irritation, material degradation, and reduced prosthesis service life.[6–9]

Titanium dioxide (TiO_2) nanoparticles have been investigated as antimicrobial modifiers for dental and prosthetic materials because of their stability, low toxicity, corrosion resistance, and photocatalytic activity.[10–15] The anatase crystalline form has higher photocatalytic activity than rutile or brookite. After ultraviolet activation, anatase TiO_2 generates reactive oxygen species, including hydroxyl and superoxide radicals, which may damage microbial cell walls, membranes, proteins, nucleic acids, and metabolic pathways.[16,17]

Although previous studies have reported the antimicrobial effects of TiO_2-containing prosthetic materials, the comparative effect of anatase TiO_2 applied as a filler versus a surface coating using slip casting on polyurethane plates remains insufficiently described.[18–25] This study tested the hypothesis that anatase TiO_2 nanoparticles reduce Candida albicans colony growth on polyurethane plates and that slip-casting surface coating produces stronger antifungal effects than filler incorporation. This study aimed to determine the effect of different concentrations of anatase TiO_2 nanoparticles applied by filler and slip-casting methods on Candida albicans colony growth on polyurethane plates.

Methods

Study design

This was an in vitro laboratory experimental study evaluating the antifungal effect of anatase TiO_2 nanoparticles applied to polyurethane plates using filler incorporation and surface coating by slip casting. The primary outcome was Candida albicans colony count expressed as colony-forming units per milliliter (CFU/mL).

Samples and grouping

A total of 54 polyurethane plate samples with dimensions of 20 × 15 × 3 mm were prepared and divided into nine groups, with six samples in each group. The study groups consisted of one untreated control group, four TiO_2 anatase filler groups, and four TiO_2 anatase slip-casting coating groups.

Table 1. Experimental groups of polyurethane plates treated with anatase TiO_2 nanoparticles

Group	Treatment method	TiO_2 concentration	n
Control	No TiO_2 anatase nanoparticles	0%	6
Filler 1%	TiO_2 anatase incorporated as filler	1%	6
Filler 2%	TiO_2 anatase incorporated as filler	2%	6
Filler 3%	TiO_2 anatase incorporated as filler	3%	6
Filler 4%	TiO_2 anatase incorporated as filler	4%	6
Coating 1%	TiO_2 anatase surface coating by slip casting	1%	6
Coating 2%	TiO_2 anatase surface coating by slip casting	2%	6
Coating 3%	TiO_2 anatase surface coating by slip casting	3%	6
Coating 4%	TiO_2 anatase surface coating by slip casting	4%	6

Materials and instruments

The materials included polyurethane components A and B, anatase TiO_2 nanoparticles, ethanol, 5% silane coupling agent, artificial saliva, Candida albicans suspension, phosphate-buffered saline, and Sabouraud Dextrose Agar. The instruments included Erlenmeyer flasks, sonicator, magnetic stirrer, rotary evaporator, Buchner vacuum apparatus, oven, vacuum chamber, sample molds, microbrush, spatula, ultraviolet light source, incubator, sterile cotton swabs, micropipette, petri dishes, Bunsen burner, and colony counter.

Preparation of polyurethane plates

Polyurethane plates were fabricated by mixing polyurethane part A and part B at a 1:1 ratio. The mixture was homogenized, placed in a vacuum chamber to remove air bubbles, poured into molds, cured, and removed for subsequent treatment and microbiological testing.

Silanization of anatase TiO_2 nanoparticles

Anatase TiO_2 nanoparticles were silanized to improve adhesion between TiO_2 and polyurethane. Thirty grams of TiO_2 anatase nanoparticles were placed into an Erlenmeyer flask containing 200 mL of pure ethanol. The mixture was sonicated at room temperature for 20 minutes and stirred for another 20 minutes. Then, 1.5 mL of 5% silane coupling agent was added and stirred at 250 rpm for 60 minutes. The solution was stored in a sealed container for 48 hours, followed by ethanol removal using a rotary evaporator at 60°C and 150 rpm for 30 minutes. Residual solvent was removed using a Buchner vacuum apparatus. The silanized nanoparticles were dried in an oven at 60°C for 20 hours and sonicated for 3 minutes to reduce agglomeration.

Filler incorporation method

For filler groups, silanized TiO_2 anatase nanoparticles were incorporated into polyurethane at concentrations of 1%, 2%, 3%, and 4%, corresponding to 0.24 g, 0.48 g, 0.72 g, and 0.96 g of TiO_2, respectively. TiO_2 was mixed with 12 g of polyurethane part B for 2 minutes until homogeneous. Subsequently, 12 g of polyurethane part A was added, maintaining a 1:1 ratio, and mixed for another 2 minutes. The mixture was placed in a vacuum chamber to eliminate air bubbles, poured into molds, cured, and stored for testing.

Surface coating by slip casting

For coating groups, polyurethane plates were first fabricated without TiO_2. After curing, the surface of each sample was treated with silane coupling agent using a microbrush and allowed to dry. Silanized TiO_2 anatase nanoparticles were mixed with ethanol to form a paste at concentrations of 1%, 2%, 3%, and 4%, corresponding to 0.24 g, 0.48 g, 0.72 g, and 0.96 g of TiO_2 in 2 mL ethanol. The paste was stirred at 300 rpm for 30 minutes until homogeneous and then applied to the polyurethane surface using the slip-casting method.

UV activation and Candida albicans biofilm formation

All filler and coating samples were irradiated with ultraviolet light at 366 nm from a distance of 15 cm for 1 hour to activate the photocatalytic properties of TiO_2. After ultraviolet irradiation, the samples were contaminated with Candida albicans by immersion in a prepared Candida albicans suspension and incubated for 48 hours at 37°C to allow fungal adhesion and biofilm formation.

Colony counting procedure

After incubation, polyurethane plates were washed and adherent Candida albicans cells were collected using sterile cotton swabs. Each swab was placed into a test tube containing phosphate-buffered saline. A 0.01 mL aliquot was inoculated onto Sabouraud Dextrose Agar using aseptic technique and incubated at 37°C for 48 hours. Colonies were counted using a colony counter and reported as CFU/mL.

Statistical analysis

Data were analyzed using statistical software [Data needs to be completed by author]. Normality was assessed using the Kolmogorov-Smirnov test, and homogeneity of variance was evaluated using Levene’s test. Because data were normally distributed but variances were not homogeneous, Welch-ANOVA was used to compare mean colony counts among groups. Games-Howell post hoc analysis was performed for pairwise comparisons. A p-value <0.05 was considered statistically significant.

Results

All 54 polyurethane plate samples were included in the final analysis. A concentration-dependent reduction in Candida albicans colony counts was observed in both filler and coating groups. The untreated control group had the highest mean colony count, while the 4% TiO_2 anatase slip-casting coating group had the lowest mean colony count. At comparable TiO_2 concentrations, slip-casting coating produced lower colony counts than filler incorporation.

Study group	n	Mean ± SD (CFU/mL)
Control group: polyurethane plate without TiO_2	6	272.00 ± 47.44
Polyurethane + TiO_2 coating 1%	6	199.66 ± 5.96
Polyurethane + TiO_2 coating 2%	6	188.50 ± 4.14
Polyurethane + TiO_2 coating 3%	6	163.00 ± 12.93
Polyurethane + TiO_2 coating 4%	6	128.83 ± 7.96
Polyurethane + TiO_2 filler 1%	6	221.67 ± 14.72
Polyurethane + TiO_2 filler 2%	6	202.33 ± 6.12

Polyurethane + TiO_2 filler 3%	6	195.67 ± 5.16
Polyurethane + TiO_2 filler 4%	6	191.00 ± 2.19

Table 2. Mean colony count of Candida albicans among study groups

The Kolmogorov-Smirnov test showed that the data were normally distributed ($p > 0.05$). Levene's test showed non-homogeneous variance ($p < 0.05$); therefore, Welch-ANOVA was used. Welch-ANOVA demonstrated a statistically significant difference in Candida albicans colony counts among the nine groups ($F = 37.664$; $p < 0.001$).

Variable	Mean square	F	p-value
Between groups	25972.741	37.664	<0.001
Within groups	689.595	-	-

Table 3. Welch-ANOVA analysis of Candida albicans colony counts

Games-Howell post hoc analysis indicated that the 4% TiO_2 anatase coating group using slip casting differed significantly from the other experimental groups and produced the greatest reduction in Candida albicans colony growth. Compared with the untreated control group, the 3% and 4% TiO_2 coating groups showed marked reductions in colony counts. These results indicate that increasing TiO_2 concentration improved antifungal activity, particularly when TiO_2 was applied as a surface coating using the slip-casting method.

Discussion

This laboratory experimental study demonstrated that anatase TiO_2 nanoparticles reduced Candida albicans colony growth on polyurethane plates. The most effective treatment was surface coating with 4% TiO_2 anatase using the slip-casting method, which produced the lowest mean colony count of 128.83 ± 7.96 CFU/mL. In contrast, untreated polyurethane plates had the highest colony count, indicating greater susceptibility to fungal colonization.

The concentration-dependent reduction in Candida albicans colony growth suggests that higher anatase TiO_2 concentrations provide greater antifungal activity. This trend was observed in both filler and coating groups. However, slip-casting coating was more effective than filler incorporation at similar concentrations. This finding may be explained by greater surface availability of TiO_2 nanoparticles in the coating groups. Because Candida albicans initially adheres to prosthetic surfaces, TiO_2 particles exposed directly at the surface are more likely to interact with fungal cells and generate reactive oxygen species at the microbial-material interface after UV activation.

The antifungal activity of anatase TiO_2 is mainly related to photocatalysis. Under UV irradiation, electrons are excited from the valence band to the conduction band, generating electron-hole pairs. These electron-hole pairs react with oxygen and water to form reactive oxygen species, including hydroxyl radicals and superoxide radicals. These reactive species may damage fungal cell walls, cell membranes, proteins, nucleic acids, and intracellular metabolic systems, leading to impaired growth or cell death.[16,17]

Candida albicans has a complex cell wall containing glucan and chitin, which may make it more resistant than several bacterial species. Nevertheless, oxidative stress induced by TiO_2-generated reactive oxygen species can disrupt fungal structures, interfere with respiratory activity, and promote cellular injury. These mechanisms support the observed reduction in colony counts after TiO_2 anatase treatment.

Silanization was also relevant because it enhanced the interaction between inorganic TiO_2 nanoparticles and the organic polyurethane matrix. Silane coupling agents can promote stable bonding through siloxane formation with TiO_2 and covalent interaction with polyurethane. Improved nanoparticle adhesion is important to maintain coating stability and reduce detachment during handling or clinical use.

These findings are clinically relevant for extraoral maxillofacial prostheses because microbial colonization may contribute to discoloration, odor, skin irritation, and reduced prosthesis service life. Modifying polyurethane with anatase TiO_2 may represent a promising strategy to improve antifungal performance. The slip-casting method may be particularly useful because it concentrates active nanoparticles on the surface where fungal adhesion occurs.

The strengths of this study include its controlled laboratory design, comparison of two application methods, evaluation of multiple TiO_2 concentrations, standardized colony counting, and use of Welch-ANOVA and Games-Howell post hoc analysis for non-homogeneous data. However, several limitations should be considered. First, the study was conducted in vitro and may not fully represent the clinical environment of extraoral prosthesis use. Second, TiO_2 activation depended on controlled UV exposure, and the practicality and safety of repeated UV activation require further evaluation. Third, this study did not assess mechanical properties, color stability, surface roughness, skin biocompatibility, nanoparticle release, or long-term performance after aging and cleaning cycles.

Further studies should evaluate the biocompatibility of TiO_2-coated polyurethane in skin-contact models, coating stability, nanoparticle release, surface roughness, color stability, tensile strength, tear resistance, and durability after repeated cleaning and environmental aging. Clinically simulated and in vivo studies are also needed before this approach can be recommended for routine fabrication of extraoral maxillofacial prostheses.

Conclusion

Anatase TiO_2 nanoparticles reduced Candida albicans colony growth on polyurethane plates in this laboratory experimental study. Surface coating with 4% anatase TiO_2 nanoparticles using the slip-casting method demonstrated the greatest inhibitory effect, producing the lowest mean colony count compared with filler incorporation and untreated control groups. These findings suggest that anatase TiO_2 surface coating may be a promising antifungal modification for polyurethane materials used in extraoral maxillofacial prostheses.

Acknowledgments

None.

Funding

None.

Author Contributions

All authors act as the guarantor of the manuscript. TTW is the main investigator of this study. TTW contributed to study conception, methodology, laboratory work, data analysis, data interpretation, and manuscript drafting. SVI contributed to supervision, methodology, data interpretation, critical revision, and final approval of the manuscript. All authors reviewed and approved the final manuscript and agree to be accountable for all aspects of the work.

Conflict of Interest

No conflict of interest.

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