Majalah Kedokteran Gigi Indonesia
Vol 9 No 2 – August 2023
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.82785

RESEARCH ARTICLE

# Candida albicans adherence on soft denture liner coated with silica and titanium nanoparticles

**Pramudya Aditama, Adella Syvia Maharani**✉

Department of Prosthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
Jl Denta No 1, Sekip Utara, Yogyakarta, Indonesia; ✉ correpondence: adellasyviamaharani@ugm.ac.id

**Submitted: 4th March 2023; Revised: 12nd April 2023; Accepted: 22nd May 2023**

**ABSTRACT**

The soft liner of acrylic resin dentures, used to coat removable partial dentures, still has drawbacks in the easy attachment of *Candida albicans* fungus. A treatment on the surface of the acrylic resin denture soft liner is needed to reduce the attachment of *Candida albicans*. Silicon dioxide ($SiO_2$) and titanium dioxide ($TiO_2$) nanoparticles have antimicrobial properties because they can induce photocatalytic production. This study aims to determine the effect of silica and titanium coating concentration on the attachment of *Candida albicans* to the soft liner of an acrylic resin denture. This study used 48 samples for 2 types of research. Each research consisted of 4 groups with 6 samples each. The samples were disc-shaped, with a diameter of 10 mm and a thickness of 2 mm. Silica and titanium coating materials of 0.5%, 1%, and 2% were obtained by mixing each nanoparticles of 0.5 g, 1 g, and 2 g in 100 ml of ethanol. After the application of the coating, the attachment test of *Candida albicans* was carried out. *Candida albicans* in SDA media were counted using a colony counter. The analysis was carried out using a one-way ANOVA test for each experiment. The ANOVA results showed an effect of silica and titanium coating concentrations on the attachment of *Candida albicans* to the soft liner of an acrylic resin denture ($F = 10.929$; $p < 0.05$ for silica, and $F = 9.830$; $p < 0.05$ for titanium). The group with a 2% silica coating concentration had the least amount of *Candida albicans* among all groups ($0.48 \pm 0.98 \times 10^7$ CFU/ml), as well as the group with a 2% titanium coating concentration ($0.30 \pm 0.83 \times 10^7$ CFU/ml). In conclusion, this study shows that the concentration of silica and the 2% titanium coating is the most effective in preventing the attachment of *Candida albicans* to the soft liner of acrylic resin dentures.

**Keywords:** acrylic resin; *Candida albicans*; silica; soft liner; titanium

## INTODUCTION

The use of acrylic resin removable partial dentures in patients with sharp alveolar ridges can cause pain during mastication. Removable partial dentures that have been used for a long time can cause changes in oral tissues, such as alveolar ridge resorption, and can cause pain or damage to the supporting tissues.[1] Alveolar ridge resorption can affect the shape and size of the ridge, one of which is a sharp alveolar ridge. One way to overcome the shortcomings of the acrylic resin removable partial denture base is to use a soft liner that is applied to the anatomical surface of the removable partial denture base. The requirement of the soft liner material to coat the acrylic resin denture can adapt well and tightly to the supporting tissue of the denture; has a stable dimension and is insoluble in oral fluids to maintain proper tissue contact; color stable during use, resistant to stains, and resistant to odors; easy to manufacture and use.[2]

A dental material, including a soft liner material on an acrylic resin denture base, must have a surface that is not easily adhered to by microorganisms. These microorganisms cause diseases around the denture and oral cavity, such as denture stomatitis.[3,4] *Candida albicans*, one of the microorganisms in the oral cavity, may increase the number of attachments to the soft liner surface of the denture. A treatment on the surface of the soft liner of acrylic resin denture is needed to reduce the attachment of *Candida albicans.*[5]

Nanoparticles are characterized by their small size, large surface area and intense interface interactions with the polymer. The nanoparticles

Majalah Kedokteran Gigi Indonesia. August 2023; 9(2): 117-122
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

can thus enhance the physical and optical properties of the polymer matrix. Nanoparticles silica and titanium have been widely applied as additives and modifications to dental materials. Silica may coat and modify hydrophobic materials, such as acrylic resin denture bases, to make the surface hydrophilic, preventing bacteria from adhering to the surface of dental materials.[6] Nanoparticle titanium has good biocompatibility, chemical stability, non-toxicity, and corrosion resistance. When used to coat a surface, titanium has the photocatalytic ability to act as an antimicrobial agent.[7] In addition, they can provide resistance to environmental stress, cracking, and aging. Anatase and rutile are nanoparticle structures that can be used as photocatalysts. Several studies have shown that anatase is more active in photocatalyst activity than rutile.[8] Using inappropriate concentrations of coating application materials can cause agglomeration and affect the durability of the coating layer on the surface of dental materials.[9] This study aims to determine the effect of silica and titanium on the soft liner of acrylic resin denture on the attachment of *Candida albicans*.

## MATERIALS AND METHODS

This research has received an ethical clearance from the Ethics Unit and Advocacy, Faculty of Dentistry Universitas Gadjah Mada number 00509/KKEP/FKG-UGM/EC/2020 and 00698/KKEP/FKG-UGM/EC/2021. This study used 48 samples for 2 types of research (silica and titanium coating). Each research consisted of 4 groups with 6 samples each. The samples were disc-shaped of acrylic resin denture with a diameter of 10 mm and a thickness of 2 mm. Silica and titanium coating materials of 0.5%, 1%, and 2% were obtained by mixing silica and titanium nanoparticles of 0.5 g, 1 g, and 2 g in 100 ml of ethanol.

The soft liner of acrylic resin denture sample was formed into discs with a diameter of 10 mm and a thickness of 2 mm (1 mm acrylic resin, 1 mm soft liner). Finishing and polishing were carried out after the research sample was formed. The research sample was divided into four groups in each experiment, with six samples each. Group I is a research sample group without a coating, group II is a research sample group with a 0.5% silica or titanium coating, group III is a research sample group with a 1% silica or titanium coating, and group IV is a research sample group with the application of 2% silica or titanium coating.

Silica and titanium were placed in a beaker and weighed using a digital balance of 0.5 g, 1 g, and 2 g, respectively. Ethanol solution as a solvent was measured using a measuring cup as much as 100 ml and mixed in a beaker containing silica and titanium, then dissolved using a magnetic stirrer until the solution was homogeneous. The dip-coating method is treated with silica coating by smearing the sample using a silane coupling agent first. The samples were treated with coating by dip-coating method into a silica and titanium coating solution, then heated in an oven at 70 ºC for 10 minutes. Furthermore, the attachment test of *Candida albicans* will be carried out.

The attachment test was carried out with *Candida albicans* cultured in culture media and then harvested and put into Sabouraud Dextrose Broth (SDB) media. After that, the initial media SDB was diluted. One hundred microliters of *Candida albicans* suspension were inserted into the microplate, and the acrylic resin disc was also inserted. After that, it was incubated for 1 hour at 37 °C. Then, the disc was removed using tweezers and rinsed with saline water for 5 seconds. Next, *Candida albicans* attached to the acrylic resin disc were knocked out at 3000 rpm for 20 seconds. Then the released *Candida albicans* were incubated again to count the number of colonies (CFU/ml) formed on the Sabouraud Dextrose Agar (SDA) medium.

Data analysis begins with the normality test using the Shapiro-Wilk test and homogeneity using the Levene test. If the data were normally distributed, it was continued with the one-way ANOVA test with 95% significance and continued with the LSD post hoc test to see which groups were significantly different for each experiment of silica and titanium.

**Table 1.** The mean and standard deviation (mean ± SD) number of attachments of *Candida albicans* in each group of silica coating

| | The Mean and Standard Deviation (mean ± SD) |
|---|---|
| Group I | 2.36 ± 0.61 x $10^7$ CFU/ml |
| Group II | 1.6 ± 0.34 x $10^7$ CFU/ml |
| Group III | 1.17 ± 0.3 x $10^7$ CFU/ml |
| Group IV | 0.48 ± 0.98 x $10^7$ CFU/ml |

**Table 2.** One-way ANOVA test results in each group of silica coating concentration

| | F | p |
|---|---|---|
| Silica coating concentration | 10.929 | 0.001* |

*p < 0.05

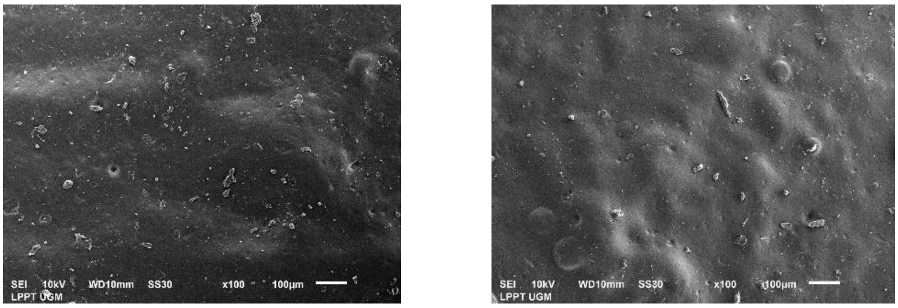
**Table 3.** Mean and standard deviation (mean ± SD) number of *Candida albicans* attachments in each group of titanium coating

| | Mean and standard deviation (mean ± SD) |
|---|---|
| Group I | 2.40 ± 0.59 x $10^7$ CFU/ml |
| Group II | 1.46 ± 0.34 x $10^7$ CFU/ml |
| Group III | 1.05 ± 0.25 x $10^7$ CFU/ml |
| Group IV | 0.30 ± 0.83 x $10^7$ CFU/ml |

**Table 4.** One-way ANOVA test results in each group of titanium coating concentration

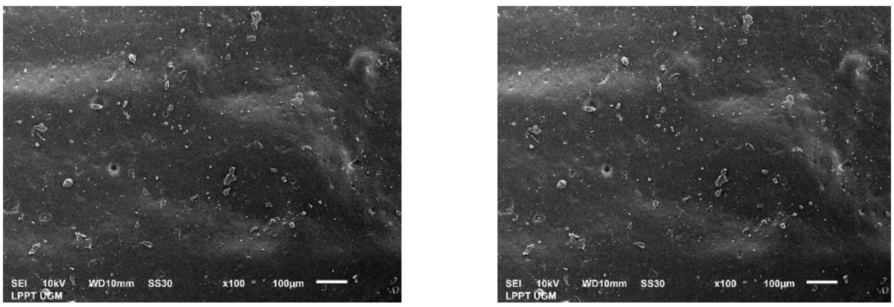
| | F | p |
|---|---|---|
| Titanium coating concentration | 9.830 | 0.001* |

*p < 0.05




(A) (B)

**Figure 1.** SEM results with 0.5% silica coating (A) and 0.5% titanium coating (B)

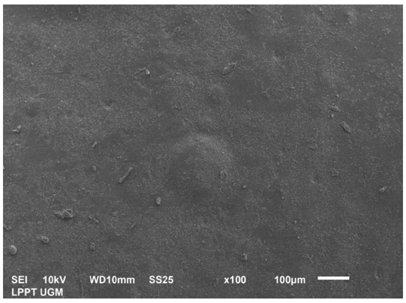



(A) (B)

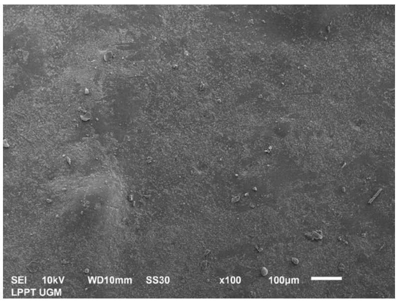
**Figure 2.** SEM results with 1% silica coating (A) and 1% titanium coating (B)

## RESULTS

The results showed that the mean and standard deviation in group IV with the application of 2% silica coating was the lowest (0.483 ± 0.983 x $10^7$ CFU/ml). Table 1 shows the results of the mean and standard deviation in all groups. One-way ANOVA test show a p-value of 0.001 ($p < 0.05$). These results indicate significant differences in each group, and silica




(A)




(B)

**Figure 3.** SEM results with silica coating 2% (A) and titanium coating 2% (B)

coating concentration affects the attachment of the fungus *Candida albicans* to acrylic resin denture soft liners. Group IV with a 2% silica coating was the most effective concentration for preventing *Candida albicans* fungus attachment. Table 2 presents the results of one-way ANOVA.

In the research using titanium coating, with the same type of group as the silica coating study above, the results obtained are the mean and standard deviation in group IV with the lowest a 2% titanium coating application ($0.30 \pm 0.83 \times 10^7$ CFU/ml) among all groups. The results of the mean and standard deviation in all groups can be seen in Table 3. One-way ANOVA test showed an F-value of 9.830 and a p-value of 0.001 ($p < 0.05$). These results indicate significant differences in each group, and titanium coating concentration has an effect on the attachment of *Candida albicans* to coastal resin dentures. Group IV with a 2% titanium coating was the most effective concentration for preventing *Candida albicans* fungus attachment. Table 4 shows the results of one-way ANOVA.

## DISCUSSION

The result of the silica study showed that the group with 2% silica coating had the highest growth inhibitor of *Candida albicans* compared to all the other groups. Silica has intrinsic hydrophilic properties because the surface of the silica contains hydroxyl groups. At higher concentrations of nanoparticle silica coating (in this study the concentrations of 2%) *Candida* growth decreased. This is because some of the coating particles clumped so the number of particles that can bind to the polymer of soft liner decreases. At low silica concentrations, only partial coating particles can bind to soft liner through silanes, so the non-binding coating particles look like mist shadows on the coating layer. This process then decreases the durability of the coating layer so the coating is easily removed.[10] The hydrogen bond is a contributing factor to nanoparticle silica agglomeration. This is due to the greater the concentration of nanoparticles in a suspension, the smaller the distance between the nanoparticles so the inter nanoparticles interact with each other and agglomerate. Hydrophilic nanoparticles have a large interaction of particles because hydroxyl groups present on the surface of nanoparticles can induce the formation of hydrogen bonds.[11] The hydrophilic nature of the surface of dental materials regulates the formation of biofilms on the surface.[10] Silica nanoparticles dissolved in ethanol can also dissolve rough acrylic resin surfaces so that the acrylic resin surface becomes smoother. Silica nanoparticles used as a coating material on acrylic resin denture soft liners can reduce the attachment of *Candida albicans*.[4] Silica can coat and modify hydrophobic materials such as acrylic resin denture soft liners so that the surface can be hydrophilic to prevent the attachment of *Candida albicans* to the surface of dental material.[11,12] Silica with 2% concentration is the most effective concentration to be used as an acrylic resin denture soft liner coating (Table 1). The amount of reactive oxygen species (ROS) on silica produced depends on the concentration used, the higher the concentration of nanoparticles, the higher the ROS that can be produced. *Candida albicans* has a thick cell wall because it consists of glucan and chitin which makes it strong. Silica dioxide produces ROS which can induce destructive effects on fungal cells and then intracellular oxidation of coenzyme A and lipid peroxidation occurs which causes a decrease in cell respiration activity resulting in the death of *Candida albicans*.[6,10,13] Based on the results of this study, it can be said that the group with 2% nanoparticle silica coating concentration had the highest percentage of inhibit *Candida albicans* growth among the other groups.

Titanium dioxide nanoparticles have various good properties such as antimicrobial, cheap, biocompatible, white color, chemically stable, free toxicity and corrosion resistant. In the literature, the addition of $TiO_2$ to the polymeric material has been shown to affect the electrical, optical, chemical and physical properties of the hybrid material.[8] Studies have shown that $TiO_2$ are effective against various microorganisms such as *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Lactobacillus acidophilus*. Due to their antimicrobial properties, $TiO_2$ have been added to various biomaterials.[14] In table 3 it is known that $TiO_2$ nanoparticles with a concentration of 2% have the highest ability to inhibit fungal growth compared to $TiO_2$ nanoparticles with concentrations of 0.5% and 1%. The high level of $TiO_2$ concentration used will affect the antifungal effect of the soft liner. Titanium dioxide at higher concentrations can cause a greater inhibitory effect on mold growth. The higher the concentration of $TiO_2$, the fewer *Candida albicans* colonies that grow.[14] Titanium coating can also maximize the finishing and polishing stages of dental material. Titanium nanoparticles can enter the cracks on the surface of dental materials due to the finishing step. Titanium nanoparticles dissolved in ethanol can also dissolve rough acrylic resin surfaces so that the acrylic resin surface becomes smoother.[13] In table 4, titanium dioxide nanoparticles effectively inhibit *Candida albicans* growth because the are broad-spectrum antimicrobials, high chemical resistance, are also able to reduce contaminants because they have photocatalyst properties.[13] The energy difference of titanium dioxide anatase 3.26eV will excite from the valence band to the conduction band and electrons and releases energy then reacts with water molecules and oxygen, then trigger the formation of ROS and hydroxyl radicals (·OH), which will form pairs of electrons (e-) and holes (h+) that can reduce and or oxidize compounds (pollutants) in the vicinity. Microorganisms will die after contact with hydroxyl radicals. Hydroxyl radicals and $O_2$ superoxide radicals play an important role in inactivating micro-organisms by oxidizing phospholipids in cell membranes, ·OH radicals are known to be 1000 times more effective in inactivating micro-organisms than common disinfectants.[8,15] The use of titanium concentrations in inappropriate coating applications can cause agglomeration, affecting the durability of titanium coatings on the surface of dental materials, and can cause titanium coating degradation.[11,16]

On scanning electron microscopy (SEM) analysis, the surface morphologies of the soft liner modified nanoparticle $SiO_2/TiO_2$ were observed by SEM. As a reference, the SEM images of soft liner with modification of 0.5% nanoparticle $SiO_2/TiO_2$ are marked in Figures 1(A) and 1(B). It can be seen that the agglomeration of nanoparticle $TiO_2$ particles is of considerable amounts. There were many free nanoparticle $SiO_2$ particles that could not be formed and coated well on the surface. As shown in Figures 2(A) and 2(B), the surface of soft liner with modification of 1% nanoparticle $SiO_2/TiO_2$ particles is covered with a rounded organic layer. It can be seen clearly in Figures 3(A) and 3(B) that the organic coating is dense, while the coating effect is quiet clear under a magnification of 100. There is still a little agglomeration in the modified nanoparticle $SiO_2/TiO_2$ particles, which may be due to the poor dispersion effect of the powder in the preliminary experiment. The SEM images of modified nanoparticle $SiO_2/TiO_2$ indicate that the coating effect of sample 2% is better than that of sample 0.5% and 1%. Further research is needed to carry out a Fourier Transform Infrared Spectroscopy (FTIR) test to see the chemical interaction between nanoparticle and polymers. It is necessary to do research on the effect of coating nanoparticles to soft liners on tissue cytotoxicity.

## CONCLUSION

The 2% concentration of silica and titanium coating was the most effective in preventing the attachment of *Candida albicans* to the soft liner of acrylic resin denture.

## REFERENCES

1. Alrahlah A, Fouad H, Hashem M, Niazy A, AlBadah A. Titanium oxide (TiO2)/ Polymethylmethacrylate (PMMA) denture base nanocomposites: mechanical, viscoelastic and antibacterial behavior. Materials (Basel). 2018; 11(7): 1-15. doi: 10.3390/ma11071096
2. Chladek G, Zmudzi J, Kasperski J. Long-Term soft denture lining materials. Materials (Basel). 2014; 7(8): 5816-5842. doi: 10.3390/ma7085816
3. Saied HM, Abdul-Hadi NF, Abdul-Razaq RW. The effect of surface treatment on tensile bond strength between soft liner and acrylic denture base and the effect of Pepsi solution on this bond with and without surface treatment. Journal of Baghdad College of Dentistry. 2011; 23(2): 17-21.
4. Hahnel S, Rosentritt M, Bürgers R, Handel G. Adhesion of Streptococcus mutans NCTC 10449 to artificial teeth: An in vitro study. Journal Prost Den. 2008; 100(4): 309–315. doi: 10.1016/S0022-3913(08)60212-7
5. Hahnel S, Rosentrit M, Bürgers R, Handel G, Lang R. Candida albicans biofilm formation on soft denture liners and efficacy of cleaning protocols. Gerodontology. 2012; 29(2): e83-91. doi: 10.1111/j.1741-2358.2011.00485.x
6. Samarayanake L. Essential Microbiology for Dentistry, 4th ed. London: Churchill Livingstone; 2012.
7. Akiba N, Azuma A, Minakuchi S. Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on Candida albicans adherence. J Med Dent Sci. 2012; 59(1): 1-7.
8. Gad MM, Abualsaud R. Behavior of PMMA denture base materials containing titanium dioxide nanoparticles: a literature review. Int J Biomater. 2019; 2019: 6190610. doi: 10.1155/2019/6190610
9. Tsuji M, Ueda T, Sawaki K. biocompatibility of a titanium dioxide-coating method for denture base acrylic resin. Gerodontology. 2016; 33(4): 539-544. doi: 10.1111/ger.12204
10. Yoshizaki T, Akiba N, Inokoshi M, Shimada M, Minakuchi S. Hydrophilic nano-silica coating agents with platinum and diamond nanoparticles for denture base materials. Dent Mater J. 2017; 36(3): 333-339. doi: 10.4012/dmj.2016-243
11. Azuma A, Akiba N, Minakuchi S. Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on Candida albicans adherence. J Med Dent Sci. 2012; 59(1): 1-7.
12. Nallaswamy D, Ramalingam K, Bhat V. Textbook of Prosthodontics. New Delhi: Jaypee; 2007.
13. Braden M, Wright PS, Parker S. Soft Lining Materials - a Review. Euro J Prosthodont Restor Dent. 1995; 3(4): 163–174.
14. Haghighi F, Mohammadi SR, Mohammadi P, Hosseinkhani S, Shidpour R. Antifungal Activity of $TiO_2$ Nanoparticles and EDTA on Candida albicans Biofilms. Original Article Infect. Epidemiol Med. 2013; 1(1): 33–38.
15. Kim BJ, Yang HS, Chun MG, Park YJ. Shore hardness and tensile bond strength of long term soft denture lining materials. The Journal of prosthetic Dentistry. 2014; 112(5): 1289-1297. doi: 10.1016/j.prosdent.2014.04.018
16. Kreve S, Reis ACD. Denture liners: a systematic review relative to adhesion and mechanical properties. ScientificWorldJournal. 2019; 2019: 6913080. doi: 10.1155/2019/6913080