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RESEARCH ARTICLE

The effect of titanium dioxide filler on soft liners on *Candida albicans* growth and surface hardness

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ABSTRACT

Soft liners are recommended in denture stomatitis, yet they are prone to microorganism colonization. Modification of soft liners can be done by adding titanium dioxide (TiO_2) because they induce photocatalytic production and increase the physical strength of the material. This research aims to examine the effect of adding TiO_2 filter in soft liners on the growth of *Candida albicans* and surface hardness. This research used disc-shaped soft liners with 2 sizes: 10 mm in diameter and 2 mm in thickness for the mold growth test and 10 mm in diameter and 6mm in thickness for surface hardness. Each of the 24 samples was divided into 4 groups: groups I, II, and III with the addition of TiO_2 at concentrations of 0.5%, 1%, and 2%, respectively, and group IV acting as the control group without TiO_2. Test of the growth of *Candida albicans* used dilution method, and calculations were made with a colony counter. Test of surface hardness used a durometer, and data were analyzed using one-way ANOVA and LSD. The results showed that among the groups, group III demonstrated the lowest growth of *Candida albicans* ($7.67 \pm 2.25 \times 10^3$ CFU/mL), while group IV exhibited the highest growth ($21.33 \pm 4.63 \times 10^3$ CFU/ml). The results of the ANOVA test showed that the addition of TiO_2 had a significant effect on the growth of *Candida albicans* ($p < 0.05$). In the LSD test, there were significant differences between the control group and all of the treatment groups. In the surface hardness test, the highest was observed in group III (29.92 ± 1.52 HA), and the lowest was in group IV (23.08 ± 2.6 HA). The results of the ANOVA test indicated the effect of adding TiO_2 on the hardness of the soft liners ($p < 0.05$). The LSD test showed significant differences between the control group and all of the treatment groups. The addition of 0.5%-2% TiO_2 concentrations to soft liners inhibited the growth of *Candida albicans*, while the 0.5% concentration showed the smallest change in surface hardness.

Keywords: *Candida albicans* growth; soft liners; surface hardness; titanium dioxide

INTRODUCTION

Denture material worn in the mouth cavity comes into contact with saliva, and the denture selectively absorbs salivary proteins and forms the acquired denture pellicle. Microorganisms attach to salivary protein receptors to form colonies. The collection of microorganisms that form a soft, non-calcified coating attached to dentures is called denture plaque. Plaque formation can occur due to insufficient cleaning of the acrylic resin surface. The salivary pellicle on the surface of the denture can lead to the colonization and proliferation of fungi, which are known to contribute to the development of denture stomatitis.[1]

According to research, the presence of *Candida* colonies is associated with cases of denture stomatitis. Smear layer examination showed that *Candida* colonies were frequently found on denture surfaces that had been used, regardless of the presence of denture stomatitis. Continuous use of dentures increases the risk of developing denture stomatitis with the presence of injury to the mucosa and the length of time the mucosa is exposed to the plaque on the denture. The use of a soft liner is highly recommended in cases of denture stomatitis to promote the healing of the tissue injury and to provide comfort to the patient.[2]

Soft liner materials are generally easily degraded and susceptible to colonization by microorganisms. Modification of the temporary soft liner material by adding an antimicrobial agent

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has the advantage of increasing the long-term durability of the soft liner clinically.[3] The addition of antifungal or antimicrobial drugs into the denture base material can progressively release antifungal properties into the oral cavity.[4] The addition of drugs into the denture liner helps break the contact between the denture biofilm and the infected tissue, thereby preventing re-infection due to contaminated dentures.[2]

TiO_2 nanoparticles have been shown to have antimicrobial properties due to the ability of TiO_2 to induce the photocatalytic production of cytotoxic oxygen radicals. TiO_2 generates strong oxidizing energy when UV radiation, water, and oxygen are present around TiO_2.[5] The irradiated titanium dioxide can decompose or oxidize organic and inorganic components. The ability of TiO_2 to decompose organic components increases the use of TiO_2 to destroy microorganisms which mostly consist of organic-based components.[6] TiO_2 nanoparticles also produce reactive oxygen species (ROS) in cells which cause a devastating effect on microbial cells. This results in a decrease in respiratory activity and ultimately leads to cell death.[7]

The addition of TiO_2 to resin materials at certain concentrations can increase impact strength, transverse strength, and surface hardness.[8] In use, soft liner materials are expected to be able to maintain their physical properties in a plastic state.[2] Hardness is a measure of a material's resistance to local plastic deformation. The hardness test was carried out to determine the strength of the surface of the material to withstand the penetration of certain loads.[9] The purpose of this study was to examine the effect of adding TiO_2 nanoparticles as a filler to soft liners on the growth of *Candida albicans* and surface hardness.

MATERIALS AND METHODS

This research received ethical clearance from the Research Ethics Commission of Faculty of Dentistry and Prof Soedomo Dental Hospital, Universitas Gadjah Mada Yogyakarta (project number 150/KE/FKG UGM/EC/2022). Molds for the construction of study samples were computerized using Prusa Slicer 2.4.1 software, and 3D printing was done with polylactic acid (PLA) material with a 0.1 mm accuracy.

Samples of soft liner discs were prepared and mixed with 0.5%, 1%, and 2% concentrations of TiO2 nanoparticles. The ratio of powder and liquid soft liner according to the manufacturer was 1:1. The percentage of TiO_2 nanoparticles was determined based on the final weight of the soft liner mixture. At a concentration of 0.5%, the weight of the TiO_2 nanoparticles was 1 g, and the weight of the powder and liquid was 99.5 g. At a concentration of 1%, the weight of the TiO_2 nanoparticles was 2 g, and the weight of the powder and liquid was 99 g. At a concentration of 2%, the weight of the TiO_2 nanoparticles was 4 g, and the weight of the powder and liquid was 98 g. A stellon pot was used for mixing. The first mixing was between soft liner liquid and TiO_2 nanoparticles to obtain homogeneity. It was followed by the addition of soft liner powder and stirred again for 30 seconds. Samples were incubated in distilled water at 37 °C for 1 day.

Candida albicans colonies were collected using sterile loops, then placed in Saboraud dextrose broth (SDB) media and incubated for 24 hours at 37 °C. The *Candida albicans* suspension obtained was added with sterile distilled water to achieve turbidity with the Brown III standard of 10^8 CFU/mL.

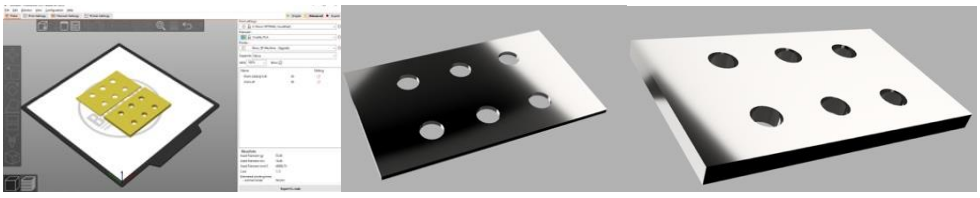


Figure 1. The process of making sample prints uses 3D printing

Twenty-four samples with a diameter of 10 mm and a thickness of 2 mm were immersed in *Candida albicans* suspension. Each sample was put into a conical tube containing 10 ml of sterile distilled water. Subsequently, the conical tube was vibrated with a vortex mixer for 30 seconds at a speed of 500 rpm, followed by dilution to a series of 10^{-3}. Three types of dilutions were made to simplify the calculation: (1) dilution 10^{-1}: 1 mL of solution was put into test tube I and added with 9 mL of distilled water; (2) dilution 10^{-2}: 1 mL of solution was taken from test tube I, put into test tube II, and then added with 9 mL of distilled water; (3) dilution 10^{-3}: 0.1 mL of solution was taken from test tube II and put in the Saboraud dextrose agar (SDA) medium. The spreader was used to spread the culture, then cultured at 37 °C for 24 hours. Calculation of *Candida albicans* used a colony counter with units of colony forming unit (CFU)/mL.

Twenty-four samples with a diameter of 10 mm and a thickness of 6 m were removed from the distilled water, allowed to dry, and then tested for surface hardness. Hardness measurement used a digital Durometer Shore A and expressed by the hardness of Shore A (HA). The instrument was kept in a vertical position while pressure was applied. The reading was taken when the foot of the instrument touched the surface of the sample. Readings were made for 5 seconds after tight contact was found. Measurements were made at 5 points on each sample and then the average surface hardness was calculated from the data obtained.

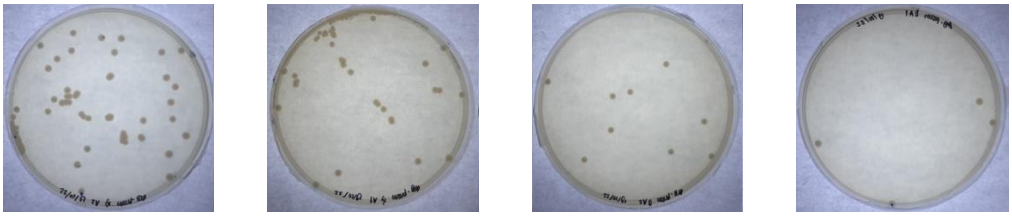
Table 1. The mean value and standard deviation of *Candida albicans* colonies on the soft liner

Groups	n	Mean ± SD (x10^3 CFU/mL)
TiO_2 0.5%	6	11.83 ± 1.60
TiO_2 1%	6	10.00 ± 2.53
TiO_2 2%	6	7.67 ± 2.25
Control	6	21.33 ± 4.63

RESULTS

The mean and standard deviation values of *Candida albicans* colonies (in units of CFU/mL) that grew on the soft liner after the addition of TiO_2 nanoparticle filler can be seen in Table 1. The difference in the number of *Candida albicans* growing on SDA media can be seen in Figure 2.

The results of the Shapiro-Wilk normality test showed that all data had a normal distribution with a significance value of 0.948 ($p > 0.05$). The results of the homogeneity test with Levene's test showed a significance value of 0.15 ($p > 0.05$), indicating that all population variances are the same and the assumption of variance between homogeneous groups has been met. The results of the one way ANOVA test showed a value of $p = 0.000$ ($p < 0.05$). These results are consistent with the hypothesis that adding TiO_2 filler to the soft liner had an effect on the growth of *Candida albicans*. The LSD post hoc test was used to determine differences in each treatment group. The significance value can be seen in Table 2. The average value and standard deviation of the surface hardness of the soft liner (in HA units) after the addition of TiO_2 filler can be seen in Table 3. The difference in the results of measuring the surface hardness of the soft liner in the treatment group can be seen in Figure 3.



(A) (B) (C) (D)

Figure 2. *Candida albicans* colonies growing on SDA (A) Control group, (B) TiO_2 0.5%, (C) TiO_2 1%, (D) TiO_2 2%

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Table 2. The results of the significance of the LSD post hoc test between groups of TiO_2 nanoparticle filler concentrations on the growth of *Candida albicans*

Concentration	TiO_2 0.5%	TiO_2 1%	TiO_2 2%	Control
TiO_2 0.5%	-	1.83	4.17*	-9.50*
TiO_2 1%	-	-	2.33	-11.33*
TiO_2 2%	-	-	-	-13.67*
Control	-	-	-	-

*. The mean difference is significant at the 0.05 level.

Table 3. The mean value and standard deviation of the surface hardness of the soft liner

Groups	n	Mean ± SD (HA)
TiO_2 0.5%	6	27.40 ± 1.88
TiO_2 1%	6	28.80 ± 2.42
TiO_2 2%	6	29.92 ± 1.52
Control	6	23.08 ± 2.16

(A) (B) (C) (D)

Figure 3. Measurement of surface hardness of soft liners (A) Control group, (B) TiO_2 0.5%, (C) TiO_2 1%, (D) TiO_2 2%

Table 4. Significance results of the LSD post hoc test between groups of filler concentrations TiO_2 nanoparticles on soft liner surface hardness

Concentration	TiO_2 0.5%	TiO_2 1%	TiO_2 2%	Control
TiO_2 0.5%	-	-1.4	-2.52*	4.2*
TiO_2 1%	-	-	-1.11	5.72*
TiO_2 2%	-	-	-	6.83*
Control	-	-	-	-

*. The mean difference is significant at the 0.05 level

The results of the Shapiro-Wilk normality test on the soft liner surface hardness data indicated that all data had a normal distribution with a significance value of 0.142 ($p > 0.05$). The results of the homogeneity test with Levene's test showed a significance value of 0.604 ($p > 0.05$). This suggests that all population variances are the same and the assumptions of variance between homogeneous groups have been fulfilled. The results of the one way ANOVA test showed a value of $p = 0.000$ ($p < 0.05$). These results are in accordance with the hypothesis that adding TiO_2 filler to the soft liner had an effect on surface hardness. The LSD post hoc test was used to determine differences in each treatment group. The significance value can be seen in Table 4.

DISCUSSION

The results of the study showed that adding TiO_2 filler to the soft liner resulted in the highest inhibition of *Candida albicans* growth at a concentration of 2% with an average number of colonies of 7.67 x 10^3 CFU/mL. The control group, which did not receive TiO_2, had the lowest number of colonies. This indicates that TiO_2 nanoparticles could inhibit the growth of *Candida albicans*. As shown in Table 1, TiO_2 nanoparticles with a concentration of 2% showed 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 may 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.[10]

Titanium dioxide that is added to the polymer does not bond with it. It remains separate from the polymer chain, and instead it is only trapped in the polymer chain network.[11] This leads to the release of the antifungal effect over time. Titanium dioxide can kill *Candida albicans* as the concentration increases.[7] The addition of TiO_2 concentration to acrylic materials has antibacterial and antifungal effects as the concentration increases.[12]The amount of ROS 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. Titanium dioxide produces ROS which can induce destructive effects on fungal cells, triggering intracellular oxidation of coenzyme A and lipid peroxidation. This process ultimately causes a decrease in cell respiration activity, resulting in the death of *Candida albicans*.[7]

The LSD test (Table 2) showed significant differences in all treatment groups compared to the control group. This effect might be due to the presence of antifungal properties in titanium dioxide. The properties of titanium dioxide nanoparticles are broad-spectrum antimicrobials, high chemical resistance, and able to reduce contaminants because they have photocatalyst properties.[13] The energy difference of titanium dioxide anatase 3.26eV exits from the valence band to the conduction band and electrons, and releases energy. This energy then reacts with water molecules and oxygen, triggering the formation of ROS and hydroxyl radicals (·OH). These radicals form pairs of electrons (e-) and holes (h+) that can reduce and or oxidize compounds (pollutants) in the vicinity. Microorganisms 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.[14]

The results showed that there was no significant difference between the number of *Candida albicans* colonies in the 0.5% and 1% TiO_2 treatment group and the 1% and 2% treatment group. This might be caused by the low level of agglomeration that occurred in the concentration range with a difference of 0.5-1%. Low filler concentrations can increase the density of polymer chains. The addition of TiO_2 filler in low concentrations allows the filler to be dispersed evenly without agglomeration. This is in accordance with the finding of Shirkavand which showed that the addition of excessive TiO_2 can increase the risk of agglomeration between nanoparticles. This can ultimately reduce the dispersion of nanoparticles in the resin material, so the effect produced by TiO2 is uneven.[15]

The highest mean surface hardness of the soft liner was found in the treatment group with the addition of 2% TiO_2 nanoparticles with an average surface hardness of 29.92 HA. The lowest surface hardness was found in the control group (without the addition of TiO_2 nanoparticles) with an average surface hardness of 23.08 HA. The increase in surface hardness that occurred in all treatment groups was still within the normal range for the soft-liner material classification. According to ISO 10139-2:2016, a soft denture lining material has a surface hardness of 25-50 HA after 24 hours of soaking in granulated water. Shore A hardness measurement is a measurement of the texture and flexibility of the material where the ideal shore A hardness value is between 25-35 SHU.[16]

The increase in hardness can be influenced by two factors: a higher filler content and the use of a silane coupling agent, which is associated with an increase in the connection between the filler and the matrix.[8] Titanium dioxide acts as a solid in the resin matrix, leading to an increase in its stiffness, reduced mobility, and volume release, which eventually appears as an escalation of violence.[11]

The LSD test (Table 4) showed significant differences in all treatment groups compared to the control group. The role of TiO_2 nanoparticle filler is to fill empty plasticizer spaces that are not chemically bonded to the polymer network. The

space will be filled with solid TiO_2 filler which will make the soft-liner material even harder. The more TiO_2 filler is added to the polymer, the more rigid the polymer matrix will be. The amount of plasticizer that is present in the liner material determines how much of the material's elasticity is lost throughout usage; the more plasticizer that is substituted at the start of polymerization, the less elastic the material is. This problem frequently arises with soft-liner products that contain acrylic.[17] The filler makes the intermolecular cross-links in the surface layer of the material larger than the internal layer of the material, resulting in an increase in hardness on the surface of the material being tested.[12]

The results showed that there was no significant difference between the surface hardness of the group added with 0.5% and 1% TiO_2 nanoparticles and the group added with 1% and 2% TiO_2 nanoparticles. This could be caused by the small difference in TiO_2 content between treatment groups, resulting in insignificant changes in physical properties. The difference in filler concentration affects the degree of saturation in the resin matrix. The addition of TiO_2 nanoparticles at a concentration of 0.5% to 1% showed an increase in mechanical strength, namely tensile, flexural and impact strength. In contrast, the addition of concentrations above the saturation point (> 2%) showed that the strength of the resin material remained largely unchanged.[15]

Finally, it is important to note that this study was limited to just one brand of soft-liner which was commercially available, and the experiment was of short duration. A longer period is needed to extrapolate the results of this study to the antimicrobial effects of soft-liner incorporated with nanoparticles. Future studies could examine the antifungal effectiveness achieved by adding TiO_2 nanoparticles in the soft-liner material.

CONCLUSION

Incorporating TiO_2 nanoparticles into the soft liner as a filler was found to decrease the growth of *Candida albicans*. Adding TiO_2 nanoparticle filler at 0.5% to 2% concentration effectively suppressed *Candida albicans* growth, with 0.5% resulting in the smallest change in surface hardness.

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

REFERENCES

1. Wirayuni KA. Akumulasi *Streptococcus mutans* pada basis gigi tiruan lepasan plat nilon termoplastik dan resin akrilik. Interdental Jurnal Kedokteran Gigi. 2017; 13(2): 28-31. doi: 10.46862/interdental.v13i2.362
2. Lima JF, Maciel JG, Arrais CA, Porto VC, Urban VM, Neppelenbroek KH. Effect of Incorporating antifungals on the water sorption and solubility of interim resilient liners for denture base relining. J Prosthet Dent. 2016; 115(5): 611–616. doi: 10.1016/j.prosdent.2015.09.029
3. Powers JM, Wataha JC. Dental Materials Foundation and Application, 11th ed. St. Louis: Elsevier; 2017. 177-178. Available at https://www.us.elsevierhealth.com/dental-materials-9780323316378.html
4. Bueno MG, de Sousa EJB, Hotta J, Porto VC, Urban VM, Neppelenbroek KH. surface properties of temporary soft liners modified by minimum inhibitory concentrations of antifungals. Braz Dent J. 2017; 28(2): 158-164. doi: 10.1590/0103-6440201701266
5. Acosta-Torres LS, Lopez-Marin LM, Nunez-Anita RE, Hernandez-Padron G, Castano VM. Biocompatible metal-oxide nanoparticles: nanotechnology improvement of conventional prosthetic acrylic resins. Journal of Nanomaterials. 2011; 2011(1): 1-8. doi: 10.1155/2011/941561
6. Sari L, Astuti. Pengaruh nanopartikel titanium dioksida pada resin sebagai material transparan anti UV dan self cleansing. Jurnal Fisika Unand. 2013; 2(1): 20–25. doi: 10.25077/jfu.2.1.%25p.2013
7. Haghighi F, Mohammadi SR, Mohammadi P, Hosseinkhani S, Shidpour R. Antifungal activity

of TiO_2 Nanoparticles and EDTA on Candida albicans Biofilms. Infection Epidemiology and Medicine. 2013; 1(1): 33–38.
8. Alwan SA, Alameer SS. The effect of the addition of silanized nano titania fillers on some physical and mechanical properties of heat cured acrylic denture base materials. Journal of Baghdad Collage of Dentistry. 2015; 27(1): 86-91. doi: 10.12816/0015269
9. Sharma DA, Shashidhara HS. A Review: flexible removable partial dentures. IOSR Journal of Dental and Medical Scienses. 2014; 13(12): 58-62. doi: 10.9790/0853-131265862
10. Putranti DT, Fadilla A. Titanium dioxide addition to heat polymerized acrylic resin denture base effect on Staphylococcus aureus and Candida albicans. Journal of Indonesian Dental Association. 2018; 1(1): 21–27. doi: 10.32793/jida.v1i1.286
11. Hashem M, Rez MFA, Fouad H, Elsarnagawy TD, Elsharawy MA, Umar A, Assery MK, Ansari SG. Influence of titanium oxide nanoparticles on the physical and thermomechanical behavior of Poly Methyl Methacrylate (PMMA): A Denture Base Resin. Science of Advanced Materials. 2017; 9(6): 938-944. doi: 10.1166/sam.2017.3087
12. Alrahlah A, Fouad H, Hashem M, Niazy A, AlBadah A. Titanium Oxide (TiO_2)/ Polymethylmethacrylate (PMMA) denture base nanocomposites: mechanical, viscoelastic and antibacterial behavior. Materials. 2018; 11(7): 1096. doi: 10.3390/ma11071096
13. Chen T, Yan J, Li Y. Genotoxicity of titanium dioxide nanoparticles. Journal of Food and Drug Analysis. 2014; 22(1): 95-104. doi: 10.1016/j.jfda.2014.01.008
14. Gatou MA, Syrrakou A, Lagopati N, Pavlatou EA. Photocatalytic TiO_2-Based nanostructures as a promising material for diverse environmental application: a review. Journals Reactions. 2024; 5(1): 135-194. doi: 10.3390/reactions5010007
15. Shirkavand S, Moslehifard E. Effect of TiO_2 nanoparticles on tensile strength of dental acrylic resins. J Dent Res Den Clin Dent Prospects. 2014; 8(4): 197-203. doi: 10.5681/joddd.2014.036
16. Bialozyt-Bujak E, Wyszynska M, Chladek G, Czelakowska A, Gala A, Orczykowska M, Bialozyt A, Kasperski J, Skucha-Nowak M. Analysis of the hardness of soft relining materials for removable dentures. Int J Environ Res Public Health. 2021; 18(18): 9491. doi: 10.3390/ijerph18189491
17. Hashem MI. Advances in soft denture liners: an update. The Journal of Contemporary Dental Practice. 2015; 16(4): 314-318. doi: 10.5005/jp.journals.10024.168