

Original Research Article

Effect of method and concentration of titanium dioxide addition on anti-biofilm ability in extraoral maxillofacial prosthetic fungus

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ABSTRACT

Background: One of the efforts to prevent adhesion and biofilm colonization on the material surface is to improve its properties by using Titanium dioxide (TiO₂) nanoparticles. TiO₂ nanoparticles have antimicrobial properties, especially against the fungus *Candida albicans* as it has photocatalytic properties that can inhibit the growth of the fungal colonies. The present study aimed to determine the effect of applying TiO₂ to polyurethane plates on the growth of *Candida albicans* fungal biofilms.

Methods: This study applied a laboratory experimental design. The subjects were divided into two groups which included the treatment and control groups. In the treatment group, there were two treatments consisting of the addition of TiO₂ filler (1%, 2%, 3%, and 4%) and the material surface coating group with TiO₂ (1%, 2%, 3%, and 4%). *Candida albicans* was cultured and grown to form biofilms on polyurethane plates in each group.

Results: The results of the statistical analysis obtained through Welch's One Way Anova showed that there was a significant difference in the number of *Candida albicans* colonies between the treatment and control groups ($P < 0.05$). Treatment with 4% TiO₂ surface coating showed the lowest number of *Candida albicans* colonies.

Conclusions: Coating the surface of the material with TiO₂ on a polyurethane plate was able to inhibit the formation of *Candida albicans* biofilms.

Keywords: Polyurethane plate, Titanium dioxide, surface coating, Biofilm, *Candida albicans*

INTRODUCTION

Maxillofacial defects occur as a result of trauma, congenital abnormalities or surgical removal of neoplastic tissue which causes maxillofacial defects, abnormalities of masticatory function, phonetic functions and psychological disorders. This defect can lead to lifting of the hard tissue, soft tissue and skeletal components of the face which are the main maxillofacial supports where reconstructive surgery cannot be performed.¹ Making a maxillofacial prosthesis aims to restore and rehabilitate maxillofacial defects. Extraoral maxillofacial prostheses aim to rehabilitate and replace damaged, deformed or missing extra oral structures, for instance in the eyes, ears,

nose, lips, areas of the facial bones and the cranium which are included in the scope of rehabilitation of extraoral maxillofacial prostheses.² Maxillofacial prostheses are made to restore appearance, function of speech, chewing and help the process of tissue healing as well as psychological trauma.³ Extraoral maxillofacial prostheses can be made from several types of materials that meet the requirements, including: physical-mechanical properties similar to the body, biocompatible, non-toxic, non-allergenic, and stable for a long time. Maxillofacial prosthetic materials that are commonly used today are copolymers, Heat cured polymethyl methacrylate, latex, polyurethane elastomers and silicone.⁴

Polyurethane elastomer is a material with very good properties and is a material commonly used in the manufacture of extraoral maxillofacial prostheses.⁵ The advantages of polyurethane are that it is not easily torn or damaged, has good elongation ability of the material, undergoes minimal shrinkage during polymerization, has a surface texture and hardness in the range of human skin, is elastic and has good edge strength, requires little instrumentation in manufacture, has optimal tensile strength as well as an easy coloring process that looks natural.⁶ However, the disadvantage is that it is sensitive to moisture and has poor color stabilization. In addition, in some cases, there is attachment of fungi and microorganisms that cause irritation.

As a medical device material, polyurethane is known to be very biocompatible, inert and has very low potential to undergo chemical changes when it is used. However, medical devices made from polyurethane are very vulnerable to becoming a growing medium for microorganisms and fungi such as *Candida albicans*.⁷ Disinfection methods for medical devices made of polyurethane using chemicals can be detrimental, pollute the environment and are less effective. In this case, the addition method using materials that produce ROS to polyurethane (reactive oxygen species), with the aim of providing a disinfection effect, is an interesting study. Titanium dioxide (TiO₂) is widely used as an ROS generating material which is very useful for preventing infection by adding it permanently to the material to obtain antimicrobial benefits.⁸

Candida albicans is a normal flora that is often found in the oral cavity, mucous tissue, digestive tract and vagina. However, it can turn into a pathogen if changes occur in its environment. In addition, *Candida albicans* has abilities and properties that tend to stick. The risk of *Candida albicans* infection or colonization generally increases due to predisposing factors such as poor oral hygiene, immunosuppression, nutritional deficiencies, long-term use of antibiotics, radiation therapy, use of dentures, people with diabetes mellitus, heavy carbohydrate diets, and heavy smoking.⁹ There are several methods to minimize the occurrence of infection in prosthesis users, including cleaning the prosthesis used. Some of these methods can be done mechanically, chemically, or a combination of the two. One method is the addition of nanoparticle materials such as TiO₂.¹⁰

TiO₂ is widely used because of several advantages including high stability, catalytic effect, efficiency, low cost, non-toxicity, high refractive index and corrosion resistance, high hardness and antimicrobial activity under a wide spectrum of configurations.¹¹ TiO₂ is known to be capable of becoming antifungal nanoparticles due to its photocatalytic properties.¹² Based on the various advantages contained in TiO₂, the authors were interested in examining the effect of TiO₂ nanoparticles with their broad spectrum, especially on *Candida albicans*. Many studies had been carried out to investigate the effect of

adding TiO₂ on the physical, mechanical and biological properties of various polymers.¹²

TiO₂ has 3 types of crystal structures which include anatase, rutile and brookite. In the photocatalytic reaction, the anatase structure provides better activity than rutile. The rutile structure has more prominent mechanical properties. Anatase crystals have better photocatalytic activity than other types of crystals because the band-gap ability of anatase is larger. Both crystalline forms of TiO₂, anatase and rutile, can absorb ultraviolet light. The range of light absorbed by rutile is greater, but the anatase form has greater photocatalytic activity.¹³

The characteristics of TiO₂ nanoparticles are broad antimicrobial spectrum, low cost, non-toxic, odorless, non-irritating, heat-resistant and high chemical resistance. TiO₂ nanoparticles are able to reduce contaminants because they have photocatalyst properties. The photocatalyst will activate when it is exposed to ultraviolet (UV) light. TiO₂ nanoparticles produce reactive oxygen species (ROS) in cells which cause destruction of microbial cells, oxidation of intracellular coenzyme A and peroxidation of some lipids which can reduce respiratory activity and then cause cell death.¹⁴ TiO₂ has a strong oxidizing ability through photocatalytic activity, so it is able to break down organic and inorganic pollutants. The ability of TiO₂ to produce reactive oxygen species (ROS) can reduce the number of microorganisms to a very small concentration without producing harmful derivative products. This ability is consistent or does not decrease during usage time and its antimicrobial ability remains optimal.¹⁵

Putranti and Fadilla also conducted another study which proved the effect of adding TiO₂ nanoparticles with concentrations of 1%, 2% and 3% which could inhibit the growth of *Candida albicans*.¹⁶ This study aimed to determine the effect of giving TiO₂ with anatase crystal phase on polyurethane plates after being exposed to ultraviolet light on the growth of *Candida albicans* colonies.

METHODS

The present study applied a laboratory experimental design. It was carried out at the Dermatology, Venereology and Microbiology Laboratory, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University from May to September 2022.

In this study, a polyurethane plate sample with a size of 20×15×3 mm was used according to the ADA Standard No.12.

The total number of samples used in this study were 54 samples divided into 8 treatment groups and 1 control group, each group consisting of 6 research samples. The treatment group consisted of group 1 consisting of 6 samples of polyurethane plates without the addition of TiO₂, groups 2 and 3 consisting of 6 samples each of

polyurethane plates with the addition of TiO₂ as filler and coating with a concentration of 1%, groups 4 and 5 consisting of 6 samples each polyurethane plates with the addition of TiO₂ as a filler and coating with a concentration of 2%, groups 6 and 7 consisting of 6 samples each, polyurethane plates with the addition of TiO₂ as a filler and coating with a concentration of 3% and groups 8 and 9 consisting of 6 samples each polyurethane plate with the addition of TiO₂ as a filler and coating with a concentration of 4%, then all samples were soaked in artificial saliva.

Before adding polyurethane, the TiO₂ nanoparticles must first go through silanization to increase the adhesion between TiO₂ and the polyurethane plate in the following ways:

- 30 g of TiO₂ nanoparticles were put into an Erlenmeyer tube containing 200 ml of pure ethanol which was used as a solvent.
- The Erlenmeyer tube was then put into the sonicator at room temperature for 20 minutes.
- Then, it was vibrated using a magnetic stirrer for 20 minutes at room temperature to obtain a homogeneous mixture.
- Then, 1.5 ml of 5% silane coupling agent was added to the mixture of TiO₂ and ethanol using a sterile syringe.
- It was vibrated using a magnetic stirrer at 250 rpm for 60 minutes.
- The mixture of all these ingredients was then poured into a closed container and left for 2 days so that the silane coupling agent could penetrate completely on the TiO₂ surface.
- Then the ethanol mixed in TiO₂ was evaporated using a rotary evaporator at a temperature of 60°C and a speed of 150 rpm for 30 minutes to separate the ethanol solvent and the silane coupling agent which had fused with TiO₂.
- A Buchner vacuum was used to remove remaining ethanol and silane coupling agents.
- The silanized nanoparticles were then dried in an oven at 60°C for 20 hours and then removed from the oven.
- Then, the TiO₂ particles were processed using a sonicator at 250 rpm for 3 minutes to break up the agglomerated particles.

Preparation of polyurethane plate samples with and without the addition of TiO₂ as a filler that has gone through silanization with a concentration of 1%, 2%, 3% and 4% in each of the 6 samples: (a) Polyurethane plate without TiO₂ nanoparticles, (b) polyurethane plate with the addition of 1% concentration of TiO₂ nanoparticles, (c) polyurethane plate with the addition of 2% concentration of TiO₂ nanoparticles, (d) polyurethane plate with the addition of 3% concentration of TiO₂ nanoparticles, (e) polyurethane plate with 4% concentration of TiO₂ nanoparticles added.

The method of adding TiO₂ as a filler was adjusted to the concentration to be tested (0.24 gr=1%, 0.48 gr=2%, 0.72 gr=3%, 0.96 gr=4% of the total weight of the polyurethane sample). The silanized TiO₂ was mixed with 12gr of part B polyurethane in a porcelain pot. Then it was stirred using a hand mixer with a screw whisk, stirring was carried out for 2 minutes until the mixture became homogeneous. Then, 12 grams of polyurethane part A was added with a ratio of part A:part B = 1:1. Next, it was stirred using a hand mixer with a screw whisk. Stirring was carried out for 2 minutes until the dough became homogeneous. Then the polyurethane mixture that had been homogenized was placed in a vacuum machine and closed for the process of releasing air bubbles. Pouring the polyurethane mixture into the mold was done after the air bubbles are completely released and poured slowly so that the dough filled the bottom of the mold. Then, the sample was removed from the mold after it hardened.

For the TiO₂ method as a coating, 12 grams of part B polyurethane in a porcelain pot was added to 12 grams of part A polyurethane with a ratio of part A:part B = 1:1. Next, it was stirred using a hand mixer with a screw whisk. Stirring was carried out for 2 minutes until the dough became homogeneous. The homogeneous polyurethane dough is placed in a vacuum machine and closed for the process of releasing air bubbles. Pouring the polyurethane mixture into the mold was done after the air bubbles were completely released and poured slowly so that the dough filled the bottom of the mold, then the sample was released from the mold after it hardened. The surface of the sample to be coated with TiO₂ was smeared with silane coupling agent liquid evenly over the entire surface of the sample using a microbrush and then allowed to dry.

TiO₂ that had gone through silanization was made into a paste by mixing each concentration group according to the concentration to be tested (0.24 gr=1%, 0.48 gr=2%, 0.72 gr=3%, 0.96 gr=4 %, of the total weight of the polyurethane sample) in 2 ml of ethanol. The paste was stirred using a magnetic stirrer for 30 minutes at a rotational speed of 300 rpm so that it became homogeneous. Furthermore, the prepared paste was layered on the surface of the polyurethane plate with a spatula using the slip casting method.

Determination of total *Candida albicans*

- All filler and coating samples were irradiated with ultraviolet light with a wavelength of 366 nm and a distance of 15 cm for 1 hour to activate the photocatalyst properties of TiO₂. Then the samples were contaminated with *Candida albicans* by placing them in an Erlenmeyer flask containing *Candida albicans* suspension. Then it was incubated for 48 hours in the incubator.
- Polyurethane plate samples went through washing.
- The polyurethane plate sample was threshed by *Candida albicans* attached to the polyurethane plate.

- After that, the polyurethane plate sample was passed through a swab using a sterile cotton bud, then put into a test tube containing PBS.
- Approximately 0.01 ml of liquid from the test tube was taken using a pipette and then poured into a petri dish with SDA media that had been made before.
- Procedures must be carried out close to fire and cleanliness must be maintained to avoid contamination with other microorganisms.
- The edge of the petri dish was heated circularly in a bunsen to make it sterile. After that, the petri dishes were incubated at 37°C for 48 hours.
- After 48 hours, the colony count was carried out with a colony counter. Colony count was carried out in units of CFU/ml.

Research data analysis was carried out using a one-way ANOVA test.

RESULTS

Based on the results of the study, the average value of *Candida albicans* colony growth in 9 treatment groups on polyurethane plates with the addition of TiO₂ can be seen in Table 1.

Table 1 shows that the average colony growth value of *Candida albicans* on the polyurethane plate with the addition of 4% TiO₂ coating was the lowest of the other 8

groups, which was 128.83 CFU/ml while the control group with the polyurethane plate without any addition was the lowest. higher than the other treatment groups was 272 CFU/ml.

The data was tested to determine normality using the Kolmogorov Smirnov test because the number of study samples was more than 50. The results of the normality test can be seen in Table 2.

Table 2 shows that the significant value is more than 0.05 ($p > 0.05$), which means that all data in each group was normally distributed. The data was then tested for homogeneity through the Levene Statistical test.

The significance value in Table 3 is 0.000 ($p < 0.05$) which indicates that the data on the number of *Candida albicans* colonies was not homogeneous. After knowing that the data were normally distributed but not homogeneous, the next step was the one way ANOVA Welch test to find out whether there were differences in the number of *Candida albicans* colonies in each group. The test results can be seen in the following table.

The significance value shown in the table is 0.000 ($p < 0.05$). So it was concluded that there was a significant difference in the 9 test groups. To find out the significant difference between the 2 groups, Howell's post hoc games test was conducted. Post hoc test results can be seen in the table below.

Table 1: Calculation of growth of *Candida albicans*.

Group	Mean (CFU/ml)	Standard Deviation
Control group	272	47.438
Polyurethane plate + TiO ₂ coating 1%	199.66	5.955
Polyurethane plate + TiO ₂ coating 2%	188.50	4.135
Polyurethane plate + TiO ₂ coating 3%	163.p0	12.931
Polyurethane plate + TiO ₂ coating 4%	128.83	7.960
Polyurethane plate + TiO ₂ filler 1%	221.67	14.720
Polyurethane plate + TiO ₂ filler 2%	202.33	6.121
Polyurethane plate + TiO ₂ filler 3%	195.67	5.164
Polyurethane plate + TiO ₂ filler 4%	191.00	2.191

Table 2: Normality test results using Kolmogorov Smirnov.

Group	N	Kolmogorov-smirnov		Description
		Statistic	Sig	
Control Group	6	0.257	0.200	Normal data
Polyurethane plate + TiO ₂ coating 1%	6	0.245	0.200	Normal data
Polyurethane plate + TiO ₂ coating 2%	6	0.308	0.077	Normal data
Polyurethane plate + TiO ₂ coating 3%	6	0.258	0.200	Normal data
Polyurethane plate + TiO ₂ coating 4%	6	0.185	0.200	Normal data
Polyurethane plate + TiO ₂ filler 1%	6	0.214	0.200	Normal data
Polyurethane plate + TiO ₂ filler 2%	6	0.315	0.063	Normal data
Polyurethane plate + TiO ₂ filler 3%	6	0.269	0.200	Normal data
Polyurethane plate + TiO ₂ filler 4%	6	0.176	0.200	Normal data

Table 3: Results of the homogeneity test using Levene Statistics.

Number of <i>Candida albicans</i> colonies	Sig
	0.000

Table 4: One way anova welch test results.

Group	N	Description
Control Group		
Polyurethane plate + TiO ₂ coating 1%	0.00 (P<0.05)	Significant
Polyurethane plate + TiO ₂ coating 2%		
Polyurethane plate + TiO ₂ coating 3%		
Polyurethane plate + TiO ₂ coating 4%		
Polyurethane plate + TiO ₂ filler 1%		
Polyurethane plate + TiO ₂ filler 2%		
Polyurethane plate + TiO ₂ filler 3%		
Polyurethane plate + TiO ₂ filler 4%		

Table 5: Results of Howell's post hoc games test.

(I) treatment	(J) treatment	Mean Difference	(I) treatment
Control	Polyurethane plate + TiO ₂ filler 1%	50.333	0.381
	Polyurethane plate + TiO ₂ coating 1%	72.333	0.126
	Polyurethane plate + TiO ₂ filler 2%	69.667	0.143
	Polyurethane plate + TiO ₂ coating 2%	83.500	0.075
	Polyurethane plate + TiO ₂ filler 3%	76.333	0.104
	Polyurethane plate + TiO ₂ coating 3%	109.000	0.023
	Polyurethane plate + TiO ₂ filler 4%	81.000	0.085
	Polyurethane plate + TiO ₂ coating 4%	143.167	0.007
	Control	-50.333	0.381
Polyurethane plate + TiO₂ filler 1%	Polyurethane plate + TiO ₂ coating 1%	22.000	0.138
	Polyurethane plate + TiO ₂ filler 2%	19.333	0.220
	Polyurethane plate + TiO ₂ coating 2%	33.167	0.025
	Polyurethane plate + TiO ₂ filler 3%	26.000	0.070
	Polyurethane plate + TiO ₂ coating 3%	58.667	0.001
	Polyurethane plate + TiO ₂ filler 4%	30.667	0.038
	Polyurethane plate + TiO ₂ coating 4%	92.833	0.000
	Control	-72.333	0.126
	Polyurethane plate + TiO ₂ filler 1%	-22.000	0.138
Polyurethane plate + TiO₂ coating 1%	Polyurethane plate + TiO ₂ filler 2%	-2.667	0.996
	Polyurethane plate + TiO ₂ coating 2%	11.167	0.065
	Polyurethane plate + TiO ₂ filler 3%	4.000	0.927
	Polyurethane plate + TiO ₂ coating 3%	36.667	0.006
	Polyurethane plate + TiO ₂ filler 4%	8.667	0.151
	Polyurethane plate + TiO ₂ coating 4%	70.833	0.000
	Control	-69.667	0.143
	Polyurethane plate + TiO ₂ filler 1%	-19.333	0.220
	Polyurethane plate + TiO ₂ coating 1%	2.667	0.996
Polyurethane plate + TiO₂ filler 2%	Polyurethane plate + TiO ₂ coating 2%	13.833	0.023
	Polyurethane plate + TiO ₂ filler 3%	6.667	0.553
	Polyurethane plate + TiO ₂ coating 3%	39.333	0.004
	Polyurethane plate + TiO ₂ filler 4%	11.333	0.057
	Polyurethane plate + TiO ₂ coating 4%	73.500	0.000
	Control	-83.500	0.075
	Polyurethane plate + TiO ₂ filler 1%	-33.167	0.025
	Polyurethane plate + TiO ₂ coating 1%	-11.167	0.065
	Polyurethane plate + TiO ₂ filler 3%	-7.167	0.277

Continued.

(I) treatment	(J) treatment	Mean Difference	(I) treatment
	Polyurethane plate + TiO ₂ coating 3%	25.500	0.044
	Polyurethane plate + TiO ₂ filler 4%	-2.500	0.902
	Polyurethane plate + TiO ₂ coating 4%	59.667	0.000
Polyurethane plate + TiO₂ filler 3%	Control	-76.333	0.104
	Polyurethane plate + TiO ₂ filler 1%	-26.000	0.070
	Polyurethane plate + TiO ₂ coating 1%	-4.000	0.927
	Polyurethane plate + TiO ₂ filler 2%	5.557	0.553
	Polyurethane plate + TiO ₂ coating 2%	7.167	0.277
	Polyurethane plate + TiO ₂ coating 3%	32.667	0.012
	Polyurethane plate + TiO ₂ filler 4%	4.667	0.566
	Polyurethane plate + TiO ₂ coating 4%	66.833	0.000
Polyurethane plate + TiO₂ coating 3%	Control	-109m000	0.023
	Polyurethane plate + TiO ₂ filler 1%	-58.667	0.001
	Polyurethane plate + TiO ₂ coating 1%	-36.667	0.006
	Polyurethane plate + TiO ₂ filler 2%	-39.333	0.004
	Polyurethane plate + TiO ₂ coating 2%	-25.500	0.044
	Polyurethane plate + TiO ₂ filler 3%	-32.667	0.012
	Polyurethane plate + TiO ₂ filler 4%	-28.000	0.032
	Polyurethane plate + TiO ₂ coating 4%	34.167	0.008
Polyurethane plate + TiO₂ filler 4%	Control	-81.000	0.085
	Polyurethane plate + TiO ₂ filler 1%	-30.667	0.038
	Polyurethane plate + TiO ₂ coating 1%	-8.667	0.151
	Polyurethane plate + TiO ₂ filler 2%	-11.333	0.057
	Polyurethane plate + TiO ₂ coating 2%	2.500	0.902
	Polyurethane plate + TiO ₂ filler 3%	-4.667	0.566
	Polyurethane plate + TiO ₂ coating 3%	28.000	0.032
	Polyurethane plate + TiO ₂ coating 4%	62.167	0.000
Polyurethane plate + TiO₂ coating 4%	Control	-143.167	0.007
	Polyurethane plate + TiO ₂ filler 1%	-92.833	0.000
	Polyurethane plate + TiO ₂ coating 1%	-70.833	0.000
	Polyurethane plate + TiO ₂ filler 2%	-73.500	0.000
	Polyurethane plate + TiO ₂ coating 2%	-59.667	0.000
	Polyurethane plate + TiO ₂ filler 3%	-66.833	0.000
	Polyurethane plate + TiO ₂ coating 3%	-34.167	0.008
	Polyurethane plate + TiO ₂ filler 4%	-62.167	0.000

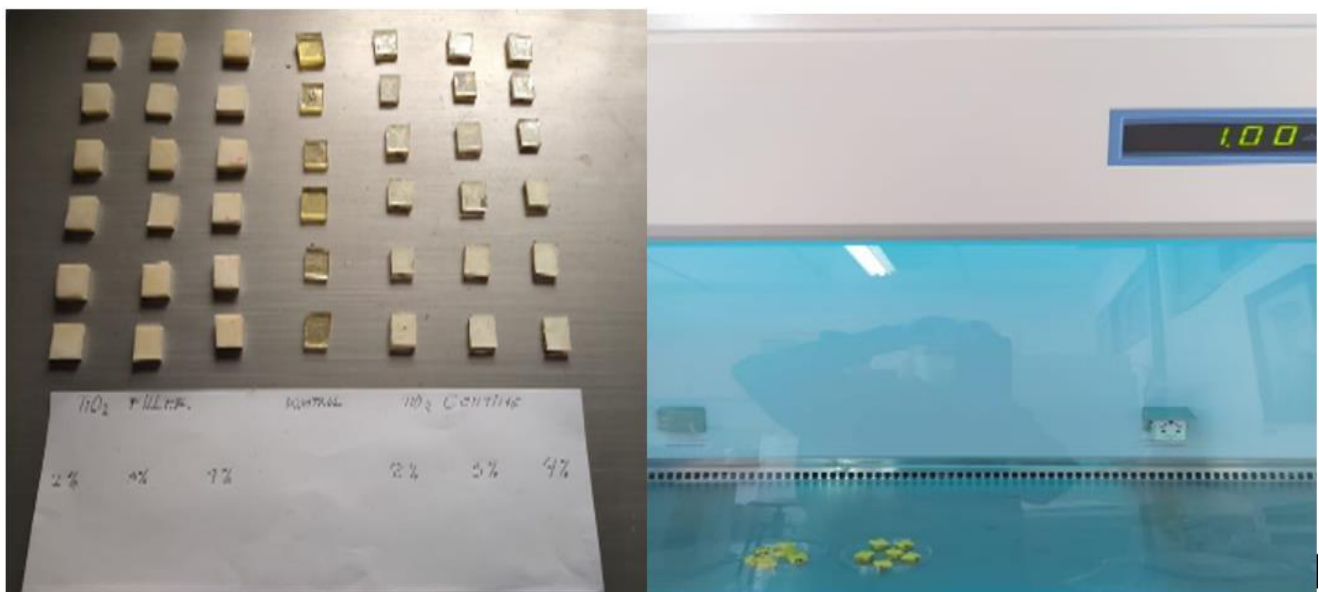


Figure 1: Polyurethane and TiO₂ sample.

In the polyurethane plate treatment group with the addition of 4% TiO₂ coating, when compared to all groups with Howell's post hoc games test, there was a significant difference in the number of *Candida albicans* colonies between the 2 groups and the mean difference in the number of *Candida albicans* colonies between the polyurethane plates with the addition of TiO₂ coating 4% and other groups found that the polyurethane plate group with the addition of 4% TiO₂ coating was the lowest. In the Howell's post hoc games test comparing the control group and other groups, there was a significant difference in the number of *Candida albicans* colonies between the control group and the polyurethane plate treatment group with the addition of 4% TiO₂ coating and the polyurethane plate treatment group with the addition of 3% TiO₂ coating. The mean difference in the number of *Candida albicans* colonies between the control group and the other groups showed that the control group was the highest.



Figure 2: *Candida albicans* colony.

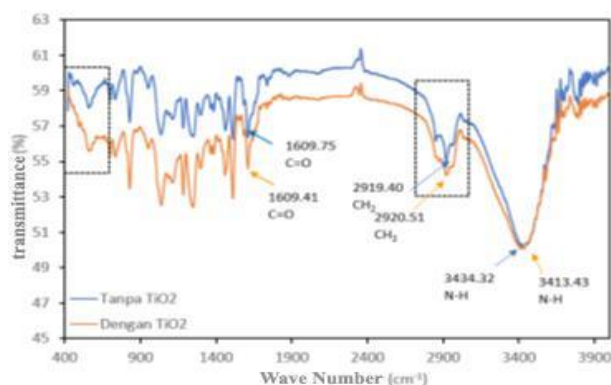


Figure 3: Spectrum FTIR test.

Thus, it proves that there was a significant difference in the number of *Candida albicans* colonies in the nine test groups. The group with the lowest number of *Candida albicans* colonies in the polyurethane plate group with the addition of 4% TiO₂ coating was 128.83 CFU/ml and the group with the highest number of *Candida albicans* in the control group was 272 CFU/ml.

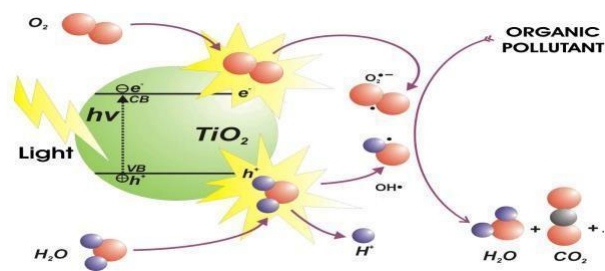


Figure 4: Schematic of TiO₂ photocatalyst process mechanism.

DISCUSSION

The results showed that there was an effect of adding 4% TiO₂ coating on the growth of the number of *Candida albicans* colonies on polyurethane plates. The polyurethane plate group with the addition of 4% TiO₂ coating concentration was the group with the lowest number of colonies, 128.83 CFU/ml. Based on the research results that have been obtained, the most effective effect is polyurethane plate samples with the addition of TiO₂ coating with a concentration of 4%. This is in line with the research conducted by Putranti and Fadilla.¹⁶ In addition, the results showed that the addition of TiO₂ to the polyurethane plate caused a decrease in the number of *Candida albicans* colonies because TiO₂ nanoparticles (TiO₂) have antimicrobial and antifungal abilities as it has photocatalytic properties.⁷ The attachment of nano TiO₂ to polyurethane was carried out by applying a silane coupling agent. Pretreatment with the application of the silane coupling agent could increase the bond strength of TiO₂. The functional group bond between TiO₂ – Si – PU forms a strong covalent bond between TiO₂ and polyurethane. This was proven in examinations using Fourier transform infrared spectroscopy (FT-IR).

The characteristics of TiO₂ nanoparticles are having a broad antimicrobial spectrum, non-toxic, odorless, non-irritating, heat resistant, low cost and high chemical resistance. TiO₂ is able to reduce contaminants because it is a photocatalyst. The photocatalyst will be active if it is exposed to Ultraviolet (UV) light. If the TiO₂ surface is exposed to photon energy from ultraviolet (UV) light, where the energy gap of TiO₂ anatase of 3.26 eV and rutile 3.0 eV will excite electrons from the valence band to the conduction band, these electrons will release energy and then react with water and oxygen molecules. Furthermore, it will trigger the formation of ROS in the form of superoxide radicals (O₂^{•-}) and hydroxyl radicals (OH[•]) which will form pairs of electrons (e⁻) and holes (h⁺) which can reduce and/or oxidize compounds (pollutants) in the vicinity. The mechanism of TiO₂ photocatalysis was as follows: microorganisms will die after contact with hydroxyl radicals (OH[•]) and superoxide radicals O₂^{•-} (ROS) produced by irradiating TiO₂. Hydroxyl radicals (OH[•]) and superoxide radicals O₂^{•-} play an important role in inactivating microorganisms by oxidizing phospholipids in cell membranes where OH[•] radicals are known to be 1000 (one thousand) times more effective in inactivating

microorganisms than common disinfectants.¹⁷ When it is used as a coating, TiO₂ is able to maintain the color and mechanical properties of a material.²

Candida albicans has a thick cell wall because it consists of glucan and chitin which makes it stronger than bacteria. TiO₂ produces ROS (reactive oxygen species) which induces destructive effects on fungal cells. Then it results in the oxidation of intracellular coenzyme A and lipid peroxidation which causes a decrease in cell respiration activity resulting in death.¹¹ Research conducted by Haghighi, showed that TiO₂ was able to kill the fungus *Candida albicans* with increasing concentrations.¹⁸ The amount of colony growth of the *Candida albicans* fungus varies in each test sample because there was a different effect of TiO₂ nanoparticles at each concentration added to the polyurethane plate.¹⁸ In another study it was proven that the addition of TiO₂ with graded concentrations or increasing concentrations has an antimicrobial effect that was directly proportional to the concentration of TiO₂ and the antimicrobial effect of TiO₂. In a study conducted by Alrahlah et al., the addition of TiO₂ at a concentration of 1%, 2% and 3% to the denture base had the highest antimicrobial properties at a concentration of 3%.¹⁵ Research conducted by Putranti and Fadilla obtained the result that the addition of TiO₂ with a concentration of 2%, 3%, and 4% was the most influential concentration of 3% compared to the highest concentration of 4%.¹⁶ In this case, the research did not go through the silanization process on TiO₂ which resulted in a less than optimal bond between TiO₂ and the polyurethane plate.¹⁶ In this study, TiO₂ went through a silanization process with the addition of a silane coupling agent to unite TiO₂ nanoparticles and polyurethane plates. Silanes were compounds whose molecules consist of functional groups bonded to organic and inorganic materials. The inorganic groups on the silane molecule reacted to produce siloxane bonds with inorganic materials from TiO₂ nanoparticles. The organic groups on the silane molecule reacted with the organic material from the polyurethane through covalent bonds. Thus it formed strong organo-functional groups between TiO₂ nanoparticles and polyurethane. Silane acted as an intermediary that binds organic materials with inorganic materials between the polyurethane plate polymer and TiO₂ material.¹¹ The silanization process formed a water-repellent bond at the interface between the two materials, because one of the factors affecting adhesion was the transfer of water to the hydrophilic surface. Water-repellent bond was a bond that had hydrophobic properties in which the surface of the polyurethane plate became more resistant to water absorption.¹⁹

Limitations

- It used the anatase type of TiO₂ so the validity of the same result will be obtained when using the same type of TiO₂.
- The highest TiO₂ concentration was 4% so it required testing using a higher TiO₂ concentration.

- The coating technique used the slip casting method, so the effectiveness of the antifungal ability correlated with the coating method. For this reason, it required another coating technique test related to its anti-fungal ability.
- The study using polyurethane plates was carried out in controlled room temperature, controlled humidity and artificial ultraviolet light so that it may have different results when used in the open space.

CONCLUSION

Based on the above research results, it was concluded that the addition of TiO₂ nanoparticles at concentrations of 1%, 2%, 3% and 4% could reduce the number of *Candida albicans* colonies on polyurethane plates. The concentration that reduced the highest number of *Candida albicans* was the addition of 4% concentration of TiO₂ nanoparticles which was carried out by adding TiO₂ by coating.

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Ethical approval: After the research code of ethics team from the Faculty of Dentistry of Gadjah Mada University carefully studied the proposed research design, this research was declared to meet the requirements or ethically feasible. However, due to the conditions of the COVID-19 pandemic when the research was taking place, a letter of ethics was issued after the conditions were declared safe by the Indonesian Government

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