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THE EFFECT OF ZINC OXIDE NANOPARTICLE CONCENTRATION AS A COATING FOR HEAT CURED ACRYLIC RESIN ON *Candida Albicans* GROWTH

I Gede Krisna Merta Yoga[1], Dyah Irnawati[2], Harsini[2], Desak Nyoman Ari Susanti[3], Dik Megaputri Handayani[4], Louise Cinthia Hutomo[5]

[1.]Dental Material Division, Undergraduate Program in Dentistry and Dentistry Profession, Faculty of Medicine, Udayana University, Bali

[2.]Department of Dental Biomaterials, Faculty of Dentistry, Gadjah Mada University, Yogyakarta

[3.]Head of the Dental Material Division of the Undergraduate Program in Dentistry and Dentistry Profession, Faculty of Medicine, Udayana University, Bali

[4]Periodontics Division, Undergraduate Program in Dentistry and Dentistry Profession, Faculty of Medicine, Udayana University, Bali

[5]Orthodontic Division, Undergraduate Program in Dentistry and Dentistry Profession, Faculty of Medicine, Udayana University, Bali

Corresponding Author: mertayoga@unud.ac.id

ABSTRACT

Introduction The most commonly used polymer material as a denture base is heat cured acrylic resin because it has high strength, accuracy, and aesthetics. However, heat cured acrylic resin has a disadvantage in biological properties, namely it does not have antimicrobial properties. **Objective** to test the effect of ZnO nanoparticle concentration as a heat-cured acrylic resin coating on the growth of *C. albicans*. **Methods** 24 acrylic resin samples were required in this study, measuring 13mm x 13mm x 2mm. The acrylic resin was coated with 3-(trimethoxysilyl)propyl methacrylate) and then coated with ZnO nanoparticles with varying concentrations of 2.5%, 5%, and 7.5% in ethanol. The ZnO nanoparticle coating was applied using the dip coating method. Samples were made in groups I, II, III, and IV. The samples were then tested for *C. albicans* growth. The data obtained were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test. **Results** showed that the mean and standard deviation of *C. albicans* growth after contact with acrylic resin coated with ZnO nanoparticles groups I to IV were 2.00±0.89; 0.83±0.75; 0.00±0.00; 0.00±0.00. The Kruskal-Wallis test results showed a significance value of 0.002, which means that the concentration of ZnO nanoparticles as a coating had a significant effect on the growth of *C. albicans* on heat cured acrylic resin ($p<0.05$). **Conclusion** of this study is that the addition of zinc oxide nanoparticles as a coating on acrylic resin can reduce the growth of *C. albicans* statistically significantly.

Keywords: *C. albicans*, ZnO nanoparticles, heat-cured acrylic resin

INTRODUCTION

Prevalence of tooth loss based on reports RISKESDAS in 2018 was 19%, this prevalence continues to increase, especially in those aged 65 years and above (30.6%)[(1)] Tooth loss can result in reduced ability to perform chewing and speaking activities and can affect a person's aesthetics. The condition of tooth loss will certainly encourage a person to make dentures to restore the function of the teeth.lost[(2)] Dentures can be broadly divided into fixed dentures and removable dentures, but the use of removable dentures is more widely chosen by the public compared to fixed dentures. Removable dentures have a component in the form of a base plate or what is commonly called a base plate or denture base. The denture base is the part of the denture that comes into direct contact with the oral mucosa. Heat cured acrylic resin is often used as a base material because it has high strength, accuracy, and aesthetics[(2)] Removable dentures have a component in the form of a base plate or what is commonly called a base plate or denture base. Base Dentures are part of the denture that is in direct contact with

the oral mucosa. The requirements for a denture base are good aesthetics because it has a color that matches the surrounding tissue, is a thermal conductor, easy to clean, easy to repair and economical price.[3] The polymer material most often used as a denture base is heat cured acrylic resin, however, heat cured acrylic resin has a weakness in its biological properties, namely it does not have antimicrobial properties[3] The antimicrobial properties of acrylic resins lead to high levels of adhesion and proliferation of microbes such as C. albicans, which can trigger denture stomatitis. Denture stomatitis is frequently found in patients using acrylic-based dentures, with a prevalence reaching 72%[4] Denture stomatitis can be prevented by optimally cleaning the acrylic resin denture base, but this is highly dependent on patient cooperation, especially in elderly patients[5] Modification of heat-cured acrylic resin with materials with antimicrobial properties can be achieved using a blending (filler) or coating method. Blending methods can alter the chemical structure of the acrylic, thereby risking reduced mechanical properties and color stability[2] Acrylic resin modification can be achieved with materials with antimicrobial properties, such as titanium oxide, aluminum oxide, or ZnO nanoparticles[6] ZnO nanoparticles also possess optical, electrical, and photocatalytic properties, low toxicity, and high UV absorption, making them excellent candidates for biomedical applications. ZnO nanoparticles are naturally known to possess strong antimicrobial properties[7] Modification of heat cured acrylic resin with materials that have antimicrobial properties can be done using the dip coating method. The advantages of the dip-coating technique include being able to close micro pores that occur due to the acrylic resin polymerization process[8]

Yoga, et al conducted research by coating heat-hardened acrylic resin with ZnO nanoparticles at concentrations of 2.5%, 5%, and 7.5% showing zinc oxide nanoparticles can reduce surface roughness and are resistant to surface abrasion[9] Kamonkhantikul, et al (2017) research mixing polymethylmethacrylate with silanized ZnO nanoparticles with concentrations of 1.25%, 2.5% and 5% showed a greater reduction in C. albicans. The best antimicrobial properties were found at a ZnO concentration of 2.5%, but ZnO as a filler of acrylic resin has the disadvantage of particle agglomeration which can cause a reduction in physical, antimicrobial, and transparency properties of acrylic resin[10]

Research by adding heat-cured acrylic resin with ZnO nanoparticles at concentrations of 2.5%, 5%, and 7.5% showed an increase in the antimicrobial properties of *C. albicans*, with the best antimicrobial properties at a concentration of 7.5% of ZnO nanoparticles[11] The aim of this research was to determine the effect of the concentration of ZnO nanoparticles as a coating for heat-cured acrylic resin on the growth of *C. albicans*.

MATERIALS AND METHODS

The research was conducted after obtaining research ethics approval from the ethics committee of the Faculty of Dentistry, Gadjah Mada University No. 43/UN1/KEP/FKG-RSGM/EC/2023. This research was conducted at the Integrated Research Laboratory of the Faculty of Dentistry, Gadjah Mada University.

Research materials and tools

The main materials and tools of this research are heat-cured acrylic resin, ZnO nanoparticles, 3-(trimethoxysilyl)propyl methacrylate, *C. albicans* fungal culture (ATCC 10231), Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB), BHI (Sigma Aldrich, USA), PBS (pH 7.4). *10), test tubes, loops, incubators*.

Preparation of acrylic resin samples

Making the sample mold

The sample mold was made from white plaster and stone plaster in a cuvette. A trapezoidal acrylic mold measuring 70 mm x 70 mm x 2 mm was embedded in the plaster mixture in the lower cuvette and allowed to set. The surface of the plaster and acrylic mold was coated with CMS, then the upper cuvette was installed, filled, covered with plaster mixture, and allowed to set. The cuvette was opened, and the acrylic mold was removed from the cuvette.

Making the arylic samples

Acrylic resin samples (ADM, England Tricodent) were made with a size of 13mm x 13mm x 2mm as many as 24 pieces. Acrylic resin was processed according to the manufacturer's specifications with a ratio of 22 g powder and 10 ml acrylic resin liquid. Acrylic resin powder and acrylic resin liquid in a stellon pot were manipulated until they reached the dough phase and then put into a cuvette. Acrylic samples in the cuvette were heat cured using a curing unit (Leleux Polypol Junior, Netherlands) at a temperature of 74 ^{o}C for 1 hour and 90 ^{o}C for 30 minutes. Next, the acrylic resin samples were cleaned, the excess was removed using a stone bur with a low-speed rotary instrument, and the dimensions were measured using sliding calipers (Mitutoyo, Japan).

Coating of zinc oxide nanoparticles on acrylic resin

Acrylic resin was coated with ZnO nanoparticles (Sigma Aldrich, USA) with varying concentrations of 2.5%, 5%,

and 7.5% in ethanol (Table 1). ZnO nanoparticle powder was weighed using a digital scale at 2.5 grams, 5 grams, and 7.5 grams. Ethanol as a solvent was measured using a measuring cup with a volume of 100 ml, then poured into a beaker glass and added with ZnO nanoparticle powder. After that, the mixture was stirred using a magnetic stirrer for 30 seconds until a homogeneous solution was obtained. Silane coupling agent (3-methacryloxypropyl trimethoxysilanes) as a coupling agent was applied to acrylic resin samples of groups II, III, and IV, then dried in the open air for 10 minutes. ZnO nanoparticle coating was applied using the dip coating method. The acrylic resin sample was placed in a petri dish, then a ZnO suspension in ethanol was poured over the sample until the entire sample was submerged (Figure 1). The sample was transferred into another petri dish, then dried in an oven at 70^{o}C for 10 minutes and then stored in a closed container.

Table 1. Coating Group Division

Group (%ZnO)	Coating Material (ZnO nanoparticles in ethanol) (%w/v)
I (0%)	0%
II (2%)	2.5%
III (5%)	5%
IV (7.5%)	7.5%

Figure 1. Coating acrylic resin samples with ZnO suspension in ethanol.

***Candida albicans* Growth Test**

Acrylic resin samples were sterilized using UV sterilization. One loop of *C. albicans* was placed in 5 ml of Sabouraud's broth media, incubated for 24 hours at 37 °C. The *C. albicans* that had been cultured in the media were then harvested and placed in a 10 ml standard McFarlan 0.5 Sabouraud's broth media into a test tube along with the acrylic resin sample. After that, it was incubated for 24 hours at 37 ^{o}C. *Candida albicans* that grew on the acrylic resin sample were threshed with a vortex mixer for 60 seconds. The acrylic resin sample was then taken from the test tube. 0.1 ml of *C. albicans* that was released in the test tube was taken using a micropipette and then placed in Sabouraud's dextrose agar, incubated for 24 hours at 37 °C. Then *C. albicans* which has been diluted to 10-5 and the number of colonies (CFU/ml) is counted[(12)]

RESULTS

The growth of *C. albicans* from each group can be determined by conducting a *C. albicans* growth test and calculating the number of *C. albicans* colonies. The mean value and standard deviation of *C. albicans* growth after contact with acrylic resin coated with ZnO nanoparticles can be seen in Table 2.

Table 2. Mean values and standard deviations of *C. albicans* concentration after contact with acrylic resin coated with ZnO nanoparticles.

Group (ZnO Concentration)	n	*C. albicans* concentration (106 CFU/mL)
I (0%)	6	2.00 ± 0.89
II (2.5%)	6	0.83 ± 0.75
III (5%)	6	0.00 ± 0.00
IV (7.5%)	6	0.00 ± 0.00

The highest average growth of *C. albicans* was in group I (0%), while the lowest average growth of *C. albicans* was in group III (5%) and group IV (7.5%), but the highest standard deviation was in group II (2.5%).

Table 3. Kruskal-Wallis test results of *C. albicans* concentration after contact with acrylic resin coated with ZnO nanoparticles.

	Results
Kruskal-Wallis	17,895
df	3
Asymp. Sig.	0.001

The results of the Kruskal-Wallis test showed a significance value of 0.001, which means that the concentration of ZnO nanoparticles as a coating had a significant effect on the growth of *C. albicans* on heat-cured acrylic resin ($p<0.05$).

Table 4. Mann-Whitney test results of *C. albicans* concentration after contact with acrylic resin coated with ZnO nanoparticles.

Group	I (0%)	II (2.5%)	III (5%)	IV (7.5%)
I (0%)	-	6.00*	0.00*	0.00*
II (2.5%)	-	-	6.00*	6.00*
III (5%)	-	-	-	18.00
IV (7.5%)	-	-	-	-

The results of the Mann-Whitney analysis showed that there was a significant difference in the average growth of *C.*

albicans between all groups, except group 3 (5%) and group 4 (7.5%).

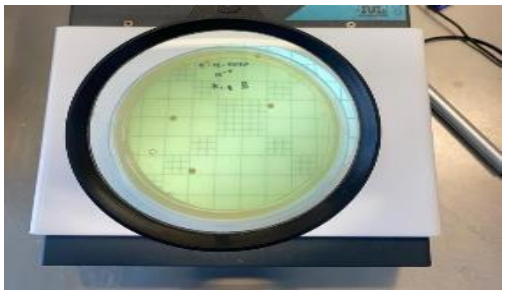

Figure 2. Colonies *C. albicans's* growth test

DISCUSSION

The antibacterial activity of ZnO nanoparticles is influenced by size, concentration, and surface modification[13] A study has been conducted on the effect of ZnO nanoparticle concentration on the growth of *C. albicans* on heat-cured acrylic resin, showing that there is a difference in the average growth between groups. This is because high concentrations will affect antibacterial activity. The effect of ZnO nanoparticle concentration related to the formation of H2O2 and high oxygen concentrations on the surface can maintain better antibacterial activity.[14] The results of Djearamane's (2022) study stated that the turbidity and number of colonies in the study showed an inhibitory effect on *C. albicans* with increasing concentrations (5, 10, 20, 40, 80, and 160 µg/mL) of ZnO nanoparticles which produced an antifungal effect. The growth inhibitory effect depends on the concentration of ZnO nanoparticles shown against *C. albicans*, there is a gradual increase in the inhibition zone and a decrease in the number of cells when the concentration of ZnO nanoparticles increases[15] The growth inhibitory effect is caused by the main mechanism, namely the formation of ROS and the release of metal ions from ZnO nanoparticles due to the interaction of ZnO nanoparticles with cell membranes, causing inhibition of cell wall synthesis, enzyme activity, DNA damage, inactivation of protein synthesis, and modification of essential protein structures. In addition to membrane dysfunction caused by the accumulation of positively charged Zn2+ from the dissolution of ZnO nanoparticles on the surface of the cell membrane, the internalization of ZnO nanoparticles disrupts microbial metabolic activity, which ultimately leads to microbial cell death[14,15]

The antimicrobial mechanism of ZnO is basically related to its photocatalytic properties. The mechanism of ZnO's antimicrobial properties is through the first active release of Zn2+ ions from ZnO nanoparticles. Zn2+ ions can inhibit active transport, amino acid metabolism, and enzymatic interference. Dissolved ions can damage bacterial cells and membranes, increasing the possibility of ZnO nanoparticle penetration into cells. ZnO nanoparticles with positive zeta potential can destroy the cytomembrane of gram-negative E. coli. Second, the production of reactive oxygen species (ROS) is considered the main mechanism of the excellent antimicrobial activity of ZnO nanoparticles against Gram-positive and Gram-negative bacteria. The formation of ROS by ZnO nanoparticles can inhibit the growth and reproduction of microorganisms. ROS can interfere with vital processes such as cellular respiration, protein synthesis, and DNA replication, thereby preventing microbial development[16] Third, direct attachment between ZnO and microbes through electrostatic interactions that destroy the integrity of bacterial cells followed by damage to cell membranes due to ZnO infiltration[17]

Surface roughness is one of the important factors affecting microbial growth. [18] The initial microbial growth generally begins in the pores on the rough surface which can provide protection against shear forces and provide more time for microbes to attach irreversibly to the substrate. The addition of ZnO nanoparticles to hot-polymerized acrylic resin can fill the pores on the surface of the acrylic resin, thereby smoothing the surface of the acrylic resin and replacing the hydrophilic polymer matrix chains, thus changing the substrate to be hydrophobic[19,20]

CONCLUSION

Based on research results The addition of ZnO nanoparticle concentration as a heat-cured acrylic resin coating can reduce the growth of *C. albicans*.

6. Conflict of Interest

The authors declare no conflict of interest.

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