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The effect of ZnO nanoparticles addition to PMMA on surface contact angle and adhesion of *Candida albicans*

O efeito da adição de nanopartículas de ZnO ao PMMA no ângulo de contato superficial e na adesão de *Candida albicans*

Dedi FARDIAZ[1], **Dyah IRNAWATI**[2], **NURYONO**[3]

1 - Universitas Gadjah Mada, Magister Dental Science Study Program, Faculty of Dentistry, Yogyakarta, Indonesia.
2 - Universitas Gadjah Mada, Department of Dental Biomaterials, Faculty of Dentistry, Yogyakarta, Indonesia.
3 - Universitas Gadjah Mada, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Yogyakarta, Indonesia.

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ABSTRACT

Background: Heat polymerized polymethyl methacrylate resin (PMMA) is a porous denture material prone to microbial attachment due to water absorption. *Candida albicans* (*C. albicans*) in PMMA can cause denture stomatitis. Zinc Oxide Nanoparticles (ZnO NPs) possess antimicrobial properties. **Objectives:** This study aimed to investigate the effect of adding various concentrations of ZnO NPs to PMMA on surface contact angle and *C. albicans* adhesion. **Material and Methods:** The PMMA samples (10x10x2mm) were prepared with ZnO NPs concentrations of 0%, 2.5%, 5%, and 7.5% (n=4). The samples were soaked in distilled water 48 hours at 37°C. The contact angle test was performed using drop-profile analysis. *C. albicans* adhesion test was evaluated through the *3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide* (MTT assay) for cell viability. The distribution profile of Zn element was observed using SEM-EDX. The release of Zn^{2+} was tested by analyzing the aqueous solution after sample immersion AAS. **Results:** The mean contact angles (°): 82.96±4.20; 82.36±0.66; 86.25±4.49; and 92.82±5.40. Results of *post-hoc* LSD showing differences only in the 7.5% ZnO NPs group. The mean viability *C. albicans* (%): 2.27±0.80; 1.55±0.50; 1.45±0.33; and 1.43±0.12. There was a tendency of decreasing means with increasing concentrations. However, this trend was not consistent with *one-way* ANOVA, which indicated no significant differences among the treatment groups. SEM-EDX demonstrated ZnO NPs distribution within PMMA's matrix. AAS results revealed no Zn^{2+} presence in distilled water. **Conclusion:** In conclusion, the addition of ZnO NPs to PMMA results in an increased contact angle, while exhibiting a statistically non-significant reduction in *C. albicans* adhesion.

KEYWORDS

C.albicans; Contact angle; Nanoparticles; PMMA; ZnO.

RESUMO

Introdução: A resina de polimetilmetacrilato polimerizada termicamente (PMMA) é um material poroso para próteses propenso à fixação microbiana devido à absorção de água. *Candida albicans* (*C. albicans*) no PMMA pode causar estomatite protética. Nanopartículas de óxido de zinco (NPs de ZnO) possuem propriedades antimicrobianas. **Objetivos:** Este estudo teve como objetivo investigar o efeito da adição de várias concentrações de NPs de ZnO ao PMMA no ângulo de contato da superfície e na adesão de *C. albicans*. **Material e Métodos:** As amostras de PMMA (10x10x2mm) foram preparadas com concentrações de NPs de ZnO de 0%, 2,5%, 5% e 7,5% (n=4). As amostras foram embebidas em água destilada por 48 horas a 37ºC. O teste do ângulo de contato foi realizado utilizando análise de perfil de gota. O teste de adesão de *C. albicans* foi avaliado através do brometo de 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazólio (ensaio MTT) para viabilidade celular. O perfil de distribuição do elemento Zn foi observado utilizando MEV-EDS. A liberação de Zn^{2+} foi testada analisando a solução aquosa, após imersão da amostra, por AAS. **Resultados:** As médias dos ângulos de contato (º): 82,96±4,20; 82,36±0,66; 86,25±4,49; e

92,82±5,40. Resultados do LSD post-hoc mostrou diferenças apenas no grupo de 7,5% de NPs de ZnO. A viabilidade média de *C. albicans* (%): 2,27±0,80; 1,55±0,50; 1,45±0,33; e 1,43±0,12. Houve tendência de diminuição das médias com o aumento das concentrações. No entanto, essa tendência não foi consistente com a ANOVA unidirecional, que não indicou diferenças significativas entre os grupos de tratamento. MEV-EDS demonstrou a distribuição de NPs de ZnO dentro da matriz de PMMA. Os resultados da AAS não revelaram presença de Zn^{2+} na água destilada. **Conclusão:** Em conclusão, a adição de NPs de ZnO ao PMMA resulta em um aumento do ângulo de contato, ao mesmo tempo que exibe uma redução estatisticamente não significativa na adesão de *C. albicans*.

PALAVRAS-CHAVE

C. albicans; Ângulo de contato; Nanopartículas; PMMA; ZnO.

INTRODUCTION

Tooth loss become an oral health problem in Indonesia with the highest prevalence of sufferers among people aged 45-65 years. The basic health research at 2018 stated that the prevalence of patients in the age range of 45-54 years was 23.6%; 55-64 years was 29%; and over 65 years was 30%. Treatment in the form of making dentures is needed to overcome problems related to tooth loss [1]. Dentures are divided into fixed and removable dentures, and removable dentures is preferred by majority of patients [2]. A removable denture consists of base structure, clasps and artificial teeth. The denture base is the part of the denture that rests on the supporting tissue, which artificial teeth and clasps are attached [3].

Polymer dentures are the most commonly used material which is easy to manipulate, lightweight, stable in the oral cavity, and more esthetics than the other materials [4]. Polymer denture bases are classified based on their polymerization method, including cold polymerized acrylic resin, light polymerized acrylic resin and heat polymerized acrylic resin. Polymethyl methacrylate (PMMA) is a types of acrylic resin that often used due to its good biocompatibility, color and texture resembling gingiva, good dimensional stability, relatively low water absorption and ease of manipulation [3].

Aside from its advantages, PMMA has a porous surface and tends to absorb water through an imbibition process so that it is easily attached by microorganisms [5]. According to research by Gad et al. [6] porous surfaces can become sites for adhesion and colonization of microorganism thereby affecting the health of the oral cavity, and triggering denture stomatitis. *Candida albicans* is capable of adhering to the PMMA surface through the formation of biofilm [7]. The formation of biofilm and bacterial growth on the PMMA surface that is already in the oral cavity, can affect the general condition of denture users [8]. Microorganism infection in the oral cavity can cause tooth decay, periodontal disease, and denture stomatitis due to *Candida* growth [9]. Early infection process *of C. albicans* in denture wearers is the adhesion of the cell wall layer to the surface through a combination of specific (ligand and receptor) and non-specific mechanisms (electrostatic poles and *van der Waals* bonds*)*, negatively charged *Candida* cell surface and positively charged acrylic resin surface*,* mutual attraction occurs [10].

PMMA has a surface contact angle of 65° which indicates a hydrophilic surface characteristic [11]. The hydrophilic surface has a high surface energy causing extensive surface wettability which makes it easier for the accumulation of bacterial plaque [12]. The study of microbial adherence involves a multifaceted analysis, encompassing crucial parameters such as surface contact angle, which influences the interaction dynamics between microorganisms and surfaces. Investigating the structural morphology of materials provides valuable insights into the substrate's topographical characteristics and its impact on microbial adhesion [13]. The wide surface wettability causes a water layer to form immediately in the contact area between the support and the physiological media so that the extracellular matrix can be properly distributed and the bacterial adhesion becomes strong [14].

A research study has been conducted to impart antimicrobial properties to polymers, including the addition of metal oxides such as ZrO_2, TiO_2, SiO_2, AI_2O_2 and ZnO [15,16]. The integration of metal oxides into PMMA to serve as antimicrobial agents involves of fillers. Zinc Oxide Nanoparticles (ZnO NPs) represent a specific category of materials capable of enhancing the strength, tensile properties, and imparting antimicrobial effects to denture bases when incorporated into the PMMA matrix [17]. The size, shape, properties and bonds formed between the ZnO NPs and the PMMA polymer matrix can improve the mechanical properties and antimicrobial effect. According to Hammani *et al*. ZnO NPs can bind to the (-COOR) group of the PMMA polymer during polymerization by forming hydrogen bonds between the carbonyl group (-C=O) and the hydroxyl group (-OH) [18]. These bonds fill spaces between linear macromolecular chains of PMMA polymer particles thereby reducing porosity [19].

Several studies explore the potential antimicrobial effect of ZnO NPs. Abdelghafar *et al*. stated that ZnO NPs has antibiofilm activity on *Staphylococcus aureus* at a concentration of 3-5% [20] . Research by Esposti *et al*. stated that 0.1 mg/μl ZnO NPs had the growth inhibition of *Streptococcus mutans* [21]. Other than that, the latest study on the potential of Zn as antimicrobial in PPMA found that Zn is effective against *Escherichia coli* and *S. aureus* [22]. Beside the use of ZnO to increase the strength of PPMA, the study assessing the effect of ZnO nanoparticles as additional material for PMMA against microbes especially for *C. albicans* is limited [23]. Therefore, this study was performed to investigate the effect of various concentrations of ZnO NPs to PMMA on surface contact angle and *C. albicans* adhesion.

MATERIALS AND METHODS

This study is a true experimental study conducted at the Integrated Research Laboratory of Faculty of Dentistry Universitas Gadjah Mada, Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada and Laboratorium Sentra Ilmu Hayati Universitas Brawijaya. This study received ethical clearance from the Ethical Commission of the Faculty of Dentistry - Universitas Gadjah Mada (UGM) under letter number 0057/KKEP/FKG-UGM/EC/2022.

Samples preparation

The concentration of ZnO NPs added in heat cured PMMA were 0%, 2.5%, 5%, and 7.5% based on previous research by Cierech et al. [23]. The composition of each gorup was presented in Table I. The samples with dimension 10 x 10 x 2 mm were made and the number of samples for each group were 4 samples.

The PMMA (ADM, England) and ZnO NPs (Sigma-Aldrich, USA) powder was put in the stellon pot and mixed with crownmess until homogeneous. The PMMA monomer liquid was added into the stellon pot, stirred, then closed until it reached the dough phase. The dough was put into the cuvette, pressed, then polymerized in curing units (Leleux Polypol Junior, Netherlands) at 74°C for 1 hour and 90°C for 30 minutes. After that, the samples were cooled to room temperature and the samples were deflasked.

The PMMA profile analysis and distribution of Zn

The Zn elements in the PMMA surface were observed using Scanning Electron Microscope Energy Dispersive X-ray (SEM-EDX) (JED-2300, USA). The samples were tested under the following conditions: 6510 (LA), volts: 15.00 kV, and pixels:1024 x 768.

The form of mass and atomic percentages were observed.

The Zn ion release test

The Zn^{2+} ions release were analyzed using Atom Absorption Spectrophotometer (AAS) (Perkin- Elmer 3110, USA). The samples were immersed in 10 ml distilled water for 48 hours at 37°C, then the sample solutions were analyzed

Table I - Study groups and material composition of PMMA and ZnO NPS

Groups	PMMA		ZnO NPS powder (grams)	Total weight of acrylic (grams)	Zn Concentration (%)
	Polymer (grams)	Monomers (mL)			
1	20	10	0	30	0
2	20	10	0.769	30.769	2.5
3	20	10	1.578	31.578	5
4	20	10	2.432	32.432	7.5

using AAS to detect the Zn^{2+} ions released. The results of ZnO NP analysis on standard blanks serve as the starting point for the results of the sample solution to be tested and the concentrations were calculated in ppm.

Contact angle test

The contact angle test was conducted by drop-profile analysis technique based on Yulianto and Rinastiti method [24]. The sample was placed in front of the camera (Nikon, Japan), then the distilled water was dripped using a 6 microliter micropipette perpendicular to the sample surface. After 10 seconds, the equilibrium between the liquid and the sample surface. The image was taken with the camera and transferred to the ImageJ software on the computer to identify the line interaction between the liquid surface and the sample surface, indicated by the formation of an angle where the magnitude can be measured in degrees (°).

C. albicans adhesion test

The *C. albicans* adhesion test consisted of four groups, with different concentrations, four independent assays were conducted for each group. *C. albicans* adhesion test followed the procedure described by Ghosemi et al. [25]. *C. albicans* suspension was prepared at a concentration of 10^6 CFU/mL in potato dextrose broth medium. Adhesion of *C. albicans* on acrylic resin samples was carried out by immersing the samples in a suspension of *C. albicans* then incubated at 30°C for 24 hours. The release of *C. albicans* into Saboraud broth was carried out by placing the sample in a test tube containing 10 mL of Saboraud broth and then vibrating it with thermolyne for 30 seconds. The released *C. albicans* was added to the potato dextrose broth. Then it was put into the incubator shaker at 30°C for 60 minutes. The substrate was extracted using *ethyl acetate* and thickened using *a rotary evaporator.* The test was continued with the MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) on a 96-well microplate and quantitative analysis was conducted to determine cell viablity as a percentage (%).

Statistical analysis

Mean and standard deviation of all data were calculated, except SEM EDX data. The contact angle and *C. albicans* viability were analyzed statistically using One Way ANOVA test with Least Significant Difference (LSD) post hoc test with 95% confidence level.

RESULTS

The contact angle test

Analysis of the aquadest contact angle on the PMMA surface used the *sessile drop test* method, and the results of the observations as a contact angle measurement is presented in Figure 1. The highest mean contact angle was found in the 7.5% ZnO NPs group, while the lowest contact angle value was in the 2.5% ZnO NPs group (Figure 2). The results of the contact angle test

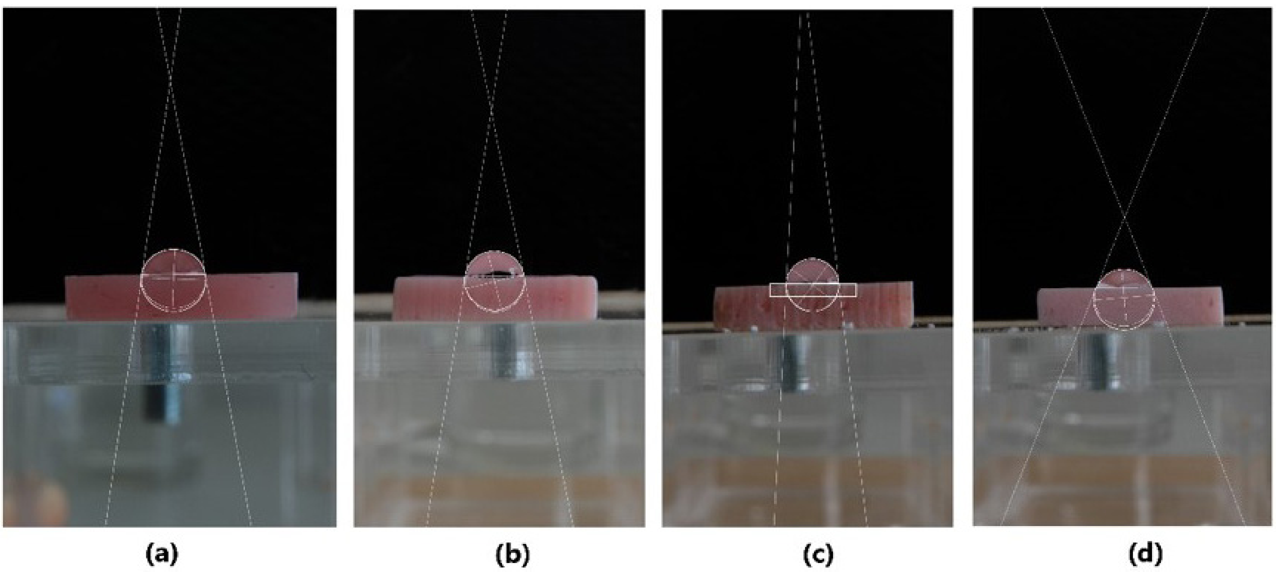

Figure 1. (a) PMMA (b) PMMA + 2.5% ZnO NPs (c) PMMA + 5% ZnO NPs (d) PMMA + 7.5% ZnO NPs.

Table II - LSD post hoc test results $_{0.05}$ contact angle test

Groups	1 0% ZnO NPs	2 2.5% ZnO NPs	3 5% ZnO NPs	4 7.5% ZnO NPs
1 (0% ZnONPs)	-	0.60	3.28	9.86 *
2 (2.5% ZnO NPs)	-	-	3.88	10.46 *
3 (5% ZnONPs)	-	-	-	6.57 *
4 (7.5% ZnO NPs)	-	-	-	-

Note: * = significant difference.

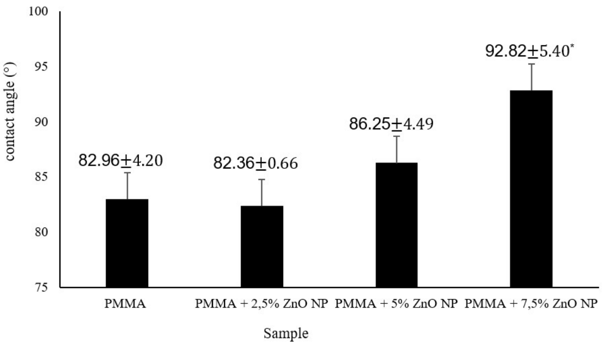

Figure 2. Mean and standard deviation of the contact angle test (°). * Mean significan different with other groups.

Table III - The mean value and standard deviation of *C. albicans* cell viability (%)

Group	Mean and standard deviation (%)	p-Value
1 (0% ZnONPs)	2.27±0.80	0.127
2 (2.5% ZnO NPs)	1.55±0.50	
3 (5% ZnONPs)	1.45±0.33	
4 (7.5% ZnO NPs)	1.43±0.12	

Table IV - Percentage of mass and atoms of Zn elements in PMMA (%)

Groups	Zn period	Zn atoms
1 (0% ZnO NPs)	0.00	0.00
2 (2.5% ZnO NPs)	3.24	0.67
3 (5% ZnO NPs)	3.31	0.68
4 (7.5% ZnO NPs)	19.69	4.67

showed the contact angle of aquadest tend to increase on PMMA as the ZnO NP concentration increased.

The results of the One-Way ANOVA showed the F value 5.451 and the significance of the test results 0.013, meaning that there was a mean difference between the four groups of ZnO NPs addition to the contact angle of water on the PMMA surface ($p<0.05$). The value obtained from the One-Way ANOVA meets the requirements for a post-hoc test Least Significant Difference $(LSD)_{0.05}$. The LSD test results have been carried out with the results presented in Table II. There is a significant difference of contact angle between 7.5% ZnO NPs group with other groups ($p<0.05$).

Adhesion test of *C. albicans*

Candida albicans adhesion test was carried out by calculating the percentage of cell viability presented in Table III. The average value of *C. albicans* cell viability in PMMA showed that with increasing ZnO NPs concentration there was a tendency to decrease the number of cell viability. The results of the one-way ANOVA test can be seen in Table III. The results of the ANOVA test showed the calculated F value 2.318 and the significance of the test results 0.127 which means that there was no significant effect between groups of ZnO NPs concentrations in PMMA on *C. albicans* cell viability ($p>0.05$).

PMMA profile analysis and distribution of Zn

The results of observations through SEM revealed that there was an interaction between ZnO NPs and PMMA showing a visual difference in the pore profile of the PMMA surface. At a concentration of 7.5% ZnO NPs it can be seen less surface pores compared to other concentrations. The addition of ZNO NP to PMMA produced a denser surface and reduce the presence of pores in PMMA. The observation results are presented in Figure 3.

The elemental content of Zn in PMMA data were presented in Table IV. The results of the analysis obtained important information about changes in the mass and atoms of Zn measured. However, when the ZnO concentration was

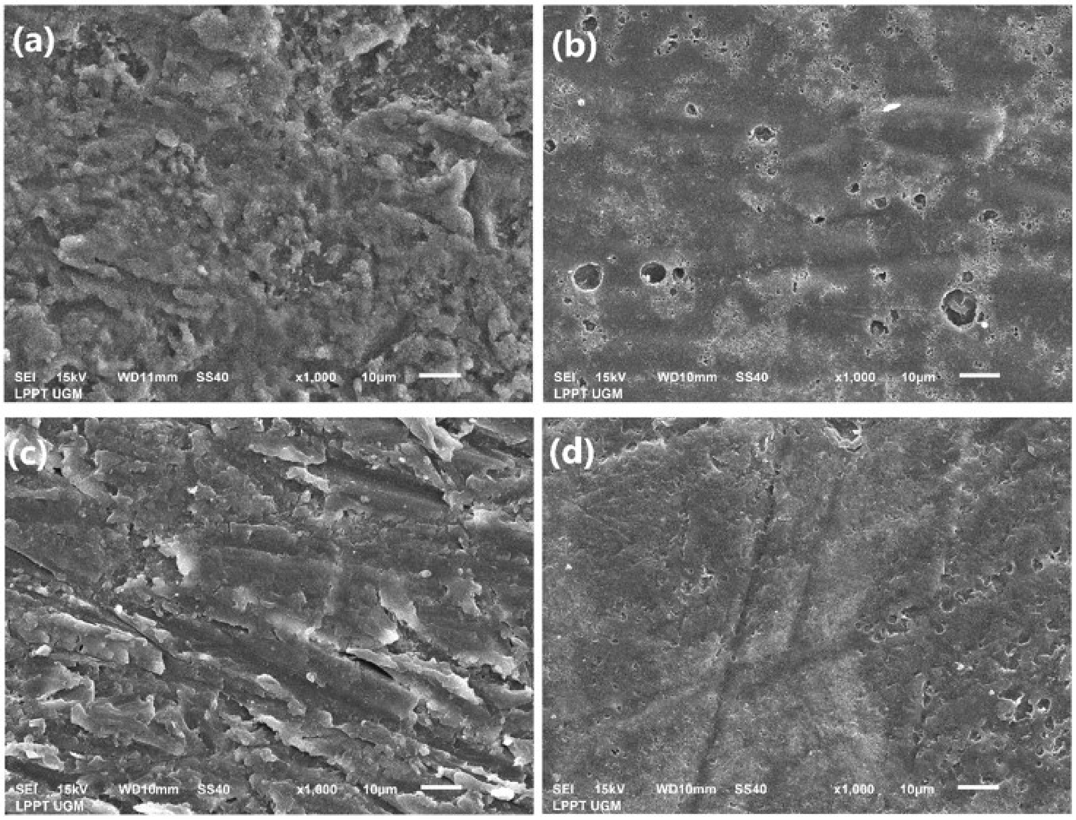

Figure 3. Analysis SEM on PMMA. (a). 0% ZnO NPs (b). 2.5% ZnO NPs (c). 5% ZnO NPs(d). 7.5% ZnO NPs.

increased to 2.5%, 5%, and 7.5% there was a significant increase in the mass and atomic number of Zn. These results indicate that the higher the concentration of ZnO in PMMA, the more mass and Zn atoms are detected.

The 7.5% ZnO NP group showed a higher mass and atomic number compared to the other groups. This shows that increasing the ZnO concentration has a significant effect on increasing the mass and number of Zn atoms in PMMA matrix. Visual observation through a pattern of dots that form a blue zone is presented in Figure 4. Observations of Zn elements in PMMA show that there are aggregations of particles scattered in the PMMA matrix. The addition of 7.5% ZnO NP to PMMA resulted in a more concentrated Zn distribution pattern. Irregular image patterns indicate that ZnO NP particles tend to gather in certain areas, forming irregular formations.

The calculation of the absorbance value in AAS showed the Zn element released from PMMA. The data were presented in Table V. Based on absorbance value in the sample it shows a substandard value, this indicates that the Zn was not detected in the solution tested.

Table V - The mean value and standard deviation of the absorbance in the AAS test

Groups	Mean and Standard Deviation
1 (0% ZnO NPs)	0.042 ± 0.0092
2 (2.5% ZnO NPs)	0.047 ± 0.014
3 (5% ZnO NPs)	0.048 ± 0.010
4 (7.5% ZnO NPs)	0.049 ± 0.012

Note: Standard 1 (0.1 mg/L) = 0.055.

DISCUSSION

In this study, the addition of ZnO NPs to PMMA affects several aspects including contact angle and *C. albicans* adhesion. The result showed that contact angle tends to increase with the concentration of ZnO NP. One-way ANOVA result indicated a significance level of 0.013, suggesting a significant effect of ZnO NP concentration on the contact angle of distilled water on the PMMA surface ($p < 0.05$). The results of this study are in accordance with the hypothesis which states that there is an effect of the addition of ZnO NP on the contact angle of distilled water on the PMMA surface. An increase in the contact angle indicates a change in surface properties to become more hydrophobic [20]. The increase in hydrophobicity

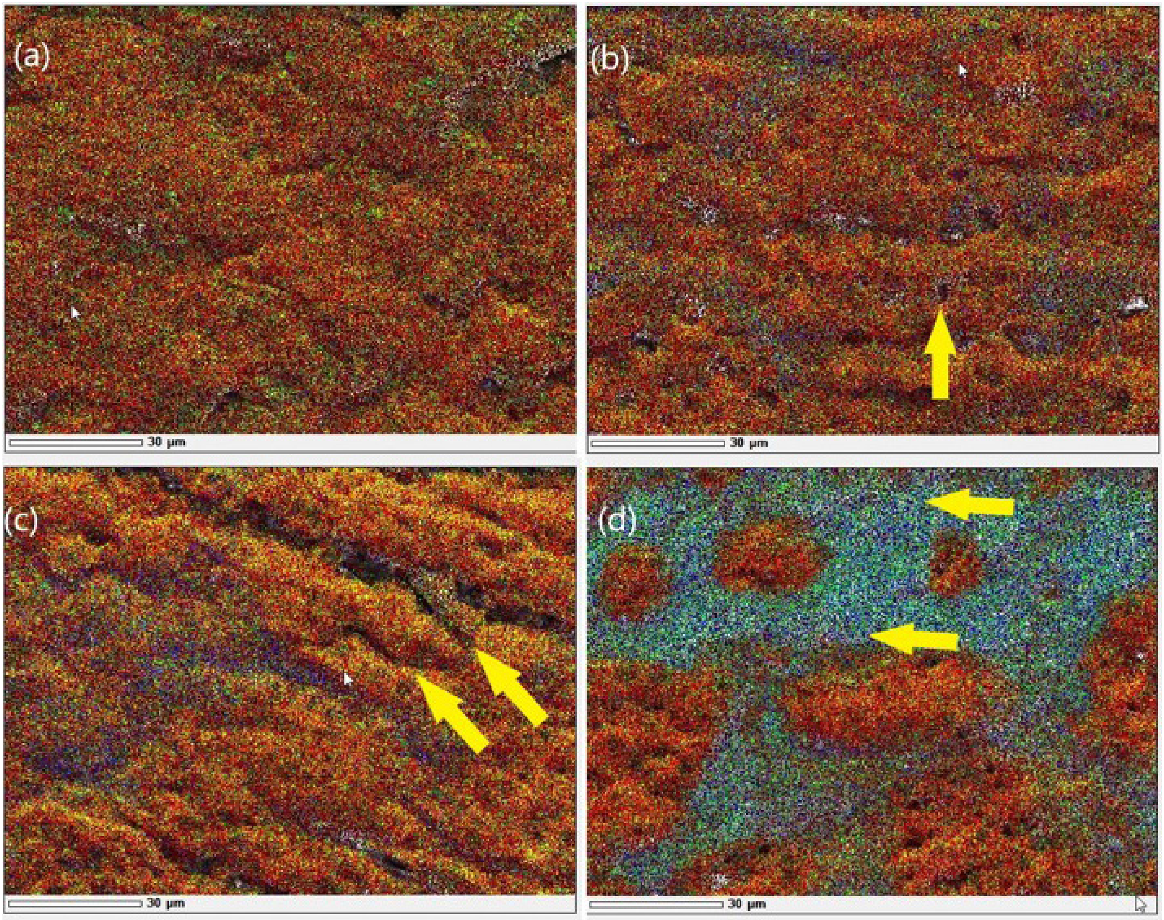

Figure 4. Distribution of Zn in PMMA. (a). PMMA (b). PMMA + 2.5% ZnO NPs (c). PMMA + 5% ZnO NPs (d). PMMA + 7.5% ZnO NPs. Yellow arrow: Zn.

occurs due to the addition of ZnO NPs which may change the morphology and surface properties of the acrylic resin. ZnO particles have hydrophobic properties so that when mixed with acrylic resin, they will form a barrier structure between the resin surface and water or other polar liquids, thereby reducing the interaction between water and the substrate surface [26,27].

Research by Saputra *et al*. stated that ZnO NP has a small particle size so that it can close the matrix gap [28]. The results of research conducted by Shanan *et al*. stated that ZnO NPs can bond with PMMA polymers through covalent bonds, forming a layer that can change the surface properties to become more hydrophobic [27]. This layer is a barrier between PMMA and aquadest. Research conducted by Esposti *et al*. stated that the deposition of microorganisms on PMMA is difficult to occur on surfaces that have low wettability [21]. Hwang *et al*., stated that on a hydrophilic surface, the surface tends to be moist and can provide a good environment for bacterial growth, whereas on a hydrophobic surface it tends to be dry and makes it difficult for bacterial growth so that it can be used to reduce or prevent bacterial growth [29].

C. albicans adhesion test showed that ZnO NP has a tendency to decrease the number of cell viability. ZnO NP 7.5% reduce the cell viability from 2.27% (0% ZnO NP group) to 1.45%. The one-way ANOVA results showed a significance test value of 0.127, indicating that the observed changes did not reach the level of statistical significance. Theoretically, the interaction between ZnO NP particles and *C. albicans* adhesion occurs through several mechanisms, including affect *C. albicans* cell metabolism and cell growth. This can happen due to ZnO NP properties that able to inhibit the activity of the cell growth enzyme, glucose-6-phosphate dehydrogenase [30]. ZnO NP can form a hydrophobic layer on the PMMA surface which can inhibit the interaction between *C. albicans* and PMMA. This is result in the reduction in cell viability due to decrease in cell growth [25].

The SEM analysis indicated Zn elements in the PMMA matrix. The surface of PMMA which was supplemented with ZnO NP looked less porous than that which was not added ZnO NP. This is in line with research conducted by Cierech *et al*. that ZnO NP has a role in filling the assembly gaps in the PMMA matrix, so that the surface is flatter [13]. The distribution of Zn elements visually presented in Figure 3 shows that higher ZnO NP concentration in PMMA, show more blue dotted zones. It can be seen that the blue dots join together to form an irregular formation. This indicates that the ZnO particles are experiencing agglomeration (clustering). The addition of 7.5% ZnO NP to PMMA causes particle aggregation which forms a collection of particles and is not evenly dispersed in the PMMA matrix. Tanase *et al*. stated that particles that have mass and are small in size can easily agglomerate to form irregular formations [31]. The occurrence of particle agglomeration, can cause a decrease in physical properties, antimicrobial properties, and a decrease in the level of transparency. Apip *et al*. stated that the antimicrobial power of ZnO NPs could be inhibited due to aggregation in the PMMA matrix [32].

The Atomic Absorption Spectrophotometer (AAS) test has been carried out in PMMA immersion. The test results showed that Zn^{2+} ions were not detected in the aquadest bath. This indicates that the Zn element was not detected in the tested solution, thereby ruling out the possibility of elemental release causing the formation of gaps or pores in the polymer matrix.

The parameter in understanding the interaction between the surface of the material and microorganisms is through the zeta potential. In this study, modification of the PMMA matrix through the addition of ZnO NP and its effect on *C. albicans* adhesion made it possible for a difference in electrical potential between the surface of the material and the cell wall *of C. albicans.* These differences result in cell adhesion to the surface of the material cannot occur. Zore *et al*. stated that the bacterial cell wall interacts with the surface of the material, zeta potential being an important factor in determining the adhesion or rejection between the two entities [33]. If the zeta potential between the bacterial cell wall and the surface of the material has the same value or is close to zero, then adhesion will occur due to the attractive force between them. However, if the zeta potential has a high value (positive or negative), the particles will repel each other, thereby inhibiting bacterial adhesion and *C. albicans* biofilm growth. In this study, the addition of ZnO NP show a decrease of *C. albicans* viability and expected to give an impactful insight in PMMA synthesis.

There are some limitations related to this study. The study used a relatively small sample size, four replications for each group. A larger sample size could provide more statistically robust results and better represent the denture user population. The samples were soaked in distilled water for 48 hours. This duration may not fully simulate the long-term conditions that dentures are exposed to within the oral cavity. Prolonged immersion times could yield different results. The study's methodology did not consider dynamic factors, such as the movement of the oral cavity and the presence of saliva, which can affect microbial adhesion and surface characteristics of denture materials. The study acknowledges the complex interaction between ZnO NPs and PMMA. The exact mechanisms and influence of various factors need further investigation. The study also did not extensively characterize the ZnO NPs used, including aspects such as particle size, shape, and surface properties. Further study analyzing size and distribution of ZnO nanoparticle is required to obtain the supporing data related the application of ZnO in the heat polymerized acrylic resin. The safety and biocompatibility of ZnO NPs in dental applications are essential, considerations and require further investigation.

On the other hand, this preclinical study provides some potential for the future studies. In this study we only focused solely on the adhesion of *C. albicans.* In real-life situations, the oral cavity harbors various microorganisms, and their interactions with denture materials may differ. This study methods then can be used in the other study involving various microorganisms. The study was conducted in a controlled laboratory setting. Clinical studies involving denture wearers could provide insights into the practical implications of ZnO NP-modified dentures. The study focused on PMMA. Denture materials can vary widely, and the interaction of ZnO NPs with different materials should be explored. This study also can be an initiation to establish studies asses the use of ZnO NP by considering potential variations in denture user populations based on factors like ethnicity, age, and overall health. Further research and fine-tuning of parameters may unlock their full potential in reducing microbial colonization. The complexity of the interaction between ZnO NPs and PMMA has been highlighted. The factors influencing this interaction, such as size, shape, concentration, and environmental conditions, need further exploration. This emphasizes the need for continued research in this area.

CONCLUSIONS

The addition of ZnO NPs resulted in an increased contact angle, indicating improved hydrophobicity. This change in surface properties may reduce water absorption, potentially enhancing the durability of PMMA dentures and minimizing the risk of microbial colonization. While we observed a trend of decreasing *C. albicans* adhesion with increasing concentrations of ZnO NPs, our statistical analysis indicated that this reduction was not significant. However, the non-significant reduction in adhesion is still noteworthy, as it suggests that ZnO NPs could potentially contribute to a decrease in denture stomatitis cases. ZnO NPs are known for their antimicrobial properties. While the study did not show a significant reduction in *C. albicans* adhesion, the antimicrobial characteristics of ZnO NPs remain promising.

Author's Contributions

DF: Conceptualization, Software, Resources, Investigation, Writing – Original Draft Preparation, Visualization. DI: Conceptualization, Metodology, Supervision, Writing – Review & Editing, Project Administration and Funding Acquisition. N: Validation, Data Curation, Formal Analysis, Writing – Review & Editing, Supervision.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions from the Ethical Commission of the Faculty of Dentistry - Universitas Gadjah Mada (UGM) under letter number 0057/KKEP/FKG-UGM/EC/2022.

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Dyah Irnawati
(Corresponding address)
Universitas Gadjah Mada, Department of Dental Biomaterials, Faculty of Dentistry, Yogyakarta, Indonesia.
Email: dyahirnawati_fkg@ugm.ac.id

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