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RESEARCH ARTICLE

The effect of adding zirconium dioxide nanoparticle to acrylic denture base on porosity and candida albicans adhesion

Angeline Antony*, Haryo Mustiko Dipoyono, Titik Ismiyati**✉**

*Specialty Study Program of Prostodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
**Departement of Prosthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
**Jl Denta No 1, Sekip Utara, Yogyakarta, Indonesia; ✉ correspondence: drg_titikis@ugm.ac.id

ABSTRACT

Acrylic resin is the most common fabricated material for denture bases. ZrO_2 nanoparticles can be used as filler to strengthen the physical properties and inhibit the adhesion of acrylic resins. This study aimed to examine the growth of Candida albicans and porosity in reinforced acrylic resin with Zirconium Dioxide (ZrO_2) nanoparticles at 2.5% and 5% a concentration targeted for denture bases application. A laboratory experiment study included twenty-seven disc-shaped samples which were divided into three groups: group I acrylic resin without ZrO_2 nanoparticles (control), group II acrylic resin with 2.5% ZrO_2 nanoparticles, and group III with 5% ZrO_2 nanoparticle acrylic resin. Samples were collected by heat polymerization while porosity observations were done using primo zeizs starr (Nikon YS100) microscope at 100x magnification. Dilution test was implemented to assess Candida albican growth. One-way ANOVA and Post Hoc LSD test statistical analysis were performed to evaluate the data ($p<0.05$). The results indicated that the mean porosity values in the control group of 2.5% and 5% were 37.4 ± 3.5, 15.8 ± 3.3, 8.0 ± 2.0 respectively while the attachment of C. albicans was 0.832 ± 0.083, 0.536 ± 0.098, 0.218 ± 0.083. One-way ANOVA confirmed a significant effect ($p<0.05$). Similarly, Post Hoc LSD test reported significant difference ($p<0.05$). The results showed that acrylic resin reinforced with ZrO_2 nanoparticles for heat-polymerized denture base effectively reduced porosity and C. albicans adhesion. Five percent of ZrO_2 nanoparticle concentration presented greater porosity reduction compared to 2.5% ZrO_2 and non-reinforced acrylic resin. Acrylic resin reinforced with ZrO_2 nanoparticles in 2.5% and 5% concentration is fungistatic.

Keywords: acrylic resin; candida albicans; denture base; porosity; $ZrO_2$2 nanoparticles

INTRODUCTION

Cavities and periodontal disease damage the teeth, cause nutritional intake problems, and is prone to the development of systemic illness. Management of these problems require application of removable dentures.[1] Removable dentures are prostheses that are used to replace missing teeth and can be removed or installed independently by the patient.[2] Application of dentures is intended to improve mastication, speech, aesthetics, and quality of life.[3] Acrylic resins are the most common materials for denture bases since 1930.[4] However, the disadvantages of acrylic resins are its nature as a color absorbing material, its resistance to shear strength and low wear, its shrinkage due to polymerization, the risk of Candida albicans fungus attachment, as well as the existence of residual monomer and porosity.[5]

The acrylic resin porosity captures food debris, which increases the number of microorganisms in the oral cavity. Candida albicans are among frequently identified organisms.[6] Gas porosity occurs inside the hot polymerized acrylic resin while contraction porosity can be identified from the resin surface.[7] Candida albicans is a commensal normal flora fungus in the human body. The fungus can be easily identified from the oral cavity and digestive tract. C. albicans adhere to and thrive on soft and hard tissues by forming a complex biofilm structure. Initial growth of Candida albicans on the denture base surface is influenced by material and physical properties, such as porosity and surface roughness.[8]

A reinforcing material has been developed to improve acrylic resin properties. It is a Zirconium dioxide (ZrO_2) nanoparticle material characterized by great strength and hardness, non-abrasiveness, non-toxicity, biocompatibility, and low thermal conductivity.[9] ZrO_2 nanoparticles has been validated through in vitro and in-vivo research and thus its application is recommended in dental and other biomedical treatments. ZrO_2 nanoparticles are also recommended for coating materials on PMMA.[10]

Researches have introduced various concentration of ZrO_2 to serve as a filler in acrylic resin. ZrO_2 with 2% and 5% concentration is a biocompatible material which increases affinity with polymers to increase the impact and transverse strength.[9] Nevertheless, adding ZrO_2 nanoparticles above 7% concentration is not recommended because it changes the acrylic resin color.[11] Furthermore, the addition of ZrO_2 to PMMA is likely to develop a denture base material that is reliable, flexible, tough, break-resistant, and provides excellent hardness.[12] ZrO_2 nanoparticles have hydrophilic properties, which are in contrast with acrylic resins. Combination of ZrO_2 and acrylic resin produces an inhomogeneous mixture, which require silane coupling agent. The silane coupling agent for mixing ZrO_2 with polymer is TMSPM (trimethacryloxypropyltrimethoxysilane).[13,14] Previous studies reported that acrylic resin with 0.5% silica nanoparticles coating reduce Candida albicans attachment to the denture bases.[15] Application of ZrO_2 nanoparticles to cold cured acrylic resin on repair denture bases demonstrated similar findings of inhibiting Candida albicans attachment.[16] This study aimed to analyze the growth of Candida albicans and porosity in reinforced acrylic resin using ZrO2 nanoparticles at 2.5% and 5% concentrations intended for denture bases.

MATERIALS AND METHODS

This research is an experimental laboratory type. The research subject consisted of samples of heat polymerized acrylic resin plates with a disk shape of 5 mm in diameter, and 2 mm thickness using 9 samples for each treatment and control group. Total subjects were 27 for 3 groups with the following details: Group I with 9 samples of heat polymerized acrylic resin without giving giving ZrO_2 nanoparticle filler as the control, group II with 9 samples of heat polymerized acrylic resin coupled with ZrO_2 nanoparticle filler of 2.5%, and group III with 9 samples of heat polymerized acrylic resin coupled with ZrO_2 nanoparticle filler of 5%.

The research materials used heat polymerized acrylic resin (QC-20, Dentstply), Red wax (Cavex Brand), Plaster cast, Cold Mould Seal (CMS) and Vaseline, Pumice, Candida albicans preparation, Saline, Aquades, Sabouraud dextrose broth (SDB), Sabouraud dextrose agar (SDA), and ZrO2 nanoparticle powder (Hongwu International Group LTD). The tools used in the research were Stellon pot, Rubber bowls, spatula, kuvet and press, Brush, Crownmess, sandpaper and Arkansas stone, digital scale, measuring glass, Magnetic Stirrer, Vortex mixer, Autoclave, Erlen Meyer Tubes, Measuring glass, Micropipette, Colony counter, Petri dish, and Microscope of primo zeisz starr.

The procedures of the research began with the distribution of ZrO_2 nanoparticle concentration. The required number of monomer was 21.7 gr divided by 0.94 gr/ml = 23 ml. Polymer + monomer = 50 gr + 21.7 gr = 71.7 gr. Subsequently, silane was applied to ZrO_2 nanoparticles as much as 1% of the filler volume followed by the making of research sample, and observation of porosity of acrylic resin samples. Observation of the presence of porosity was done using a primo Zeisz Starr microscope with a magnification of 100 times. The surface of each sample was divided into 4 to view areas using a pencil. The porosity in each viewing area was calculated using a microscope. The amount of porosity obtained was calculated using the formula: Total porosity = Total porosity in all viewing areas/4.

Candida albicans obtained from the laboratory were transferred into SDB medium in microtubes and then mixed and incubated for 24 hours. After that, the result was centrifuged for 15 minutes with a power of 3000 rpm and equalized with Mc Farland's turbidity standard of 0.5. The exposure of Candida albicans to acrylic resin samples knocked out Candida albicans fungus attached to acrylic resin samples, diluted Candida albicans, cultured the Candida albicans fungus attached to acrylic resin samples, and calculated the number of Candida albicans colonies with colony forming units per ml (CFU/ml).

The process obtained ratio-scale data. Normality test was done by Shapiro-Wilk test and obtained normally distributed data. Homogeneity test was done by Levene's test, and generated homogeneous data variance. The data were normally distributed and homogeneous, and thus they were further processed using parametric test of one-way ANOVA with a significance level of 95% and Least Significant Difference-test (LSD).

RESULTS

The average and standard deviation of porosity on the denture base of acrylic resin are presented in Table 1. Table 1 shows that the average calculation of the highest amount of porosity is in the control group (37.4 ± 3.5). The decrease emerged after the addition of ZrO2 nanoparticles concentrations of 2.5% and 5%.

To examine whether the average denture base of heat polymerized acrylic resin with and without the addition of ZrO2 nanoparticles as filler is significantly different in statistics, one-way ANOVA was performed. Prior to the ANOVA one way test, the data variants were first tested in terms of normality (Table 2) and homogeneity.

Homogeneity test and Lavene's test obtained the result of P = 0.223. The normality test of porosity average data with Shapiro-Wilk test obtained the p value of each group of p >0.05, which indicated that the data were distributed normally. Homogeneity test of data variant with Levene's test obtained the value of p>0.05, which means that variants between the groups are homogeneous. Therefore, the requirement to perform parametric test has been fulfilled. On this basis, one way ANOVA was performed as presented in Table 3.

The ANOVA one way test result of porosity of the denture base of acrylic resin added by ZrO2 nanoparticle filler (Table 3) showed significant difference between groups. The control group, acrylic resin group + ZrO2 filler concentration of 2.5% and 5% were known to have significant value (p<0.05). Afterwards, Least Significance difference of post hoc test was performed to find out which group had significant difference (Table 4).

Post hoc test of LSD result in Table 4 showed that there is a significant difference in the amount of porosity in all groups, namely control group (acrylic resin) with acrylic resin group + ZrO2 of 2.5% and acrylic resin group + ZrO2 of 5%, acrylic resin group + ZrO2 of 2.5% with acrylic resin group + ZrO2 of 5% (p<0.05).

The average and standard deviation of the number of Candida albicans attachment on the denture base of acrylic resin is presented in Table 5. Table 5 showed that the average calculation of the highest number of C. albicans attachment was in the control group. The decrease occurred after the addition of ZrO2 nanoparticles concentrations of 2.5% and 5%, and the highest decrease was in the 5% filler group.

To test whether the average value of C. albicans attachment on the denture base of heat polymerized acrylic resin with and without the addition of ZrO2 nanoparticles as filler is significantly different in statistics, one way ANOVA test was performed. Prior to one way ANOVA test, at first the normality (Table 6) and homogeneity of data variants were tested.

Homogeneity test result with Lavene's test obtained a value of p = 0.777. The normality test result of average value data of C. albicans attachment with Shapiro-Wilk test obtained the p value of each group of p>0.05, which means that the data were distributed normally. Homogeneity test of data variant with Levene's test obtained p>0.05, which means that variant between groups were homogeneous. Thus, the requirements for parametric test have been fulfilled. Then, one way ANOVA test is carried out with the presented result in Table 7.

The one-way ANOVA test result of Candida albicans attachment on the denture base of acrylic

Table 1. The average and standard deviation of the amount of porosity on the denture base of acrylic resin added by ZrO_2 nanoparticle filler

Groups	Average ± standard deviation
Control (acrylic resin)	37.4 ± 3.5
Acrylic resin + ZrO_2 Filler of 2.5%	15.8 ± 3.3
Acrylic resin + ZrO_2 Filler of 5%	8.0 ± 2.0

Table 2. Normality test result (Shapiro-Wilk) of porosity data on the denture base of acrylic resin added by ZrO2 nanoparticle filler

Groups	Shapiro-Wilk p
Control (acrylic resin)	0.168
Acrylic resin + ZrO2 filler of 2.5%	0.514
Acrylic resin + ZrO_2 filler of 5%	0.176

Table 3. One way ANOVA test result of the amount of porosity on denture base of acrylic resin with addition of ZrO_2 nanoparticle filler

	Sum of df quadrate	df	The average of quadrate	F	Sig.
Between groups	4181.556	2	2090.778	224.904	0.000*
In group	223.111	24	9.296		
Total	4404.667	26			

Note: *significant difference on p<0.05

Table 4. Post hoc LSD test result on the amount of porosity on the denture base of acrylic resin added by ZrO_2 nanoparticle filler

Group	Control (Acrylic resin)	Acrylic resin +	Acrylic resin + ZrO_2
Control (Acrylic resin)	-	21.55*	29.44*
Acrylic resin + ZrO_2 filler of 2.5%		-	7.88*
Acrylic resin + ZrO_2 filler of 5%			-

Note: *significant difference on p<0.05

Table 5. The average and standard deviation value of the amount of C. Albicans attachment on denture base of acrylic resin added by ZrO_2 nanoparticle filler (CFU/ml)

Group	Average ± standard deviation
Control (acrylic resin)	0.8322 ± 0.08258
Acrylic resin + ZrO_2 filler of 2.5%	0.5356 ± 0.09863
Acrylic resin + ZrO_2 filler of 5%	0.2178 ± 0.08393

Table 6. Normality test result (Shapiro-Wilk) of C. Albicans attaschment data on denture base of acrylic resin added by ZrO_2 nanoparticle filler

Group	Shapiro-Wilk p
Control (acrylic resin)	0.733
Acrylic resin + ZrO_2 filler of 2.5%	0.738
Acrylic resin + ZrO_2 filler of 5%	0.334

Table 7. The result of one way ANOVA of C. Albicans attachment on denture base of acrylic resin added by ZrO_2 nanoparticle filler

	Sum of df Quadrate	df	Average of Quadrate	F	Sig.
Between groups	1.700	2	.850	108.064	.000*
In group	.189	24	.008		
Total	1.888	26			

Note: *significant difference on p<0.05

Table 8. The result of LSD post hoc on attachment of Candida albicans on denture base of acrylic resin added by ZrO_2 nanoparticle filler

Groups	Control (Acrylic Resin)	Acrylic resin + ZrO_2 of 2.5% filler	Acrylic resin + ZrO_2 of 5% filler
Control (acrylic resin)	-	0.29667*	0.61444*
Acrylic resin + ZrO_2 of 2.5% filler			0.31778*
Acrylic resin + ZrO_2 of 5% filler			

Note: *significant difference on $p<0.05$

resin with ZrO2 filler table (Table 7) obtained the result that shows significant differences between groups. The control group, acrylic resin group + ZrO2 filler concentration of 2.5% and 5% are known to its significance value ($p<0.05$).

Furthermore, post hoc test of least significance difference (LSD) was conducted to find out which groups had significant difference (Table 8). The results of the post hoc test of LSD in Table 8 showed that there was a significant difference in the number of Candida albicans attachments in all groups, i.e. control group (acrylic resin) with acrylic resin group + ZrO2 2.5% and acrylic resin group + ZrO2 5%, acrylic resin group + ZrO2 2.5% with acrylic resin group + ZrO2 5% ($p<0.05$).

DISCUSSION

The results of this study indicated that average porosity value in the control group was greater than that of the treatment group. The polymerization of acrylic resin constantly produced residual monomer. Evaporation of the residual monomer caused the formation of pores on the surface. Therefore, acrylic resin in the control group without ZrO_2 addition established large amount of porosity. Less porous surface was identified from the treatment group which mixed 2.5% and 5%. ZrO_2 concentration to the acrylic resin

The addition of ZrO_2 nanoparticles in Group 2 and 3 reduced the amount of porosity. The findings are associated with introduction of silene coupling agent to increase adhesion between materials with different properties. The silene coupling agent bonds different surface energies between hydrophobic acrylic resin and hydrophilic ZrO_2 into a homogeneous mixture.[13,14.15] Homogeneity occurs due to the mixing of ZrO_2 nanoparticle with micro-powder acrylic resin compound.[17] A homogeneous mixture represents the tight arrangement of the particles, reduced porosity, and improved mechanical strength. These results are relevant with other research findings reporting that homogeneity of mixture affects the mechanical properties, including strength and hardness.[18,19,20]

In this study, 5% concentration of ZrO_2 generated less porosity than the 2.5% nanoparticle concentration. It is inferred that more filler concentration decreases the visible porosity. This finding was correlated with silene agent. Addition of ZrO_2 caused the nanoparticles to be well distributed and to equally fill the acrylic resin polymer chain. Therefore, higher concentration of ZrO_2 increased the opportunity to effectively fill the polymer since the amount of porosity decreased. Previous study reported that 5% ZrO_2 nanoparticles were biocompatible materials in increasing compatibility with polymers to enhance the impact and transverse the strength of the denture.[9]

The porosity of the acrylic resin denture base with ZrO_2 nanoparticle filler demonstrated significant differences between groups. The control group, acrylic resin with ZrO_2 in a concentration of 2.5% and 5% and had a significant value. In the control group, residual monomer caused extensive amount of porosity, while on the other groups with 2.5% and 5% nanoparticles, the ability to fill the polymer chain depended on the strength of the ZrO_2 concentration. Larger concentrations had the opportunity and strength to crosslink with larger polymer chains.

Decreased C. albicans attachment is associated with reduced porosity of the acrylic resin denture base. This is relevant with previous study which indicated that the porosity of denture surface facilitates adhesion and proliferation of microorganisms and affects mechanical and chemical procedures for denture cleaning.[10] In this context, reduced porosity is equivalent with decreased candida attachment.

The attachment mechanism of C. albicans to acrylic resin was not only affected by porosity but also influenced by hydrophilic property of the acrylic resin surface. Transforming hydrophilic surface properties into a hydrophobic inhibit the attachment of C. albicans.[21] The different findings between the control group and two intervention groups related to ZrO_2 nanoparticles serve the function in increasing antimicrobial properties of a medium to prevent the attachment of C. albicans. Similar research confirmed that higher percentage of ZrO_2 addition counteract the C. albicans attachment.[16] Besides that, the antimicrobial activity of ZrO_2 nanoparticles depends on the crystalline plane of nanoparticles and zirconia complex.[22]

This study discovered that less porous surface contributes to the probability of C. albicans attachments. The limitation of this study was the fact that the addition of ZrO_2 nanoparticle filler in hot polymerized acrylic resin should modify the color into a slightly brighter but, based on visual observation, the color changes was not significant since the concentration of ZrO_2 filler in this study did not exceed 5%. Significant color changes can be observed in ZrO_2 nanoparticle above 7% concentration. The finding is consistent with other research, which mentioned that addition ZrO_2 nanoparticles above 7% greatly affected the resin appearance.[11]

The minimal inhibitory concentration on ZrO_2 reinforced acrylic resin with 2.5% concentration was 35.6%, while that at 5% concentration was 73.8%. This study proved that targeted application of acrylic resin reinforced with ZrO_2 for denture base at a concentration of 2.5% and 5% was only fungistatic. Maximum yielded result at 5% concentration was only able to inhibit <99%. In line with the findings of this study, antifungals properties are categorized as fungistatic when the inhibition is below 99%, while fungicidal characteristic is represented by ability of inhibition beyond 99%.[23]

CONCLUSION

Acrylic resin reinforced with ZrO_2 nanoparticles targeted for heat-polymerized denture base applications reduced surface porosity and C. albicans adhesion. 5% ZrO_2 nanoparticle concentration demonstrated greater reduction of porosity compared to 2.5% ZrO_2 concentration and acrylic resin denture without ZrO_2. Acrylic resin reinforced with ZrO_2 nanoparticles at a concentration of 2.5% and 5% is fungistatic.

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