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RESEARCH ARTICLE

The effect of Zirconium Dioxide nanoparticles concentrations as filler on heat cured acrylic resin denture base toward viability of fibroblast cells (in vitro study)

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ABSTRACT

Heat cured acrylic resin is the most commonly used denture base material. Zirconium dioxide (ZrO_2) nanoparticles can be applied as an additional filler to increase mechanical strength and reduce residual monomer. This research aimed to analyze the effect of ZrO_2 nanoparticles concentrations as filler on heat cured acrylic resin denture base toward the viability of fibroblast cells. Twenty four disc-shaped heat cured acrylic resin plates (with diameter of 5 mm and width of 2 mm) were divided into four groups (n=6). They consisted of group I as the control (acrylic resin), group II of acrylic resin with 2.5% ZrO_2, group III of acrylic resin with 5% ZrO_2, and group IV of acrylic resin with 7.5% ZrO_2. Cell viability was obtained by MTT assay and ELISA plate reader. The result was examined with one way ANOVA followed by LSD post hoc assessment. It was revealed that the highest cell viability percentage on an experimental group was of 2.5% ZrO_2 with as high as 97.49% value. One way ANOVA test and LSD post hoc test showed a significant difference between groups ($p<0.05$). Based on this research, it is conclusive that the use of ZrO_2 nanoparticles concentration as filler on heat cured acrylic resin denture base affects the viability of fibroblast cells. ZrO_2 nanoparticles 2.5% show higher fibroblast cell viability than that of 5% and 7.5% ZrO_2 nanoparticles concentrations.

Keywords: acrylic resin; fibroblast; filler; viability; zirconium dioxide nanoparticles

INTRODUCTION

Tooth loss, which affects appearance, is the main reason why patients need a denture. One of the problematic effects of tooth loss is nutrition intake intervention, which leads to systemic problems. Therefore, it is necessary to have immediate treatment for tooth loss as a way to maintain the function of mastication, phonetics, and esthetics.[1] One of the prostheses to replace missing teeth is a denture, which is supported by the healthy teeth, oral mucosa, or combination of teeth and mucosa.[2] The primary purpose of denture treatment is to restore masticatory function, phonetics, esthetics, and to maintain tissue health to prevent further injury of the oral structure.[3]

Acrylic resin is a polymer chain which consists of methyl methacrylate units. This material is highly preferred for removable denture fabrication.[4] The most widely used acrylic resin in the denture fabrication is heat-cured acrylic resin. The usage of acrylic resin as a denture base can reach more than 98% effectiveness.[5] In general, the acrlyic resin has an excellent esthetic result, precision, good stability in the oral environment, ease of fabrication and adjustment, economical cost, and is repairable.[6,7] However, in addition to its advantages, acrylic resin is known to have some adverse impacts such as producing residual monomers which have cytotoxic effect to surrounding tissue and low mechanical properties which lead to fracture of denture base.[8]

The dental filler is a material that plays a role in sustaining the mechanical strength of resin material. Filler volume affects the strength of resin material. The higher the volume of a filler, the higher the strength of resin material, despite the likelihood of fracture.[9] Therefore, in order to increase the mechanical properties of resin material, filler must be modified.[10]

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Zirconium dioxide (ZrO_2) is a widely used metal oxide to increase denture base strength, because it has great mechanical strength and good surface properties.[11] The addition of ZrO_2 nanoparticles with different concentrations of 2.5%, 5%, and 7.5% of PMMA in sequence significantly increases the flexural strength, transverse strength, and thermal stability.[12,13] Nanoparticle is a particle with the average diameter and dimension of around 10-9 m. Its small size makes nanoparticles have unique physical, chemical, mechanical, electrical, and magnetic properties such as freedom to infiltrate the cell.[14] The underlying mechanism of nanoparticle material of cytotoxicity is Reactive Oxygen Species (ROS) formation. Formation of ROS in massive amounts will induce oxidative stress, which may lead to cell failure to maintain normal physiological function. Oxidative stress may lead to cellular component failure and cell death.[15] Injection of 50% and 100% of ZrO_2 nanoparticles into intraperitoneal of rats showed that ZrO_2 plays a significant role in increasing Reactive Oxygen Species (ROS), induced the free radicals alternately. Free radicals may cause cell injury of the rats' liver and kidney.[14]

Acrylic denture base must fulfill the biocompatibility requirements because it contacts the oral mucosa for a long time.[9] Several metals studied and known to be tolerated by humans are Fe, Mn, Cr, Ni, Mo, Ag, Ti and Al in predetermined concentrations.[16] In addition to metals, the study on local hydroxyapatite and chicken scratch collagen revealed that these materials do not cause acute toxicity in fibroblast cell cultures and, instead, encourage the growth of fibroblasts. Moreover, they also do not cause systemic toxicity to liver cell and kidney cells.[17]

Ideally, the denture base is not cytotoxic. Cytotoxicity test of material is intended to see the potential of a material to injure the cell by measuring the percentage of its cell viability.[9] In vitro test is needed to measure the cell viability.[5] The most commonly used viability test in dentistry is a fibroblast cell.[18] On this basis, this research aims to review the concentration effect of Zirconium dioxide (ZrO_2) as a filler in the heat-cured resin acrylic denture base on fibroblast cell viability.

MATERIALS AND METHODS

Materials used in this research were heat-cured acrylic resin (QC-20, Dentsply), ZrO_2 nanoparticles (Hongwu International Group LTD), silane (Ultradent, South Jordan), M199 as a cell culture media, *3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide* (MTT) and fibroblast cell culture (Vero cell line) (LPPT UGM, Yogyakarta).

This research is experimental laboratory research with 24 subjects of disc-shaped heat-cured acrylic resin with 5 mm diameter and 2 mm height. The specimens were divided into four groups (n=6), control group (heat-cured acrylic resin), group of heat-cured acrylic resin which was added with 2.5% ZrO_2 nanoparticle filler, group of heat-cured acrylic resin which was added with 5% ZrO_2 nanoparticle filler, and group of heat-cured acrylic resin which was added with 7.5% ZrO_2 nanoparticle filler.

One percent of silane of filler weight was added into ZrO_2 nanoparticles using a magnetic stirrer for 20 minutes. Then it was measured using a sonicator for 30 minutes. Afterward, it was left to dry for 14 days in the room temperature.[19] Silane treated with ZrO_2 nanoparticles was mixed with acrylic resin polymer powder according to the concentration group. The materials were mixed using a vortex mixer for 20 minutes to obtain homogenous nanoparticles distribution on the acrylic resin. Table 1 showed composition of silane application on the ZrO_2 nanoparticles. Table 2 showed composition of polymer, monomer and ZrO_2 nanoparticles.

Table 1. Composition of silane application on the ZrO_2 nanoparticles

	Groups	ZrO_2 (g)	Silane (g)	Silane (ml)
I	Control	0	0	0
II	ZrO_2 2.5%	1.25	0.0125	0.01
III	ZrO_2 5%	2.5	0.025	0.018
IV	ZrO_2 7.5%	3.75	0.0375	0.028

Acrylic resin specimens were made with standard packing procedure and heat-cured acrylic resin polymerization QC-20 (Dentsply, Germany) using conventional water bath technique.[9] Isolation and preparation of Vero cell cultures were carried out. The viable cells were counted under the binocular microscope with 100x magnification. After

Table 2. Composition of polymer, monomer and ZrO_2 nanoparticles

Groups	Polymer (g)	Monomer (g)	Monomer (ml)	ZrO_2 (g)
I Control	50	21.7	23	0
II ZrO_2 2.5%	48.75	21.7	23	1.25
III ZrO_2 5%	47.5	21.7	23	2.5
IV ZrO_2 7.5%	46.25	21.7	23	3.75

that, the viable cells were diluted into suspension with cell density $2x10^4$ cells/100 µL.[20] Cultured cells were incubated for 24 hours, and each acrylic resin samples in the wells was incubated for 24 hours.[21,22] Culture media was thrown and all samples were taken out from the wells. 100 µL *3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide* (MTT) solution were put in each well, then incubated for 4 hours on 37 °C. Subsequently, 100 µL SDS-HCl (safety data sheet – Hydrochloric Acid) were added. The microplate was positioned into an ELISA plate reader with 550 nm wavelength. The absorbance value of OD (Optical Density) was obtained from the counting of the viable fibroblast cell amount. This formula counted cell's viability

$$\frac{\text{Treated groups OD} - \text{media OD}}{\text{Control OD} - \text{media OD}} \times 100$$

The data obtained the ratio with normal distribution and homogeneous data variants, and it was followed by one-way ANOVA parametric tests with 95% level of significance. The ANOVA test result showed a significant difference among the groups ($p<0.05$). Post Hoc LSD test result showed a significant difference in each group ($p<0.05$).

RESULTS

The mean and standard deviation of the fibroblast cell viability after exposure of ZrO_2 of 2.5%, 5%, and 7.5% on the acrylic resin plate are presented in Table 3. Table 3 showed that the highest mean of cell viability was from group of ZrO_2 nanoparticles with 2.5% of concentration by 97.49 ± 1.45 and the lowest cell viability was the control group with 86.15 ± 2.03.

According to the results of the average viability of fibroblast cells in Table 3, the acrylic resin group

Table 3. Mean and standard deviation of fibroblast cell viability (%) after exposure of ZrO_2 of 2.5%, 5% and 7.5% on the acrylic resin plate

Groups	Mean ± SD
Control (acrylic resin)	86.15 ± 2.03
Acrylic resin + ZrO_2 2.5%	97.49 ± 1.45
Acrylic resin + ZrO_2 5%	92.67 ± 1.58
Acrylic resin + ZrO_2 7.5%	90.27 ± 2.51

with ZrO_2 concentration of 2.5%, 5%, and 7,5% can be included in the non-cytotoxic category because the viable cells were more than 90%. the control group was categorized in the mild cytotoxic category because the viable cells were around 60-90%.[23] According to ISO 10993-5 (2009), all research groups (control group, acrylic resin with ZrO_2 nanoparticles 2.5%, 5%, and 7.5%) were categorized in no cytotoxic category because the viable cells were more than 70%.[21]

The normality test using Shapiro-Wilk showed a significance level of more than 0.05 ($p>0.05$), which means that the data were normally distributed. The homogeneity test using the Levene test showed that $p=0.640$ ($p>0.05$). Thus, it can be concluded that the data variance was homogenous.

Both tests result revealed that the data were qualified to be tested on a parametric test. Using one-way ANOVA as the parametric test, this study revealed that there was a significant difference between control groups, acrylic resin of ZrO_2 nanoparticles of 2.5%, 5%, and 7.5% groups. LSD post hoc test was done to know the difference between the mean of fibroblast cell viability that was influenced by ZrO_2 nanoparticles concentration.

The result of the LSD post hoc test indicated that there were significant differences between fibroblast cell viability among the groups, control groups with acrylic resin + ZrO_2 2.5%, 5%, and 7.5%; acrylic resin + ZrO_2 5%, with acrylic resin

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ZrO_2 of 5% and 7.5%; acrylic resin + ZrO_2 5% and 7.5%; acrylic resin + ZrO_2 5% with acrylic resin + ZrO_2 of 7.5% ($p<0.05$).

DISCUSSION

The research on the effect of ZrO_2 (zirconium dioxide) nanoparticle concentrations as filler on heat-cured acrylic resin denture base toward the viability of fibroblast cells showed that the percentage of fibroblast cell viability was at its highest in the treatment group of 2.5% concentration with an average value of 97.49% because the addition of ZrO_2 nanoparticles with lower concentration will obtain a more homogenous acrylic resin structure[24] and faster polymerization,[25] and produce lower residual monomer.[24,25]

The residual monomer is a factor that influences the the viability of cells.[26] Lower ZrO_2 nanoparticles concentration leads to a softer surface of acrylic resin,[27] and better cell response, cell proliferation and adhesion, as well as an increase in fibroblast cells viability.[28] Moreover, silane as a coupling agent influences the escalation of fibroblast cell viability because silane has a good biocompatibility.[29]

The lowest cell viability percentage was seen on the control group (acrylic resin) with mean of 86.15%. This result demonstrated that the control group was more cytotoxic than acrylic resin group with the addition of filler ZrO_2 nanoparticles of 2.5%, 5%, and 7.5%. This result was attributed to the residual monomer that influences the fibroblast cell viability.[30]

One-way ANOVA and LSD post hoc tests showed that there was a significant difference ($p<0.05$) in the 4 treatment groups (control group, acrylic resin groups with ZrO_2 nanoparticle concentration of 2.5%, 5%, and 7.5%). This may be attributed to the differences of ZrO_2 nanoparticles concentration that were added in acrylic resin denture base. The greater of nanoparticles concentration in the suspension, the lower of cells viablitiy, and the smaller of the distance between nanoparticles lead to mutual interaction between the nanoparticles.[14]

The interaction of nanoparticles will lead to the nanoparticles agglomeration and non-homogenous mixture of polymer, monomer, and the higher level of ZrO_2 nanoparticles, causing the decrease of the polymerization process and the increase of monomer amount.[31] The increasing concentration of ZrO_2 nanoparticles is related to acrylic resin surface roughness. Since the concentration of ZrO_2 increases, the surface roughness of acrylic resin also increases.[27] Residual monomer and the rough acrylic resin surface will decrease the fibroblast cell viability.[26,28]

The highest viability cells were found on ZrO_2 nanoparticles of 2,5% as the acrylic resin denture base filler. The low concentration of nanoparticles makes faster polymerization, lower residual monomer, homogenous acrylic resin structure, and smoother acrylic resin surface.

CONCLUSION

The concentration ZrO_2 nanoparticles as filler on heat-cured acrylic resin denture base affects fibroblast cells's viability. ZrO_2 nanoparticles of 2.5% show higher viability of fibroblast cell than 5% and 7.5% ZrO_2 nanoparticle concentrations.

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