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RESEARCH ARTICLE

Effect of addition titanium dioxide nanoparticles as acrylic resin denture base filler on cytotoxicity

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ABSTRACT

Denture base material should have a good level of biocompatibility. Acrylic resin is frequently used as a denture base material, however it has a disadvantage of producing residual monomer. Residual monomer is known to have a cytotoxicity effect. Titanium dioxide (TiO_2) nanoparticles are used as fillers due to their biocompatibility and ability to enhance the mechanical properties of acrylic resin. The addition of the material to acrylic resin could affect the amount of residual monomer. The aim of this study was to examine the effect of the addition of TiO_2 nanoparticles as acrylic resin denture base filler on the cytotoxicity in fibroblast cells. The samples consisted of 24 heat cured acrylic resins in disc shape (5 mm in diameter and 2 mm in thickness), divided into 4 groups (n = 6): three groups given treatment with 0.5%, 1%, 2% TiO_2, respectively and one control group. Cell viability was measured with MTT assay. The results were tested with one way ANOVA with 95% confidence level followed by LSD post hoc test. The results showed that the highest percentage of cell viability was found in the treatment group of 0.5% TiO_2 with value of 91.83 ± 1.75%, while the lowest value was seen in the treatment group of 2% TiO_2 with value of 79.38 ± 3.34%. Significant differences were shown between the treatment groups of 0.5% and 2% TiO_2, as well as between the control and treatment group with 2% TiO_2. The conclusions of this research are the addition of TiO_2 nanoparticles as acrylic resin denture base filler has an effect on cytotoxicity; the addition of 0.5% TiO_2 nanoparticles filler results in lower cytotoxicity on fibroblast cells compared to the addition of 1% and 2% TiO_2.

Keywords: acrylic resin denture base; cytotoxicity; fibroblast cell; titanium dioxide nanoparticles

INTRODUCTION

Denture base is a part of prosthesis that rests on the soft tissue and to which artificial teeth are attached. It is supported by the supporting tissue or residual alveolar ridge.[1] Acrylic resin is a widely used material for making denture base.[2] Polymethyl methacrylate (PMMA) is the basic material of acrylic resin. The advantages of acrylic resin are good aesthetics, low water absorption and solubility, easy to repair, and simple processing techniques.[3] However, acrylic resin also has several disadvantages including low impact resistance, fatigue failure, low thermal conductivity, and producing residual monomers.[4,5]

Many techniques have been used to improve the mechanical properties of acrylic resins, including the addition of materials such as fibers or particles.[6] Many other attempts were made to increase the strength of acrylic resins by modifying the structure of PMMA by copolymerization with rubber, or reinforcement by incorporation of different forms and types of filler like metallic wire, fibers, and the use of metallic oxides. The development of nanotechnology and nano-sized materials encourages the use of nano-sized fillers to enhance denture base resins thus producing a polymer with improved mechanical and physical properties as compared to those filled with micro-sized particles.[5]

Titanium dioxide (TiO_2) is one of the nanoparticles used because of its photocalytic properties, high refractive index, thermal stability, and non-reactive properties.[7] Titanium dioxide nanoparticles also exhibit antibacterial properties

and could increase the mechanical properties of acrylic resin.[8] Titanium dioxide nanoparticles show low cytotoxicity and do not increase the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) which is a biomarker of oxidative stress and carcinogenesis.[9]

A research of TiO_2 nanoparticle filler with a concentration of 0.5, 1 and 2% showed that there was an increase in tensile strength of acrylic resin containing 1% TiO_2.[8] Addition of 1% TiO_2 could increase impact strength while addition of 5% TiO_2 could increase microhardness in conventional acrylic resin materials.[6] The addition of TiO_2 to heat-cured acrylic resin could increase impact strength, transversal strength and surface hardness.[10]

Denture base material must be an inert material. In a condition that is not mixed or not cured, denture base material must be harmless to technician. In addition, denture base material must not irritate patients.[2] The supporting soft tissue is located between the denture base and alveolar bone. Fibroblasts are the dominant cells in the gingival connective tissue and because acrylic resin is located close to the epithelium, a molecule weighing lower than 100 kDa could penetrate the connective tissue below, giving path to the resin monomer to reach the connective tissue cells.[11] Citotoxicity test of Transbond XT adhesive containing 1 wt% TiO_2 nano-particles to human gingival fibroblasts (HGF) and mouse L929 fibroblasts proved to have comparable and even lower toxicity than the pure adhesive, indicating its potential safety for intraoral applications.[12] Previous research has demonstrated that the cytotoxic effect of the resin monomer (containing MMA) on human PDL fibroblasts is dose dependent. Cytotoxicity patterns could be influenced by polymerization method and the chemical composition of the resin.[11]

In general, nanoparticles are assumed to cause greater toxicity than micro-sized particles because the number of particles that could enter the body increases as particle size decreases. The cytotoxicity of nanoparticles is also determined by other physico-chemical factors including size, concentration, chemical composition, and crystal structure.[12] Acrylic resins are formed by polymerization of methyl methacrylate but this polymerization could leave some amount of residual monomers in denture base. The residual monomer leaks out for some time which causes various cytotoxic effects on the surrounding tissues.[4] This study aimed to examine the effect of the addition of TiO_2 nanoparticles as acrylic resin denture base filler on cytotoxicity.

MATERIALS AND METHODS

This was a laboratory experimental study with 24 disc-shaped heat-cured acrylic resin specimens with a diameter of 5mm and thickness of 2 mm, divided into 4 groups (n = 6), three groups treated with 0.5% TiO_2 filler, 1% TiO_2 filler, 2% TiO_2 filler, respectively and one control group. The materials used in this study were: heat-cured acrylic resins (QC-20, Dentsply), 25 nm TiO_2 nanoparticles (Sigma-Aldrich) and fibroblast cell culture. Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. ELISA reader (Bio-rad, Swiss) was used in this study to read the absorbance. The study was approved by the ethics commission of the Faculty of Dentistry, Universitas Gadjah Mada (No.001475/KKEP/FKG-UGM/EC/2018).

One percent silane was added into TiO_2 nanoparticles, the mixture was stirred using a magnetic stirrer for 20 minutes, followed by sonication using sonicator for 30 minutes, then left to dry at room temperature for 14 days.[10,13] Acrylic resin specimens were produced according to manufacture recommendation (2.3:1 polymer:monomer ratio)

Table 1. Composition of polymer, monomer, and TiO_2 nanoparticle

	Polymer (g)	Monomer (g)	Monomer (ml)	TiO_2 (g)
Group I (0.5% TiO_2)	9.95	4.33	4.61	0.07
Group II (1% TiO_2)	9.90	4.31	4.58	0.14
Group III (2% TiO_2)	9.80	4.26	4.53	0.29
Group IV (control)	10	4.35	4.63	0

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(QC20, Dentsply). Monomers were first converted from milliliters to grams by considering the mass density of methyl methacrylate (0.94 g/ml).[14] The composition of the polymer, monomer, and TiO_2 filler can be seen in Table 1.

Nanoparticles of TiO_2 which had been given surface treatment with silane and polymer were mixed using a vortex mixer for 20 minutes, to obtain nanoparticles dispersion on acrylic resin. Fibroblast cultures were carried out in media consisting of fetal bovine serum and penicillin-streptomycin. Dilution was performed to make a suspension with a cell density of $2x10^4$ cells / 100 µL.[15]

Cell cultures were incubated for 24 hours. Subsequently, the 96-well microplate was given an acrylic resin sample and incubated for 24 hours.[16] Procedure of MTT assay was performed after the microplate was incubated in a dark room for 24 hours. The microplate was then inserted into the ELISA plate reader with a wavelength of 550 nm. The absorbance value (optical density) was obtained from the formation of formazan crystals. Cytotoxicity was measured based on cell viability relative to the controls with the following formula:

$$\% \text{ viable cell} = \frac{\text{OD treatment} - \text{OD medium}}{\text{OD control} - \text{OD medium}} \times 100$$

OD: optical density

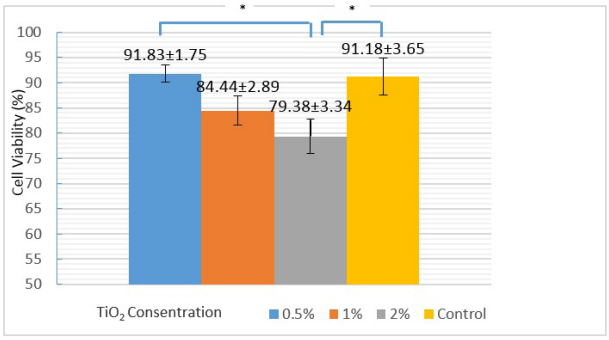
One way analysis of variance (ANOVA) with a 95% significance level was done for all the tested groups followed by Low Significant Difference (LSD).

RESULTS

The mean and standard deviation of the percentage of fibroblast cell viability after exposure to acrylic resin with TiO_2 filler of 0.5%, 1%, and 2% can be seen in Table 2. The data in the table were also presented in the form of bar charts in Figure 1.

Table 2. Results of mean and standard deviation of cell viability (%)

Groups	n	Mean (%) ± SD
0.5% TiO_2	6	91.83 ± 1.75
1% TiO_2	6	84.44 ± 2.89
2% TiO_2	6	79.38 ± 3.34
Control	6	91.18 ± 3.65



* The mean difference is significant at the 0.05 level

Figure 1. Cell viability of tested groups

The research data were analyzed by one-way ANOVA parametric test. The requirements to be met in to carry out one-way ANOVA test were normal distribution of data and homogeneity of data variance. Shapiro-Wilk normality test results showed that the data in each concentration group had a normal distribution. This is indicated by a significance value greater than 0.05 ($p>0.05$). The result of homogeneity test with Levene's test showed a significance value>0.05, which means that all population variances were the same and the assumption homogeneous groups as fulfilled. The data were normally distributed and homogeneous, thus fulfilling the requirements to conduct a one-way ANOVA test.

One-way ANOVA test results showed a value of $p = 0.024$ ($p<0.05$). These results indicated that there were significant differences in the treatment group. This showed the effect of adding TiO2 filler with a concentration of 0.5%, 1%, and 2% on the viability of fibroblast cells. To find out the differences in each treatment group, a post hoc LSD test was conducted and the results can be seen in Figure 1.

The results of the post hoc LSD test showed that there were significant differences in the cell viability between the control group and the 2% TiO_2 treatment group, as well as between 0.5% TiO_2 treatment group and 2% TiO_2 group ($p<0.05$) (Figure 1). There was no significant difference in the cell viability between the treatment group of 0.5% TiO_2 and the control, 1% TiO_2 and the control, 0.5% TiO_2 and 1% TiO_2, and 1% and 2% TiO_2.

DISCUSSIONS

The results showed that the highest percentage of cell viability was shown in 0.5% TiO_2 group with mean of cell viability of 91.83 ± 1.75%. A high level of cell viability due to the addition of low concentrations of TiO_2 filler results in high polymerization. High polymerization rates result in low residual monomer production. Low residual monomers result in low cytotoxicity. Titanium dioxide nanoparticles with silane tend to bind to the monomers; the polymerization of the group added with 0.5% TiO_2 is higher because the residual monomers tends to bind with TiO_2 nanoparticles with the help of silane and then polymerized. Heat-cured acrylic resin produces a residual monomer of 0.2-0.5%.[14] Addition of filler material in low concentrations results in a high degree of polymerization, then resulting in lower amount of residual monomer.[17] The amount of residual monomer in acrylic resin is an important factor that can cause cytotoxicity.[11]

The lowest percentage of cell viability was seen in the 2% TiO_2 group with mean of cell viability of 79.38 ± 3.34%. Addition of excessive nanoparticles can cause the agglomeration of nanoparticles which then interfere with the reaction of the polymerization of acrylic resin. A decreased polymerization rate in the addition of 2% TiO_2 may also be caused by more monomers which tend to bind more to silane than to TiO_2 nanoparticles, thus they are not polymerized due to excessive addition of nanoparticles. Interventions in polymerization reactions due to the addition of high nanoparticle concentrations can result in increased residual monomer production. An increase in residual monomer can cause cytotoxic effects which decrease cell viability. An increase in TiO_2 nanoparticle concentration can increase the agglomeration of nanoparticles which then interfere with polymerization.[18] The greater the concentration of nanoparticles in a suspension, the smaller the distance among nanoparticles, therefore nanoparticles will interact with each other and experience agglomeration.[19] Addition of excessive TiO_2 nanoparticles can interfere with the polymerization reaction of acrylic resin which results in an increase of unreacted monomers in polymers.[20] An increase in the amount of material added to acrylic resin will affect the polymerization of acrylic resins therefore higher residual monomers are produced.[17]

Significant differences were shown between 0.5% TiO_2 treatment group and 2% TiO_2 treatment group, as well as between the control and 2% TiO_2 treatment group. This was because differences in the amount of TiO_2 in the 1.5-2% concentrations added to acrylic resin plates could trigger the agglomeration of nanoparticles and could increase the risk of uneven mixing of polymers, monomers, and nanoparticles which decreased polymerization, resulting in an increase in the amount of residual monomers. The results of this study were in accordance with the results of the mechanical test, i.e. the addition of TiO_2 nanoparticles with a concentration of 0.5% -1% increased mechanical strength, namely tensile strength, flexural strength, and impact strength, while the addition of such nanoparticles with concentrations above the saturation point such as 2% -5% decreased the strength of acrylic resin because there was an increase in the amount of residual monomer which could interfere with the acrylic resin matrix.[6,8] Low filler concentration can increase the density of PMMA matrix chains while excessive addition of TiO_2 could increase the risk of agglomeration among nanoparticles which can reduce the dispersion of nanoparticles in resin acrylic, thus increasing the amount of residual monomer.[8]

The cytotoxic effect of acrylic resins can be influenced by the composition of each material, the proportion of powder/liquid or the polymerization methods of each resin.[21] Acrylic resin is formed by polymerization of methyl methacrylate but it does not occur thoroughly, leaving a residual monomer in denture base. The release of residual monomer for some time may cause various cytotoxic effects on the surrounding tissue.[4] The cytotoxic effects of methacrylate monomers occur due to the alterations of cell membranes, including migration of small lipid-soluble methacrylates into the lipid bilayer and solubilization of this layer.[22]

Further research is needed on the effect of adding TiO_2 nanoparticles to acrylic resin denture base plates on the amount of residual monomers.

Research needs to be done on the effect of adding TiO_2 nanoparticles on acrylic resin denture base plate using direct counting method. A scanning electron microscope test is needed to see the agglomeration of nanoparticles that occur in samples.

CONCLUSION

Addition of TiO_2 nanoparticles as acrylic resin denture base filler has an effect on the cytotoxicity of acrylic resin denture bases. The addition of 0.5% TiO_2 nanoparticle filler resulted in lower cytotoxicity on fibroblast cells compared to the addition of 1 and 2% TiO_2 fillers.

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