THE EFFECT OF SILVER VERSUS TITANIUM DIOXIDE NANOPARTICLES ON POLY METHYLMETHACRYLATE DENTURE BASE MATERIAL

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ABSTRACT

Materials and methods: Three groups of heat cured acrylic resin specimens were prepared; the first was Group C made of conventional heat cured acrylic resin, the second group was Group Ag where the acrylic resin specimens were incorporated with 5% Silver nanoparticles, and the third group Ti had its specimens incorporated with 5% Titanium oxide nanoparticles. The specimens were tested for microbial colonization using direct culture and serial dilution methods. The specimens were then further tested for surface roughness.

Results: There were higher bacterial counts in specimens of group C (control) 51.12 ± 4.7198 than those with Ag nanoparticles 32.44 ± 2.7701 and TiO2 nanoparticles 31.92 ± 2.2159. The difference was statistically significant between the control group (C) and the Ag nanoparticles group and between the control group and the TiO2 nanoparticles group. However, there was a statistically non-significant difference between the Ag nanoparticles group and TiO2 nanoparticles group. Statistical analysis by ANOVA for repeated measures showed statistically non-significant difference between the three groups regarding the surface roughness.

Conclusions: Ag and TiO2 nanoparticles had a similar antimicrobial effect when added to PMMA, The addition of 5% of Ag and TiO2 nanoparticles has not affected the surface roughness of PMMA.

KEYWORDS: Titanium, Silver, Nanoparticles

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INTRODUCTION

Up to date, there is no material that can fulfill the role of polymethyl methacrylate (PMMA) particularly in the field of removable prosthodontics. PMMA is the mostly used denture base material as it is light in weight, insoluble in the oral fluids and easily repaired. It also has a low cost and superior esthetics. However, it has several limitations as it undergoes polymerization shrinkage and has inferior mechanical properties. Recently, several nanosized fillers have been added to improve the properties of PMMA. [1] Nanoparticles have a very high surface area and they affect the mobility of the polymer chains at their interfaces. Thus, the addition of a small quantity of nanoparticles drastically transforms the properties of a polymer. [2,3]

Silver (Ag) nanoparticles are smaller than other fillers and possess distinct physical, chemical, and biological properties. [4] They have been used to enhance the mechanical and thermal properties of denture base materials. They also possess an antimicrobial activity as they prevent the adherence of both bacteria and fungi on the surfaces of denture bases. [5] It has been recommended to incorporate lower concentrations of Ag nanoparticles into denture base materials as they impart a black color to the denture bases when used in higher concentrations. [6] The use of PMMA incorporated with Ag nanoparticles promotes better oral health in patients with removable prosthesis thus improves their quality of life. [7]

Titanium dioxide (TiO2) nanoparticles are also used for reinforcement of denture base materials. They are biocompatible, non-toxic, and chemically stable. They also have high strength, resist corrosion, and have an antimicrobial activity. They alter the electrical, optical, chemical, and physical properties of polymers. They also cause drastic improvement of the mechanical properties of denture base materials when compared to unmodified acrylic resins. [8-10] It was reported that the enhancement of the properties of the polymers was only achieved with lower concentrations of TiO2 and that higher concentrations had an adverse effect on the final hybrid polymer. [8]

The texture of the surface of a denture base of a removable prosthesis should be smooth with minimal surface roughness as it directly contacts the oral mucosa. A rough surface is unhygienic and tends to retain plaque and microorganisms which affect the oral health. It was reported that the recommended threshold to reduce the plaque accumulation is 0.2μm and that accumulation of microorganisms increases significantly when the surface roughness is more than 2μm. [11]

The favorable mechanical properties achieved with the addition of different nanofillers renders them ideal for improvement of the qualities of denture base materials. However, the effect of these fillers on the surface properties of the denture bases as well as their antimicrobial activity should be in consideration. Hence, this study was conducted to compare the effect of adding Ag and TiO2 nanoparticles on the antimicrobial effect and surface roughness of PMMA denture base material. The first null hypothesis was that no difference would be found in the antimicrobial action of the denture base material by addition of Ag and TiO2 nanoparticles. The second null hypothesis was that no difference would be found in the surface roughness of the denture base material by addition of Ag and TiO2 nanoparticles.

MATERIALS AND METHODS

The denture base material used in this study was heat cured poly methyl methacrylate (Acrostone, Acrostone Dental Factory, El Salam City, Egypt). Two types of nanoparticles were used in this study, Silver (Ag) and Titanium Dioxide (TiO2). They were prepared According to the manufacturer's instructions.

Silver Nanoparticles Preparation

Silver (Ag) nanoparticles were prepared using chemical reduction method. Microwave irradiation of silver nitrate (AgNO3) solution in ethanolic medium using polyvinylpyrrolidone (PVP) as a stabilizing agent was carried out. Ethanol is used because it acts as a reducing agent in the presence of microwaves. The solvent was then evaporated at mild temperature to obtain the Silver in powdered form. [12]

Titanium Dioxide Nanoparticles preparation

Anatase particles were prepared by precipitation from homogenous solution using titanium (IV) isopropoxide as precursor in aqueous solution acidified with nitric acid to PH 2 using a water to titanium mole ratio of about 200. [13]

Nanocomposite mixture Preparation

Three groups of specimens were constructed, each group consisting of n25 = samples.

Group C was considered the Control group and the specimens of this group were constructed out of conventional Heat Cured PMMA (Acrostone, Acrostone Dental Factory, El Salam City, Egypt) without the addition of Nanoparticles.

Group Ag had its PMMA powder incorporated with 5% by weight silver nanoparticles prior to monomer addition and specimen construction. Silver particle average size was less than 45nm and their shape was spherical.

Group Ti had its PMMA powder incorporated with 5% by weight titanium nanoparticles also prior to monomer addition and specimen construction. Titanium particle average size was less than 15 nm and their shape was spherical.

Both nanocomposite mixture groups were prepared by gradual addition of the nanoparticles to the PMMA powder under continuous stirring until a homogenous mixture is obtained.

Specimen construction

Base plate wax (Cavex Set up Wax; Cavex, Haarlem, the Netherlands) was cut into 15×2±0.01 mm. These discs were then flasked in dental stone (Fujirock EP; GC, Leuven, Belgium). Separating medium was applied on the stone surface, then the upper half of the flask was seated and filled with another layer of stone. After setting, the flasks were opened, and the wax discs were removed, thus leaving the molds empty for the denture base material.

All three previously prepared PMMA nanocomposite powder mixtures had their monomer added to them according to the manufacturer's instructions. Once the mixtures reached the dough stage, they were packed into the molds and pressed using a hydraulic press. The flasks were then placed in a curing unit and cured at 74°C for 90 min and then to 100°C for 30 min. The flask was removed and left to cool gradually, and then opened and the acrylic resin specimens were finished and polished using a tungsten carbide bur. (Fig. 1)
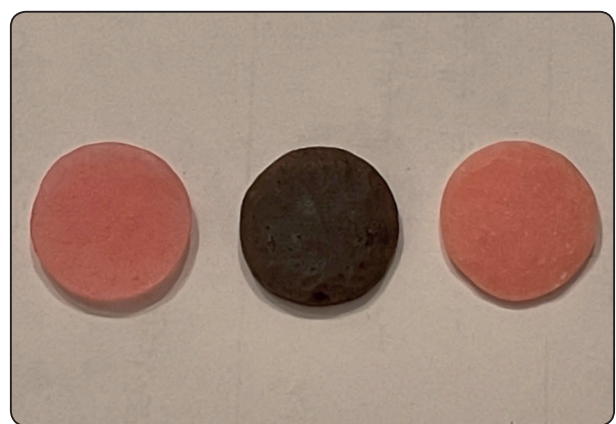

Fig. (1) Specimens from the three groups: Control (C), Silver (Ag) and Titanium (Ti).

Investigations

Microbial investigation

For the C. albicans adherence assay: Disinfection of the specimens with 70% alcohol was carried out and then ultrasonic cleaning with sterile distilled water. These were then soaked in artificial saliva containing 2×10^6 cells of C. albicans (ATCC 10231) at 37°C for 48 hours. The specimens were then placed in sterile tubes containing 1 ml of Sabouraud dextrose broth for 24 hours and vortexed for 10 minutes. (Fig.2)

The tubes were centrifuged for 5 minutes at 4500 rpm followed by the extraction of the acrylic resin specimens and clustered pellet. The C. albicans in the detached cell suspension was counted by direct culture tests and serial dilutions to determine colony forming units (CFUs) as follows:

A) Direct culture test:

One hundred microliters (100 µl) of the centrifuged pellet were taken and cultured in plates filled with SD agar and incubated for 24 hours at 37∘C. After incubation, colonies of C. albicans were counted in the plates using a marker pen colony counter. Colonies covering the whole surface of the plate were considered overgrowth.
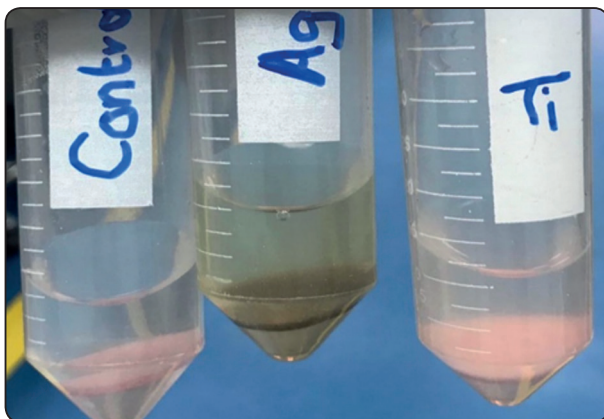
B) Serial dilution method:

Five tubes were filled with 900 µl of normal saline. One hundred microliters of the concentrated pellet were added to the first tube (1/10) and mixed. A total of 100 µl was aspirated from the first tube and added to the second tube ($1/10^2$). The second tube was then mixed, and 100 µl were aspirated from it, before being added to the third tube ($1/10^3$). The solution in the third tube was mixed, followed by aspirating 100 µl and adding it to the fourth tube ($1/10^4$), which were mixed as well. 100 µl were added to the fifth tube ($1/10^5$) from the fourth tube and mixed. Finally, 100 µl were aspirated from the fifth tube and, after proper mixing, discarded. At this stage, all tubes contained 900 µl of diluted concentration of the broth. SD agar plates were cultured with 10 µl of diluted concentration of each tube. The plates were incubated for 24 hours at 37 ∘C. Colonies were counted as previously mentioned in the direct culture test. (Fig. 3)

Surface roughness

The surface roughness (Ra value) of 25 specimens from each group was measured using Perthometer (Mahr, Germany). Each sample was placed on the provided platform so that the Perthometer probe is in slight contact with its surface. The probe

Fig. (2) Acrylic resin specimens of Control, Silver and Titanium groups in falcon tubes containing artificial saliva.
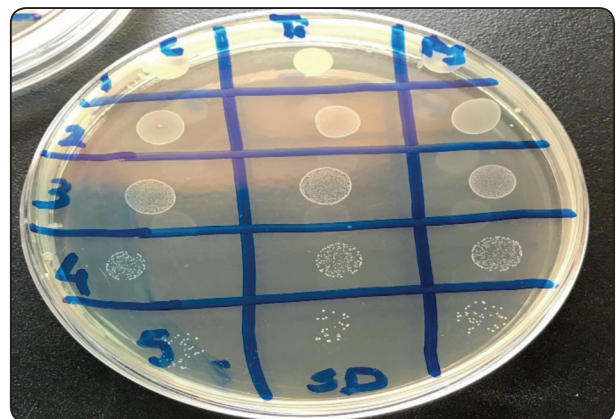

Fig. (3) Plate count of different dilutions for the Control (C), Silver (Ag) and Titanium (Ti)

was passed over the entire specimen surface, and 3 readings were recorded per specimen. An average of these readings was taken as the value of surface roughness for every specimen.

G power software for windows version 3.1.9.4 was used to calculate the sample size. The results were collected and statistically analysed. The collected data were tested for normality by checking distribution of data and calculating the mean values and normality curves. Numerical Data were presented by mean, and standard deviation (SD). ANOVA for repeated measures was used for comparison between the groups. Tukey's HSD was used as a Post Hoc test. The significance level was set at $P \leq 0.05$.

RESULTS

Table 1 shows that there were higher bacterial counts in group C (control) 51.12±4.7198 than in the other two groups with Ag nanoparticles 32.44±2.7701 and TiO2 nanoparticles 31.92±2.2159. Statistical analysis by ANOVA for repeated measures showed statistically significant difference between the three groups. Tukey's HSD was used as a Post Hoc test which showed there was a statistically significant difference between the control group and the Ag nanoparticles group and between the control group and the TiO2 nanoparticles group. However, there was a statistically non-significant difference between the Ag nanoparticles group and TiO2 nanoparticles group.

The results for the comparison of the surface roughness in the three groups were presented in Table 2. Although the specimens of the group with Ag nanoparticles show increased surface roughness (Ra values) 1.1719±0.2049 than both the group with TiO2 nanoparticles 1.1683±0.1983 and group C (control) 1.121±0.1759. Statistical analysis by ANOVA for repeated measures showed statistically non-significant difference between the three groups.

TABLE (1) Shows the mean, standard deviation, and P value of ANOVA for repeated measures test for comparison of the number of bacteria in the three groups.

Groups	Mean	Std. Deviation	P value
Group C (Control)	51.12 [a]	4.7198	< .00001
Group Ag	32.44 [b]	2.7701	
Group Ti	31.92 [b]	2.2159	

Similar letters indicate non-significance.

TABLE (2) Shows the mean, standard deviation, and P value of ANOVA for repeated measures test for comparison of the surface roughness in the three groups.

Groups	Mean (μm)	Std. Deviation	P value
Group C (Control)	1.121	0.1759	.584924
Group Ag	1.1719	0.2049	
Group Ti	1.1683	0.1983	

DISCUSSION

The addition of nanoparticles to PMMA denture bases has proved to have many beneficial effects. [14] Among the most widely used materials in this field are Silver and Titanium oxide nanoparticles. To the best of our knowledge, no previous studies have compared the effect of these two materials when incorporated into PMMA powder.

For standardization purposes, 5% by weight of both Silver and Titanium Oxide nanoparticles was added to the study groups. Silver nanoparticles were proven to have an antimicrobial effect when used at concentrations of 5% by weight and were therefore used in this concentration. [15] For the Ti group, 5 % by weight TiO2 nanoparticles were added to the PMMA powder. Studies have mentioned that this is the highest concentration that can be incorporated

into PMMA without causing toxicity, although it might affect other mechanical properties. [8,16]

The first null hypothesis was rejected as the results of this study showed significantly reduced numbers of bacterial colonies in the PMMA specimens incorporated with Ag and TiO2 nanoparticles in comparison to the control group. These results coincide with the results of several previous studies. [15-17]

A study reported that Ag nanoparticles inhibited the formation of Candida albicans nanofilm on denture bases and had an antifungal activity. The incorporation of 5% Ag nanoparticles inhibited the adhesion of Candida albicans on the surface of the denture bases. [15] Another study proposed the use of Ag nanoparticles with nystatin or chlorhexidine digluconate for treatment of denture stomatitis. [16] Furthermore, a study confirmed that Ag nanoparticles inhibit bacterial growth on the surface of PMMA and release Ag ions that induce an antibacterial action against Escherichia coli and S. epidermidis. [17]

It has been reported that the antimicrobial activity of Ag nanoparticles is due to the formation of reactive oxygen species (ROS) or due to the release of free silver ions from the denture bases. The mode of action depends on the method of synthesis, Ag nanoparticle dispersion and Ag concentration in the PMMA. [17]

Similarly, TiO2 nanoparticles have an antimicrobial effect specially on candida species. In fact, it has been found that the antimicrobial activity is not only limited to various bacterial strains but also on fungi and viruses. [18] The antimicrobial activity of TiO2 nanoparticles is due to its surface properties as well as the photocatalytic production of cytotoxic oxygen radicals. [19] Studies have shown that the antimicrobial effect of TiO2 nanoparticles was intensified when exposed to UV-A light. [20] The results of this study showed that there was no significant difference in the antimicrobial activity of the Ag and TiO2 nanoparticles.

The effect of the added nanoparticles on the surface roughness depends on the quantity of the filler as well as the degree of homogeneity within resin matrix. The interface between the nanoparticles and the PMMA matrix, as well as the agglomeration of the nanoparticles may also influence the surface roughness of the hybrid PMMA. [7] Although the Ag nanoparticles slightly increased the surface roughness of PMMA specimens more than the TiO2 nanoparticles. Yet, the difference between the surface roughness of the specimens of the three groups was statistically nonsignificant. Thus, the second null hypothesis was accepted.

These results are similar to a previous study where there was a statistically nonsignificant difference between the denture base specimens without nanoparticles and those with silver nanoparticles with different concentrations. [15] However, they are different from those of other studies where there was a significant increase in the surface roughness of specimens of PMMA incorporated with TiO2 nanoparticles when compared to pure PMMA. [21,22] The dissimilarity of the results may be due to the reduced size of the TiO2 nanoparticles used in this study (less than 15 nm). According to a previous study, the size of the filler particles affects the surface characteristics of resin composites where the Ra value increases as the size of the filler particles increased. [23]

According to the results of this study, there is no difference regarding the antimicrobial effect and the surface roughness of PMMA with the addition of 5% Ag and TiO2 nanoparticles. Further studies are recommended to evaluate and compare the effect of other concentrations on different properties of PMMA.

CONCLUSIONS

- Ag and TiO2 nanoparticles had a similar antimicrobial effect when added to PMMA.

- The addition of 5% of Ag and TiO2 nanoparticles has not affected the surface roughness of PMMA.

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