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Research Article

Comparison of Antifungal Properties of Acrylic Resin Reinforced with ZnO and Ag Nanoparticles

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ABSTRACT

Background: metalic-Nanoparticles (NPs) is new antifungal materials versus the resistant fungi as *Candida albicans* (*C. albicans*) that is the main factor of acrylic-denture candidosis. Whenever Ag NPs as a strong antifungal metalic-NPs exhibit toxic effect on human cells, the aim of this study was evaluation the antifungal effects of ZnO NPs in acrylic resin (polymethyle methacrylate (PMMA)) as a more biocompatible material on *C. albicans* in comparison to acrylic resin (PMMA) containing Ag NPs.

Methods: In this laboratory study, eleven 10-member groups of acrylic resin specimens with ZnO NPs and Ag NPs (totally 110 specimens) were used. The weight percent of NPs 0.5, 2.5, 5, 10, and 20%, that were added to the monomer in each phase and after mixing with powder, flasking carried out in a conventional manner and in a flask with 12 cylindrical cavities with a diameter of 10 mm and thickness of 4 mm in internal space to produce discoid specimens, after deflasking the specimens were finished, polished, cleaned ultrasonically for 5 min in ethanol, rinsed with sterile water and dried in warm air. Afterward they inucleated by 0.1 ml of an inoculating suspension containing 10^3 concentration of *C. albicans* (ATCC 10231) and were incubated for 24 hours. Then, the sample was washed with 4.8 ml of 0.9%NaCl solution, and 0.1mL of the washing NaCl solution was taken and cultured on tutrient agar, then incubated for another 24 hours and colony numbers were counted and the data was analyzed by one way ANOVA.

Results: Ag & Zno NPs could significantly decrease population of *C. albicans* after 24 hour of exposure time, meanwhile Ag NPs exhibited stronger antifungal effect than ZnO NPs ($P<0.001$).

Conclusion: Zno NPs can inhibited the *C. albicans*. To suggest this technique as a reliable method and determination of technical particle, the efficiency of ZnO NPs should be evaluated in clinical trials.

Introduction

One of the most common oral infections is denture–dependent candidosis that derived mainly from *Candida Albicans* (*C. albicans*) in patients that wear denture fabricated from polymethyle methacrylate (PMMA).[1-4] PMMA is one of the most broadly materials due to its biocompatibility, and current studies have revealed a growing attention in its applications as a medicinal carrier. This material can be used as adjuvant for vaccines or carrier of many drugs as antibiotics and antioxidants through different methods of administration.[5] The conventional treatment is not completely efficient and/or permanent because of several reasons including inadequate design or drug resistance microorganisms.[6,7] Nanotechnology as a new science could present effective materials in this context to promote the properties the denture and producing some antimicrobial andante adhesive properties on them. Indeed, metalic NPs impregnated in PMMA can enhance mechanical, physical, microbicidal and antifungal properties of the resin. Ag NPs as metalic NPs in addition to strong antifungal and antibacterial properties because of ion release has long-term antibacterial activity without possible resistance in microorganisms.[8-10]

ZnO NPs have antimicrobial and antifungal effects,

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as well as other advantages, such as lower price, less toxicity, white appearance, and ability to block the ultraviolet ray, as compared with Ag NPs. ZnO NPs showed killing ability versus a vast group of gram negative and positive bacteria [11] and fungi and particularly *C. albicans*.[12] Moreover exploring the mechanical effect of composite resin modified by ZnO NPs determined increasing compressive and shear without altering the flexural strength of composite resin,[13] from the other hand toxicity of Ag NPs to human cell and brown dicoloration of PMMA result in suspecting the use of this material any more.[14-16]

Overall, Lower toxicity of ZnO NPs, their killing ability of *C. albicans*, white appearance and ability to reinforce of composite resin is some advantages of this material. As a result of these reasons and due to the fact that the addition of ZnO NPs to PMMA used in making a denture base is yet to be examined, the present study was carried out to evaluate the antifungal properties of ZnO NPs in acrylic resin and comparison with Ag NPs.

Materials and Methods

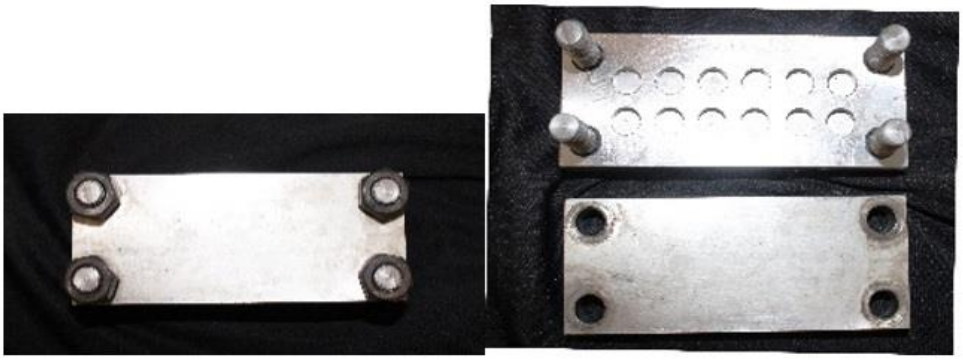
Preparation of test specimens

PMMA specimens with five different concentrations of Ag NPs and five different concentrations of ZnO NPs were prepared. These study specimens included one pure PMMA as blank and two groups of PMMA+Ag NPs and PMMA+ZnO NPs respectively each of which consisted of 10 specimens and in sum 110 specimens. Ag NPs material was of 99.99% purity with spherical particle in size of 20 nm and ZnO NPs of 99% purity and spherical particle in size of 10-30 nm were purchased from (US Research Nanomaterials, Inc).

The certain amount of NPs from two materials separately were weighted, mixed with monomer, homogenized in an ultrasonic set (Heilscher ultrasonics Gmbh, UP200H, Germany) for 5 minutes and mixed with weighted PMMA powder. The weight percent was used in every procedure consisted of: 0.5, 2.5, 5, 10 and 20 %. After preparing the mixture, flasking procedures carried out by a prepared flask with 12 cylindrical cavities in internal space, every with 4 mm depth and 10 mm diameter as depicted in Figure 1.[16] The flasks was put into a tank with water in ambient temperature, warmed to 74°C within one hour, remained for 7 hours in the temperature, transferred to a boiling water and remained for 30 minutes in the condition.[17] Then the specimens were de-flasked and after finishing and polishing, evaluated precisely to assure to be free from every possible pores, cracks and defects (Figure 1).

Morphological studies with Scanning Electron Microscopy

The morphological characterization of specimens was explored by SEM before the microbiologic experiment using Scanning Electron Microscopy (SEM) (Elektronen optik, Dortmund, Germany) to assure the homogeneity of NPs.



(1a)

(1b) ZnO Ag

Figure 1. (1a): Cylindrical cavities for preparing specimens (1b): Prepared specimens.

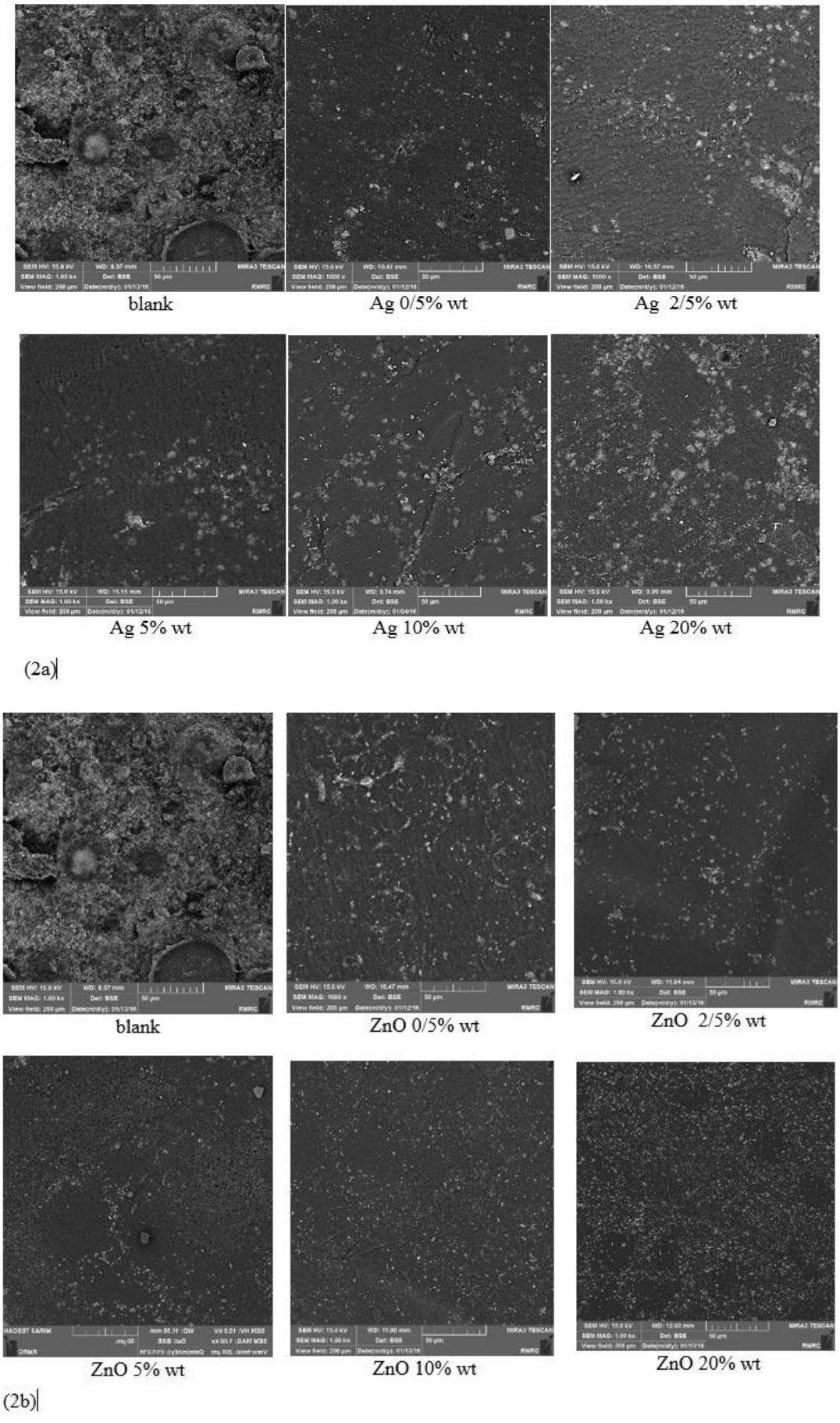


Figure 2. SEM pictures demonstrating the surface characteristics of (2a) : Ag NPs and (2b): ZnO NPs in different concentrations.

Antifungal test

The standard *C. albicans* strain (ATCC 10231) used in the present study was purchased in lyophilized form from Iran's Pasteur Institute, which was activated by culturing in sterile trypticase soy broth (Liofilchem, Italy) and incubating for 48 hours at 30°C. Single colonies from the grown plate were transferred into 4 mL of Nutrient brothand incubated overnight at 30°C. A centrifugation procedure at 3000 rpm for 15 minutes was used to harvest the cells. Then the cells were rinsed twice and re-suspended in Ringer solution to reach on optical density of approximately 0.1 at 600 nm with a spectrophotometer (Coleman, USA) or concentrations of approximately 10^8 CFU/mL. Cell number of prepared innoculum was confirmed by standard pour plate technique and the prepared inoculum used for the in vitro antimicrobial tests according to the method used by Zhang et al with some modifications:[18]

The fungi suspension was diluted by sterile 0.9% NaCl solution using a ten-fold dilute method to obtain a concentration of 10^3cfu/mL.

Samples with a dimension of 10 × 10 × 4 mm^3 were cleaned ultrasonically for 5 min in ethanol, rinsed with sterile water and dried in warm air. Then, samples were put into sterile well bottomed tubes with one sample in each, and 0.1 mL the fungi suspension was dropped on top of the samples and the tubes were sealed. Accordingly, all samples with fungi were incubated at a condition of 30°C for 24 hours. Then, the sample with fungi was washed with 4.8 mL 0.9%NaCl solution, and 0.1mL of the washing NaCl solution was taken and added into the nutrient agar medium, spread evenly and incubated for another 24 hours. Colony numbers were counted on a colony counting instrument (Gallenhamp UK).

Statistical Analysis

All experiments were performed for ten samples and representative results were presented as the means ± standard deviations. The ANOVA analyses for quantitative assays were performed and $p<0.05$ was considered to be statistically significant.

Results

Morphological characterization of prepared specimens

SEM images of the prepared specimens were showed in Figure 2. As can be seen wrinkled specimens with rough and approximately porous surface characteristics were obtained in blank PMMA specimens (×100, Figure 2a and b). On the other hand, incorporation of AG NPs or ZnO NPs resulted in more homogeny appearance in SEM pictures (Figure 2a and Figure 2b) respectively and smoother and integrated beads than their blank counterparts were achieved.

Anti-fungal activity of prepared specimens

Figure 3a shows the fungal colonies count after exposure to different PMMA+Ag NPs and 24 h incubation on the nutrient agar medium. The corresponding counts and microbicidal rates are shown in Table 1 as well. There were many fungal colonies on the blank control sample indicating that the blank PMMA did not have antimicrobial properties. In the contrary, a few fungal colonies however were found on Ag samples, indicating that PMMA+Ag NPs lowered the fungal count. As can be seen from Table 1 the number of colonies were decreased by increasing Ag concentration in the samples, while the calculated microbicidal Rate increased with the increasing of Ag content, demonstrating that the addition of Ag improved the antimicrobial properties of samples in a dose dependent way. For example, the microbicidal rates were 78%, 82.5%, 83.4%, 90.4 and 93.3 for 0.5, 2.5, 5, 10 and 20 % of Ag respectively.

On the other hand, the antimicrobial effect of PMMA+ZnO NPs was lower when compared with their Ag counterparts as shown in Figure 3b. Furthermore, at concentrations below 5%, ZnO did not show considerable anti-microbial properties as the obtained MRs were below 32%. Nevertheless the antimicrobial activity increased at concentrations of 5% and up. Interestingly, and contrary to Ag samples, in the case of ZnO the concentration dependent activity was not observed.

Table 1. Number of fungal colonies after 24 hours incubation on the surface of PMMA+Ag NPs and PMMA+ZnO NPs in study groups (mean ± standard deviations) with increasing concentrations of Ag and ZnO.

Sample group	Ag Concentration (%)	Number of microorganisms (Mean ± SD, n=10)	MR%*	ZnO Concentration (%)	Number of microorganisms (Mean ± SD, n=10)	MR%
1	0	586 ± 42	-	0	586 ± 42	-
2	0.5	127 ± 20	78	0.5	398 ± 51	32
3	2.5	102 ± 14	82.5	2.5	413 ± 32	29.5
4	5	97 ± 12	83.4	5	151 ± 30	74
5	10	56 ± 14	90.4	10	122 ± 17	79
6	20	39 ± 12	93.3	20	106 ± 12	81.9

*MR; Microbicidal Rate calculated as mean number of microorganisms in test group / mean number of microorganisms in control group.

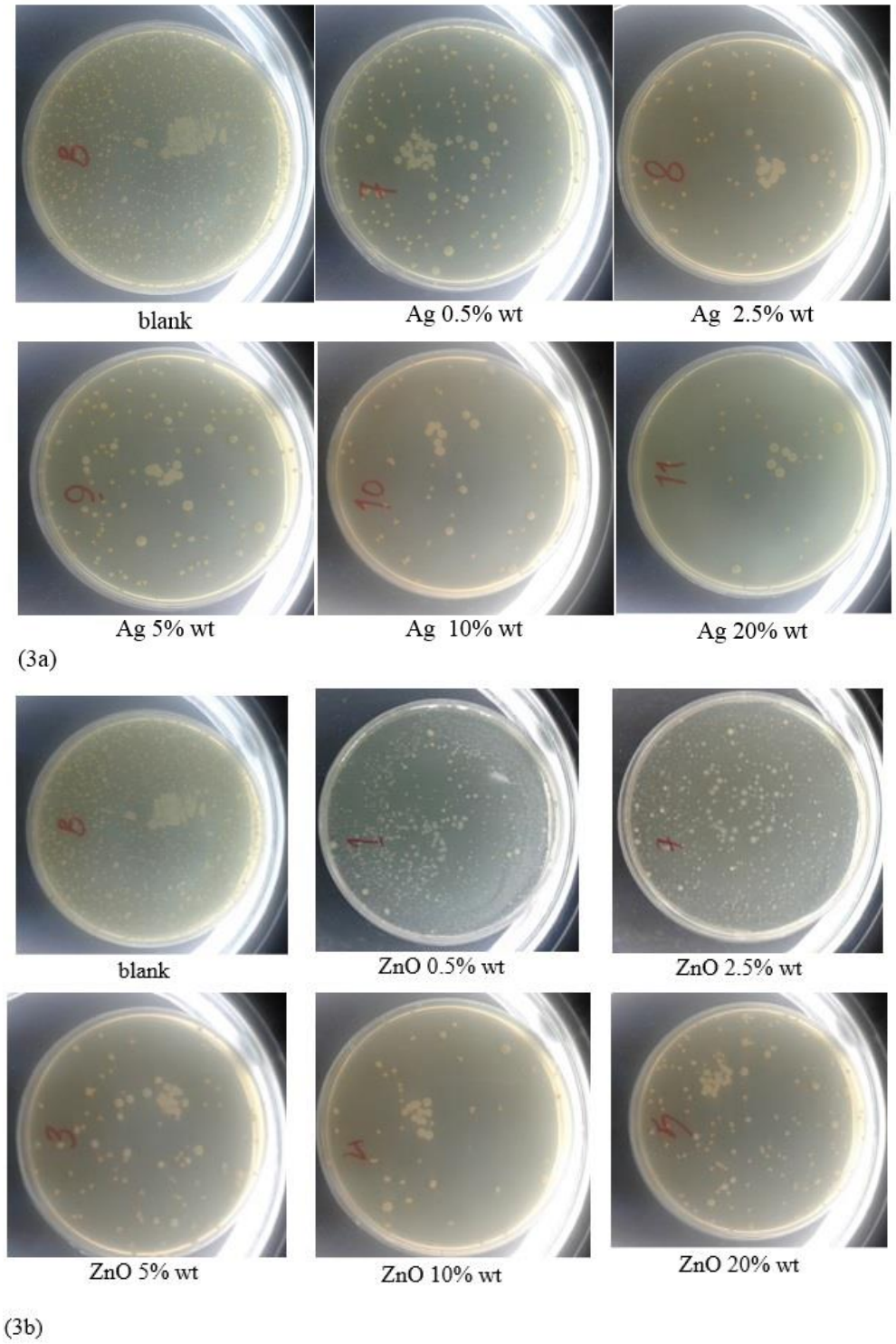


Figure 3. Plate pictures related to cultures of *C. alnicans* after exposure to (3a): PMMA+Ag NPs and (3b): PMMA+ZnO NPs specimens.

Discussion

The aim of this study was to evaluate the antifungal effects of ZnO NPs and Ag NPs used to modify acrylic dentures. Our finding demonstrates significant reduction in *C. Albicans* number by increasing nano-Ag and nano-ZnO concentration in acrylic resin. Figure 4 confirmed statistically this claim as the differences between colonies counts in PMMA+ZnO NPs and PMMA +Ag NPs specimens and that of blank are significant ($P \leq 0.001$). This result is in agreement with previous studies about the antifungal effect of Ag NPs in PMMA.[20] Current study showed in comparison to Ag NPs, higher concentration of ZnO NPs is required to inactivate or kill the *C. Albicans* as shown in Figure 4 ($P \leq 0.001$). The statistical graphs are shown in

figures 4 (a, b and c). This finding is similar to other study about the antimicrobial effect of metallic-oxides NPs [21-22] as the report of the median minimum inhibitory concentration (MIC) values for bacteria were 7.1, 200 and 500 mg/L for Ag, CuO and ZnO NPs, respectively. Whenever the toxic effect of ZnO NPs on mammalians cells was less than Ag NPs, and the respective median LC_{50} (lethal concentration, 50%) values for these cells were 11.3, 25 and 43 mg/L. This can show whenever ZnO NPs exhibit weaker antifungal properties than Ag NPs, it has less harmful effect on human and environment. From other hand brown discoloration of denture reinforced by Ag NPs is other disadvantage of this material can prevent using that in dentures.[23]

Ag in metal and metal oxide forms are antimicrobial materials that was reviewed in Maleki Dizaj et al. 2014.[19] Our finding is in good correlation with the published data in this regard and showed antifungal activity of Ag samples with a concentration dependent manner as the MR of the samples were significantly different from each other (p<0.001).

According to reports, Ag nanoparticles can make pits in the microbial membrane and then fragment the cell. It has also been recognized that Ag nanoparticles can interact with disulfide or sulfhydryl groups of enzymes that lead to disruption of metabolic processes which in turn cause the cell death.[19] Scientific reports also shown that antifungal activity of Ag NPs occurred by disrupting the structure of the cell membrane and inhibiting the normal budding process due to the destruction of the membrane integrity.[24] The mechanisms of killing the fungi by ZnO NPs in different species is different, as it can alter the cellular function that result in deformation of fungal hyphae from one side and also it can disturb in development of conidia and conidiaphores and killing of hyphae.[25] One study claimed both nano and bulk ZnO showed comparable toxicity to yeast *Saccharomyces cerevisiae* (8-h EC50 121–134 mg ZnO/l and 24-h EC50 131–158 mg/l respectively),[26] whenever it was shown the bulk form of ZnO is more less effective to *C. Albicans* and other fungi in comparison to MgO, CaO and ZnO NPs could well inhibit the growth of fungi consisted of *C. Albicans.*[27,28] The synergism of antifungal and antioxidant effects of ZnO NPs that enhances antifungal effect of these NPs material is the other subject of discussion in the literature. One study reported ZnO-thiram composites can facilitate internailization of ZnO NPs to fungi cell and in this manner oxidation process can kill the cell.[29]

Another proposed possibility about antimicrobial activity of ZnO NPs is the generation of hydrogen peroxide as a main factor of the antibacterial activity.[19] It is also believed that, ZnO NPs antifungal activity is because of affecting cellular functions, which caused deformation in fungal structure.[30]

Conclusion

As conclusion, ZnO NPs in PMMA effectively inhibited the *C. Albicans*, whenever its effect is not as powerful as Ag NPs. Because the long term and sustainable efficiency of this material as well as lower toxicity and its easy handling that is important to the treatment of chronic oral disease derived from the fungi, ZnO NPs in the range of 2.5 to 5 % W/W can be considered as a good candidate to incorporate in denture resins.

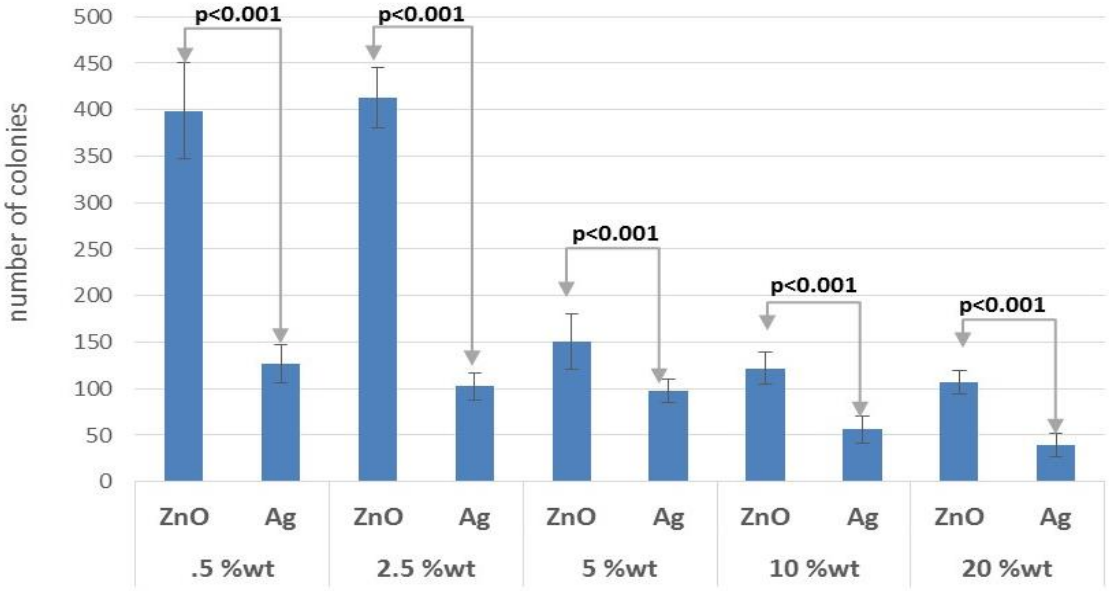


Figure 4. Comparison of antifungal property of Resin acrylics include Silver and Zinc oxide NPs indifferent concentration.

Conflict of interests

The authors claim that there is no conflict of interest.

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