RESEARCH PAPER

Correlation Between Nanoparticles Concentration, Prosthetic Material with Antifungal Activity of Silver Nanoparticles

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Received: 20th Feb, 2026 | **Revised:** 4th Mar, 2026 | **Accepted:** 25th Mar, 2026 | **Available Online:** 10th Apr, 2026

ABSTRACT

Objective: To assess the effect of silver nanoparticles (AgNps) concentration and different prosthetic materials (Denture base, Denture liner, Denture adhesive) with antifungal activity against Candida Albicans.

Materials and Methods: Total 120 specimens which divided equally into the. Group I: Heat cured acrylic resin denture base. Group II: Chemical cured acrylic resin denture liner. Group III: Cream type denture adhesive. Each group was further divided into four equal subgroups (ten specimens each) according to the concentration of silver nanoparticles; 0% (Control), 0.1%, 0.3% and 0.5%. Antifungal activity was done by counting the colony forming unit (CFU).

Results: The data showed that MIC for denture base was 0.3% for denture base, 0.5% for denture liner and 0.1% for denture adhesive. Two-way ANOVA showed highly significant effect of different prosthetic material as well as Ag-Nps concentration on CFU ($p < 0.0001$).

Conclusion: The minimum inhibitory concentration (MIC) required for complete inhibition is affected by the material type and concentration.

Keywords: Candida Albicans, Denture adhesive, Denture Liner, PMMA, Silver Nanoparticles.

How to cite this article: Mohamad A, Harby N, Badr W, Zaki S, Helal O, Dahab I, AbdelShafi MA, Bilal A, Hassan M. Correlation Between Nanoparticles Concentration, Prosthetic Material with Antifungal Activity of Silver Nanoparticles. Int J Drug Deliv Technol. 2026;16(29s):482-488. DOI: 10.25258/ijddt.16.29s.61

Source of support: Nil.
Conflict of interest: The authors declare no conflict of interest.

Introduction

Candida species can be found in many ecological situations such as the surface of fruit, rotten woods, soil, sea water, or associated with mammals and insects especially bees(1). It is a commensal organism in skin, female genitourinary system, gastrointestinal tract and oral cavity in about 30-60% of the healthy population(2), and counts for about 80% of the yeasts in the oral cavity. Candida albicans is distributed evenly throughout the oral cavity but the most common site is the dorsum of the tongue, also, it can occupy plaque surface. The colonization of the mouth by Candida albicans occurs at birth and decrease in early childhood and increases in the middle and late age (3). Candidiasis is influenced by a wide array of etiological factors, including inadequate denture hygiene, continuous denture use, immunosuppressive conditions, reduced salivary secretion, antibiotic therapy, high-carbohydrate diets, age extremes, smoking, diabetes mellitus, and malignancies (4). Additional factors are deficiencies in vitamin B12 and iron, as well as the administration of psychotropic medications (5). Owing to its multifactorial origin, management encompasses several strategies, with antifungal agents serving as the cornerstone of therapy(6) Complementary measures include nightly removal of dentures, diligent plaque control, and the use of antiseptic mouth rinses (7), Denture hygiene can be further optimized through brushing, soaking in commercial cleansing tablets, immersion in antimicrobial mouthwash solutions, or ultraviolet light (8).

Incorporation of antifungal agents in denture is effective. Furthermore, the incorporation of antifungal compounds directly into denture materials has demonstrated marked effectiveness in preventing Candida colonization(9).

A nanomaterial is defined as a natural, incidental, or engineered substance comprising particles that may exist in an unbound form, as aggregates, or as agglomerates, with at least 50% of the particles having one or more external dimensions within the 1–100 nm size range[(10)]. Nanoparticles may be natural or synthetic. Natural nanomaterials are produced by many living organisms as bacteria and fungi or prepared from plant extract(11). Nanoparticles are used in dentistry to enhance the properties of dental implants, adhesive agents, composite, denture base, bone grafts, hypersensitivity and bleaching agents(12-16)

Silver has been employed in dentistry and medicine since the 1800s due to its anticaries and antimicrobial properties. In the 1900s, prior to the development of antibiotics, silver compounds were commonly used to treat tetanus, gonorrhea, and even the common cold. They have also served as topical antibacterial agents for managing skin infections, burns, and chronic ulcers(17). More recently, the use of silver nanoparticles (Ag-NPs) in dentistry has garnered significant attention, as dental composites incorporating Ag-NPs exhibit superior antibacterial activity (18). Some authors evaluated the effect of an irrigant solution containing nano silver on Enterococcus faecalis and Staphylococcus aureus, and they found that there is no growth of any of them observed at different time intervals(19). Other reported the use of silver nanoparticles with the dentin adhesive inhibit the growth of the residual bacteria at tooth restoration interface, which decrease recurrent caries(20). Silver nanoparticles have an activity against Streptococcus mutants when added to endodontic cement, resin cement, and glass ionomer cement(21). It shows favorable clinical and radiographic outcome when used as an obturation material for primary Molars(22).

The effectiveness of antifungal agents depends on their administration at proper concentration. Using these agents at sub-lethal levels not only fails to control microbial growth but may also promote undesirable effects, including increased microbial tolerance, the development of antibiotic resistance, and a greater propensity for biofilm formation. Therefore, it is critical to be aware of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of disinfectants. The MBC represents the lowest concentration of an antimicrobial capable of killing more than 99.99% of the bacterial population.(23)

The null hypothesis states that:

The concentration of silver nanoparticles and the type of prosthetic material (denture base, denture liner, or denture adhesive) have no impact on the growth of *Candida albicans*, irrespective of the prosthetic material employed.

Material and Methods

Study Design

A total of 120 specimens were prepared for this study and evenly allocated into three main groups. Group I consisted of heat-cured acrylic resin denture bases (Acrostone heat-cured denture base, Acrostone, Cairo, Egypt). Group II included chemically cured acrylic resin denture liners (Mollosil, Detax GmbH, Ettlingen, Germany). Group III comprised cream-type denture adhesives (GlaxoSmithKline, England). Each main group was further subdivided into four subgroups of ten specimens each, corresponding to the concentration of silver nanoparticles: 0% (control), 0.1%, 0.3%, and 0.5%. The silver nanoparticles utilized in this study were spherical, measured less than 20 nm in diameter, and were stabilized with polyvinylpyrrolidone (Nanostreams, Egypt).

Preparation of denture base material containing nanoparticles

Wax patterns were prepared with dimensions 64 x 10 x 3.3 mm according to ISO standard number 20795-1:2013 followed by investment of wax pattern in mold and wax elimination by immersion of mold in boiling water. The nanoparticles were measured with Analytical balance device and incorporated into the polymer powder. Mixing of the material according to manufacturer instructions, the polymer to monomer ratio will be 3:1 by volume or 2.5:1 by weight, the material was mixed until reaching the dough stage followed by packing of denture base material in the molds. The material is cured by immersing the flasks in a 75ºC water bath for 2 hours, then a 100º C water bath for 1 hour and 30 minutes. The specimens were retrieved from mold then finished and polished using grit sandpaper

Preparation of liner material containing nanoparticle

The chemical cured soft liner will be mixed according to manufacturer instructions (3g of polymer with 1.8 ml of monomer) with the nanoparticles powder and packed into the molds after reaching the dough stage

Preparation of adhesive material containing nanoparticles

Heat cured acrylic resin specimens not containing nanoparticles are prepared and disinfected as stated before. The nanoparticles were incorporated into adhesive and painted on the surface of denture base material.

Disinfection of Specimens

All specimens were disinfected by immersing in glutaraldehyde 2% for 2 minutes and rinsed with sterile water

Preparation of bacterial cultures

Standard Candida albicans cultures were prepared and introduced in broth media and incubated for 24 hours at 37°C. Candida albicans were transferred to the surface of specimens in a sterile condition in laminar flow cabinet. Variable pipette was used to inoculate the bacteria to the surface of specimens and left for 24 hours at 37°C in microbiological incubator, and then specimens are dipped into test tube containing broth media and gently shake for 2 minutes by the vortex mixer. The fungal count was done by the plate count technique by transferring the fungi from the test tube to Brain Heart Infusion media and it is spread over the plates, and the numbers of colony forming units (CFU) were calculated.

Statistical analysis:

Two-way ANOVA was done to assess the effect of different prosthetic material and different nanoparticles concentration versus Candida albicans CFU using SPSS© version 20 for windows. The level of significance was set at $p<0.0001$.

Results

The results are shown in table 1 and figure 1. The data showed that MIC for denture base was 0.3% for denture base, 0.5% for denture liner and 0.1% for denture adhesive. Two-way ANOVA showed highly significant effect of different prosthetic material as well as Ag-Nps concentration on CFU ($p<0.0001$) (Table 2). Multiple comparisons using post-hoc Tukey test showed highly significant difference between the different prosthetic materials ($p<0.0001$) (Table 3). All Ag-Nps concentrations showed statistically significant difference when compared with the control group but not with each other (Table 4).

Table 1: Fungal Growth against different groups. Positive signs indicate growth of Candida Albicans, meanwhile negative signs indicate absence of growth

	Group I: Denture Base		**Group II: Denture Liner**		**Group III: Denture Adhesive**	
	CFU (Mean ± SD)	**Sensitivity**	**CFU (Mean ±**	**Sensitivity**	**CFU (M**	**Sensitivity**

			SD)		ean ± SD)	
Control	9211697 ± 364912.2	+	8514796 3 ± 4196 31.1	+	3901 40 ± 1736 0.6	+
0.1% Silver Nanoparticles	5317 ± 988.5	+	288 ± 10.1	+	Nil	-
0.3% Silver Nanoparticles	508 ± 99.4	+	Nil	-	Nil	-
0.5 % Silver Nanoparticles	Nil	-	Nil	-	Nil	-

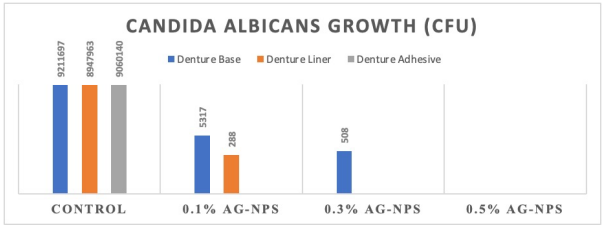

Figure 1:Candida Albicans Growth in different groups used in this study

Table 2: Two-Way ANOVA results (p<0.0001)
Figure 2: Left: Candida albicans growth in control group. Right: No growth in 0.5% AG-NPs

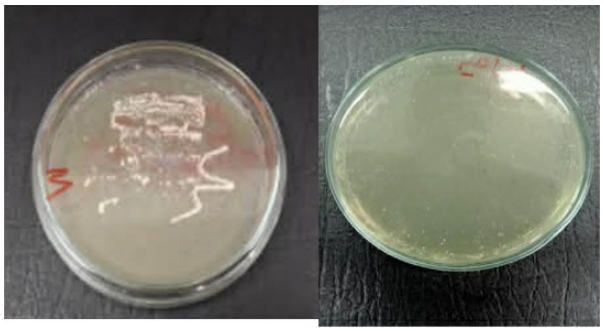

Table 2: Two-way ANOVA results for the effect of concentration and material type on Candida albicans growth

*Dependent Variable: CFU		
Source	F	Sig.
Prosthetic Material	2266.143	.000
Concentration	9301.117	.000

Table 3: Post Hoc-Tukey test for multiple comparisons of Different prosthetic Materials Candida albicans (CFU)

Prosthetic Material		Sig.
Denture Base	Denture Liner	.000
	Denture Adhesive	.000
Denture Liner	Denture Adhesive	.000

Table 4:Post Hoc-Tukey test for Different Ag-Nps Concentration

Concentration		Sig.
Control vs	0.1%	.000
	0.3%	.000
	0.5%	.000
0.1% vs	0.3%	1.000
	0.5%	1.000
0.3% vs	0.5%	1.000

Discussion:

The use of antifungal agents incorporated into denture liners or tissue conditioners to inhibit *Candida albicans* colonization should be reserved for patients at elevated risk, including those with xerostomia, a history of denture stomatitis, or physical disabilities affecting oral hygiene (24). A major limitation of conventional antimicrobial agents is the emergence of multidrug resistance. In contrast, the high surface-area-to-volume ratios and distinctive chemical and physical properties of various nanomaterials are thought to enhance their antimicrobial efficacy. Notably, nanoparticles are

capable of withstanding extreme conditions, such as high-temperature sterilization, which would typically inactivate conventional antibiotics(25). Numbers of factors contribute to antibacterial effect of nanoparticles. The smaller size of nanoparticle, the greater the antimicrobial effect. Indeed, spherical nanoparticles show greater antibacterial activity and concentration(26). The results agree with the studies that showed that silver nanoparticles are concentration dependent(27, 28).

The results show that the denture adhesive has greater antifungal activity against Candida albicans. This is in agreement with the previous results conducted that showed that the dental adhesives decrease the adherence of Candida albicans to heat cured acrylic resin denture base (29). (29). It is attributed to the decrease of the pH of the environment to the acidic level up to 100 hours which inhibit the growth of Candida albicans(30). A greater release of nanoparticles is observed from denture liners and adhesives compared to heat-cured acrylic resin denture bases, with the amount of release generally increasing with higher nanoparticle concentrations in denture bases and adhesives, whereas denture liners display the opposite trend. This phenomenon can be explained by the dual sorption theory, the intrinsic properties of the polymer, and water sorption behavior. In heat-cured denture bases, increasing nanoparticle concentration within the monomer results in a higher proportion of nanoparticles becoming entrapped within the polymer network during the curing process, leaving fewer free nanoparticles on the surface. Additionally, water diffusion through the polymer chains is limited due to the low hydrophilicity of heat-cured denture base polymers. Consequently, the remaining free nanoparticles outside the polymer network are readily leached out upon exposure to water(31)

The disk diffusion method is the most employed technique for antibiotic susceptibility testing in microbiology laboratories due to its low cost, simplicity, and applicability across a wide range of bacterial species and antibiotics. This method offers flexibility in selecting antibiotic disks, allowing clinical laboratories to tailor combinations based on the bacterial species and the origin of the isolate. Its straightforward interpretation facilitates the identification of atypical phenotypes and potential contamination. However, a primary limitation of this method is its inability to provide the MIC of the tested antibiotics (32)

The cytotoxic effect of nanoparticles against human body is studied. It has been reported that nanoparticles can cause adverse effect on lung, central nervous system, cardiovascular and gastrointestinal tract (33). Cytotoxicity depends on the shape, size of and the dose of the nanoparticles. The toxic dose of copper nanoparticles with range under 20 nm against mouse embryonic fibroblast is 1000 ug/ml or greater(34). However, other researchers showed that tissue conditioner containing silver nanoparticles with average size 50-80 nm and concentration up to 0.5% showed no cytotoxic effect on human gingival fibroblasts, and within the limitations of his study it was concluded that tissue conditioners containing nanoparticles shows good cytocompatibility and further studies are needed to confirm that results (35). Shifting to green synthesis is a natural alternative to synthetic antifungal agents. Natural extracts such as Musa paradisiaca l, and Avicennia marina, Berzelia lanuginose, Helichrysum cymosum, and Searsia crenata showed marked antifungal activity against Candida albicans (36-38).

Conclusion

The incorporation of silver nanoparticles (AgNPs) into dental denture bases materials, liners, and adhesives, significantly enhances their antifungal activity against *Candida albicans* in a concentration-dependent manner. The minimum inhibitory concentration (MIC) required for complete inhibition was 0.3% for denture base, 0.5% for denture liner, and 0.1% for denture adhesive. The antifungal activity varied by the prosthetic material, and the denture adhesives containing AgNPs demonstrated the greatest antifungal effect followed by denture liners and denture bases. These findings indicate that AgNPs can be an effective strategy for the prevention of fungal colonization and denture-related stomatitis in patients at risk.

Study limitations:

The study was conducted in vitro using standardized laboratory conditions, which may not fully replicate the complex oral environment found in vivo. Indeed, only *Candida Albicans* were tested, whereas other species may also play a role in denture stomatitis. There was no direct assessment of silver nanoparticle toxicity, since the cytotoxic potential of prosthesis containing Ag-NPs was cited from previous literature.

Acknowledgement

The author declares that there are no acknowledgements

Conflict of Interest

The authors report no conflict of interest.

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