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Antifungal and cleansing efficacy of gold nanoparticle reinforced denture cleanser- An *in vitro* study

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Abstract:
The oral cavity represents a dynamic microbial enivironment. Therefore, it is of interest to evaluate the AuNP reinforced denture cleanser and investigated the antifungal and cleansing efficacy of denture cleansers incorporated with and without AuNPs. AuNP reinforced denture cleanser were prepared and evaluated for antifungal zone of inhibition. Denture cleansers with AuNP's had a significant improvement in their antifungal and cleansing efficacy. Thus, the AuNPs decreased the concentration of cleanser and prevented fungal colonisation on the denture surface.

Keywords: Antifungal, cleansing efficacy, denture cleanser, gold nanoparticle (AuNPs)

Background:
The oral cavity represents a dynamic microbial enivironment in which microorganisms continuously interact with host tissues and prosthetic surface. In completely edentulous individuals, removable dentures provide additional surfaces that can facilitate the adhesion, accumulation and maturation of microbial biofilms, compromising both bacterial and fungal species **[1-3].** Traditional denture cleansers, though effective in removing debris and hasaantibacterial effect by mechanochemical methods. Traditional cleansers may have little effect on completely eliminating biofilms and preventing microbial recolonization **[4].** In recent years, nanotechnology as a promising approachand enhances the quality of healthcare **[5].** Among various nanomaterials, gold nanoparticle (AuNPs) has considerable attention to their distinctive physochemical properties and antimicrobial potential **[6].** Their nanoscale dimensions and surface area to volume facilitate interactions with microbial cells, leading to disruption of cellular membranes and inhibiton of microbial growth **[7].** Furthermore, AuNPs have demonstrated relatively less cytotoxicity in biomechanical applications. Biofilms deliver a shielding atmosphere for microorganisms, permitting them to persist despite traditional cleaning efforts **[8].** The incorporation of AuNPs into denture cleansers should pose remarkable antibacterial and antifungal properties and their combined effect to disrupt biofilms is yet to be assessed **[9].** Therefore, it is of interest to evaluate the AuNP reinforced denture cleanser and investigated the antifungal and cleansing efficacy of denture cleansers incorporated with and without AuNPs.

Materials and Methods:
This present *in vitro* study conducted in the Department of Prosthodontics and Microbiology, Chennai, India. The material used in the study, Clinsodent (ICPA Health Products), goldsalt (Sisco Research laboratories, SRL precursor), DPI Heat cure material(The Bombay Burmah Trading Corporation Ltd,).

Synthesis of AuNP reinforced denture cleanser:
Gold nanoparticles were synthesized by Turkevich citrate reduction method to yield AuNPs with final stock concentration of 1% w/v and the particles were characterised **[10].** Denture cleanser powder containing potassium persulphate and sodium perborate (Clinsodent, ICPA Health Products) was added in a ratio of 2 milligrams of powder and 18 milliliters of sterile deionized water. For the test formulation (Group A) 20 µg of 1% AuNP suspension was combined with an equal volume of denture cleanser slurry (1:1 v/v). The mix was placed in a bath sonicator at room temperature (approximately 22–25°C) and sonicated for 30 minutes to promote dispersion and interaction between AuNPs and cleanser components **[11]**. Denture cleanser without incorporation of NP was used as control group B. After sonication the final test mixture was stored at 4°C.

Zone of inhibitionassay:
Candida albicans (standard strain) was cultured and adjusted to a standardized inoculum density (0.5 McFarland standard). The inoculum was spread onto Mueller–Hinton agar plates with 2% glucose and 0.5 µg per mL methylene blue to facilitate fungal growth and better visualization. Using a sterile 5 mm diameter core borer, three wells were aseptically prepared in each agar plate. The wells were assigned as follows: Well 1 (Test group): denture cleanser combined with AuNPs, Well 2 (Control group): denture cleanser without AuNPs, Well 3 (Negative control): sterile distilled water. 50 µL of the respective test solution was filled in each well. The plates were incubated at 37 °C for 24 hours. A Vernier caliper was used to assess the diameter of the inhibited zones in millimeters to assess the antifungal activity. To ensure reproducibility each experiment was run in triplicate. Minimum Inhibitory Concentration were determined using the

microdilution in a 24-well plate **[12].** In each row of wells, 500 µL of sterile sabouraud broth, followed by the addition of 100 µL of the Group A test specimens stock solution to the first well. A two-fold serial dilution was then performed by transferring 100 µL from the first well to the next, mixing thoroughly and repeating through the series, discarding the final 100 µL to maintain equal volumes. A standardized inoculum, adjusted to ~5 × 10^5 CFU/mL, was added to bring the final volume to 200 µL. A positive control of bacteria were included without antibiotic and antimicrobial agent. The plate was incubated at 37°C for 20 hours. After incubation, wells were examined for turbidity and the MIC was identified. The same procedure is repeated for control samples (Group B). A total of 100 specimens of polymethyl methacrylate (PMMA) sample, in dimension 64 mm × 10 mm × 3.3 mm were fabricated using the lost wax technique. The specimens were subsequently polished andrandomly allocated into three groups; Group A (Test group) was immersed in the denture cleanser containing AuNP, while Group B (Control Group) were immersed in the denture cleanser without AuNP, both at the predetermined MIC dilution for 8 hours. Group A and Group B were subjected to MIC of denture cleansers and denture cleanser with AuNP. Group C was neutral control without immersion in any cleansers. They were contaminated with artificial saliva at 37 degrees Celsius for 24 hours. Specimens from Group A, B and C were immersed in sterile sabouraud dextrose broth inoculation with a standard suspension of *Candida albicans* and incubated at 37degree celsiud for five days under controlled conditions to facilitate biofilm formation **[13].** Following incubation, the specimen were gently agitated to detach lossely adherent microbial cells, after which swab samples were inoculated onto sterile sab ouraud dextrose agar plates using the lewn culture technique and incubated at 37 degree celsiusfro 24-48 hours. Following incubation the colonies formed on the gar plates were counted to determine the colony formation units (CFU). The CFU/ml of the original samples was calcukated using the dilution factor and the volume of the diluted sample plated, accosding to the formula: CFU/ml= (Number of colonies × Dilution factor)/Volume plated. Descriptive statistics was conducted to assess the mean and standard deviation. One-Way ANOVA and Post hoc Tukey was used to compare the groups. P<0.05 was significant.

Results:

The antifungal efficacy of the gold nanoparticle indenture cleanser was evaluated using the zone of inhibition test. The mean zone of inhibition for the test group (denture cleanser with AuNPs-Group A) was 27.535 ± 0.727 mm, whereas the control group (denture cleanser without AuNPs-Group B) exhibited a significantly smaller inhibition zone of 23.57 ± 0.959 mm (P<0.001). The minimum inhibitory concentration, Group A was determined 1.25µg of AuNP, with 0.125 µg of cleanser at a 10^{-6} dilution, the MIC for Group B was 0.25 µg of cleanser at 10^{-5} dilution. Based on the findings, the specimens were subjected to microbial culture using the respective MIC concentration of both groups. The cleansing efficiency was evaluated, denture cleanser at the MIC dilution of 10^{-5} and 10^{-6}. Hence, a concentration of 0.25 µg of denture cleanser (corresponding to 2.5 µg of AuNP for test group)(10^{-5} dilution) and a concentration of 0.125 µg of denture cleanser (corresponding to 1.25 µg of AuNP for test group) (10^{-6} dilution) was evaluated. The mean number of microbial colonies in the Group C samples was 63.24±1.08. The mean number of microbial colonies in the Group B samples was 27.75±2.10 and Group A samples was 7.57±1.48 for lower concentration 0.125 µg of denture cleanser after immersing (**Table 1**). The mean number of microbial colonies in the Group B sample s was 22.02±1.03 and the Group A samples was 4.41±0.75 for higher concentration of 0.25 µg of denture cleanser. Group A - Denture cleanser with incorporation of Gold Nanoparticle, Group B - Denture cleanser without incorporation of Gold Nanoparticle, Group C- without immersion, N-samples size. Post hoc Tukey HSD analysis further confirmed lowest mean of 4.41±0.755 for specimen immersed in 0.25 µg cleanser with 2.5µg AuNPs (**Table 2**).

Table 1: Comparative analysis of the cleansing efficiency of denture cleansers on the denture surface using ANOVA

Concentration	Variables	N	Mean (No Of Colonies)	Std. Deviation	Std. Error of Mean	P value
0.125 µg of denture cleanser(corresponding to 1.25 µg of AuNP in test group)	Group C	20	63.24	1.081	0.241	<0.001**
	Group B	20	27.75	2.105	0.47	
	Group A	20	7.57	1.481	0.331	
0.25 µg of denture cleanser(corresponding to 2.5 µg of AuNP in test group)	Group C	20	63.24	1.081	0.241	<0.001**
	Group B	20	22.02	1.032	0.237	
	Group A	20	4.41	0.755	0.169	

Group A - Denture cleanser with incorporation of Gold Nanoparticle, Group B - Denture cleanser without incorporation of Gold Nanoparticle, Group C- without immersion, N-samples size.

Table 2: Intergroup - Comparative analysis of the cleansing efficiency of denture cleansers on the denture surface using Tukey HSD

Dependent Variable (No Of Colonies)	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
0.125 µg of denture cleanser(corresponding to 1.25 µg of AuNP in test group)	**Group C**	**Group B**	37.40*	.539	**<0.001****	36.10	38.69
	Group C	**Group A**	57.58*	.539	**<0.001****	56.28	58.87
	Group B	**Group A**	20.18*	.539	**<0.001****	18.88	21.47
0.25 µg of denture cleanser(corresponding to 2.5 µg of AuNP	**Group**	**Group**	41.22*	.305	**<0.001****	40.48	41.95

in test group)	**C**	**B**					
	Group C	**Group A**	58.83*	.305	**<0.001****	58.09	59.56
	Group B	**Group A**	17.61*	.305	**<0.001****	16.87	18.34

**The mean difference is significant at the 0.05 level*

Discussion:

The denture cleanser with AuNP significantly improved the antifungal and cleansing efficacy compared to denture cleanser without AuNP and hence the null hypothesis was rejected. The antifingal effect of the AuNP-incorporated denture cleanser was evident, that the mean zone of inhibition significantly higher than control group **[10-13].** The antimicrobial properties of AuNPs primarily due to their capacity to damage microbial cell membranes and increase oxidative stress, which eventually results in microbial cell death **[14].** The antifungal potential of AuNPs suggests their role as a promising antimicrobial agent in oral healthcare applications **[2].** The present study further supported this evidence by demonstrating a significant reduction in microbial activity when AuNPs were incorporated into the denture cleanser. Incorporation of AuNPs, a potential enhancement in antifungal activity. Previous research has reported an MIC of 24.37µg per ml for plain AuNPs against *candida albicans*, which decreased to 2.437µg per ml when combined with indolicidin **[15].** The lower MIC observed in the present study may be attributed to the combined antifungal effects of the cleanser and AuNPs **[16].** Previous studies have also suggested that nanomaterials can inhivit microbial abhesion and biofilm formation on dental prosthetic surfaces **[17].** Various antimicrobial agents have been incorporated into denture cleanser to improve their antimicrobial efficacy **[18].** Silver nanoparticles (AgNPs), for instance, have been extensively investigated for their antimicrobial properties and effectiveness against *Candida albicans* **[17].** However, prolonged exposure to AgNPs has been associated with potential cytotoxic effects, which may limit their suitability for long-term applications. Similarly, zinc oxide nanoparticle (ZnONPs) has been incorporated into denture cleansing formulations because of their antimicrobial properties; however, their uze may be associated with denture discoloration and irritation of the oral tissues **[19].** In comparison AuNPs have been reported to exhibit good biocompstibility and potential to modukate inflammatoey responses, supporting their investigation for biomedical and dental applications. The cleansing efficacies of the denture cleanser were evaluated based on the microbial colonies on the denture surface in our study. The mean number colony count on the denture base resin was significantly reduced following immersed in the AuNP-incorporated cleanser compared with both the cleanser without AuNPs and before immersion. Chlorhexidine-based denture cleansers have also been used due to their broad-spectrum antimicrobial activity, but their extended use can stain the denture and disruptoral microbiome **[20, 21].** Certain agents have been associated with cytotoxic effects and allergic reactions in suspectible individuals **[22].** In addition, prolomged exposure of denture cleanser in PMMA can cause surface roughness **[23].** Effervescent washing granules containing 1.2ZnSLS showed antifungal effectiveness against *Candida albicans* equivalent to that of commercial effervescent tablets, according to Piboonchaisit *et al.* **[24]**. A low concentration of silanized or hybrid nanoparticles would be a potential strategy to avoid denture stomatitis, according to Bunyan *et al.* **[25]**. The extract of *T. conoides* seaweed and the commercial denture cleaning Fittydent were shown by Varsha *et al.* to be beneficial in lowering the number of *C. albicans* colonies **[26]**. AuNPs have been investigated as potential alternatives to their reported biocompatibility and relatively low cytotoxicity compared with certain other metal nanoparticles **[19].** Furthermore, the biological effects of AuNPs can vary depending on their physicochemical characteristics, particularly particle size and morphology. Spherical AuNPs have generally demonstrated lower cytotoxicity than nanoparticles with rod or star shaped morphologies **[18].** Therefore, AuNPs may represent a potential alternative to conventional antimicrobial agents for incorporation into denture cleansing frmulations. The *in vitro* experimental condition of the present study were limited in their ability to replicate the comples oral environment , including salivary flow, pH variations and the presence of a microbial community. Also, the research was limited to short-term evaluation of the antifungal and cleansing effects of the AuNP-incorporated cleanser. Further studies on the long duration stability and the cumulative effects of AuNP exposure on denture surface and oral tissues are required. Experimental research on animal and human populationare needed to confirm the reproducibility of these findings. As the first study to explore the effectiveness of AuNP in denture cleanser, it opens new possibilities for nanoparticle-based antimicrobial strategies in prosthodontics. Although the incorporation of AuNPs demonstrated promising outcome, the present study did not evaluate the potential for nanoparticle aggregation or the effects of varying AuNPs concentration. The incorporation of AuNPs in denture cleansers presents a promising strategy to improve oral hygiene among denture wearers, to prevent candidal-denture stomatitis.

Conclusion:

Denture cleansers with AuNP's had a significant improvement in their antifungal and cleansing efficacy. The AuNPs decreased the concentration of cleanser and prevented fungal colonisation on the denture surface. Further studies should focus on *in vivo* evaluations to assess the clinical benefits, safety and long-term impact of AuNP-enhanced denture cleansers.

Conflict of interest statement:

There is no conflict of interest among the authors

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