

Comparative Evaluation of the Antifungal Efficacy of Tissue Conditioners Incorporated With Graphene Oxide Nanoparticles (GoNPs), Silver Nanoparticles (AgNPs) and Copper Nanoparticles (CuNPs) in Inhibiting the Growth of Candida Albicans: An Invitro Study

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ABSTRACT

Background: Candida-induced stomatitis is a significant concern for most medically compromised denture-wearing patients. The combination of a tissue conditioner with an antifungal agent can effectively enhance the health of compromised tissues. This study aimed to evaluate and compare the antifungal efficacy of tissue conditioners incorporated with nanoparticles of graphene oxide, silver nanoparticles, and copper nanoparticles in inhibiting the growth of Candida albicans.

Materials and Methods: Graphene oxide, silver nanoparticles, and copper nanoparticles were added at 5% by weight to the tissue conditioner material to form four groups: A, B, C, and D. Antifungal susceptibility was assessed using the well diffusion method with SDA on 90-mm plates. A total of five plates for each category were prepared and incubated at 37 °C for seven days. The tissue conditioner liquid and powder, each containing 5% GoNPs, 5% AgNPs, and 5% CuNPs respectively, were mixed and then filled into the punched-out wells. The diameter of the inhibition zone (DIZ) was measured with a metallic scale at the end of one day and seven days. The collected data were subjected to statistical analysis using one-way ANOVA, Tukey's multiple post hoc test, and the dependent t-test.

Results: The addition of nano-graphene oxide in the tissue conditioner showed a statistically significant increase in antifungal activity against Candida albicans. The acrylic-based tissue conditioner with GO NPs exhibited the highest antifungal activity, followed by the tissue conditioner with CuNPs, and the least with the tissue conditioner containing AgNPs, both after the 1st day and the 7th day. In contrast, the control group showed no signs of an inhibition zone on day 17.

Conclusion: Antifungal activity is present. Thus, graphene oxide, silver, and copper nanoparticles can be used to reduce fungal growth and have the potential to treat candida-induced denture stomatitis through drug delivery via tissue conditioners.

Keywords: Antifungal, Tissue Conditioners, Graphene Oxide.

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Denture stomatitis (DS) can affect both partial and complete denture wearers, although it is commonly seen on the palatal mucosa beneath a maxillary complete denture. DS rarely affects the lower arch, possibly due to the washing effect of saliva and the cleansing action of the tongue, whereas in the upper arch, the prosthesis may have a better peripheral seal and thus create its own microenvironment. Institutionalized patients may find it difficult to follow the prescription for the daily application of the antifungal agent onto the denture and consequently rely on nursing or other healthcare professionals. One possible strategy is to incorporate an antifungal agent into a tissue conditioner, which may be used to improve the denture fit and reduce trauma by cushioning the tissues against applied loads. Recent studies have demonstrated the antifungal efficacy of various nanomaterials, such as magnesium oxide (MgO), zinc oxide (ZnO), and silver nanoparticles when mixed with tissue conditioners.

Nano graphene oxide (nGO) is known to have antimicrobial activity through surface contact. Sharp nanosheets cause perturbation of the cell membrane, electrolyte leakage, and eventually cell death. Silver has a long history of use as an antimicrobial agent. Silver can interfere with sulfhydryl groups on enzymes involved in metabolism and proteins in the tissue structure of microorganisms, and it also inhibits cell replication by binding to and denaturing bacterial DNA and RNA. The present study aims to evaluate and compare the antifungal efficacy of tissue conditioners incorporated with nanoparticles of graphene oxide, silver nanoparticles, and copper nanoparticles in inhibiting the growth of *Candida albicans*. The null hypothesis states that there is no significant difference in antifungal efficacy when tissue conditioners incorporated with nanoparticles of graphene oxide, silver nanoparticles, and copper nanoparticles inhibit the growth of *Candida albicans*.

MATERIALS AND METHODS

Preparation of GoNPs-incorporated tissue conditioner

Graphene oxide nano particles (GoNPs) powder were added in 5% by weight relative to the acrylic based tissue conditioner powder and thoroughly mixed.

Preparation of AgNPs-incorporated tissue conditioner

AgNPs powder were added in 5% by weight relative to the acrylic-based tissue conditioner powder and thoroughly mixed.

Preparation of CuNPs-incorporated tissue conditioner

CuNP powder was added in 5% by weight relative to the acrylic-based tissue conditioner powder and thoroughly mixed. Four groups were considered in the study: Group A (commercially available tissue conditioner), Group B (tissue conditioner + graphene oxide nanoparticles), Group C (tissue conditioner + silver nanoparticles), and Group D (tissue conditioner + copper nanoparticles). The obtained data were subjected to statistical analysis using one-way ANOVA, Tukey's multiple post hoc test, and dependent t-tests test.

RESULTS

The acrylic-based tissue conditioner with GO NPs showed the highest antifungal activity, followed by the tissue conditioner with CuNPs, while the least activity was observed with the tissue conditioner containing AgNPs, both after the 1st and 7th days. In contrast, the control group displayed no signs of an inhibition zone on either day 1 or day 7. The mean diameter of the inhibition zone for group A (control), group B (Ag NPs), group C (Cu NPs), and group D (GO NPs) after day 1 was 0, 14.15, 16.99, and 20.82, respectively. After 7 days, the mean values decreased to 0, 13.53, 16.08, and 20.15, respectively.



Figure 1: Mean diameter of inhibition zone among all the groups after day 1.

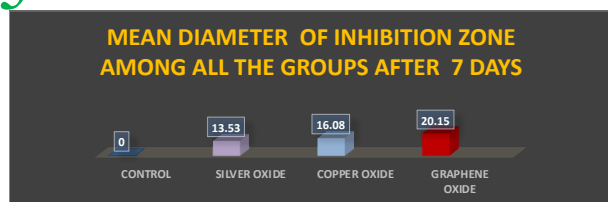


Figure 2: Mean diameter of inhibition zone among all the groups after day 7.

A dependent t-test was conducted to compare inhibitory efficiency after 1 and 7 days, showing statistically significant differences for each group with values for group A.

Table 1 Comparative analysis of Mean Diameter of Inhibition among all the groups at different time intervals using Dependent t-test

GROUPS	DAY	MEAN±SD	T Value	P value
Silver nanoparticles	DAY 1	14.15±0.20	18.27	0.00*
	DAY 7	13.53±0.27		
Copper nanoparticles	DAY 1	16.99±0.28	30.85	0.00*
	DAY 7	16.08±0.23		
Graphene Oxide nanoparticles	DAY 1	20.82±0.46	15.36	0.00*
	DAY 7	20.15±0.46		

* $p \leq 0.05$ is considered as statistically significant

Table 2,3 shows the statistical comparison of the mean diameter of the inhibition zone of the groups after day 1 and 7 by one way ANOVA test($p < 0.05$). F value indicates one-way ANOVA value i.e., on day 1 is 4854.88 and day 7 is 4457.45.

Table 2 Comparative analysis of Mean Diameter of Inhibition among all the groups after 1 day using One Way ANOVA

GROUP	MEAN±SD	F VALUE	P VALUE
A	0.00±0.00	4854.88	0.000*
B	14.15±0.20		
C	16.99±0.28		
D	20.82±0.46		

* $p \leq 0.05$ is considered as statistically significant

Table 3 Comparative analysis of Mean Diameter of Inhibition among all the groups after 7 days using One Way ANOVA

GROUP	MEAN±SD	F VALUE	P VALUE
A	0.00±0.00	4457.45	0.000*
B	13.53±0.27		
C	16.08±0.23		
D	20.15±0.46		

* $p \leq 0.05$ is considered as statistically significant

Post -Hoc Tukeys test analysis revealed statistically significant mean difference ($p = 0.00$) between each pair of groups after 1 and 7 days

Table 4 Pairwise comparison of diameter of Zone of inhibition after 1 day using Post-Hoc Tukey's Test

Comparison Groups	Mean difference	P Value
Group A Vs Group B	14.15	0.00*
Group A Vs Group C	16.99	0.00*
Group A Vs Group D	20.82	0.00*
Group B Vs Group C	2.84	0.00*
Group B Vs Group D	6.66	0.00*
Group C Vs Group D	3.82	0.00*

* $p \leq 0.05$ is considered as statistically significant

Table 5: Pairwise comparison of diameter of Zone of inhibition after 7 days using Post-Hoc Tukey's Test

Comparison Groups	Mean difference	P Value
Group A Vs Group B	13.53	0.00*
Group A Vs Group C	16.08	0.00*
Group A Vs Group D	20.15	0.00*
Group B Vs Group C	2.55	0.00*
Group B Vs Group D	6.62	0.00*
Group C Vs Group D	4.06	0.00*

* $p \leq 0.05$ is considered as statistically significant

DISCUSSION

The precise pathogenies of DS are not known, but infection with *Candida* is most likely to be associated with mucosal trauma induced by ill-fitting dentures, sub-optimal oral hygiene, the nocturnal wearing of dentures, and xerostomia³. Research suggests that trauma results in an inflammatory reaction, which creates a favorable environment for *C. albicans* to invade the tissues and initiate an inflammatory reaction. Candidal growth has also been associated with the use of soft denture liners, often used to improve the fit of dentures.

To overcome these limitations, antifungal agents have been incorporated into TCs. Douglas and Walker reported incorporating the antifungal agent nystatin into TCs in 1973; similar studies have been published since, classifying antifungal agents based on natural and synthetic origins, Natural agents, such as oils may possess effective antifungal properties⁴.

Biopolymers as Antimicrobial and Antifungal Agents: Di Giulio M et al. (2018) have proved that graphene oxide shows antimicrobial and antibiofilm efficacy against chronic wound microorganisms,

including *Candida albicans*. Its blade-like configuration physically damages the microbial cellular membrane and causes leakage of intercellular substances, leading to cell death. Wrapping and photothermal ablation mechanisms also lead to cell damage. Chemical production of ROS also causes the disintegration of cell membranes and eventual cell death⁵.

Biocide-releasing polymers (silver zeolites, silver nanoparticles, and platinum nanoparticles) act as carriers for biocides and do not participate in polymerization. These materials can release their biocides near the cells in high local concentrations. Polymeric surfaces can be coated with silica, titanium dioxide, and poly (dimethyl siloxane). These coatings can alter the hydrophobic nature of PMMA to a hydrophilic one. In contrast to polymeric biocides and biocide-releasing polymers, surface coatings can only affect the surface properties of PMMA^{6,7}.

In the present study, *Candida albicans* (American Type Culture Collection catalogue number 90028 strains) was obtained and subcultured on Sabouraud dextrose agar (SDA) in a tube, then incubated at 37°C. After 24 hours of incubation, the *Candida albicans* suspension was mixed with sterile saline to achieve a density of 0.5 McFarland for concentration standardization. Antifungal susceptibility was assessed using the well diffusion method on SDA within 90-mm diameter Petri plates. The plates were inoculated with *Candida albicans* using an inoculation loop. Once the inoculum dried, 6mm diameter and 5mm depth wells were created with a sterile cork borer. Tissue conditioner liquid and powder containing 5% GoNPs, 5% AgNPs, and 5% CuNPs were mixed respectively according to the manufacturer's instructions, and then filled into the punched-out wells.

Five (5) plates for each category were prepared and incubated at 37°C for seven days. At the end of the seventh day, the diameter of the inhibition zone (DIZ) was measured using a digital vernier caliper. In the present study, the mean diameter of the inhibition zone for group A(control), group B(Ag NPs), group C(Cu NPs), and group D(Go NPs) after day 1 was 0, 14.15, 16.99, and 20.82, respectively. The mean values decreased after 7 days to 0, 13.53, 16.08, and 20.15, respectively.

The acrylic-based tissue conditioner with GO NPs showed the highest antifungal activity, followed by tissue conditioner with CuNPs and least with tissue conditioner with AgNPs both after the first day and the seventh day, whereas the control group showed no sign of an inhibition zone on days 1 and 7.

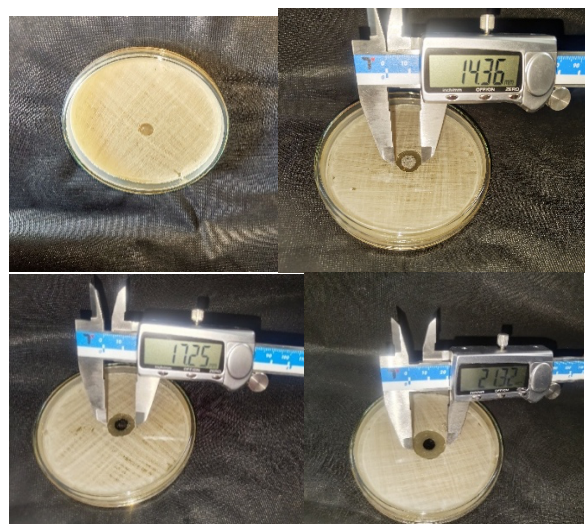


Figure 1: showing DIZ on day 1 of control, silver nanoparticles, copper nanoparticles, graphene oxide nanoparticles



Figure 2: showing DIZ on day 7 of control, silver nanoparticles, copper nanoparticles, graphene oxide nanoparticles

Clinical significance the current study rejects the null hypothesis. Antifungal activity is present, Thus, Graphene Oxide, Silver, Copper nanoparticles can be used to reduce the fungal growth and have potential to treat candida induced denture stomatitis by drug delivery through tissue conditioners.

Limitations

Based on the limitations of the methodology followed within this study, the following were the conclusions drawn:

Graphene Oxide (GO), Silver, Copper nanoparticles exhibit antifungal activity.

Tissue conditioner with GO NPs showed highest antifungal activity on 1st day

Tissue conditioner with GO NPs showed highest antifungal activity on 7th day

This in vitro study included observation for 7 days. In many treatment modalities, the use of soft liner can extend up to several weeks. Therefore, along with the cytotoxicity test, the durability of sustained drug delivery should be evaluated for further clinical use.

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