

Original Article

Long term antifungal efficacy of silver-zinc zeolite nanoparticles incorporated in two soft denture liners - An *in vitro* assessment

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ABSTRACT

Background: There is generally a lack of compliance in patients who report with oral candidiasis, as they are advised to temporarily stop wearing the prosthesis and are prescribed topical antifungals which are generally unpleasant to taste and follow a rigorous schedule. Furthermore, with the alarming evidence of drug resistance, there is a need for an enhanced drug and drug delivery system. The aim of the study was to determine the dose-dependent antifungal efficacy of silver-zinc zeolite nanoparticles (SZZ-NPs) when incorporated in two brands of soft denture liners against *Candida albicans*.

Materials and Methods: A total of 72 samples were made to determine the *in vitro* antifungal efficacy of SZZ-NPs and fluconazole by measuring the mean inhibition diameter (MID). Two concentrations of SZZ-NPs were compared (0.5%, 2% w/w) with fluconazole 5%w/w which is routinely prescribed. The antifungals were incorporated in two types of commercially available soft denture liners (Visco gel, GC soft denture liner). The MID's were measured at day 1, day 7, day 15, and day 30. The values obtained ($P < 0.001$) were analyzed with one-way ANOVA, Tukey's *post hoc*, and independent t-test.

Results: A statistically significant difference ($P < 0.001$) was noted among all the antifungal agents at all the time intervals tested. The anti-fungal efficacy of SZZ-NPs 2% w/w incorporated in GC soft denture liner was significantly superior ($P < 0.001$) to all groups tested and it retained its antifungal efficacy even on day 30 (MID: 18.33 ± 2.44).

Conclusion: SZZ-NPs 0.5%w/w, 2%w/w, and fluconazole 5%w/w can be incorporated with soft denture liners against *C. albicans*. Fluconazole 5%w/w is the recommended choice for short-term antifungal efficacy, while SZZ-NPs 2%w/w is recommended when long-term antifungal efficacy is needed. GC soft denture liner was the recommended choice.

Key Words: *Candida albicans*, drug delivery systems, drug resistance, nanoparticles, zeolites

Received: 20-Oct-2020
Revised: 07-Sep-2021
Accepted: 09-Nov-2021
Published: 28-Feb-2022

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INTRODUCTION

Denture-induced stomatitis is commonly encountered during the use of acrylic dentures. *Candida albicans* (*C. albicans*) is the predominant

pathogen, associated with this opportunistic infection.^[1,2] Maintenance of oral hygiene, adequate denture cleansing, and the therapeutic use of topical

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How to cite this article: Ferreira AN, D'Souza K, Aras M, Chitre V, Parsekar S, Pinto MJ. Long term antifungal efficacy of silver-zinc zeolite nanoparticles incorporated in two soft denture liners - An *in vitro* assessment. Dent Res J 2022;19:12.

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antifungals aid in the prevention, progression, and elimination of the infection. The high recurrence rate of denture-induced candidiasis can be attributed to the rapid clearance of the topical antifungal by saliva and tongue movements.^[3] Geriatric denture wearers often have poor vision and limited motor skills and are unable to clean the dentures effectively, thus creating an ideal environment for biofilm formation.^[3,4]

Triazole antifungals are routinely prescribed in cases of oral candidiasis and have to be applied at the site twice daily for 3–4 weeks. This tedious anti-fungal application regime coupled with its unpleasant taste has reduced patient compliance. Numerous studies have also reported drug resistance, due to its indiscriminate use.^[5]

To overcome these shortcomings, the incorporation of antifungal agents in various dental materials was considered. Silver zinc zeolites nanoparticles (SZZ-NPs) are broad-spectrum antimicrobials made of crystalline aluminosilicate, in which silver and zinc ions are lodged within the void spaces. These ions are continuously released in small amounts to exert an anti-microbial effect against aerobic, anaerobic bacteria, and fungi alike.^[6,7] Since silver rarely caused resistant microorganisms to develop,^[8] it can be postulated that SZZ-NPs can be incorporated in soft denture liners as a method of drug delivery system and have the potential to reduce the possibility of biofilm formation by pathogenic fungi.

The purpose of this *in vitro* study was to evaluate and compare the antifungal efficacy of SZZ-NPs 0.5%w/w and 2%w/w with fluconazole 5%w/w when incorporated in two brands of commercially available soft denture liners against *C. albicans* over the course of 30 days. The null hypothesis was that there would be no significant difference between the antifungal efficacy of SZZ-NPs 0.5%w/w, SZZ-NPs 2%w/w, and fluconazole 5%w/w and the two denture liners, at the time intervals tested.

MATERIALS AND METHODS

This *in vitro* study was carried out aseptically using standard barrier techniques.

Preparation and inoculation of agar plates

C. albicans (ATCC MYA-2719) was inoculated into Sabouraud dextrose broth and incubated at 37°C. After 8 h, the *C. albicans* suspension was standardized by dilution with sterile broth to a density visually

equivalent to barium sulfate standard; McFarland tube number 5. A drop of diluted *C. albicans* solution was placed on each sterile Sabouraud agar plate, and a lawn culture was made. The agar punch wall method was used to prepare three wells (5-mm deep, 6 mm in diameter) in each agar plate using a glass capillary tube.

Weighing and incorporating the antifungal agent with denture liner

Viscogel (Dentsply, Germany) and GC (GC Soft Liner, Japan) was dispensed according to the manufacturer's instructions and weighed. SZZ-NPs 0.5% w/w, 2% w/w (Irgaguard B5000 Reena chemicals, India), and fluconazole 5% w/w (Adcock Ingram Healthcare, India) was then incorporated in the powder of the soft denture liners. All specimens were weighed on a precise digital scale.

The antifungal agent was mixed using a plastic spatula and incorporated into the denture liners in various concentrations^[9] (SZZ-NPs 0.5, 2% w/w, Fluconazole 5%w/w). The mix was dispensed into the punch holes of the inoculated Petri plates and then incubated for 30 days at 37°C. The mean inhibition diameter (MID) for each test punch hole was measured in millimeters, using a graduated metal ruler [Figure 1]. A total of 72 samples were made and divided into groups with 18 samples each. Group 1 and group 2 had SZZ-NPs incorporated with 0.5%w/w and 2% w/w, respectively. Group 3 was incorporated with 5% w/w fluconazole and group 4 was the control and left unmodified. Each group was further divided into two subgroups depending on the soft denture liner used ($n = 9$). Triplicates were done of each concentration and material to check the repeatability

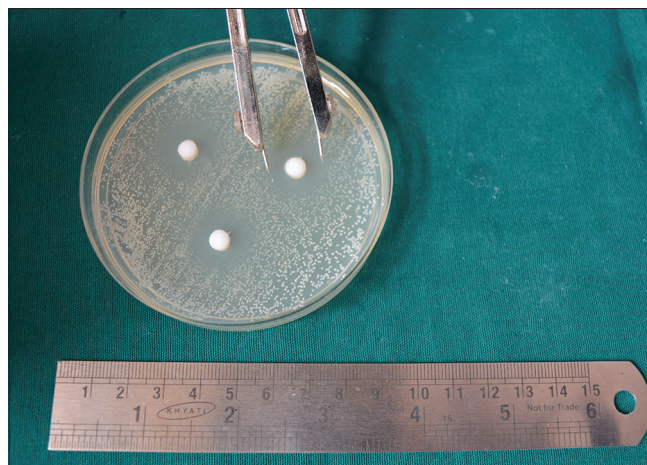


Figure 1: Mean inhibition diameter was measured in millimeters, across each test punch hole using a graduated metal ruler.

of the antifungal effect. MID was measured on days 1, 7, 15 and 30, for all groups tested [Figure 2].

RESULTS

The data obtained were analyzed using the SPSS (Statistical Package for the Social Sciences, version 22, Chicago, IL, USA) software. The comparison between the antifungal agents when incorporated in denture liners, was done using One-way ANOVA test and Tukey's *post hoc* test and are represented in Table 1 and Figure 3. At the end of day 1, the mean MID was the highest with fluconazole 5% w/w. By Day 7 and 15, SZZ-NPs 2%/w/w showed a higher MID mean value among all the antifungal agents tested followed closely by fluconazole 5%/w/w. The mean of SZZ-NPs 2% was also found to be the highest on day 30 followed by SZZ-NPs 0.5%/w/w. A statistically highly significant difference ($P < 0.001$) was noted among all the antifungal agents at all the time intervals.

An independent *t*-test was used to compare Viscogel and GC at the various time intervals for each of the antifungal agents and is described in Table 2 and Figure 4. Accordingly, for SZZ-NPs 0.5%/w/w, GC denture liner was found to have the highest mean values at days 1, 7, 15, and 30. A similar outcome was noted with respect to SZZ-NPs 2%/w/w and fluconazole 5%/w/w. The mean values obtained in the control group were 6.00 at all-time intervals. A significant difference ($P < 0.001$) was noted between the values for Viscogel and GC on day 1 and a highly significant difference ($P < 0.001$) on day 30 when SZZ-NPs 0.5%/w/w was incorporated. The values for antifungal agent SZZ-NPs 2%/w/w were highly significant ($P < 0.001$) at all the time intervals. A similar trend was observed with fluconazole 5%/w/w, except that on day 30 no significant difference was noted between the 2 denture liners tested.

DISCUSSION

In this study, there was a significant difference ($P < 0.001$) between the antifungal efficacy of SZZ-NPs and fluconazole against *C. albicans* when incorporated in two commercially available soft denture liners. The differences between the denture liners (Viscogel and GC) when incorporated with the antifungal agents, were not significant at all the time intervals tested. Hence, the null hypothesis was partially accepted and partially rejected.

Figureiral *et al.*^[10] in their clinical study established that the treatment of patients with denture stomatitis using fluconazole provided short-term relief. He also observed that patients had developed a certain level of antimicrobial resistance to fluconazole. SZZ-NPs are broad-spectrum anti-microbial agents, in which silver and zinc antimicrobial ions are present in the void spaces of their crystalline aluminosilicate structure. These anti-microbial ions are continuously released in small amounts and come into contact with environmental microorganisms. They suppress their development by inactivating vital microbial enzymes, interrupting RNA replication, and blocking their respiration by an oxidative process.^[6,7,11] Zeolites containing silver and zinc ions can be successfully incorporated into dental materials as they have sustained antimicrobial activity, low toxicity, are odorless and tasteless while being chemically stable against temperature and humidity changes.^[12,13]

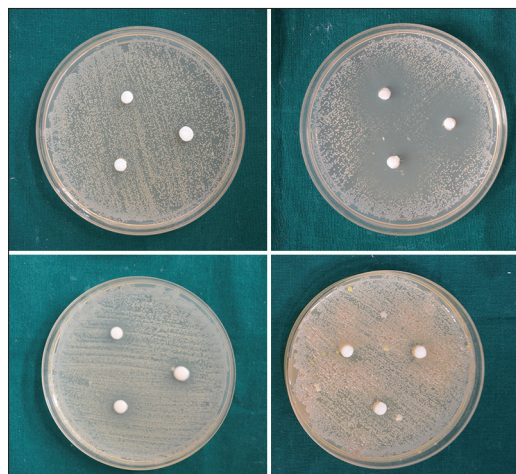


Figure 2: Mean inhibition diameters were recorded at day 1, day 7, day 15, and day 30.

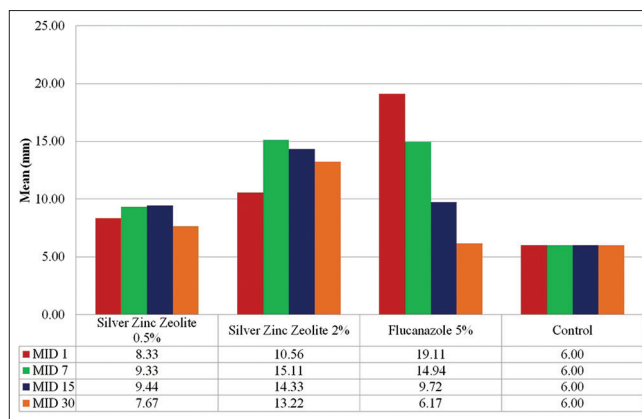


Figure 3: Mean values of antifungal agents at various time intervals.

Table 1: Comparison of antifungal agents used with the denture liners at various time intervals

Duration	Antifungal agent	Mean (mm)±SD	F	Significant	Interpretation
MID 1	SZZ-NPs 0.5%	8.33±0.97	344.38	0.000*	Control< SZZ-NPs 0.5%< SZZ-NPs 2%< Fluconazole 5%
	SZZ-NPs 2%	10.56±1.50			
	Fluconazole 5%	19.11±1.91			
	Control	6.00±0.00			
MID 7	SZZ-NPs 0.5%	9.33±1.53	55.32	0.000*	Control< SZZ-NPs 0.5%< Fluconazole 5%< SZZ-NPs 2%
	SZZ-NPs 2%	15.11±3.16			
	Fluconazole 5%	14.94±3.69			
	Control	6.00±0.00			
MID 15	SZZ-NPs 0.5%	9.44±1.10	32.40	0.000*	Control< SZZ-NPs 0.5% < Fluconazole 5%< SZZ-NPs 2%
	SZZ-NPs 2%	14.33±4.49			
	Fluconazole 5%	9.72±2.16			
	Control	6.00±0.00			
MID 30	SZZ-NPs 0.5%	7.67±0.59	26.45	0.000*	Control< Fluconazole 5%< SZZ-NPs 0.5%< SZZ-NPs 2%
	SZZ-NPs 2%	13.22±5.55			
	Fluconazole 5%	6.17±0.38			
	Control	6.00±0.00			

MID: Mean inhibition diameter; SZZ-NPs: Silver Zinc Zeolite-Nanoparticles; SD: Standard deviation

Table 2: Comparison of denture liners (viscogel and GC) with the antifungal agents used at the various time intervals

Duration	Denture liner	Mean±SD							
		SZZ-NPs 0.5% (mm)	Significant	SZZ-NPs 2% (mm)	Significant	Fluconazole 5% (mm)	Significant	Control (mm)	Significant
MID 1	Viscogel	7.77±0.83	0.01*	9.66±1.00	0.007*	17.44±0.88	0.000*	6.00±0.00	-
	GC	8.88±0.78		11.44±1.42		20.77±0.83		6.00±0.00	
MID 7	Viscogel	8.88±0.92	0.229	12.66±1.00	0.000*	11.77±1.71	0.000*	6.00±0.00	-
	GC	9.77±1.92		17.55±2.6		18.11±1.83		6.00±0.00	
MID 15	Viscogel	8.66±0.86	0.001*	10.33±0.26	0.000*	7.88±0.78	0.000*	6.00±0.00	-
	GC	10.22±0.66		18.33±2.44		11.55±1.33		6.00±0.00	
MID 30	Viscogel	7.44±0.52	0.115	8.11±0.78	0.000*	6.00±0.00	0.063	6.00±0.00	-
	GC	7.88±0.6		18.33±2.44		6.33±0.5		6.00±0.00	

MID: Mean inhibition diameter; SZZ-NPs: Silver Zinc Zeolite-Nanoparticles; SD: Standard deviation

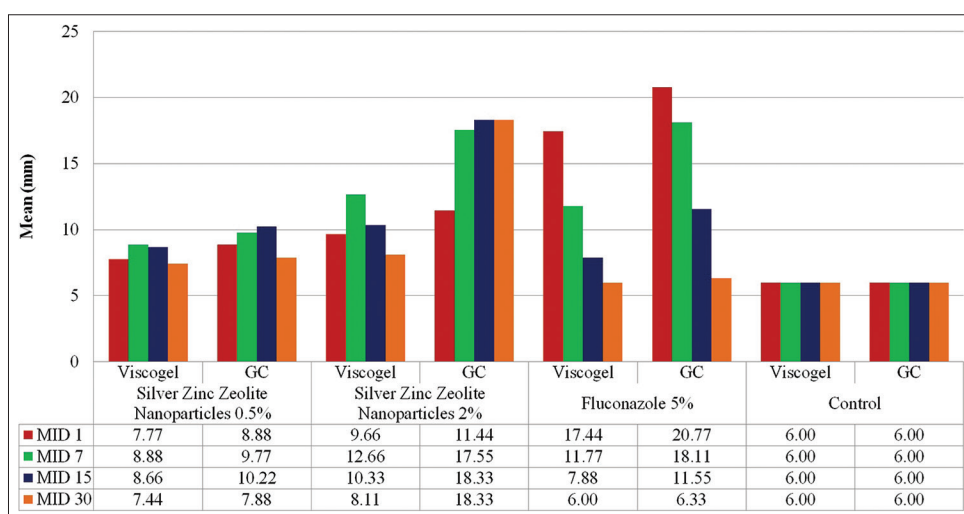


Figure 4: Mean values of Viscogel and GC at various time intervals.

These modified soft denture liners play a vital role in denture plaque control as saliva has no effect on its antifungal property.^[14] Various authors have concluded that this method of drug delivery system

has a significant inhibitory effect on the growth of *Candida* species by allowing a continuous presence of drug at the site, and in minimum concentrations.^[15-17] The minimum concentration of fluconazole required to have antifungal efficacy against *C. albicans* is 5% w/w, and increasing the concentration had no significant effect.^[9] Also, it was noted that 0.5% w/w SZZ-NPs was the minimum concentration required to have antifungal efficacy against *C. albicans*^[18] and increasing the concentration of zeolite beyond 2.5%, resulted in a significant decrease in mechanical properties.^[13] It is generally recommended to add 0.2%–2% zeolite by weight.^[7,19] Our study parameters were thus determined based on the findings from these studies.

Commonly used denture liners which did not have any inherent antifungal activity were chosen. This was demonstrated by the growth of *C. albicans* during the entire duration of the study in the control group.

The present study assessed the antifungal efficacy at four-time intervals. The short time duration of 1 and 7 days, was decided as denture liners continue to flow for 7 days and were clinically effective throughout this period.^[20] The longer time duration of 15 and 30 days was decided upon, as most denture liners are replaced every 2–4 weeks depending on the patient's oral hygiene.^[9]

Among the antifungals tested, 0.5% SZZ-NPs showed the lowest antifungal efficacy on day 1, but it steadily increased by day 15, and then declined by day 30. 2% SZZ-NPs on the other hand, showed inferior antifungal efficacy compared to 5% fluconazole on day 1, but by day 7, it was superior to all antifungals tested. It retained its antifungal efficacy even on day 30. On day 1, fluconazole 5% showed the highest antifungal efficacy, but it rapidly declined and by day 30, the antifungal activity seen was minimal. These differences could be attributed to the differences in the rates of release of each antifungal agent, fluconazole having a faster release and shorter half-life (20-50 h) compared to SZZ-NPs which has a porous structure and allows for the sustained cation release of antimicrobial metals and secondary regeneration by ion-exchange when metals are depleted.^[21] Another *in vitro* study also reported prolonged anti-microbial effects on *C. albicans* and bacteria causing nosocomial respiratory infection, lasting nearly 4 weeks with tissue conditioners incorporated with silver zeolites.^[22]

The differences in the amount of antifungal resistance in various other studies were probably due to the innate features of the tested strains. This study used the ATCC MYA-2719 strain of *C. albicans* as its biofilm mass was comparable to that of clinical isolates.^[23-25] Agar punch well technique and SDA was used, which ensured accuracy of the MID measurements, as is highly specific for *C. albicans*.

The antifungal efficacy of the various antifungals tested, showed superior values when incorporated with GC comparatively. This is consistent with another study by Matsuura *et al.*^[22] which compared the prolonged antimicrobial effect of tissue conditioners containing silver-zeolites and found that VG lost their antifungal effects on *C. albicans* in four weeks. This phenomenon is credible given that some components of tissue conditioners, like ethanol, are time dependently released from the material.^[26]

There is no report in the literature on the antifungal efficacy of these modified denture liners over the entire intended span of denture liner use (30 days). No other study compared the difference in the antifungal efficacy between various brands of soft denture liners. However, it was limited to two brands of soft denture liners. Further research is warranted by means of *in vivo* clinical trials.

CONCLUSION

Within the limitations of the study, it can be concluded that SZZ-NPs 0.5%w/w, 2%w/w and fluconazole 5%w/w can be incorporated with soft denture liners against *C. albicans*. The antifungal efficacy depends not only on the antifungal agent and its concentrations but also on the brand of soft denture liner used. Fluconazole 5%w/w is the recommended choice for short-term antifungal efficacy, while SZZ-NPs 2%w/w is recommended when long-term antifungal efficacy is needed. GC soft denture liner was the recommended choice.

Acknowledgment

Dr. Phelen Da Costa, MDS Public Health Dentistry, for her immense help with the statistical data and analysis.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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