

An *In Vitro* Assessment of the Antifungal Efficacy of Two Commercially Available Soft Denture Liners Modified with Silver Zinc Zeolite Nanoparticles

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Abstract

Statement of the Problem: The treatment of oral candidiasis, involves adjusting the old prosthesis, use of temporary soft denture liners and initiation of anti-fungal therapy. However, due to lack of patient's compliance, copious amount of saliva and increased evidence of drug resistance, there is a need for an improved drug and drug delivery system.

Aims: Assess the antifungal efficacy of two different brands of modified denture liners (Visco gel-Dentsply and Soft denture liner-GC) containing silver zinc zeolite towards *Candida albicans*.

Methods and Material: The mean inhibition diameter (MID) was used to test the antifungal efficacy of 2% SZZ and 5% fluconazole in two commercially available soft denture liners: Visco gel and GC. MIDs were measured at day 1, day 3, day 7 and day 15, while carrying out the monitoring daily. Data was statistically analysed using one way ANOVA and post hoc Bonferroni test.

Results: 2% w/w SZZ in GC soft denture liner was found to be the most effective ($p < 0.001$).

Conclusion: Within the limitations of the study, it can be concluded that SZZ and fluconazole mixed with denture liners can be used against *C. albicans*. 5% fluconazole is the recommended choice for short term antifungal efficacy, while 2% SZZ is recommended when prolonged antifungal efficacy is indicated. GC soft denture liner was the recommended choice in both these cases.

Keywords: Silver Zinc Zeolite Nanoparticles; Soft Denture Liner; *Candida albicans*; Antifungal Efficacy

Abbreviations

SZZ: Silver Zinc Zeolite; SDA: Sabouraud Dextrose Agar; MID: Mean Inhibition Diameter

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Introduction

The most common sequelae of wearing a removable prosthesis is chronic atrophic candidiasis/denture stomatitis/denture sore mouth (11 - 67%) [1]. Although it has a multifactorial etiology, *Candida albicans* has been established as the primary pathogenic microorganism [1-3]. *C. albicans* acquires pathogenicity in patients with immunodeficiency and/or chronic local irritation.

Topical therapy using Triazole antifungal drugs (fluconazole and itraconazole) is the first line of treatment for oral candidiasis [4]. The success of these drugs in the oral cavity depends on patient compliance. The presence of saliva and the cleansing action of the oral musculature further tend to reduce the concentration of drugs to sub-therapeutic levels [5,6]. Various studies have shown that antifungal agents can be incorporated in soft denture liners. This method of drug delivery system has been proven to have a significant inhibitory effect on the growth of *Candida* species by allowing a continuous presence of drug at the site, and in minimum concentrations [7-9]. Prolonged or recurrent use of antifungal drugs leads to the development of resistant species making it necessary to seek new therapeutic approaches [6,10]. These shortcomings led to the addition of antimicrobial zeolites in the soft denture liner [11-15].

Zeolites are aluminum silicate crystalline structures that have void spaces measuring 3 - 10Å. Antimicrobial cations, such as silver and zinc, may be lodged within the void spaces of the zeolites and be exchanged over time with other cations from their environment [14,16-18]. As this ion availability occurs, the free cations come into contact with the environmental microorganisms, suppressing their development by inactivating vital microbial enzymes, interrupting RNA replication and blocking their respiration by an oxidative process [19-21].

Purpose of the Study

The purpose of this *in vitro* study was to evaluate the antifungal efficacy of two commercially available soft denture liners containing Silver Zinc Zeolite (SZZ) and fluconazole against *Candida albicans* over a long duration of time (15 days). The null hypothesis was that there would be no significant differences between the antifungal efficacy of fluconazole and SZZ and the denture liners used.

Materials and Methods

Materials used in the study (Figure 1)

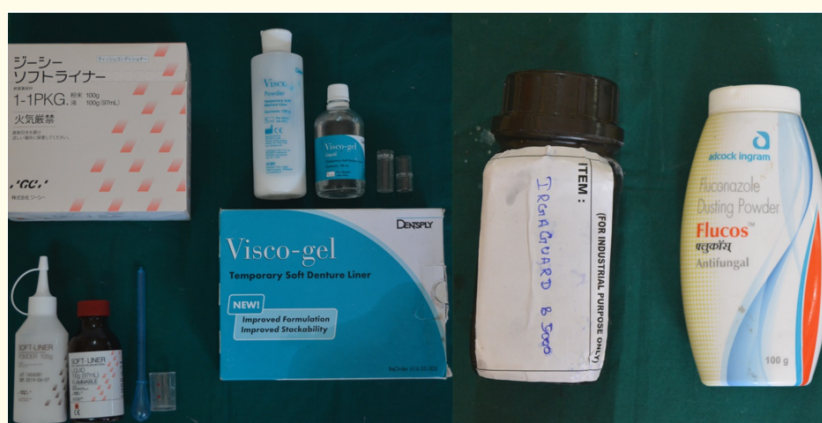


Figure 1: Materials used in the study.

- Soft denture liners: Visco gel, Dentsply Germany; GC Soft Liner, Tokyo-Japan
- Anti-fungal agents: Pure form of fluconazole (Adcock Ingram Healthcare Pvt. India); Silver Zinc Zeolite (Irgaguard B5000 Reena chemicals, India).

Microorganism used for study

- *C. albicans* (standard strain).

Incubation media used

- Sabouraud dextrose broth.
- Sabouraud dextrose agar (SDA).

The study was conducted under strict aseptic conditions.

Preparation of inoculum

Standard strain of *C. albicans* was inoculated into Sabouraud dextrose broth and incubated at 37°C. After 8 hours, the *C. albicans* suspension was standardized by dilution with sterile broth to a density visually equivalent to barium sulphate standard; McFarland tube number 5.

Inoculation of agar plates with *C. albicans*-agar punch well method

0.5 ml of diluted *C. albicans* solution was dropped on each sterile Sabouraud agar plate, and a lawn culture was made (Figure 2). Three wells (5-mm deep, 6 mm in diameter) were created using a glass capillary tube in each agar plate.



Figure 2: Inoculation of sabouraud dextrose agar in petri plates with *C. albicans* inoculums.

Weighing the specimens

The powder and liquid of the soft denture liners were dispensed according to the manufacturer’s instructions and weighed. 2% of SZZSZZ and 5% of fluconazole (by weight) was separately weighed and added to the powder of the soft denture liners. All specimens were weighed on a precise digital scale.

Incorporating the antifungal agent with denture liner [22]

The antifungal agent was hand mixed with denture liner in % w/w concentrations for 30 seconds following the manufacturer’s instructions. The mix was loaded into a sterile syringe and then dispensed into the punch holes in the inoculated petri plates (Figure 3). Plates were incubated for a total of 15 days at 37°C. Mean inhibition diameter (MID) for each test punch hole was measured in millimeters across the punch hole at 24 hours, 72 hours, 7 days and 15 days (Figure 4). Triplicates were done of each concentration and material to check the repeatability of the antifungal effect (N = 9).



Figure 3: Each antifungal agent mixed and dropped into the punch holes.



Figure 4: MID measurements carried out periodically at 24 hours, 72 hours, 7 days and 15 days.

Sample size

A total of 54 samples were made.

Group 1: SZZ at 2% concentration (N = 18).

Group 2: Fluconazole at 5% concentration (N = 18).

Group 3: No antifungal agent-control group (N = 18).

Each group was further divided into two subgroups (N = 9) depending on the soft denture liner used

Results

The inhibition diameters of SZZ and fluconazole in viscogel and GC soft denture liner were recorded (Table 1). Both antifungal agents demonstrated clear inhibition against *Candida albicans*. The control group did not exhibit any antifungal activity against *C. albicans* (Figure 5). These differences were statistically significant (P < 0.0001).

Duration (Days)	Antifungal agent	N	Mean	SD	P Value
1	SZZ 2% in GC	9	12.33	1.689	< 0.001
	SZZ 2% in Viscogel	9	10		
	FLU 5% in GC	9	21.78	2.282	
	FLU 5% in Viscogel	9	17.89		
3	SZZ 2% in GC	9	13.11	1.815	<0.001
	SZZ 2% in Viscogel	9	10.22		
	FLU 5% in GC	9	20.89	3.578	
	FLU 5% in Viscogel	9	14.56		
7	SZZ 2% in GC	9	18.33	3.632	<0.001
	SZZ 2% in Viscogel	9	12.89		
	FLU 5% in GC	9	18.67	3.650	
	FLU 5% in Viscogel	9	12.44		
15	SZZ 2% in GC	9	18.78	4.574	<0.001
	SZZ 2% in Viscogel	9	10.67		
	FLU 5% in GC	9	12.11	2.338	
	FLU 5% in Viscogel	9	8		

Table 1: Statistical analysis of MID values observed for the antifungal agents used in the study.

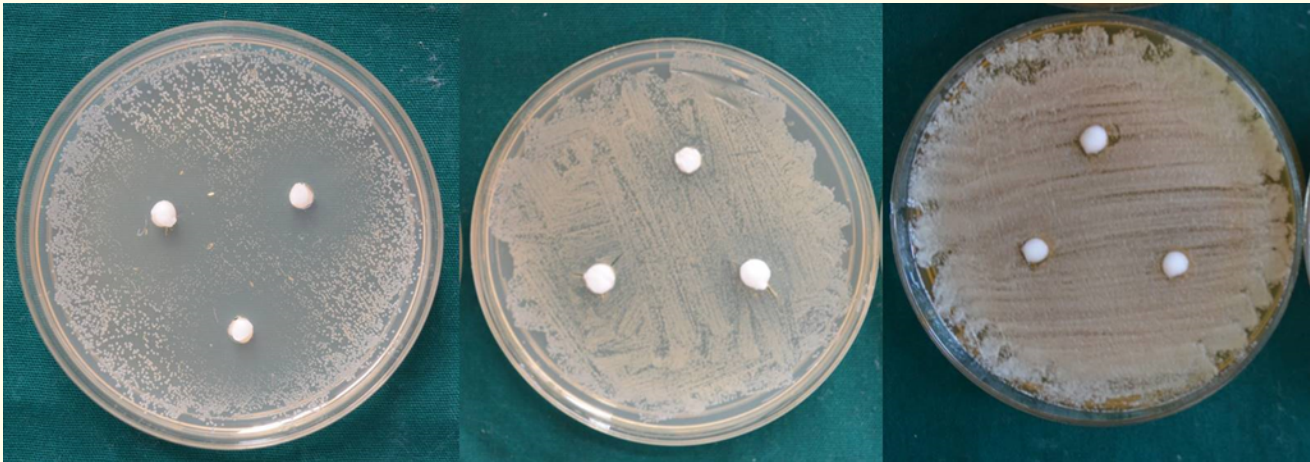
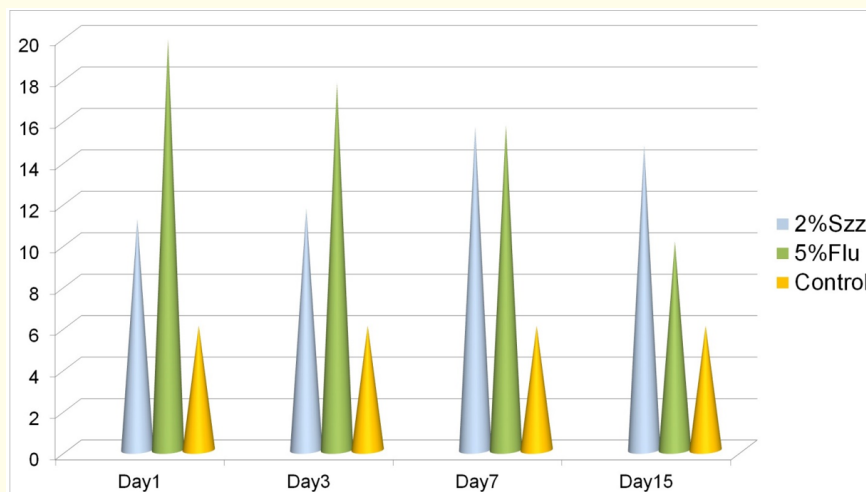


Figure 5: Control group exhibited no antifungal activity.

At day 1, the Inhibition diameter for the three different groups was in the range of 6 mm and 24 mm. The ANOVA followed by post hoc Bonferroni showed that 5% fluconazole showed the highest antifungal efficacy ($p < 0.001$) with MID (19.83 mm), followed by 2% SZZ (11.17 mm). On day 3 the antifungal efficacy of 5% fluconazole decreased significantly ($p < 0.001$) however it still showed the highest MID (17.72 mm), followed by 2% SZZ (11.67 mm). On day 7, the MID of 2% SZZ was the highest (15.61 mm), followed by 5% fluconazole (15.56 mm) ($p < 0.001$). The MID of SZZ was seen to increase steadily from day 1 to day 15, while that of fluconazole declined steadily ($p < 0.001$). By day 15, the MID of 2% SZZ was the highest (14.72 mm) (Graph 1). For all the antifungal agents used at all-time intervals tested, The MID showed a higher value in GC as compared to Viscogel from Day1 to Day15 (Figure 6 and graph 2).



Graph 1: MID vs antifungal agent.

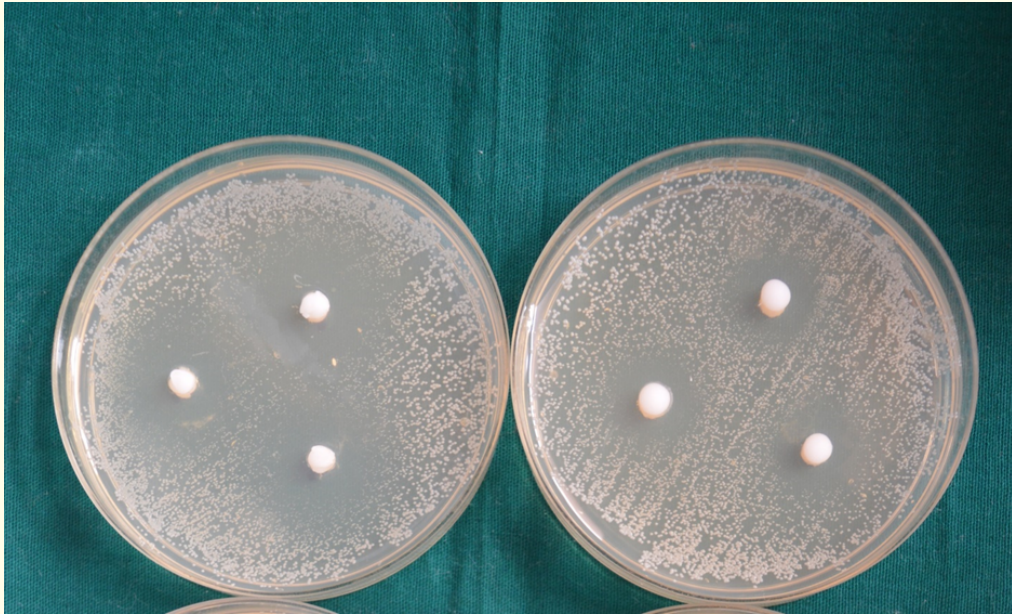
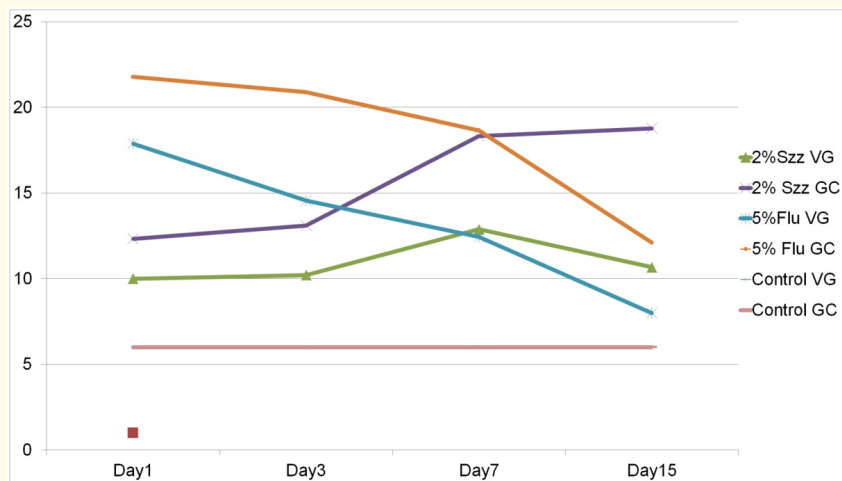


Figure 6: MID showed a higher value in GC as compared to Viscogel.



Graph 2: Brand and antifungal agent interaction.

Discussion

This study used the agar punch well technique to evaluate and compare the efficacy of two antifungal agents, SZZ and fluconazole when incorporated into two commercially available soft denture liners; viscogel and GC against *C. albicans*. The agar punch core ID assay was used in this study, thus ensuring accuracy of the MID measurements. SDA which is highly specific for *C. albicans*, further ensured accuracy of the results. Commonly used denture liners which did not have any inherent antifungal activity were chosen as observed by the growth of *C. albicans*. Denture liners continue to flow for a period of 7 days and suggested that they are clinically effective throughout this period [23]. Most denture liners have maximum effect from 24 - 72 hours are replaced every 2 weeks depending on the patients oral hygiene [22]. The study time parameters were therefore decided upon in this study.

The antifungal efficacy was tested at Day1, Day3, Day7 and Day15. Both the antifungal agents tested were effective against *C. albicans*. On comparing the MIDs SZZ showed a low MID on Day1, which steadily increased to Day15 and its antifungal efficacy was present till Day15 whereas Fluconazole was highly effective on Day1 after which there was a steady decline in its efficacy. This could be due to the difference in the rates of release of each antifungal agent, fluconazole having a faster release and shorter half-life (20 - 50 hours) compared to SZZ which produces sustained cation release.

Increasing the concentration of zeolite in percentages > 2.5% resulted in a significant decrease in mechanical properties [24]; therefore the study evaluated SZZ concentrations below 2.5% w/w. Among the brands tested, the samples mixed with GC showed a prolonged antifungal efficacy than those mixed with VG. This is consistent with another study by Matsuura, *et al.* [13] 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 plausible given that some components of tissue conditioners, for example, ethanol, are time dependently released from the material [25].

Limitations of the Study

Only two commercially available denture liners have been tested in this study, thus the results obtained may vary with other denture liners as *in vitro* results cannot be extrapolated *in vivo*, further investigation is needed by means of *in vivo* clinical trials.

Conclusion

Within the limitations of the study, it can be concluded that SZZ and fluconazole mixed with denture liners can be used against *C. albicans*. 5% fluconazole is the recommended choice for short term antifungal efficacy, while 2% SZZ is recommended when prolonged antifungal efficacy is indicated. GC soft denture liner was the recommended choice in both these cases.

Conflict of Interest

The authors declare no conflicts of interest.

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All authors gave their final approval and agreed to be accountable for all aspects of the work.

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