

Antifungal Efficacy of Silver Zinc Zeolite Nanoparticles in Denture Liners - An *In Vitro* Study

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Abstract

Aims: Assess the dose dependant antifungal efficacy of denture liners containing silver zinc zeolite towards *Candida albicans*.

Materials and Methods: The mean inhibition diameter (MID) was used to test the antifungal efficacy of silver zinc zeolite (SZZ) and fluconazole when incorporated in a soft denture liner (GC). Two concentrations of SZZ (0.5% and 2%) and one of fluconazole (5%) were used. MIDs were measured at day 1, day 3, day 7 and day 15, while carrying out the monitoring daily.

Statistical Analysis Used: One way ANOVA and post hoc Bonferroni test.

Results: 2% w/w SSZ in GC soft denture liner was found to be the most effective ($p < 0.001$) and superior to 0.5%w/w SSZ and 5%w/w fluconazole as it retained its antifungal efficacy even on day 15.

Conclusion: Within the limitations of the study it can be concluded that silver zinc zeolite added to GC soft denture liner can be used against *Candida albicans* as well as strains resistant to conventional therapies.

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

Abbreviations

SDA: Sabouraud dextrose agar

Introduction

The most common sequelae of wearing a removable prosthesis is chronic atrophic candidiasis/denture stomatitis/denture sore mouth (11 - 67%) [1]. Newton classified the severity of denture stomatitis as type I (localized erythematous), type II (diffuse erythematous) and

type III (hyperplastic granular) [2]. Although it has a multifactorial etiology, *Candida albicans* has been established as the primary pathogenic microorganism [1,3,4]. *C. albicans* is a normal commensal of the oral microflora which acquires pathogenicity in immunodeficiency and/or chronic local irritation.

Topical therapy is the first line of treatment for oral candidiasis [5]. Triazole antifungal drugs (fluconazole and itraconazole) are commonly used in the treatment of denture stomatitis. However, the success of topically applied drugs in the oral cavity may be compromised by lack of patient compliance. Furthermore, the diluent effect of saliva and the cleansing action of the oral musculature tends to reduce the concentration of drugs to sub-therapeutic levels [6,7]. Various studies have shown that antifungal agents can be incorporated in soft denture liners and 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 [8-10]. Prolonged or recurrent use of antifungal drugs leads to the development of resistant species. This makes it necessary to seek new therapeutic approaches [7,11]. These shortcomings led to the addition of antimicrobial zeolites in the soft denture liner [12-16].

Zeolites are aluminum silicate crystalline structures that present void spaces measuring 3 - 10 angstroms in their structure. 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 [15,17-19]. 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 [20-22]. The efficacy of antimicrobial zeolites against aerobic and anaerobic bacteria and fungi has been demonstrated [17,21,23,24].

Purpose of the Study

The purpose of this *in vitro* study was to evaluate the antifungal efficacy of a commercially available soft denture liner containing different percentages of Silver Zinc Zeolite (SZZ) 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 when incorporated in the denture liner.

Materials and Methods

Materials used in the study (Figure 1)



Figure 1: Materials used in the study.

- Soft denture liner: 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 using standard barrier techniques. All laboratory procedures, including plating of agar plates, were carried out in a laminar airflow cabinet hood.

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

Diluted *C. albicans* solution (0.5 ml) 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 for all concentrations of materials to be tested (Figure 3).

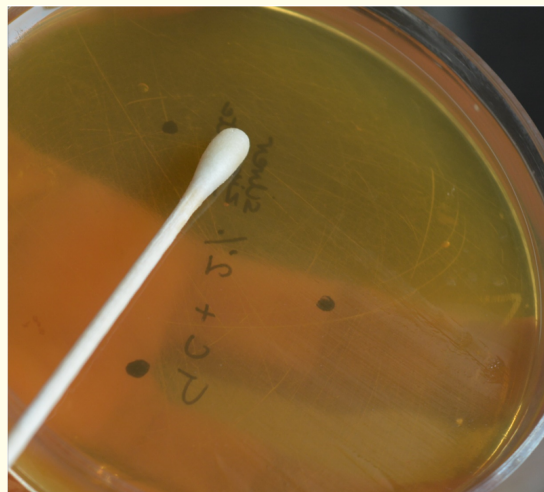


Figure 2: Inoculation of Sabouraud dextrose agar in petri plates with *C. albicans* inoculums.

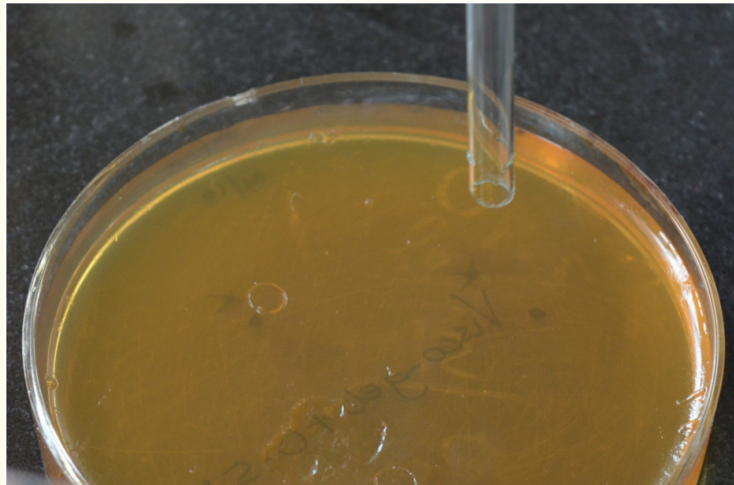


Figure 3: Punch holes made using a glass capillary tube.

Weighing the specimens

The powder and liquid were dispensed according to the manufacturer’s instructions and weighed. 0.5%, 2% of SZZSZZ and 5% of fluconazole (by weight) was separately weighed and added to the powder of the soft denture liner. All specimens were weighed on a precise digital scale.

Incorporating the antifungal agent with denture liner [25]

The antifungal agent was hand mixed with denture liner in various % w/w concentrations for 30 seconds according to 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 4). 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, using a graduated metal ruler (Figure 5). Triplicates were done of each concentration and material to check the repeatability of the antifungal effect (N = 9).

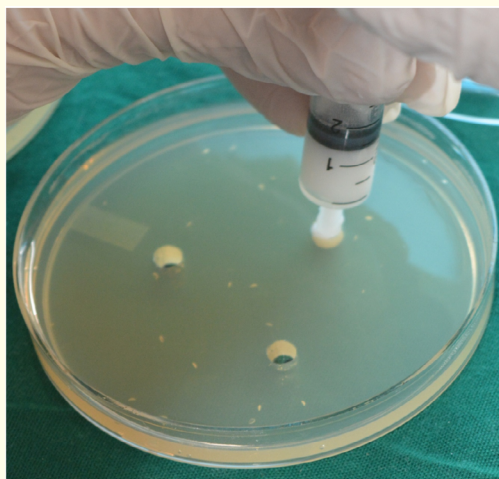


Figure 4: Each antifungal agent mixed and dropped into the punch holes.



Figure 5: MID measurements carried out periodically at 24 hours, 72 hours, 7 days and 15 days.

Sample size

A total of 72 samples were made.

- Group 1: SZZ at 0.5% concentration (N = 18).
- Group 2: SZZ at 2% concentration (N = 18).
- Group 3: Fluconazole at 5% concentration (N=18).
- Group 4: No antifungal agent- control group (N = 18).

Results

The inhibition diameters of SZZ and fluconazole in GC soft denture liner were recorded. Both antifungal agents demonstrated clear inhibition against *Candida albicans* (Figure 6). These differences were statistically significant ($P < 0.0001$). The control group did not exhibit any antifungal activity against *C. albicans*.

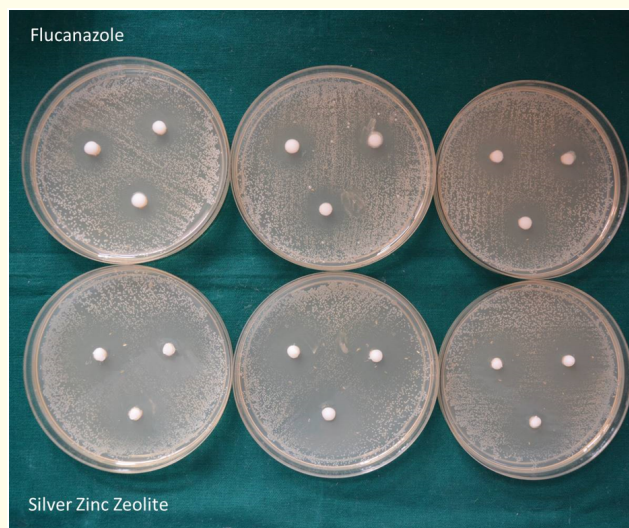
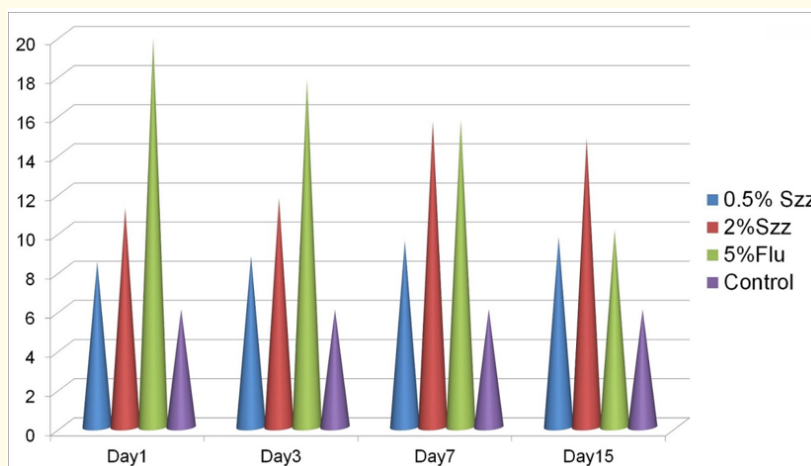


Figure 6: Both antifungal agents demonstrated clear inhibition against *Candida albicans*.

At day 1, the inhibition diameter for the four different groups lies 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) and 0.5% SZZ (8.44 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) and 0.5% SZZ (8.72 mm). On day 7, the MID of 2% SZZ was the highest (15.61 mm), followed by 5% fluconazole(15.56 mm) and 0.5% SZZ (9.50 mm) ($p < 0.001$). The MID of SZZ (0.5% and 2%) was seen to increase steadily from day 1 to day 15, while that of 5% fluconazole declined steadily ($p < 0.001$). By day 15, the MID of 2% SZZ was the highest (14.72 mm), followed by 5% fluconazole (10.06 mm), and 0.5% SZZ (9.67 mm). SZZ revealed a significant dose dependent antifungal efficacy against *Candida albicans* (Table 1 and graph 1). Higher concentrations of SZZ (2%) showed a higher MID compared to lower concentration (0.5%)

Duration (Days)	Antifungal Agent	N	Mean	SD	P Value
1	SZZ 0.5%	18	8.44	1.097	
	SZZ 2%	18	11.17	1.689	< 0.001
	FLU 5%	18	19.83	2.282	
3	SZZ 0.5%	18	8.72	0.826	
	SZZ 2%	18	11.67	1.815	< 0.001
	FLU 5%	18	17.72	3.578	
7	SZZ 0.5%	18	9.50	1.543	
	SZZ 2%	18	15.61	3.632	< 0.001
	FLU 5%	18	15.56	3.650	
15	SZZ 0.5%	18	9.67	0.970	
	SZZ 2%	18	14.72	4.574	< 0.001
	FLU 5%	18	10.06	2.338	

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



Graph 1: MID vs antifungal agent.

Discussion

This study used the agar punch well technique to evaluate and compare the efficacy of two antifungal agents, SZZ and fluconazole when incorporated in varying concentrations into a commercially available soft denture liner against *C. albicans*. The agar punch core ID assay was used in this study, thus ensuring accuracy of the MID measurements. Use of SDA which is highly specific for *C. albicans*, further ensured accuracy of the results. Commonly used denture liner which did not have any inherent antifungal activity was 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 [26]. Most denture liners have maximum effect from 24 - 72 hours are replaced every 2 weeks (depending on the patients oral hygiene) [25]. The study time parameters were therefore decided upon in this study.

The various concentrations of the two antifungal agents were tested at Day 1, Day 3, Day 7 and Day 15. Both the antifungal agents tested were effective against *C. albicans*. On comparing the MIDs SZZ showed a low MID on Day 1, which steadily increased to Day 15 and its antifungal efficacy was present till Day 15 whereas Fluconazole was highly effective on Day 1 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.

The anti-fungal efficacy increased with increased dose of SSZ (2% w/w > 0.5% w/w). The minimum concentration of fluconazole required to have antifungal efficacy against *C. albicans* is 5% w/w increasing the concentrations showed no significant antifungal activity [25]. The minimum concentration of SZZ required to have antifungal efficacy against *C. albicans* is 0.5% w/w [26]. Increasing the concentration of zeolite in percentages > 2.5% resulted in a significant decrease in mechanical properties [27]; therefore the study evaluated SZZ concentrations below 2.5% w/w.

Limitations of the Study

Only one commercially available denture liners has 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.

Conflict of Interest

The authors declare no conflicts of interest.

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