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Antifungal Efficacy of a Permanent Silicon Soft Liner Containing Silver Nanoparticles

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A B S T R A C T

Objectives: This study aimed to evaluate the in vitro antifungal efficacy of addition of silver nanoparticles (SNPs) to Mucopren® silicone soft liner material.

Materials and Methods: Twenty disc samples (8 × 2 mm) of Mucopren® silicone soft liner containing 0wt% (control), 0.5wt%, 1wt%, 2wt%, and 3wt% SNPs were fabricated. Samples were powdered and added to 150 mL of Sabouraud dextrose agar culture medium and placed on separate culture dish plates. Each plate was inoculated with 10^6 colony forming units per milliliter (CFUs/mL) of Candida albicans (PTCC 5027) according to the CLSI protocol, and incubated at 37°C. The colony count was verified at 24 h, and the antifungal effect of the samples was evaluated according to the percentage of viable cells in the 2 subgroups with/without thermocycling. Data were analyzed using SPSS version 20 via ANOVA and t-test ($P<0.05$).

Results: All experimental groups showed higher antifungal activity than the control group, and this effect was dose-dependent ($P<0.05$). The lowest colony count was recorded in the 3wt% group. Thermocycling had no significant effect on the antifungal efficacy, except in 0.5wt% concentration of SNPs ($P=0.013$).

Conclusion: Addition of SNPs to Mucopren soft liner conferred antifungal effects. Further mechanical stability and toxicity studies are still required.

Keywords: Antifungal Agents; Candida albicans; Denture Liners; Stomatitis, Denture; Nanoparticles

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INTRODUCTION

Soft liners are commonly used to enhance the adaptation of ill-fitting dentures, allow healing of traumatized underlying tissues, and increase the retention of intraoral and extraoral prostheses [1]. These materials are polymers with a glass transition temperature below the room temperature.

The clinical success of a soft liner depends on its viscoelastic properties and its adaptation to the residual ridge upon the application of occlusal loads. This inherent property makes them suitable for use in the oral cavity for several diagnostic and therapeutic purposes [1]. Irrespective of the material, soft liners are divided into 2 groups of short-term, also

known as tissue conditioners, and long-term. The latter has an acrylic or silicon base, and is often used in patients with thin sharp ridges, extensive ridge resorption, severe bony undercuts, and congenital or acquired palatal defects [1].

About 65% of denture users develop denture stomatitis [2]. Candida albicans (C. albicans) is frequently considered the main responsible culprit [2, 3]. This fungal microorganism adheres to both the denture base and soft liner. Various solutions have been suggested to overcome this problem [1-5]. Some of them are based on chemical or mechanical elimination of C. albicans biofilm from the surface of the liner. Improvement of oral hygiene and application of topical antimicrobial agents have also been proven to be effective [1]. Nystatin and fluconazole added to the composition of soft liners at various concentrations and forms (powder, suspension, etc.) have shown promising results as well [6]. However, studies are limited, and no consensus has been reached on their routine incorporation [6, 7].

Silver and its compounds have long been used as an antimicrobial agent providing good tissue response and low toxicity [8]. Silver nitrate has been used for the treatment of visceral diseases, salivary gland fistula, and bone abscess. Silver sulfadiazine is a wide-spectrum antibiotic, which is commonly used for the treatment of burns [9]. Advances in science and technology of nanoscale materials led to the introduction of silver nanoparticles (SNPs) [8-10]. Since SNPs have high toxicity against most microorganisms, they are considered a good choice of antimicrobial agent [9].

SNPs cause the death of fungi by different mechanisms such as interference with the permeability of their cell membrane [11]. Moreover, SNP incorporation into dental materials decreases the adherence of microorganisms, which is optimal in the oral environment. SNPs added to soft liners may then serve as a hidden antifungal agent [10]. This is clinically favorable and therefore, is gaining attention [10,12-14].

Recently, the fungicidal activity of SNPs was documented to be equal to that of other antifungal agents such as amphotericin B and fluconazole [12]. Although very few studies have evaluated the antimicrobial effect of SNPs in tissue conditioners [10, 12-14], none has investigated this effect in long-term silicon liners. Besides, it has not been clarified how long this antimicrobial effect would last. Thus, this in vitro study aimed to assess the antifungal properties of Mucopren long-term silicone soft liner containing SNPs.

MATERIALS AND METHODS

Sample fabrication:

The soft liner selected in this study was Mucopren cold-cure silicon long-term liner (Kettenbach, GmbH & Co. Eschenburg, Germany) supplied as two pastes. A total of 20 discs containing different concentrations (0wt% as the control group, 0.5wt%, 1wt%, 2wt%, and 3wt%) of SNPs (Nanoshel; Nanon, Tehran, Iran) 80-100 nm in size (40 nm average) with 99.9% purity were prepared [10].

For the fabrication of each sample, the base and the catalyst pastes with equal volumes were injected on 2 separate pads using an impression gun (Kettenbach, GmbH & Co. Eschenburg, Germany). Measuring the weight of the base, the catalyst, and the pad separately by a digital scale with 0.000 g accuracy (Kern, Balingen, Germany), the weight of the required amount of SNPs was calculated to obtain Mucopren samples containing 0.5wt%, 1wt%, 2wt% and 3wt% SNPs. The weighted amount of SNPs was mixed with the base of the soft liner for 100 s, and then the mixture (SNPs + base paste) was added to the catalyst and mixed for another 60 s. To assess the quality of mixing and ensure adequate dispersion of SNPs, transverse sections were made in the 0.5wt% samples by cutting (direct sectioning by a sharp tool) and tearing (random sectioning by tearing), and they were evaluated under a scanning electron microscope (KYKY-EM3200, KYKY Technology; Shanghai, China) as seen in Figure 1. A stainless-steel mold (2 × 8 mm) was designed and fabricated by laser cutting.

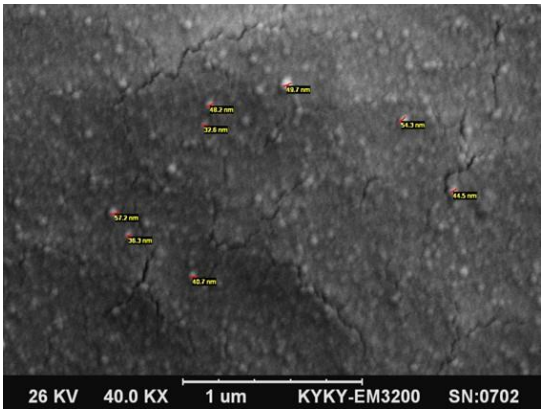

Fig. 1. Scanning electron microscopic micrograph of 0.5wt% sample indicating the dispersion of SNPs

The prepared mixture (SNPs + base + catalyst) was poured into the mold standing on a glass slab; then, another slab was gently placed on top of it to obtain a uniform surface. According to the manufacturer's instructions, the samples were separated from the mold after 15 min (Fig. 2), randomly divided into 2 subgroups, and incubated at 37°C (Pars Azma, Tehran, Iran). Half of the samples underwent thermocycling (Dorsa, Tehran, Iran) for 5000 cycles between 5°C and 55°C with a dwell time of 30 s and a transfer time of 30 s.

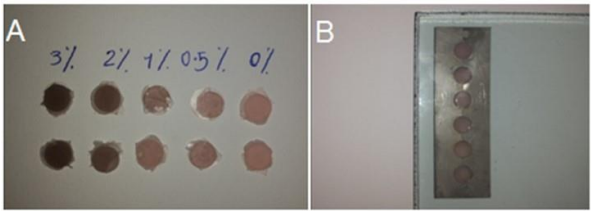

Fig. 2. Preparation of samples: (A) samples with different percentages of SNPs; (B) fabrication of samples in the mold between two glass slabs

Microorganisms and antimicrobial assay:

For the agar dilution technique, all samples were UV-irradiated (Eppendorf AG; Hamburg, Germany) at 260 nm wavelength for 16 h. To assess the antifungal efficacy, each disc sample was powdered and added to the Sabouraud dextrose agar culture medium. Next, standard strain C. albicans (PTCC 5027) was obtained (Pasteur Institute, Tehran, Iran). Then, 10^6 colony forming units per milliliter (CFUs/mL) of C. albicans (PTCC 5027) was added to each agar plate according to the CLSI protocol.

From the fresh C. albicans culture, one colony was transferred to 100 mL of tryptic soy broth liquid medium, and the absorbance was read every 15 min by a spectrophotometer (Eppendorf, Hamburg, Germany) at 600 nm. One milliliter of the medium containing C. albicans was then transferred to another vial. Serial dilution was performed to prepare 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. Then, 100 µL of the dilutions was incubated in potato dextrose agar culture medium for 24 h at 37°C. After 24 h, the number of colonies in the plate multiplied by the reverse dilution coefficient, gave the number of C. albicans in 1 mL. A plate containing pure culture medium inoculated with C. albicans served as the negative control (Fig. 3G); 256 µg/mL of amphotericin B (Cipla Ltd., Mumbai, India) was added to another plate to serve as the positive control (Fig. 3F). After incubation for 24 h at 37°C, the C. albicans colony count was determined. The reduction in viable cells was calculated by comparison with the control samples according to the CLSI protocol. The antifungal effect of the samples was evaluated according to the percentage of viable cells in the 2 subgroups with/without thermocycling.

Statistical analysis:

Data were analyzed using SPSS version 20. The frequency of colonies in groups with 0wt%, 0.5wt%, 1wt%, 2wt%, and 3wt% SNPs was reported as the mean and standard deviation values. As the interaction effect of the two variables (percentage of SNPs and thermocycling) was significant according to two-way ANOVA (P=0.008), one-way ANOVA and the post-hoc Tukey's HSD test were used to determine the effect of nanoparticle percentage on thermocycled and non-thermocycled subgroups. Independent sample t-test was used to determine the effect of thermocycling on each SNP percentage. The level of significance was set at 0.05.

RESULTS

The antifungal effect of soft liners containing various concentrations of SNPs against C. albicans was demonstrated as the mean number of viable cells (CFUs) after 24 h of incubation (Table 1).

Table 1. Minimum (min), maximum (max), mean, standard deviation (SD), standard error (SE) of mean and P-value of colony counts in different nanoparticle concentrations (wt%) with and without thermocycling.

Nanoparticle concentration	Thermo-cycling	Minimum	Maximum	Mean	SD	SE of mean	P*
0.5	no	339880	421780	386570	31701.46	12942.06	0.013
	yes	326090	364750	343563.33	14240.33	5813.59	
1.0	no	244799	302550	281223.33	23967.34	9784.62	0.744
	yes	255400	301280	285230	16702.04	6818.58	
2.0	no	184700	220520	201148.33	12080.84	4931.98	0.276
	yes	188500	202500	195001.66	4941.75	2017.46	
3.0	no	132700	162000	148810	10410.30	4249.98	0.814
	yes	132350	155900	147485	8512.91	3475.38	

*P-values are relevant to the effect of thermocycling

When compared with the colony count at 0-hour, the control group (0wt% SNPs) did not show any antifungal effect against the tested strain. As the concentration of SNPs increased, the colony count decreased (Figs. 3A to 3E and Fig. 4) (P<0.001). The greatest antifungal efficacy was noted in samples containing 3wt% SNPs. The effect of thermocycling on the colony count was not significant except for the 0.5wt% concentration (P=0.013; Fig. 5). The positive control group (256µg/mL amphotericin B) showed 0 colony count while the negative control group (pure culture medium) showed no antifungal property (CFUs:10^6; Figs. 3F and 3G).

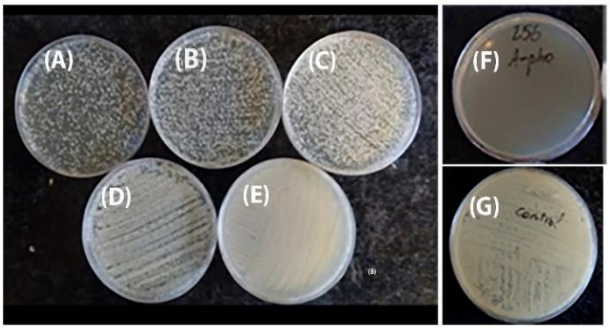

Fig. 3. Plates containing 0wt% (A), 0.5wt% (B), 1wt% (C), 2wt% (D) and 3wt% (E) SNPs, amphotericin B as the positive control (F), and pure culture medium as the negative control (G)

DISCUSSION

In the present study, Mucopren soft liner containing 0.5 wt%, 1 wt%, 2 wt%, and, 3wt% concentrations of SNPs yielded fungicidal properties against C. albicans.

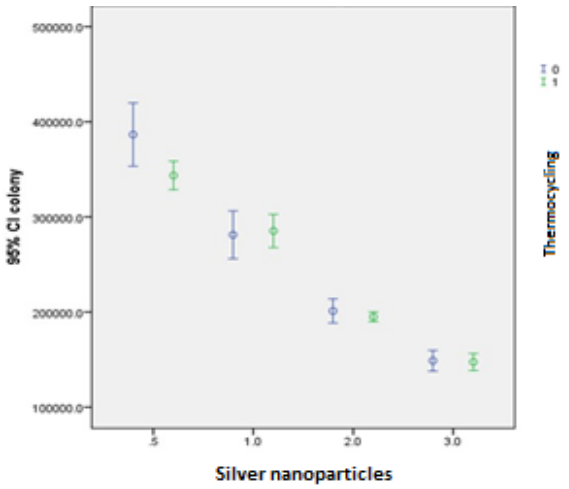

Fig. 4. Error bar of the mean and 95% confidence interval of the study groups (x: nanosilver concentration in groups (wt%), y: average colony count)

Thermocycling had no significant effect on this property except in 0.5wt% concentration.

Normally, soft liners do not impose an inhibitory effect on microorganism growth and proliferation. Studies have shown increased colonization of pathogenic micro-organisms in presence of soft liners [15-17]. Pavan et al. [15] evaluated the colonization of C. albicans, Staphylococcus aureus, and Pseudomonas aeruginosa on Molloplast B and Ufi Gel P soft liners and found no growth inhibition zone on culture plates containing these soft liners. None of the 6 soft liners used in the study by Bulad et al. [16] had any inhibitory effect on C. albicans compared with nystatin.

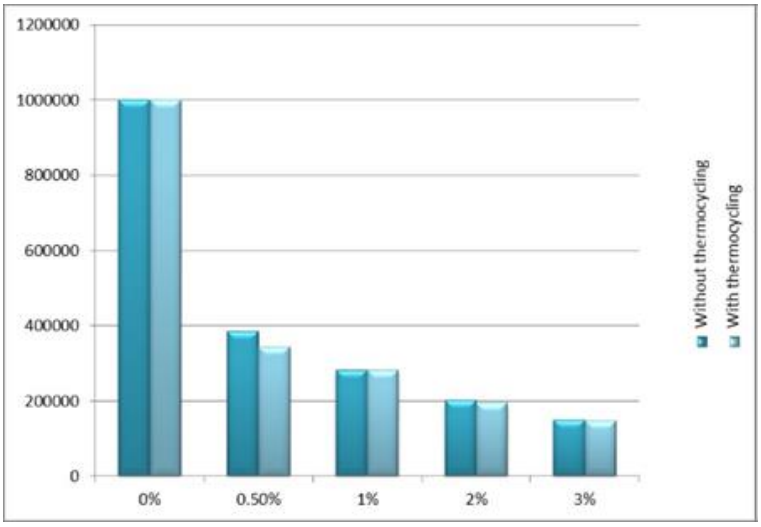

Fig. 5. Comparison of antifungal efficacy of Mucopren containing different SNP concentrations (wt%) with and without thermocycling (x: nanosilver concen-tration in groups, y: average colony count)

In the current study, Mucopren alone had no fungicidal effect. The porous nature of the liners, especially silicon ones, facilitates water sorption and distribution of nutrients. Debris accumulated in these porosities enhances fungal growth [1]. Due to the presence of ethanol and plasticizers, acrylic soft liners are more resistant to fungal growth [10]. The results of this study were different from the findings of some previous studies [10,14]. Nam [10] evaluated the antimicrobial effect of addition of 0.1wt%, 0.5wt%, 1.0wt%, 2.0wt%, and 3.0wt% SNPs to GC soft liner against C. albicans, Staphylococcus aureus, and Streptococcus mutans. They showed that the bacterial activity was completely inhibited by the addition of ≥ 1% concentration of SNPs, while for the fungicidal activity, ≥2% concentration of SNPs was required.

The tissue conditioner selected in their study was GC soft liner (GC cooperation, Tokyo, Japan) supplied as powder and liquid. Mucopren is a silicon soft liner in the form of two pastes. Therefore, there are some differences in the type of soft liner used and sample fabrication between the two studies.

In 2011, a pilot study investigated the antifungal effect of the addition of 10, 20, 40, 80, 120, and 200 ppm concentrations of SNPs to Ufi Gel SC soft liner, and reported similar results to the current study. However, the SNPs in their study had a mean size of 22.8 nm and some other differences existed between the two studies in terms of the method of assessment of antifungal property [14].

In general, thermocycling is performed to simulate thermal aging in the oral environment [18]. Effects of thermocycling on the physical properties of soft liners such as tensile and shear bond strength have been evaluated in some previous studies [18-20]. The addition of 0.5wt%, 1wt% and 2wt% SNPs to a heat-cure acrylic liner (Vertex) was evaluated in 2015 by Issa and Abdul-Fattah [13]. Simulating the oral environment, they assessed the antifungal efficacy at 7 and 30 days after immersion in artificial saliva. Interestingly, a reduction in colony count was reported after 30 days. The authors attributed this fungicidal activity increase to the oxidation of SNPs and the formation of active and highly reactive silver ions [13]. In this study, thermocycling was performed for 5000 cycles in distilled water between 5-55°C. No significant difference was noted in colony count between the thermocycled and non-thermocycled groups, except for the 0.5% concentration of SNPs. It indicates that the SNPs were well dispersed in the silicon and were not lost during thermocycling and therefore, they maintained their antifungal activity. The results of the

present study implicate that SNPs incorporated into long-term silicon liners might act as a hidden antifungal agent in the denture base of edentulous patients and therefore can be used as an alternative treatment for denture stomatitis resistant to conventional treatments or geriatric medically compro-mised denture wearers. The current study used 0.5wt%, 1wt%, 2wt% and 3wt% SNPs, which were the more commonly used concentrations of SNPs in previous studies [10-15]. However, further investigations are required to clarify the optimal concentration of SNPs regarding the silver content, the possible disruption of oral microflora, the biocompatibility of silver, and mechanical stability of the final product for proper and safe clinical application.

CONCLUSION

Addition of SNPs to Mucopren conferred antifungal activity and can be considered for prevention of fungal contamination of soft liners. Thermocycling did not reduce the fungicidal properties of SNPs incorporated in the liner except in 0.5wt% SNP group.

CONFLICT OF INTEREST STATEMENT

None declared.

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