

INCORPORATION OF SILVER NANOPARTICLES IN SOFT DENTURE LINERS; ANTIFUNGAL EFFICACY, CYTOTOXICITY AND TENSILE BOND STRENGTH



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Abstract

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. Irrespective of the material, soft liners are divided into 2 groups of short-term, also known as tissue conditioners, and long-term.

Silver and its compounds have long been used as an antimicrobial agent providing good tissue response and low toxicity. The SNPs added to soft liners may serve as hidden antifungal agents, decreasing microorganism adherence, which is optimal in the oral environment. Despite their advantages, the use of soft denture liners is associated with several problems. Silver nanoparticles have been suggested to increase the antimicrobial properties of soft-liners; however, their safety and physical properties remain a matter of debate.

Purpose/Aim

Although very few studies have evaluated the antimicrobial effect of SNPs in tissue conditioners, none has investigated this effect in long-term silicon liners. Besides, it has not been clarified how long this antimicrobial effect would last.

Despite the positive antimicrobial results of the incorporation of antifungals into soft lining materials, adverse effects are reported on their structural properties and especially tensile strength.

On the other hand, the interactions between cells and nanoparticles have been shown to result in DNA damage, cancers, developmental toxicities associated with future growth retardation, deformity, or even fetal death. Therefore, the necessity of designing new studies aimed at overcoming the toxicity of SNPs concerning their antimicrobial activities is emphasized.

Therefore, this study aimed to assess the effect of addition of silver nanoparticles (SNPs) to a silicone soft liner on antifungal efficacy, cytotoxicity and its tensile bond strength to denture base resin.

Methods and Materials

20 disc (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 106 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 (figure1).

To verify the cytotoxicity, The SNPs with over 98% purity were added to Mucopren in 0.5, 1, 2, and 3 weight percentage (wt%) concentrations and manually homogenized (figure 2). The mixture of the pieces of Mucopren plus SNPs and SNPs alone were placed in 96-well plates containing Dulbecco's Modified Eagle Medium culture, FBS, and antibiotics with L929 fibroblasts. Cell viability and biocompatibility were determined after 1, 2, and 3 days of incubation using the methylthiazol tetrazolium assay. Optical density was read by an ELISA reader at 570 nm and compared to those of positive and negative controls.

To evaluate the bond strength, SNPs were added to Mucopren cold cure soft liner in 0 (control), 0.5, 1, 2, and 3 wt% concentrations and bonded in 120 stainless steel molds with processed heat cure acrylic resin blocks. Liner/resin combination samples were divided into two groups. The first half was stored for 2 days in distilled water at 37°C and then subjected to tensile bond strength, while the other half were thermocycled 3000 times before testing. Mean bond strength, expressed in mega pascals (MPa), was determined in the tensile test with the use of a universal testing machine at a crosshead speed of 5 mm/min (figure 3). Data were analyzed using SPSS version 20 via ANOVA and t-test ($P < 0.05$).

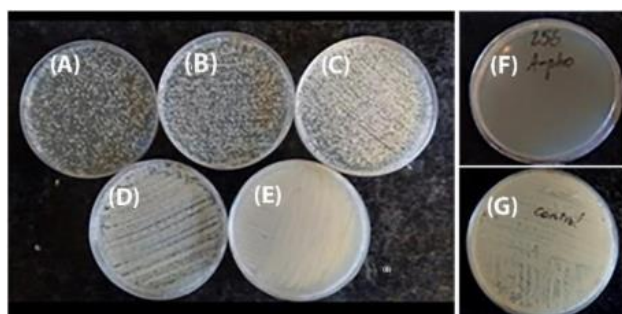


Figure 1. Plates containing 0wt% (A), 0.5wt% (B), 1wt% (C), 2wt% (D) and 3wt% (E) SNPs, amphotericin B as the positive control (F), and pure culture medium as the negative control (G)

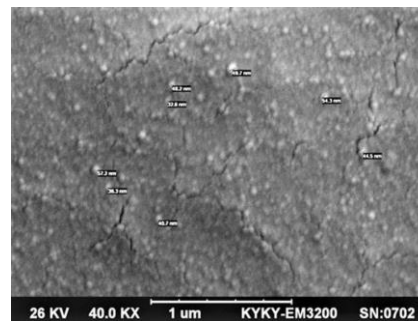


Figure 2. Electron microscopic micrograph of a sample containing silver nanoparticles

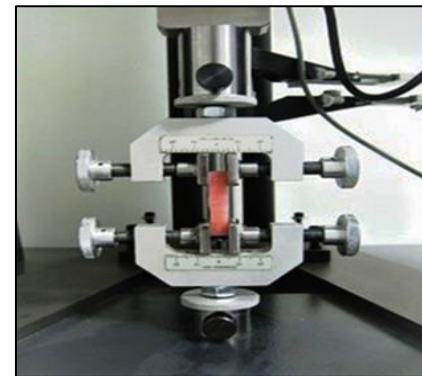


Figure 3. Micro-Tensile Bond Strength Test, Using the Universal Testing Machine

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 ($P = 0.013$).

Among Mucopren mixed with different SNPs concentration, the cell toxicity had no significant difference in the same days, and cell toxicity decreased over time ($P = 0.016$). The SNPs alone were less cytotoxic than Mucopren incorporated SNP samples ($P > 0.05$).

Addition of SNPs and thermocycling both caused a significant reduction in the tensile bond strength of Mucopren to acrylic resin; however, in the thermocycled group, the bond strength increased with the increase in the concentration of SNPs ($p < 0.001$).

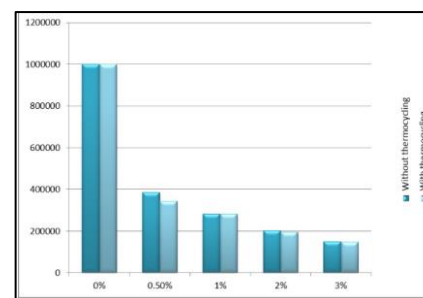


Table 1. Comparison of antifungal efficacy of Mucopren containing different SNP concentrations with and without thermocycling (x: nanosilver concentration in groups, y: average colony count)

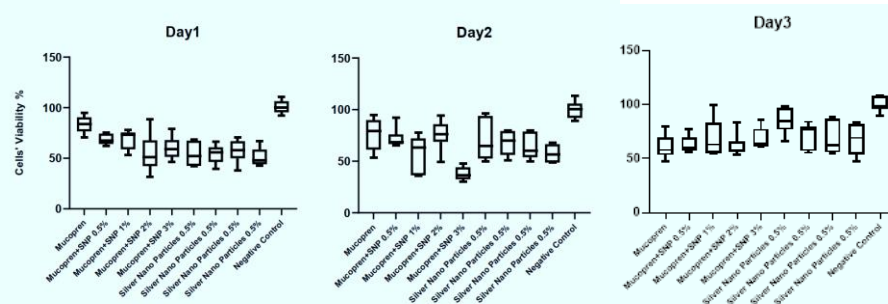


Figure 5. Results of cell viability in different groups in comparison to those in control group after 1, 2, and 3 days of incubation

Table 1. Results of cell viability in different groups in comparison to those in control group after 1, 2, and 3 days of incubation

| Concentration | Mean Bond Strength without Thermocycling in MPa | Mean Bond Strength with Thermocycling in MPa | Mean Difference | 95% Confidence | P-value |
|---------------|---|--|-----------------|----------------|---------|
| 0 | 1.19±0.12 | 0.32±0.07 | -0.87 | -0.96 -- -0.78 | <0.001 |
| 0.5 | 1.05±0.16 | 0.38±0.11 | -0.68 | -0.79 -- -0.56 | <0.001 |
| 1 | 0.97±0.11 | 0.45±0.08 | -0.52 | -0.60 -- -0.43 | <0.001 |
| 2 | 0.84±0.11 | 0.43±0.07 | -0.41 | -0.32 -- -0.49 | <0.001 |
| 3 | 0.60±0.11 | 0.50±0.08 | -0.11 | -0.19 -- -0.02 | 0.02 |

Conclusions

The addition of 0.5, 1, 2, and 3wt% concentrations of SNPs to Mucopren soft liner conferred antifungal effects. Thermocycling did not reduce the fungicidal properties of SNPs incorporated in the liner except in 0.5wt% SNP group. This incorporation did not cause a significant change in its cell toxicity in an in vitro condition, but reduced its tensile bond strength to denture acrylic resin.