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Antifungal efficacy of nanoparticle-reinforced denture soft liners: A comparative *in vitro* study

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Abstract:
Colonization of soft denture lining materials by fungi especially *Candida albicans* has been associated with denture stomatitis. Therefore, it is of interest to evaluate the antifungal activity of a soft liner containing silver, titanium dioxide and boron nitride nanoparticles. The colony forming units were analyzed using one-way ANOVA to determine the group differences ($p < 0.05$) and the surface morphology was evaluated using Scanning Electron Microscopy (SEM). The incorporation of AgNPs in soft denture liners was the most promising strategy against *C. albicans* colonization, followed by BN NPs and TiO2 NPs. Thus, data shows that AgNP reinforced soft liners may be a potential method to reduce denture associated candidal infection.

Keywords: Antifungal agent, titanium oxide, nanoparticles, denture liners, polyethyl methacrylate, *Candida albicans*

Background:
One of the most difficult dental treatments is the fabrication of a complete denture. Such a course of treatment gets increasingly challenging if there is significant resorption of the residual ridges, particularly the mandibular one **[1]**. The base of a complete denture, which is closely fitted to the oral mucosa, plays a major role in clinical dental applications by giving the prosthesis stability, support and retention. The most common material for fabrication of denture base is poly (methyl methacrylate) (PMMA). Due to its extreme durability, this hard, rigid material is used to manufacture prostheses for the majority of edentulous patients **[2]**. According to Hamid a soft, elastic and pliable material that makes up all or part of a denture's fit or intaglio surface is called a denture soft lining material **[2]**. In order to equally disperse the masticatory stresses that the prosthesis transmits to the underlying tissues, it might act as a cushion between the tissues and the hard denture base. The development of microorganisms is one issue with wearing soft denture liners **[3, 4]**. Microorganisms first stick to the lining's surface before entering the substance. Research has showed that the use of soft lining materials can accelerate the growth of bacteria and fungi, which are promoted by the materials' structure and the environment beneath the denture**.** Soft lining materials have been shown to retain more *Candida albicans* than acrylic and rougher surfaces have also been shown to improve adherence and retention **[5]**. The use of nanoparticles like Ag, ZnO, TiO_2 and SiO_2 in dental materials to enhance their characteristics has received attention lately **[6].** Therefore, it is of interest to evaluate and compare the antifungal efficacy of boron nitride, titanium dioxide and silver nanoparticle incorporated soft liners against *Candida albicans*.

Materials and Methods:
The purpose of this experimental *in vitro* investigation was to assess the antifungal qualities of soft liners containing silver nanoparticles, titanium dioxide and boron nitride. This study was performed in Maulana Azad National Institute of technology (MANIT) Bhopal and dextrose technologies Pvt. Ltd, Bangalore. Study sample consisted of 40 samples, 10 samples in each group. The samples were split up into different groups: Group A had 10 control samples, Group B had 10 boron nitride reinforced soft liners, Group C had 10 silver nanoparticles reinforced soft liners and Group D had 10 titanium dioxide reinforced soft liners.

Study procedure:
Mixing nanoparticles suspension into the soft liner monomer:
The nanoparticle incorporation protocol involved preparing suspensions at specific weight concentrations to ensure uniform dispersion within the soft-liner monomer matrix. Boron nitride nanoparticles were added at a concentration of 1.5 wt%, while silver nanoparticles and titanium dioxide nanoparticles were incorporated at 2 wt% each. The concentrations were based on the values reported in the literature. The values given in the literature served as the basis for the concentrations. Using a probe sonication device set to 120 W and 60 KHz; the mixture was dispersed into distinct nanocrystals for 3 minutes. The bowel containing the monomer and NPs suspension was cooled in an ice-water bath to remove the heat produced by sonication, which could alter the volume of the suspension or cause deterioration.

Preparation of moulds:
The specimens were prepared using metal dies with a cross section of 10 mm by 10 mm and a thickness of 5 mm. They were then placed in addition silicon which was then placed into dental plaster mixture poured in metal dental flask. Dental plaster was allowed to set and dewaxing procedure was performed to obtain the mould with desired measurements.

Preparation of the specimens:
To prevent any particle agglomeration, the monomer suspension containing NPs was added right away and combined with the PEMA powder using the normal powder or liquid ratio of 2.25gm in 2ml monomer. The loaded soft liner was proportioned and mixed with these nanoparticles. As advised by the manufacturer, the soft liner was packed into the molds after it reached the dough stage and the two-step curing process was carried out in a thermostatically controlled water bath. The flask holding the samples was heated to 70 °C in the first phase and kept there for 90 minutes. In the second step, the temperature was increased to 100 °C and kept there for 30 minutes. This was

followed by the cooling of flask for 30 minutes at room temperature and then they were completely cooled for 15 minutes using tap water before deflasking. After removing the specimens from the flask, any surplus material adhered to the samples was cut off using a sharp blade and any sample flash was further removed using fine grit sandpaper. Lastly, the specimens were sterilized in an autoclave.

Isolation of *candida albicans*:
Soft liner samples were treated with different nanoparticles and incubated with *Candida albicans* on YGCA (Yeast Glucose Chloramphenicol Agar) at 25°C for 24–48 hours. Phosphate-buffered saline (PBS) was used to wash the samples after incubation in order to eliminate non-adherent cells. Adhered cells were dislodged by vortexing and the suspension was serially diluted and plated to determine the CFU (colony forming units). Ten replicates were performed for each group.

Results:

Table 1: Depicting the colony forming units in individual sample

Samples	CFU			
	Control Group	Boron Nitrate Np's	Silver Np's	TiO_2 Np's
1.	1.2×10^3	0.3×10^3	0.3×10^2	0.9×10^3
2.	1.3×10^3	0.2×10^3	0.3×10^2	1.0×10^3
3.	1.2×10^3	0.3×10^3	0.3×10^2	0.9×10^3
4.	1.3×10^3	0.2×10^3	0.3×10^2	1.0×10^3
5.	1.2×10^3	0.3×10^3	0.3×10^2	0.9×10^3
6.	1.3×10^3	0.2×10^3	0.3×10^2	1.0×10^3
7.	1.2×10^3	0.3×10^3	0.3×10^2	0.9×10^3
8.	1.3×10^3	0.2×10^3	0.3×10^2	1.0×10^3
9.	1.2×10^3	0.3×10^3	0.3×10^2	0.9×10^3
10.	1.3×10^3	0.2×10^3	0.3×10^2	1.0×10^3

One-way study of Variance (ANOVA) was used in the statistical study to identify significant differences ($p < 0.05$) between groups. The null hypothesis assumed no difference in mean CFU counts among the different nanoparticle treatments. To determine which treatments varied substantially from the control, a post-hoc Tukey's HSD test was employed **(Table 1).** The average CFU counts for each treatment group were as follows: Control: 1.25×10^3 - Boron Nitrate NPs: 0.25×10^3 - Silver NPs: 0.3×10^2 - Titanium Dioxide NPs: 0.95×10^3 Silver nanoparticles demonstrated the strongest antifungal effect, with statistically significant ($p < 0.05$) reductions in CFU compared to all other groups. Boron Nitrate nanoparticles also showed moderate efficacy. Titanium Dioxide nanoparticles were less effective but still provided some reduction compared to the control. The surface morphology of the soft liner samples incorporated with nanoparticles (Boron Nitride, Titanium Dioxide and Silver Nanoparticles) was examined using Scanning Electron Microscopy (SEM). To get rid of any leftover medium or unattached particles, soft liner specimens were first cut into ~5 mm^2 pieces and thoroughly cleaned with phosphate-buffered saline (PBS). After fixing the samples in 2.5% glutaraldehyde for two hours at room temperature to maintain their structural integrity, they underwent graded ethanol dehydration (30%, 50%, 70%, 90% and 100%) for 10 minutes each. The samples were then vacuum-dried in a desiccator to remove any remaining moisture**.**

Discussion:
In contemporary removable prosthodontics, soft denture liners play a crucial function. However with many advantages, still the soft liners use in dentistry is very limited as it exhibits multiple clinical failures. According to Chladek *et al.* (2024) **[7]**, certain denture soft lining materials encourage *Candida albicans* development *in vitro*, while Ferreira *et al.* (2022) **[8]** reported that two denture soft lining materials encouraged oral yeast development and presence. Additionally, plaque buildup and *Candida albicans* colonization may result from the increased porosity of denture soft lining materials Abuhajar *et al.* 2022 **[9].** One of the key drivers behind the escalating problem of chemical agent–induced antimicrobial resistance is the extensive and indiscriminate use of antimicrobial compounds. A promising strategy to counter resistant pathogens involves the application of nanoantibiotics nanoparticles engineered with intrinsic antibacterial properties. (Antifungal Activities of Silver Nanoparticles (AgNPs) Against Selected Phytopathogenic Fungi), Oribhabor and Ugwuoke (2025) **[10]**. Alarmingly, the World Health Organization projects that antimicrobial resistance (AMR) could surpass all other causes of death by 2050, positioning it as the foremost global health threat. Murray *et al.* (2022) **[11]** nanotechnology has advanced quickly in recent years and is being used in many different industries, including computers, biosciences, information technology and medicine. Nanocarrier systems are particularly advantageous for antifungal agents that exhibit poor solubility, limited transport and inadequate absorption Palaria *et al*. (2022) **[12]** such drugs, constrained by these pharmacokinetic challenges, are especially well suited to delivery through nanocarrier platforms. A nanocarrier system when used in situ significantly enhances solubility and bioavailability, while effectively prolonging drug release, offering a novel therapeutic approach. Tiwari *et al.* (2023) **[13]** metal nanoparticles tend to cluster on cellular surfaces, exerting mechanical stress on cell walls and membranes Xia *et al.* (2024) **[14]** various nanoparticles such as silver, boron nitride, titanium dioxide, zeolite *etc.* have been reported with antifungal and antibacterial properties. Silver nanoparticles (AgNPs) are regarded as the most promising class of nanoantibiotics, owing to their potent antibacterial and antiviral activities. Rodrigues *et al.* (2024); Sati *et al.* (2025) **[15, 16]** TiO2 NPs also showed a value-added property of antimicrobial activity. According to earlier research, TiO2 NPs showed notable cytotoxic effects against both Gram-positive and Gram-negative bacteria, as well as algae. The photocatalytic process of TiO2 NPs causes fatal damage to microorganisms. Silver (Ag) is more toxic than many other metals against a wide range of sessile bacteria and fungi that colonize on plastic surfaces and it has a long history of use in medicine due to its well-known antimicrobial properties, low toxicity profile and well-tolerated tissue response **[17].** There is an electromagnetic attraction between the negatively charged metal oxide nanoparticles **[18].** Boron Nitride has bacteria and fungi suppression mechanisms and one of them is chemical ROS

formation **[19].** Important biological components like proteins, nucleic acids and phospholipids can be harmed by reactive oxygen species (ROS), which can lead to cell lysis. Although a lot of research has targeted at the antifungal qualities of nanoparticles on soft liners, there hasn't been a comparison on effect of various nanoparticles on antifungal efficacy of soft liners. The purpose of our study was to assess the antifungal capabilities of various nanoparticles added to dental soft liners against *Candida albicans*. The tested groups included: Control (unaltered soft liner), boron nitrate nanoparticles (BN NPs), Silver Nanoparticles (Ag NPs) and Titanium Dioxide Nanoparticles (TiO_2 NPs). When compared to all other groups, silver nanoparticles showed the highest antifungal activity, reducing CFU in a statistically significant ($p < 0.05$) manner. AgNPs' capacity to release silver ions, which can damage microbial cell walls, obstruct DNA replication and produce reactive oxygen species that cause cell death, is thought to be the cause of their antifungal action **[19, 20]**. Studies have demonstrated that AgNPs, when incorporated into soft liners like Mucopren® silicone, exhibit dose-dependent antifungal activity. For instance, a study found that adding 3 wt% AgNPs resulted in the lowest colony count of *C. albicans*, indicating significant antifungal effectiveness. Similarly, another study reported that acrylic soft liners containing 0.2% and 0.3% AgNPs maintained antifungal rates of 63.38% and 75.51% respectively **[19]**. Nam reported the introduction of ≥ 1% concentration of SNPs entirely suppressed bacterial activity, but a minimum of ≥ 2% concentration of SNPs was necessary for fungicidal activity **[21].** Nanoparticles of boron nitrate also have shown a moderate level of effectiveness. In comparison to the control, titanium dioxide nanoparticles were less effective but still produced some decrease. Studies have suggested that incorporation of TiO_2 NPs into a heat-cured soft denture liner demonstrated antifungal activity, with the mean viable count of *C. albicans* decreasing significantly as the concentration of TiO_2 NPs increased. At 2 wt% concentration, the viable count reduced to 1.00, compared to 139.80 in the control group which support the concentrations as we have used in our study **[22].** Further, incorporation of nanoparticles into soft liners provides an innovative approach for controlled and sustained release of therapeutic agents directly at the site of action **[23].**

Conclusion:

Incorporating AgNPs into soft denture liners offers a promising strategy to combat *Candida albicans* colonization and associated complications. While TiO2 NPs and BN NPs also present antifungal properties, AgNPs currently provide the most effective solution. Future research should focus on optimizing nanoparticle concentrations to enhance antifungal activity while maintaining the desired material properties and ensuring patient safety.

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