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Evaluation of Physico-mechanical and Antimicrobial Properties of a Silicone-Based Soft Denture Liner Modified with Zinc Oxide Nanoparticles and Fluconazole: An In Vitro Study

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ABSTRACT

Purpose: This in vitro study evaluated the physico-mechanical and antimicrobial properties of a silicone-based soft denture liner modified with zinc oxide nanoparticles (ZnO-NPs).

Methods: Forty disc-shaped specimens were fabricated from a silicone soft denture liner and divided into four groups according to ZnO-NP concentration (0%, 5%, 7%, and 10% w/w). Antimicrobial activity was assessed against Candida albicans (ATCC 10231) and Staphylococcus aureus (ATCC 25923) using disc diffusion and minimum inhibitory concentration assays. Shore A hardness and flexibility were evaluated according to ISO 10139-2 standards. Zinc ion release was measured after immersion in artificial saliva. Data were analyzed using one-way ANOVA ($\alpha = 0.05$).

Results: Zinc oxide nanoparticle modification showed no antifungal activity against C. albicans. However, significant antibacterial activity against S. aureus was observed, with the 7% concentration demonstrating optimal inhibition. Physico-mechanical properties improved progressively with increasing ZnO-NP concentration. Zinc ion release was minimal at 5% and 7% concentrations and remained within acceptable limits.

Conclusions: Zinc oxide nano-particles at a 7% concentration enhanced antibacterial performance and mechanical properties of the soft denture liner while maintaining low ion release. These findings suggest that ZnO-NP modification may improve the functional performance of denture liners for prosthodontic applications.

Keywords: Soft denture liner; zinc oxide nanoparticles; Candida albicans; Staphylococcus aureus; antimicrobial; mechanical properties

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INTRODUCTION

Edentulism continues to represent a significant oral health challenge, particularly among the elderly population, with profound effects on mastication, speech, facial aesthetics, and overall quality of life.[1,2] Although implant-supported prostheses have transformed modern prosthodontic rehabilitation, complete dentures remain the most widely used treatment option for many patients, especially those with systemic conditions, anatomical limitations, or financial constraints.[3] Consequently, improving the comfort, function, and longevity of conventional dentures remains an important focus in prosthodontic research. To address patient discomfort and enhance denture adaptation, soft denture liners have been widely incorporated into clinical practice. These resilient materials act as a cushion between the denture base and the underlying mucosa, allowing for more even distribution of functional stresses and reducing trauma to compromised oral tissues.[4,5] Despite their clinical benefits, soft liners are associated with several well-documented limitations, including gradual hardening, loss of elasticity, debonding from the denture base, and surface degradation over time.[6] Such changes not only compromise their mechanical performance but also create favorable conditions for microbial adhesion and biofilm formation. Among the microorganisms implicated in denture-related infections, Candida albicans plays a central role in the development of denture stomatitis, a common inflammatory condition affecting denture wearers.[7,8] The porous structure and deteriorating surface of soft liners facilitate microbial colonization, allowing these materials to act as reservoirs for pathogens. Over the years, numerous approaches have been explored to mitigate this issue, particularly through the incorporation of antifungal agents such as nystatin and fluconazole into denture liners and tissue conditioners.[9,10] While these strategies have demonstrated effectiveness in reducing fungal growth, their long-term application remains limited by drug leaching, potential reduction in mechanical properties, and concerns regarding the development of microbial resistance. In recent years, the application of nanotechnology in dentistry has gained increasing attention as a means of overcoming these limitations. Among various nanomaterials, zinc oxide nanoparticles (ZnO-NPs) have emerged as promising candidates due to their broad-spectrum antimicrobial activity, biocompatibility, and potential to enhance the mechanical properties of polymer-based materials.[11,12] The antimicrobial action of ZnO-NPs is generally attributed to mechanisms such as the generation of reactive oxygen species, release of zinc ions, and disruption of microbial cell membranes.[13] In addition to their antimicrobial effects, these nanoparticles can function as reinforcing fillers within polymer matrices, contributing to improved hardness, strength, and durability.[14] Several studies have investigated the incorporation of ZnO-NPs into denture base resins and soft lining materials, reporting varying degrees of success in enhancing antimicrobial activity and physico-mechanical properties.[15,16] However, the effectiveness of ZnO-NPs against Candida albicans, particularly when incorporated into silicone-based soft denture liners, remains inconsistent. One possible explanation is that embedding nanoparticles within a polymer matrix may restrict their availability, limiting ion release and reducing direct interaction with microbial cells.[17] Furthermore, the optimal concentration of ZnO-NPs that achieves a balance between antimicrobial efficacy and acceptable material properties has yet to be clearly defined.

METHODS

Study Design

This in vitro experimental study was carried out at the College of Dentistry, Hawler Medical University, Kurdistan Region, Iraq. The study protocol was reviewed and approved by the Scientific Research Ethical Committee at the College of Dentistry, Hawler Medical University (Reference No.: HMUD, 2425107; Date of Approval: 14 January 2025).

The research was designed to assess the effect of incorporating zinc oxide nanoparticles and fluconazole into a denture liner material on its physico-mechanical and antifungal properties. Specimens were prepared according to the manufacturer's instructions and allocated into control and experimental groups. The experimental specimens were modified with different concentrations of zinc oxide nanoparticles, while fluconazole was used for antifungal comparison. The prepared samples were subjected to antifungal evaluation, Shore A hardness testing, flexibility assessment, ion release analysis, and surface characterization.A commercially available A-silicone chemically cured soft denture liner (Mollosil®, DETAX GmbH, Germany) was used as the base material for all groups. The material consists of base and catalyst compo-

nents mixed in a 1:1 weight ratio according to the manufacturer's guidelines. Zinc oxide nanoparticles (ZnO-NPs) (purity 99+%, average size ≈20 nm; Nanografi, Istanbul, Türkiye) were selected as the inorganic additive. Before incorporation, ZnO-NPs were sonicated in ethanol for 10 minutes to minimize agglomeration and ensure uniform dispersion, then sterilized using dry heat at 180 °C for one hour to preserve nanoparticle integrity. Pharmaceutical-grade fluconazole powder was handled aseptically and stored under controlled dry conditions to prevent degradation. The denture liner was modified with 5%, 7%, and 10% (w/w) concentrations of ZnO-NPs or fluconazole, while the 0% group served as the unmodified control. These concentrations were selected based on a preliminary pilot study designed to balance antifungal efficacy and material stability. Equal amounts of base and catalyst were weighed using a digital precision balance. The additives were first blended manually into the base component for one minute (120 cycles/min) to achieve initial dispersion, followed by mechanical mixing for three minutes using a sterilized Lentulo spiral mounted on a slow-speed handpiece to ensure uniform distribution. The catalyst paste was then incorporated and mixed for 30 seconds according to the manufacturer's instructions. The selected ZnO-NP concentrations (5%, 7%, and 10% w/w) were based on previous literature and preliminary observations aiming to balance antimicrobial efficacy with acceptable mechanical performance and minimal ion release. All samples were prepared at room temperature (22 ± 2 °C) under controlled laboratory conditions. The mixed material was spread into uniform silicone sheets and allowed to polymerize completely. Disc-shaped specimens were obtained using a manual puncher (5 ± 0.5 mm diameter, 1 mm thickness) as described by 18. Before testing, specimens were disinfected in 2% chlorhexidine gluconate for five minutes, rinsed with sterile distilled water, and exposed to ultraviolet light for 30 minutes on each side to ensure surface sterility. Mechanical characterization included Shore A surface hardness and flexibility, assessed using the three-point bending test, following ISO 10139-2:2016 standards for soft lining materials. All specimens were conditioned in distilled water at 37 °C for 24 hours before testing to simulate intraoral conditions. Antifungal activity against Candida albicans (ATCC 10231) and antibacterial activity against Staphylococcus aureus (ATCC 25923) were evaluated using agar disc diffusion and minimum inhibitory concentration assays. Standardized microbial suspensions (0.5 McFarland) were inoculated on appropriate agar plates, and specimens were placed aseptically on the surface before incubation at 37 °C for 24 hours. Inhibition zones were measured in millimeters. MIC evaluation was performed by incubating specimen extracts in broth cultures and assessing turbidity after 24 hours. Zinc ion release was quantified using inductively coupled plasma mass spectrometry after seven-day immersion of the specimens in artificial saliva at 37 °C. Filtrates were analyzed for Zn^{2+} concentration in parts per million. Characterization of the modified denture liner specimens was performed to assess nanoparticle distribution, crystalline phase stability, and potential chemical interactions within the polymer matrix. Surface morphology and nanoparticle dispersion were evaluated using a high-resolution scanning electron microscope after sputter-coating each specimen with gold for 60 seconds. Micrographs were obtained at magnifications ranging from 500× to 5000× Figure 1.

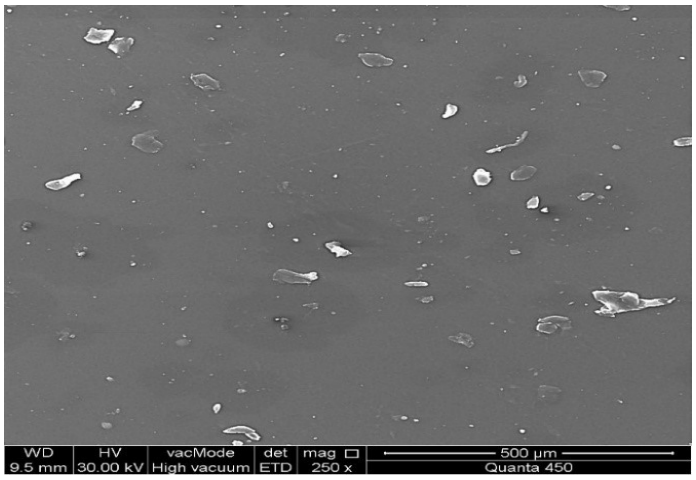

Figure 1. Scanning electron microscopy (SEM) image of zinc oxide nanoparticles

Crystallographic analysis was conducted using X-ray diffraction with Cu-Kα radiation (λ = 1.5406 Å) operating at 40 kV and 30 mA. Diffraction patterns were collected over a 2θ range of 10°–80° to confirm the presence and structural integrity of ZnO nanoparticles within the silicone-based material Figure 2. Chemical characterization was performed using ATR-FTIR spectroscopy within the 4000–400 cm^{-1} range to detect functional groups associated with the silicone matrix (Si–O–Si), zinc oxide (Zn–O), and fluconazole. Spectral compari-

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sons across groups were used to determine whether the incorporation of additives resulted in any detectable chemical interactions or modifications to the base material.

Figure 2. Energy-dispersive X-ray spectroscopy (EDS) spectrum of zinc oxide

RESULTS

No antifungal activity was observed for any of the ZnO-NP–modified groups (5%, 7%, and 10%) against Candida albicans. In the agar disc diffusion test, no inhibition zones were detected in any group, and the minimum inhibitory concentration (MIC) testing showed persistent turbidity in all wells, confirming continued fungal growth. Repeated assays produced identical outcomes. These findings indicated that ZnO nanoparticles, within the tested concentrations, did not exert antifungal effects. (Table 1)

In contrast, the ZnO-NP–modified specimens demonstrated concentration-dependent antibacterial activity against Staphylococcus aureus. The 5% and 7% groups produced measurable inhibition zones of 10 mm and 12 mm, respectively, whereas no inhibition was observed in the 10% or control groups. Statistical analysis confirmed significant differences between the 5% and 7% groups compared with the control ($P < 0.05$) -(Figure 3).
ZnO-NPs demonstrated significant antibacterial activity against Staphylococcus aureus at 5% and 7%, likely due to oxidative stress-induced membrane damage.[19]

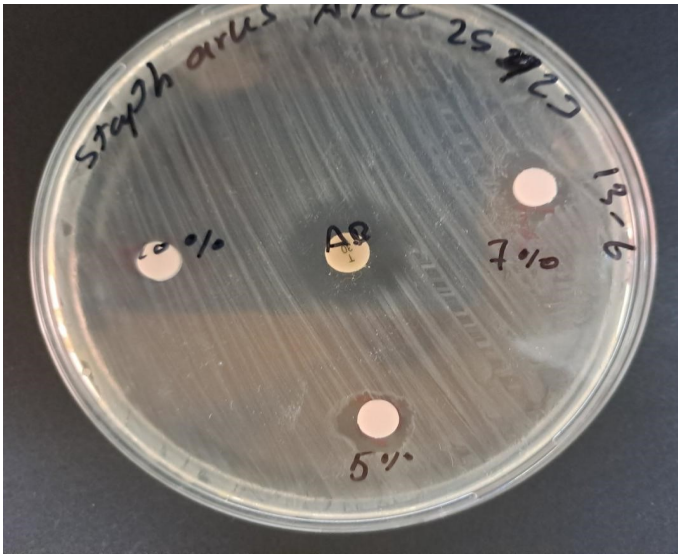

Figure 3. Antibacterial activity of ZnO-modified denture liners against S. aureus via disc diffusion assay

Table 1. Agar Disc Diffusion Test Results (n = 10 Per Group)

Groups	ZnO Concentration	Fluconazole	C.albicans Inhibition Zone	S.aureus Inhibition Zone	Interpretation
1	0% (control)	0% (control)	No zone	No zone	No antimicrobial effect
2	5%	5% Clear zone	No zone	Small zone (≤10 mm)	Mild antibacterial activity
3	7%	7% Clear zone	No zone	Clear zone (10–12 mm)	Effective antibacterial activity
4	10%	10% Clear zone	No zone	Small zone (≤5 mm)	Mild antibacterial activity, no added antifungal effect

MIC and turbidity testing supported these results: the 7% ZnO-NP concentration resulted in complete inhibition, with clear wells and markedly reduced optical density, establishing it as the MIC threshold. Partial inhibition was observed in the 5% group, while the 10% ZnO-NP group showed no added benefit, likely due to nanoparticle agglomeration reducing bioavailability. All ZnO-NP groups remained ineffective against C. albicans. (Table 3)

Table 3. MIC and Turbidity Test Results (n = 10 Per Group)

Groups	ZnO Concentration	C.albicans Inhibition Zone	S.aureus Inhibition Zone	Interpretation
1	0% (control)	Present (turbid)	Present (turbid)	No inhibition
2	5%	Present (turbid)	Moderate turbidity	Partial inhibition
3	7%	Present (turbid)	No visible turbidity	Complete inhibition (MIC threshold)
4	10%	Present (turbid)	No visible turbidity	No added benefit beyond 7%

Physico-mechanical testing demonstrated that Shore A hardness increased progressively with rising ZnO-NP concentration. The lowest value was recorded in the control group (30.5 ± 0.84HA), while the highest was observed in the 10% group (33.4 ± 0.40HA). Only the 10% ZnO-NP group differed significantly from the control ($P < 0.05$), indicating that substantial reinforcement of the silicone matrix occurred at higher nanoparticle loading. Flexibility also improved in a concentration-dependent manner, increasing from 20.1 ± 0.05 m/N in the control group to 26.1 ± 0.03 m/N in the 10% ZnO-NP group. All ZnO-modified groups showed significant differences from one another ($P < 0.05$), confirming the consistent impact of ZnO incorporation on enhancing elastic performance. These results align with previous studies.[20,21] and highlight the clinical potential of ZnO-modified denture liners for improved durability and patient comfort. Inductively Coupled Plasma Mass Spectrometry(ICP-MS) analysis revealed a concentration-dependent release of Zn^{2+} ions following seven days of immersion in artificial saliva. No zinc ions were detected in the control group. The 5% and 7% ZnO-NP groups showed values below the quantification limit (<0.01 ppm), indicating negligible ion release. The 10% group demonstrated a detectable level of 0.03 ppm, although this remained far below cytotoxic concentrations. All measurements were reproducible, and no spectral or matrix interferences were encountered during analysis.

DISCUSSION

The present study evaluated the antimicrobial and physico-mechanical properties of a silicone-based soft denture liner modified with zinc oxide nanoparticles (ZnO-NPs) and fluconazole. The findings demonstrated that ZnO-NP incorporation improved antibacterial activity, hardness, and flexibility; however, no detectable antifungal activity against Candida albicans was observed. These findings indicate that the biological performance of ZnO-NPs within a silicone matrix may differ from that reported for free nanoparticles in suspension. The antifungal evaluation revealed that none of the ZnO-NP concentrations (5%, 7%, and 10%) produced inhibition zones against Candida albicans, and turbidity persisted in all tested groups. These findings are in agreement with Rao et al., who reported limited antifungal activity of ZnO-NPs incorporated into silicone soft denture liners. Similarly, Cierech et al. suggested that the incorporation of nanoparticles within polymeric materials may restrict ion release and reduce direct interaction with fungal cells. In contrast, previous investigations have reported significant antifungal activity of ZnO nanoparticles against Candida albicans.[19,22] The discrepancy between these findings may be attributed to differences in nanoparticle concentration, particle size, synthesis method, testing protocol, and the ability of the silicone matrix to limit nanoparticle bioavailability. The minimal zinc ion release observed in the present study further supports this explanation. In contrast to the

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antifungal findings, ZnO-NP–modified specimens demonstrated measurable antibacterial activity against Staphylococcus aureus. The 7% ZnO-NP group exhibited the largest inhibition zone and the most favorable antibacterial performance, whereas the 5% group showed moderate inhibition. These findings are consistent with previous reports describing the antibacterial efficacy of ZnO nanoparticles against Gram-positive bacteria.[12,13,19] The antibacterial action of ZnO-NPs has been attributed to the generation of reactive oxygen species, release of Zn^{2+} ions, and disruption of bacterial cell membranes.[13,19] The superior performance observed at 7% concentration suggests that this level provided an optimal balance between nanoparticle availability and dispersion within the silicone matrix. Interestingly, the antibacterial effect decreased at the 10% concentration. Similar findings have been reported in nanoparticle-modified dental materials, where excessive nanoparticle loading resulted in agglomeration and reduced antimicrobial efficiency.[15,17,20] Agglomerated particles exhibit lower effective surface area and reduced interaction with microorganisms, thereby limiting their antimicrobial activity. This observation highlights the importance of optimizing nanoparticle concentration rather than assuming that higher concentrations necessarily produce superior antimicrobial performance. The minimum inhibitory concentration (MIC) and turbidity assay findings further supported the antibacterial results. The 7% ZnO-NP group demonstrated complete inhibition of bacterial growth and the lowest optical density values, whereas the 5% group showed partial inhibition. These findings indicate that 7% ZnO-NP represented the most effective concentration against Staphylococcus aureus under the conditions of the present study. Similar concentration-dependent antibacterial behavior has been reported by Raghupathi et al. and Pushpalatha et al. Regarding the physico-mechanical properties, the incorporation of ZnO-NPs resulted in progressive increases in Shore A hardness. The highest hardness value was observed in the 10% group, while the control group demonstrated the lowest value. These findings agree with those reported by Cierech et al. and Rao et al., who observed increased hardness following nanoparticle incorporation into denture materials. The increase in hardness may be attributed to the reinforcing effect of inorganic nanoparticles, which restrict polymer chain mobility and improve resistance to surface deformation.Flexibility values also increased with increasing ZnO-NP concentration. The highest flexibility was recorded in the 10% group, indicating that nanoparticle incorporation positively influenced the elastic behavior of the silicone liner. Similar observations have been reported in previous studies evaluating nanoparticle-modified soft denture materials.[16,20] The improvement in flexibility may be explained by enhanced stress distribution within the polymer matrix and improved filler–matrix interaction. Clinically, improved flexibility may contribute to better load distribution and increased patient comfort. ICP-MS analysis demonstrated minimal zinc ion release from all modified groups. Zinc release remained below the quantification limit in the 5% and 7% groups and reached only 0.03 ppm in the 10% group. These findings are consistent with previous reports demonstrating controlled zinc ion release from ZnO-containing dental materials.[17,20] The low release values observed in the present study suggest acceptable biocompatibility and support the safety of ZnO-NP incorporation at the investigated concentrations. The combined findings of this study indicate that ZnO nanoparticles primarily functioned as antibacterial and reinforcing agents rather than antifungal additives when incorporated into silicone-based soft denture liners. Among the investigated concentrations, the 7% ZnO-NP group demonstrated the most favorable balance between antibacterial efficacy, physico-mechanical performance, and controlled ion release. These findings are consistent with previous reports demonstrating that optimal nanoparticle concentrations provide superior performance compared with either lower or excessively high concentrations.[15,19,20] The present study was limited by its in vitro design and the use of planktonic microorganisms rather than mature oral biofilms. Furthermore, the long-term effects of aging, thermal cycling, and continuous ion release were not evaluated. Therefore, future studies should investigate biofilm-based models, long-term aging conditions, and combined antifungal–nanoparticle systems to further enhance the clinical performance of soft denture liners.Clinically, ZnO-NP–modified liners may help reduce bacterial colonization and improve material longevity; however, their inability to inhibit fungal growth suggests limited effectiveness against denture-related infections such as denture stomatitis. Therefore, combined antimicrobial strategies may be more suitable.

This study is limited by its in vitro design and the use of planktonic microorganisms, which do not fully represent oral biofilm conditions. Additionally, nanoparticle dispersion within the matrix was not directly evaluated. Future research should focus on improving nanoparticle distribution, exploring hybrid antimicrobial systems, and conducting biofilm-based and in vivo studies.

CONCLUSION

This study was limited by its in vitro design and short-term evaluation period. Therefore, the long-term durability, additive stability, and biological performance of the modified liners under dynamic oral conditions could not be fully assessed. Although the incorporation of additives demonstrated promising antimicrobial and physico-mechanical benefits, further optimization and in vivo studies are required to confirm their long-term performance and clinical relevance. Future investigations should focus on combined antifungal–nanoparticle systems and evaluate their effectiveness under biofilm conditions, long-term aging protocols, and clinical settings. Although the incorporation of additives demonstrated improvements in antibacterial activity and physico-mechanical properties, further optimization and in vivo studies are required to confirm long-term performance and clinical relevance. These findings are consistent with previous reports.[18,19,25]

Suggestions for Further Studies

In light of current findings, it is recommended that future research investigate biosynthesized ZnO nanoparticles, which may offer improved dispersion, reduced cytotoxicity, and enhanced bioactivity compared to their chemically synthesized counterparts.[26] Functionalizing ZnO-NPs with natural polymers such as chitosan could further mitigate agglomeration and foster more effective microbial interactions[27] Moreover, combining ZnO-NPs with complementary nanomaterials—such as titanium dioxide or silver nanoparticles—may yield synergistic effects, enhancing both antimicrobial efficacy and mechanical performance.[28]

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Conflict of Interest

The authors declare no conflicts of interest.

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