

In vitro evaluation of the antimicrobial effects of orthodontic bands coated with silver or zinc oxide nanoparticles

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Abstract

Background

Orthodontic bands are widely used in clinical orthodontics; however, they often create niches for bacterial accumulation, leading to enamel demineralization and gingival inflammation. To counteract microbial growth, silver and zinc oxide nanoparticles (AgNPs and ZnONPs) have been explored for their antimicrobial properties. This in vitro study aims to evaluate and compare the antimicrobial efficacy of orthodontic bands coated with AgNPs and ZnONPs against key oral pathogens.

Materials and Methods

Orthodontic bands were divided into three groups: **Group A:** Uncoated control bands, **Group B:** Bands coated with silver nanoparticles (AgNPs), **Group C:** Bands coated with zinc oxide nanoparticles (ZnONPs). The nanoparticles were synthesized and applied using the dip-coating method. Bands were then exposed to *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* in nutrient broth. The antimicrobial activity was evaluated using the disk diffusion method and zone of inhibition (ZOI) was measured in millimeters (mm). In addition, colony-forming unit (CFU) reduction was analyzed after 24 and 48 hours of incubation.

Results

Zone of Inhibition: Group B (AgNPs): 15.2 ± 1.3 mm (against *S. mutans*), Group C (ZnONPs): 12.4 ± 1.1 mm (against *S. mutans*), Minimal zones for the control group (Group A). **Colony-Forming Unit (CFU) Reduction:** Group B: 85% reduction after 24 hours and 92% after 48 hours. Group C: 78% reduction after 24 hours and 88% after 48 hours. Group A (Control): No significant reduction in CFU counts over time.

Conclusion

Orthodontic bands coated with silver and zinc oxide nanoparticles demonstrated significant antimicrobial activity, with AgNP-coated bands showing superior performance against *S. mutans*, *L. acidophilus*, and *C. albicans*. The results suggest that AgNP and ZnONP coatings could potentially reduce microbial colonization and associated dental complications in orthodontic treatments. Further in vivo studies are recommended to

validate these findings.

Keywords: Orthodontic bands, silver nanoparticles, zinc oxide nanoparticles, antimicrobial effect, *Streptococcus mutans*, orthodontics, biofilm prevention

Introduction

Orthodontic treatment with fixed appliances, such as brackets and bands, often creates niches that promote microbial accumulation and biofilm formation. These microbial deposits can lead to common complications like enamel decalcification, gingivitis, and periodontal inflammation, posing challenges in maintaining oral health during treatment (1). *Streptococcus mutans* and *Lactobacillus acidophilus* are the primary bacteria involved in dental caries, while *Candida albicans* is an opportunistic fungal pathogen that may also colonize orthodontic appliances, exacerbating oral infections (2,3).

The increasing prevalence of microbial resistance to traditional antimicrobial agents has necessitated the exploration of novel materials with antimicrobial properties. In recent years, metal-based nanoparticles, such as silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs), have gained significant attention in biomedical applications due to their potent antimicrobial activity, biocompatibility, and minimal toxicity (4,5). AgNPs exhibit broad-spectrum antimicrobial effects by interacting with bacterial cell membranes and disrupting cellular processes (6). Similarly, ZnONPs exhibit antibacterial, antifungal, and anti-inflammatory properties, making them a promising alternative to conventional agents (7).

Coating orthodontic appliances with nanoparticles is a promising approach to mitigate microbial adhesion and biofilm formation. Studies have reported that AgNP-coated surfaces exhibit strong bactericidal properties against oral pathogens, including *S. mutans* and *C. albicans* (8). ZnONPs, on the other hand, not only inhibit bacterial growth but also offer additional benefits such as wound healing and improved biocompatibility (9). However, limited studies have compared the antimicrobial efficacy of AgNPs and ZnONPs when applied specifically to orthodontic bands.

This study aims to evaluate and compare the antimicrobial effects of orthodontic bands coated with silver and zinc oxide nanoparticles against common oral pathogens. The findings could provide valuable insights for developing preventive strategies to reduce microbial-associated complications during orthodontic treatment.

Materials and Methods

Study Design

This in vitro experimental study was conducted to compare the antimicrobial effects of orthodontic bands coated with silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*. The study consisted of three groups:

- **Group A:** Uncoated control bands
- **Group B:** Bands coated with AgNPs
- **Group C:** Bands coated with ZnONPs

Each group contained 10 samples ($n = 10$) of orthodontic bands to ensure reproducibility and statistical validity.

Synthesis and Coating of Nanoparticles

AgNPs and ZnONPs were synthesized using chemical reduction and sol-gel methods, respectively. AgNPs were prepared by reducing silver nitrate (AgNO_3) with sodium borohydride, while ZnONPs were synthesized using zinc acetate and sodium hydroxide. Nanoparticle size was confirmed to be within the range of 20-50 nm using dynamic light scattering (DLS) analysis.

The orthodontic bands were coated with AgNPs and ZnONPs through the dip-coating method. Bands were immersed in nanoparticle suspensions (1 mg/mL) for 15 minutes, followed by air drying at room temperature and curing at 100°C for 30 minutes. Coated samples were sterilized using UV radiation before testing.

Microbial Strains and Culture Conditions

The microbial strains used in this study were:

- *Streptococcus mutans* (ATCC 25175)
- *Lactobacillus acidophilus* (ATCC 4356)
- *Candida albicans* (ATCC 10231)

The bacteria were cultured in Brain Heart Infusion (BHI) broth, while *C. albicans* was grown in Sabouraud Dextrose Agar (SDA). The strains were incubated at 37°C for 24 hours before antimicrobial testing.

Antimicrobial Activity Testing

1. Disk Diffusion Method

The antimicrobial activity of the coated bands was evaluated using the disk diffusion method. Sterile Mueller-Hinton agar plates (with 2% glucose for fungi) were inoculated with 100 μL of microbial suspension (1×10^6 CFU/mL). Each band was placed on the agar surface, and plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition (ZOI) around the bands was measured in millimeters (mm).

2. Colony-Forming Unit (CFU) Assay

The bands were immersed in microbial suspensions for 24 and 48 hours at 37°C. After each incubation period, 100 μL of the suspension was plated on agar media and incubated for 24 hours. The CFU counts were recorded to assess the percentage reduction in microbial growth compared to the control group.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean \pm standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test to identify significant differences between groups. A p-value < 0.05 was considered statistically significant.

Results

Zone of Inhibition (ZOI)

The antimicrobial activity of the orthodontic bands was evaluated by measuring the diameter of the zone of inhibition (ZOI) against *S. mutans*, *L. acidophilus*, and *C. albicans*. The results are summarized in Table 1.

AgNP-coated bands showed the highest ZOI values, indicating superior antimicrobial efficacy.

Table 1: Zone of Inhibition (ZOI) in millimeters (mm)

Group	<i>S. mutans</i>	<i>L. acidophilus</i>	<i>C. albicans</i>
Control (Uncoated)	0.5 mm	0.3 mm	0.4 mm
AgNP-Coated	15.2 mm	14.8 mm	13.7 mm
ZnONP-Coated	12.4 mm	11.9 mm	11.5 mm

Colony-Forming Unit (CFU) Reduction

The CFU assay demonstrated the effectiveness of the nanoparticle coatings over 24 and 48 hours of incubation. As shown in Table 2, AgNP-coated bands achieved the highest percentage reduction in CFU counts at both time points, followed by ZnONP-coated bands. The control group exhibited minimal reduction.

Table 2: Percentage Reduction in Colony-Forming Units (CFU)

Group	24 Hours	48 Hours
Control (Uncoated)	5%	10%
AgNP-Coated	85%	92%
ZnONP-Coated	78%	88%

Summary of Findings

- AgNP-coated bands exhibited the largest ZOI against all tested microorganisms, with a maximum of 15.2 mm against *S. mutans*.
- ZnONP-coated bands also showed effective antimicrobial activity, though slightly lower than AgNP-coated bands.
- The CFU reduction assay confirmed that AgNP-coated bands provided the highest microbial reduction, with 92% reduction at 48 hours.
- The uncoated control group demonstrated negligible antimicrobial activity throughout the study.

These results suggest that AgNP and ZnONP coatings significantly enhance the antimicrobial properties of orthodontic bands, with AgNPs showing superior efficacy.

Discussion

The present study demonstrates the antimicrobial efficacy of orthodontic bands coated with silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) against key oral pathogens. The findings suggest that both coatings significantly reduce microbial growth, with AgNP-coated bands showing superior performance compared to ZnONP-coated bands. This is consistent with prior research highlighting the broad-spectrum antimicrobial properties of AgNPs against bacteria and fungi (1).

AgNPs exert their antimicrobial effect by interacting with bacterial cell membranes, leading to membrane

rupture, protein denaturation, and inhibition of DNA replication (2). The larger zone of inhibition (15.2 mm) and higher colony-forming unit (CFU) reduction (92% at 48 hours) in the AgNP group confirm their strong bactericidal and fungicidal effects. These results are consistent with studies that have reported AgNPs' ability to disrupt biofilms and inhibit the growth of oral pathogens such as *Streptococcus mutans* and *Candida albicans* (3,4). Furthermore, silver nanoparticles are known for their slow release of silver ions, providing prolonged antimicrobial effects, which is beneficial for orthodontic treatment, where appliances remain in the oral cavity for extended periods (5).

ZnONPs, while not as effective as AgNPs, also demonstrated significant antimicrobial activity, with a maximum zone of inhibition of 12.4 mm and an 88% CFU reduction at 48 hours. ZnONPs act by generating reactive oxygen species (ROS) and disrupting bacterial membranes (6). In addition to their antimicrobial properties, ZnONPs offer added benefits such as anti-inflammatory effects and the promotion of wound healing, which could be advantageous in orthodontic patients experiencing soft tissue irritation (7). Previous studies have shown that ZnONPs can inhibit the growth of oral pathogens, though their efficacy may vary depending on nanoparticle concentration and pathogen type (8).

The minimal antimicrobial activity observed in the control group highlights the importance of surface modification for reducing microbial adhesion on orthodontic appliances. Orthodontic treatment creates niches that promote biofilm formation, leading to enamel decalcification and gingival inflammation if not controlled (9). Incorporating antimicrobial coatings such as AgNPs and ZnONPs on orthodontic bands could help mitigate these complications by preventing microbial colonization.

Although both nanoparticles demonstrated significant antimicrobial activity, AgNPs outperformed ZnONPs across all tested parameters. This aligns with previous studies reporting the superior antimicrobial efficacy of silver nanoparticles compared to other metal-based nanoparticles (10). However, the potential cytotoxicity of silver nanoparticles warrants further investigation, as excessive silver ion release can adversely affect human cells (11). Future studies should focus on optimizing the concentration of nanoparticles to ensure a balance between antimicrobial efficacy and biocompatibility.

Limitations

This study was conducted under in vitro conditions, which may not fully replicate the complex environment of the oral cavity. Factors such as saliva flow, pH variations, and patient behavior may influence the effectiveness of nanoparticle coatings in vivo. Therefore, further in vivo studies are required to validate the clinical applicability of these findings.

Conclusion

The results of this study indicate that orthodontic bands coated with AgNPs and ZnONPs exhibit significant antimicrobial properties, with AgNPs demonstrating superior efficacy. The use of such nanoparticle coatings holds promise in reducing microbial-related complications during orthodontic treatment. However, long-term in vivo studies are needed to assess their safety and durability in clinical settings.

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