Comparative Evaluation of Zn-Nano Particles' Anti-Bacterial Properties on Titanium Surface - In Vitro Study

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***Abstract*—Introduction: Dental implants are widely used in restorative dentistry, but their long-term success can be compromised by peri-implant diseases, especially peri-implantitis. Bacterial colonization of implant surfaces, particularly by *Staphylococcus aureus*, is a key factor in disease initiation. Modifying titanium surfaces with antibacterial coatings such as zinc nanoparticles may help reduce this risk.**

Material and methods: Twenty commercially pure titanium discs (10 mm × 2 mm) were prepared and divided into two groups: control (uncoated, n=10) and test (ZnO-coated, n=10). Zinc coating was carried out using electro-deposition at 2.5 V and 85 °C for 2 hours, followed by sintering at 400 °C. Antibacterial activity was assessed by colony-forming unit (CFU) counts and measurement of inhibition zones on BHI agar. Statistical analysis was performed with significance set at $p \leq 0.05$.

Results: ZnO-coated discs showed a significantly larger inhibition zone (2.69 ± 0.47 mm) compared to controls (1.92 ± 0.32 mm, $p < 0.001$). CFU counts were lower in the test group, though not statistically significant.

Conclusion: Coating titanium with ZnO nanoparticles enhanced antibacterial activity against *S. aureus*, likely due to the release of Zn^{2+} ions and generation of reactive oxygen species that disrupt bacterial cell membranes. This surface modification holds promise for reducing peri-implant microbial colonization, thereby lowering the risk of peri-implantitis and improving implant longevity. Further in vivo studies are required to confirm biocompatibility, long-term stability, and clinical applicability.

I. INTRODUCTION

Dental implants and implant-supported prostheses have revolutionized restorative dentistry by providing a functional and esthetic solution for missing teeth. However, despite their widespread use and high success rates, the long-term success of these implants can be significantly compromised by biological complications, particularly peri-implant diseases. Among these, peri-implantitis—a pathological condition characterized by inflammation of the peri-implant mucosa and progressive loss of supporting bone—is a growing concern. As implant therapy becomes more common, the incidence of peri-implant diseases also increases, prompting the need for preventive and therapeutic strategies aimed at mitigating associated microbial colonization and inflammation[2].

The Sixth European Workshop on Periodontology reported that peri-implantitis affects approximately 28–56% of implant patients, reflecting the magnitude of this clinical issue[3]. The condition is often preceded by peri-implant mucositis and, if left untreated, can lead to implant failure. One of the critical aspects of peri-implantitis is its microbial etiology. The microbial profile associated with peri-implantitis is complex and resembles that found in periodontitis, consisting predominantly of anaerobic Gram-negative bacteria[4]. Species such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* are commonly found in peri-implant lesions, contributing to the inflammatory cascade and tissue destruction[5].

Interestingly, *Staphylococcus aureus*, although not traditionally categorized as a periodontal pathogen, has been frequently isolated from infected implant sites. It acts as an early colonizer of the implant surface and appears to facilitate the subsequent colonization of more pathogenic bacteria. This suggests that *S. aureus* may play a pivotal role in the initiation and progression of peri-implantitis. Its capacity to form biofilms and resist host immune responses further emphasizes the need for materials and surfaces that can effectively counteract such microbial adhesion and proliferation[6].

To address these challenges, research has increasingly focused on modifying the surface properties of dental implants to prevent bacterial adhesion while maintaining or enhancing biocompatibility. Titanium, the material most commonly used in dental implants due to its favorable mechanical and biological properties, can be further improved by surface modifications. Among the various strategies, antibacterial surface coatings using antibiotics, antimicrobial peptides, and metal-based nanoparticles (such as silver (Ag), copper (Cu), and zinc (Zn)) have demonstrated promising results[7].

Of particular interest is zinc—a trace element essential for numerous biological functions, which also exhibits significant antimicrobial activity. Zinc-based nanoparticles have shown efficacy in disrupting bacterial membranes, inhibiting biofilm formation, and altering microbial metabolism. Furthermore, zinc has a relatively low cytotoxicity profile compared to other metals like silver, making it a more biocompatible choice for biomedical applications. Zinc nanoparticles (ZnNPs) are now being investigated in diverse fields including electronics, cosmetics, food packaging, and medical biotechnology, particularly for their antimicrobial properties[8].

Several methods have been developed for the synthesis of ZnNPs and their integration onto titanium surfaces, including chemical reduction of metal salts in solution, plasma immersion ion implantation, thermal aggregation, and sputter coating via physical or chemical vapor deposition. These techniques aim to create a surface that is not only hostile to microbial colonization but also conducive to cellular adhesion and osseointegration[9].

In the clinical context, an ideal antibacterial coating for dental implants must fulfill two essential criteria: (1) it should effectively reduce bacterial colonization, and (2) it must do so without compromising the biocompatibility of the implant surface. This dual requirement poses a significant challenge, as many potent antimicrobial agents may also exert cytotoxic effects on host tissues. Hence, a careful balance between antimicrobial efficacy and biological safety is critical[10].

In this study, we explore the application of zinc nanoparticles as a surface coating for titanium discs. Preliminary findings suggest that titanium coated with zinc not only improves surface roughness and wettability—factors crucial for cell attachment and proliferation—but also exhibits enhanced corrosion resistance and antibacterial activity against key pathogens implicated in peri-implant infections. This dual benefit positions Zno coated titanium as a promising material for future clinical applications in implantology, with the potential to significantly reduce the incidence of peri-implant diseases and improve long-term implant success.

II. METHODOLOGY

(1) Preparation of titanium discs

Cp Titanium discs of 2mm thickness and 10 mm diameter were used for this study. These were procured directly from the manufacturer. A total of 20 such discs were prepared. The discs were then divided into 2 group control (n=10) and testing (n=10) and thoroughly disinfected using 2% Glutaraldehyde solution by immersion for 30 mins.

Figure 1: Commercially pure Titanium discs

(2) Coating of titanium surfaces

The test group then coated by pure zinc (Zn) particles. They were pressed in a bench press; to produce a 10 × 10 × 2mm plates that will act as the anode. Platinum wires were used to hang the electrodes in the solution. A thermometer was used to monitor the temperature during the process. The deposition process was carried out with a power supply unit by applying an electrode potential of ~2.5 V at 85 °C temperature stabilized by a thermostatic water bath for 2 h, during the deposition process a continuous stirring were carried out by a magnetic stirrer. After the deposition, specimens were taken out from the electrolytic bath, rinsed with deionized water, and would be left to dry for 24 h on a clean bench. The coated CpTi specimens were then sintered at 400 °C for 2 h in an electric furnace with a heating rate of 5 °C/min and was gradually cooled to room temperature inside the furnace.

Figure 2: Preparation of discs

(3) Evaluation of anti-bacterial properties

The titanium discs coated with zinc nanoparticles & control group were then subjected to evaluation of anti-bacterial properties. Antibacterial activity of the coated titanium surfaces was assessed against Staphylococcus aureus (Gram positive). All the titanium discs coated with Staphylococcus aureus were collected after 24 h cultures on Nutrient broth and were regulated to 0.5 McFarland standard turbidity. The discs were incubated for 24 hours. Swabs from the discs were used to inoculate BHI agar plates followed by incubation for another 24 hours. The colony forming units were calculated and results were analyzed statistically.

Figure 3: Evaluation of anti-bacterial properties

III. OBSERVATIONS & RESULT

Tables 1, 2, 3 & graph show the ZnO nanoparticles antimicrobial properties as compared to the control group which show increased antimicrobial activity in ZnO group.

Table 1: Assessment of control group.

	Mean	Std. Deviation
Colony Forming Units	704.5	117.80
Full Zone Measurement	3.32	0.458
Zone Of Inhibition	1.92	0.322

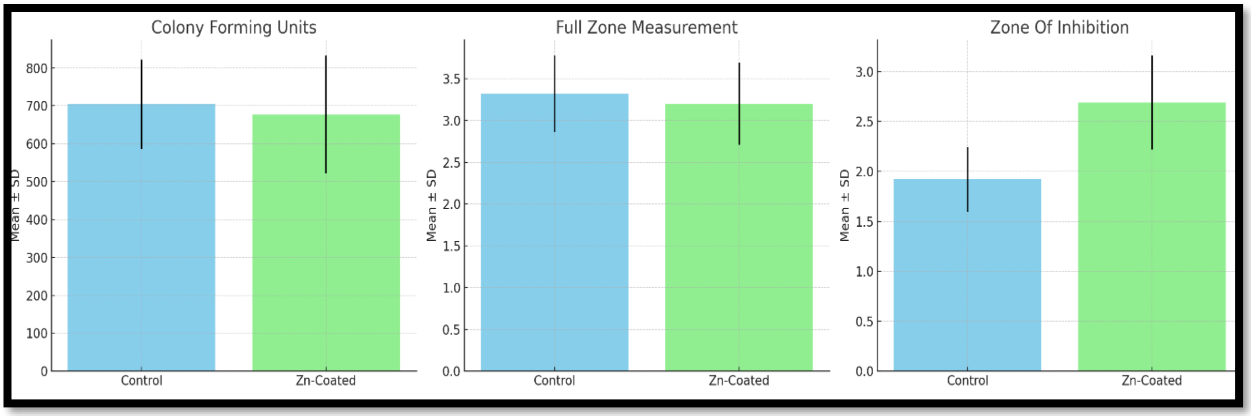
Table 2: Assessment of anti-microbial properties of Zn nanoparticles coated on the titanium surfaces.

	Mean	Std. Deviation
Colony Forming Units	677	155.55
Full Zone Measurement	3.20	0.492
Zone Of Inhibition	2.69	0.470

Table 3: Comparative evaluation between control group and experimental group having anti-microbial properties of Zn nanoparticles coated on the titanium surfaces.

$p \leq 0.05$ – Significant, CI = 95 %

		Mean	Std. Deviation	F-value	p-value, S/NS
Colony Forming Units	Control	704.5	117.80	0.199	0.661, NS
	Case	677	155.55		
Full Zone Measurement	Control	3.32	0.458	0.318	0.580, NS
	Case	3.20	0.492		
Zone Of Inhibition	Control	1.92	0.322	18.243	<0.001, HS
	Case	2.69	0.470		



Graph 1: Comparison of Colony Forming Units, Full Zone Measurement and Zone of Inhibition between control group and nano Zn-coated group

IV. DISCUSSION

This in vitro study clearly demonstrated that coating titanium surfaces with zinc oxide (ZnO) nanoparticles significantly boosts their antibacterial properties. Compared to uncoated titanium, the ZnO-coated samples exhibited larger zones of bacterial inhibition and more severe damage to bacterial cell membranes. These observations confirm the strong antimicrobial potential of ZnO when applied as a nanocoating.

Our findings are in line with recent research highlighting ZnO's effectiveness as an antimicrobial agent in biomedical applications. For example, Tudorache et al. (2025) found that PMMA/ZnO-Ag coatings on dental titanium abutments effectively reduced bacterial colonization, attributing this to ZnO's ability to release reactive oxygen species and zinc ions (13). Similarly, Ji et al. (2025) reported that adding ZnO to the surface of 3D-printed Ti-6Al-4V implants not only improved antibacterial activity but also enhanced surface wettability and corrosion resistance—both key factors for successful implant integration (14).

ZnO nanoparticles combat bacteria through multiple mechanisms. They produce reactive oxygen species (such as hydrogen peroxide and hydroxyl radicals), which disrupt bacterial cell structures and functions. Additionally, the release of Zn^{2+} ions interfere with microbial enzymes and metabolic systems, leading to cellular breakdown (5). The physical structure of the nanoparticles—often sharp-edged and charged—also contributes to membrane disruption through direct contact.

The importance of these antimicrobial effects has been emphasized in other studies as well. Fathyunes et al. (2024) showed that incorporating ZnO into plasma electrolytic oxidation coatings enhanced both bacterial resistance and biocompatibility on Ti-6Al-4V alloys (15). Likewise, Fatemi et al. (2025) demonstrated that ZnO combined with hydroxyapatite in nanocomposite coatings significantly improved both antimicrobial performance and bone integration (16). These findings support our results and reinforce the idea that ZnO is both a powerful antibacterial agent and a promising material for implant surface modifications (7).

A key strength of our study was the successful deposition of a uniform and firmly adherent ZnO coating on the titanium surface. This uniformity is crucial for ensuring stable performance and minimizing cytotoxicity, a point also highlighted by Nahidh et al. (2024), who emphasized the importance of consistent nanoparticle dispersion for long-term antibacterial efficacy (17).

In summary, our results add to the growing body of evidence supporting ZnO nanoparticles as a valuable tool for enhancing the antibacterial capabilities of titanium implants. Moving forward, further research is needed to evaluate their effectiveness in vivo, examine long-term safety, and optimize nanoparticle characteristics for maximum therapeutic benefit without affecting host tissue compatibility.

V. CONCLUSION

This in vitro study aimed to evaluate the antibacterial efficacy of zinc nanoparticles (Zn-NPs) when applied to titanium surfaces, commonly used in dental and orthopedic implants. Titanium discs were coated with Zn-NPs using a controlled deposition technique, and their surface characteristics were analyzed.

The antibacterial activity was assessed against common oral pathogens, including *Streptococcus mutans* and *Staphylococcus aureus*, using standard microbiological assays such as zone of inhibition, colony-forming unit (CFU) counts, and biofilm quantification.

The results demonstrated a significant reduction in bacterial adhesion and growth on Zn-NP-coated titanium surfaces compared to uncoated controls. These findings suggest that Zn-NPs enhance the antibacterial properties of titanium, potentially reducing the risk of peri-implant infections and improving long-term implant success.

The conclusion of the study is that using zno particles coating over titanium help in significant reduction of the microbial activity which in turn help in reduction of the peri-implantitis, bone resorption & increases the longevity of the implant. Further in vivo studies are recommended to validate these findings and explore biocompatibility and long-term performance.

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