

Application Potential of ZnO Nanoparticles in Orthopedic

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Abstract: Orthopedic implants are susceptible to bacterial infections such as *S. aureus* and *E. coli*, which increase the risk of postoperative infection. Surface modification can effectively address this issue by imparting new properties to the implants without affecting their mechanical strength and Young's modulus. Zinc oxide nanoparticles have been widely studied due to their excellent bactericidal properties, osteogenic characteristics, and biological functions. The article aims to offer an extensive analysis of its bactericidal, cytocompatibility, and osteogenic advantages as well as potential limitations, and explore the feasibility of their application in orthopedic implants. It aims to provide new ideas and methods for solving implant-related infections and promoting bone repair.

Keywords: ZnO nanoparticles, Cytocompatibility, Antibacterial Properties, Osteogenesis, Cytotoxicity

1. Introduction

In the field of modern medicine today, orthopedic surgery plays a pivotal role in the treatment of skeletal diseases and injuries. Many materials, such as metals, alloys, and ceramics, are used in orthopedic applications due to their excellent physical, mechanical, and tribological properties [1]. However, some problems with these traditional materials may seriously affect the post-implantation results, such as the delayed bone formation caused by titanium-based orthopedic implants [2]. Additionally, implant-related infections (IRI) are the most usual and complicated challenges in the field of clinical orthopedics. As the demands for orthopedic implants continue to rise, so does the risk of infection. The infection rate for primary implant surgery generally ranges from 2% to 5%, but after revision surgery, this proportion may double. Such a high risk of infection underscores the urgent need for orthopedic implants to have bactericidal properties [3].

Surface modification technology can provide orthopedic implants with new properties, such as bactericidal and osteogenic characteristics, without altering their inherent Young's modulus, mechanical, and physical properties, by applying coatings to the implants. This offers solid support for the development of orthopedic implants [3].

Zinc oxide nanoparticles (ZnO-NPs) have been increasingly gaining attention in the field of biological research due to their osteogenic qualities, low toxicity, strong cytocompatibility, and antibacterial qualities. ZnO-NPs can exert bactericidal effects by releasing reactive oxygen species (ROS) and Zn^{2+} . Zinc is a vital trace element required by organisms, playing a crucial role in the activation reactions of over 300 enzymes within the body [4]. In the human body, the content of zinc is approximately 2 to 3 grams, with 57% located in skeletal muscle and 29% in bones. Zinc is so

important because of its ability to encourage bone formation, mineralization, and growth throughout tissue culture procedures [5]. Furthermore, ZnO-NPs also have good cell compatibility, making them a promising coating material for Ti-based orthopedic implants.

This article aims to synthesize existing research results, conduct an in-depth investigation of the advantages and disadvantages of ZnO-NPs, and comprehensively explore their feasibility as a coating for orthopedic implants. This effort provides new ideas and references for the development of orthopedic implants.

2. Antibacterial Properties of ZnO-NPs

2.1. Antibacterial Mechanism

ZnO-NPs possess excellent antibacterial properties, and their bactericidal mechanisms mainly include three aspects: 1. Bacteria are eradicated by ZnO-NPs via electrostatic interactions with the cell wall's negatively charged elements; 2. ZnO-NPs eradicate bacteria through the emission of ROS; 3. Bacteria are eradicated by ZnO-NPs through the release of Zn^{2+} (Figure 1) [6].

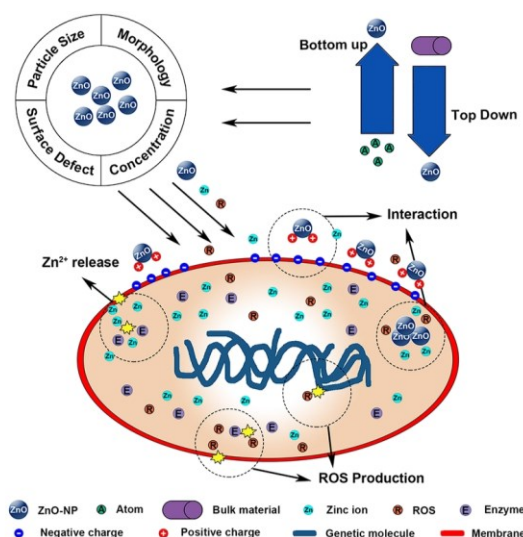


Figure 1: Strategies for preparing ZnO-NP, its antibacterial properties, and influencing elements [6]

ZnO-NPs typically carry a positive charge in water, while the average Zeta potentials of untreated *Escherichia coli* and *Staphylococcus aureus* are -44.2mV and -35.6mV, respectively. Compared to Gram-positive bacteria, Gram-negative bacteria have an additional layer of negatively charged lipopolysaccharide (LPS), which results in a more negative potential for *E. coli* than for *S. aureus* [7-9]. This potential difference enables electrostatic attraction between ZnO-NPs and bacteria [7], allowing ZnO-NPs to approach bacterial cell membranes and accumulate on their surfaces.

The build-up of ZnO-NPs on bacterial cell membranes can disrupt the cell structure, leading to leakage of cellular contents, interference with normal cellular functions, and ultimately causing cell death. Furthermore, when bacteria come into contact with ZnO-NPs, their Zeta potential changes, altering the surface charge distribution of the cell membrane and consequently affecting its stability and permeability [10].

Surface defects on ZnO-NPs play a crucial role in modulating their antimicrobial properties. Physical defects, such as sharp edges and irregular protrusions, can compromise bacterial viability by exerting mechanical stress that directly disrupts cell walls and membranes, ultimately impairing cellular integrity and function. Chemical defects-induced surface electronic states may enhance the production of ROS and Zn^{2+} [11].

Figure 2 can illustrate the chemical processes by which ROS are generated at zinc oxide defect sites [12]. The generated ROS damage cell membrane phospholipids, lipoproteins, and nucleic acids, leading to oxidative stress and ultimately cell death [13, 14].

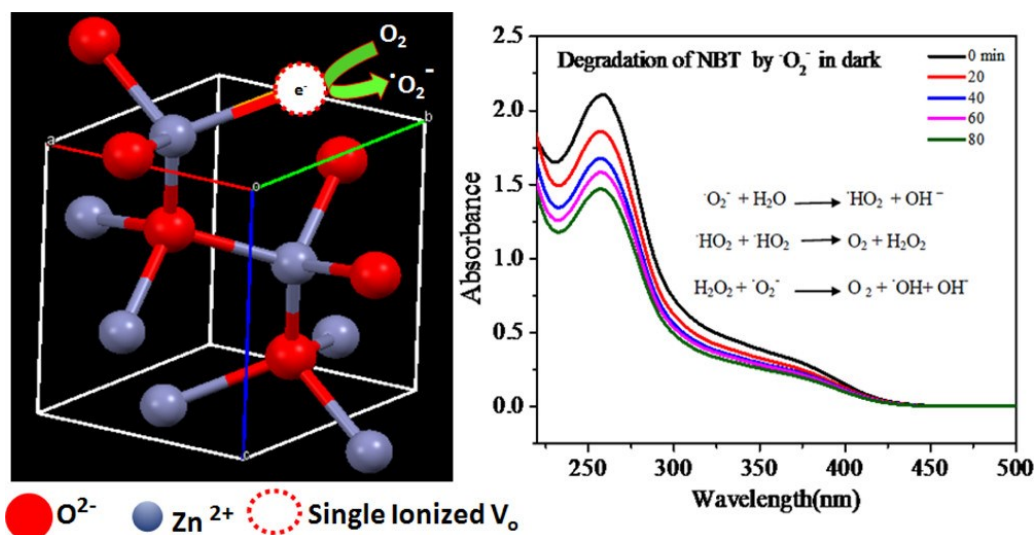


Figure 2: Schematic of the formation of superoxide radicals at surface defects of ZnO-NPs and the generation of ROS by superoxide radicals [15].

In the extracellular ROS derived from Zinc nanorods (ZNR), the hydroxyl radical ($\cdot\text{HO}_2$), as the most active oxidant, oxidizes and destroys the main components of the bacterial cell wall, peptidoglycan, and cytoplasmic membrane, along with the superoxide anion ($\cdot\text{O}_2^-$) [16, 17]. Moreover, elevated levels of intracellular ROS, resulting from the entry of extracellular H₂O₂ and Zn²⁺ into the cytoplasm, intensify the harm to phospholipids and cytoplasmic membranes due to extracellular ROS and further harm intracellular proteins and DNA, hastening bacterial demise [18].

By attacking bacterial walls, Zn²⁺ can either inactivate metabolic and transmembrane processes [19] or attach the surface of the cell wall and interacting with proteins and enzymes, destroying the cell membrane by altering the secondary and tertiary structures of the protein [20]. Studies have also shown that zinc can both inhibit bacterial growth and produce ROS-mediated killing [21].

2.2. Influencing Factors

The research from Jones et al. [22] suggests that ZnO-NPs may have antimicrobial properties against diverse microorganisms, including gram-positive as well as gram-negative bacteria. In contrast to other metal oxide nanomaterials, ZnO demonstrated notable growth suppression against *S. aureus*, leading to its selection for additional study. Research revealed that nanoparticle dimensions play a crucial role in their antimicrobial properties; tinier nanoparticles (around 8 nm across) can suppress growth by nearly 95% at a minimal concentration (1 mM), whereas bigger nanoparticles (about 50-70 nm) only reduce *S. aureus* activity by 50% at a 5 mM concentration, paralleling findings in other bacteria, thus confirming nanoparticle size's substantial effect on antibacterial actions. Furthermore, Heinlaan et al. [23] demonstrated the toxicity of Zn²⁺ emitted by ZnO-NPs to *Vibrio fischeri* bacteria.

Synthesizing the results of multiple experiments, ZnO-NPs' antibacterial activity depends on the size and concentration of particles. Smaller particles exhibit more potent antibacterial activity. The greater surface area and higher concentration, shows the more potent the antibacterial activity [24].

3. Cytocompatibility of ZnO-NPs

3.1. Cytocompatibility Mechanism

G. Ambarasan et al. [25] carry out vitro studies on the ZnO/CuO-Cs-1wt.% nano material and the results are depicted in Figure 3. After a 72-hour treatment, cell viability increased to ($168.52 \pm 14.46\%$), accompanied by normal cell morphology and extended filopodia. This may be due to the cytocompatibility of stable and slowly released free radicals (e.g. $\cdot\text{O}_2^-$ and $\cdot\text{OH}^-$) from the ZnO/CuO-Cs-1wt.% nano, as well as its ability to accelerate wound healing characteristics. In addition, this Quercetin/C-ZnO/CuO nanocomposite may promote cell proliferation by controlling the release of metal ions [26, 27].

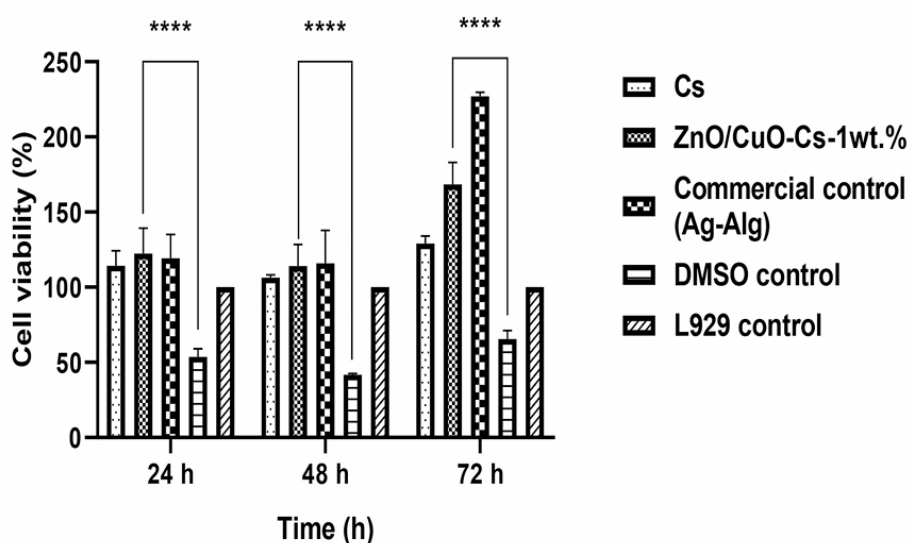


Figure 3: Examining the compatibility of the mouse fibroblast cell line with varying incubation periods [25].

Vascular endothelial growth factor (VEGF) is the foremost angiogenic mediator identified to date [28]. ROS and H_2O_2 produced by ZNR can induce the expression of VEGF in cells. Numerous researches have demonstrated that H_2O_2 leads to VEGF production in a variety of human cells and organs, including keratinocytes [29], retinal pigment epithelial cells [28], and endothelial cells [30]. ROS is also capable of facilitating the creation of new blood vessels through the stimulation of various stages such as migration, cell proliferation as well as tubular formation.

Hassan et al. [27] through chicken chorioallantoic membrane (CAM) experiments showing that ZnO nanorods at concentrations of $10\mu\text{g/ml}$ and $20\mu\text{g/ml}$ could significantly increase the length, size, and number of connections of blood vessels, promoting vascular maturation (Figure 4). Histopathological examinations further confirmed that ZNR at a concentration of $20\mu\text{g/ml}$ could induce good neovascularization, with an increase in vascular branching. Hassan et al. [27] used wound healing experiments to indicate that the wound healing rates of wounds treated with $10\mu\text{g/ml}$ and $20\mu\text{g/ml}$ of ZNR were notably greater than the levels observed in the control group, reaching 63% and 84%, respectively, after 14 days. Histological examinations revealed that ZNR could promote angiogenesis, epithelial regeneration, and new tissue formation during the wound healing process, reduce inflammatory cell infiltration, and increase collagen deposition.

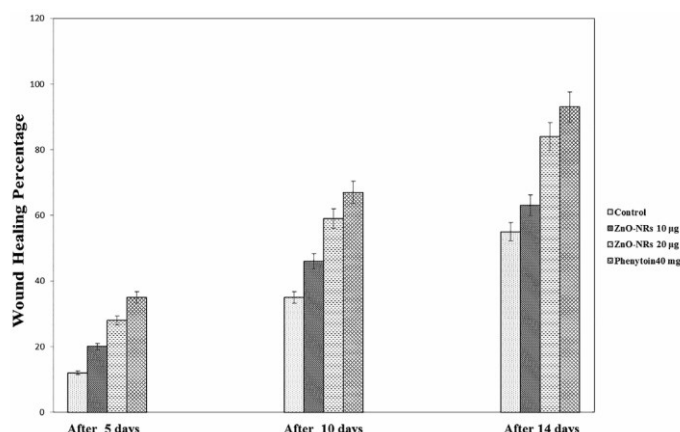


Figure 4: Percentage of wound healing following ZNR and phenytoin remediation as opposed to control (non-treated) [27].

Therefore, ZnO-NPs can express good cytocompatibility by producing ROS and H_2O_2 , and also have functions such as promoting wound healing and neovascularization, which can provide a promising application prospect for Ti-based orthopedic implants.

3.2. Influencing Factors

The emission of Zn^{2+} from ZNR varies over time [31]. Conversely, the increase in ZnO-NR expression relies on the concentration. Hassan et al.'s findings [27] demonstrated that whereas the released Zn^{2+} has no effect, the ROS generated by ZnO-NRs is concentration-dependent.

4. Osteogenic Properties

4.1. Osteogenic Mechanisms

The osteogenic properties of ZnO-NPs are ascribed to the emission of Zn^{2+} [6]. On the one hand, Zn^{2+} is involved in a variety of enzymatic catalytic activation processes [4]. On the other hand, Zn^{2+} can encourage bone growth, mineralization, and formation [32, 33]. ZnO-NPs and water molecules interact to form Zn-OH groups, which serve as apatite nuclei and quicken the mineralization process [34]. Zn^{2+} can also motivate the mitogen-activated protein kinase (MAPK) pathway, encouraging the region of the osteocalcin gene to express [35, 36]. Yamaguchi et al. [37] noted that at concentrations between 10 and 250 $\mu\text{mol/L}$, Zn^{2+} may prevent osteoclasts from differentiating. They suggested that by lowering tumor necrosis factor- α levels in vivo, Zn^{2+} inhibits the nuclear factor- κB signaling pathway, thus decreasing the production of osteoclasts and increasing the proliferation of osteoblasts, both of which are involved in the formation of bone.

According to Westhauser et al. [38], doping ionic dissolution products in Zn-MBG nanoparticles substantially increased the production of collagen and the calcification of the extracellular matrix in addition to upregulating the expression of OPN and OCN. As studied by Khader and Arinzeh et al. [39], the number of mesenchymal stem cells (MSCs) on composite scaffolds containing ZnO-NPs was higher than that in the control group alkaline phosphatase, serving as an early marker gene for osteogenic differentiation, exhibited greater activity in the ZnO-NPs group after cultivation compared to the control group. Moreover, hydroxyproline assays indicated that the production of collagen by the 10% ZnO NPs composite scaffold was significantly higher than that of the control group when cultured in osteogenic induction medium, thereby further proving that the addition of ZnO-NPs can promote osteogenic differentiation.

4.2. Influencing factors

Wang et al.'s [40] research found that the osteogenic capability of ZnO-NPs exhibits concentration dependence, as shown in Figure 5. They noted that when MG63 cells were cultivated with 10 µg/mL of ZnO-NPs, the expression of collagen and alkaline phosphatase was significantly higher than when the cells were cultivated at a 5 µg/mL concentration. Furthermore, within the non-toxic range of application, higher ZnO-NPs resulted in better osteogenesis.

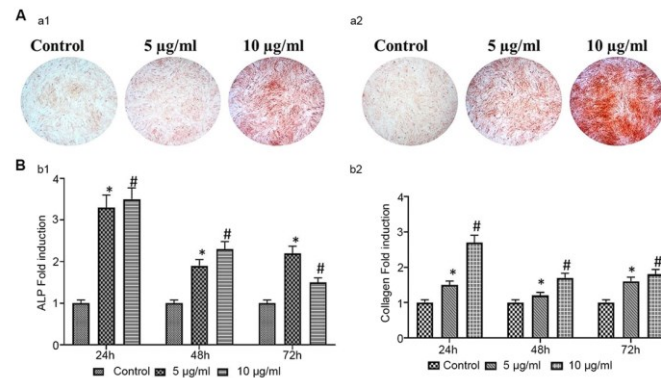


Figure 5: The impact of ZnO-NP concentration on osteogenesis activity [6]. (A) Calcium mineralization in osteoblasts treated with varying concentrations of ZnO-NPs for (a1) 5 and (a2) 7 days using alizarin red stain. Osteoblasts treated with varying concentrations of ZnO-NPs for 24, 48, and 72 hours showed (B) mRNA expression; (b1) ALP; and (b2) collagen.

5. Cytotoxicity

5.1. Cytotoxicity Mechanism

Despite the advantages of ZnO-NPs in promoting osteogenic properties and bactericidal expression, facilitating wound healing, and new blood vessel formation through the release of Zn^{2+} [6, 22-24, 27], there are still some qualitative and quantitative limitations that hinder their development. For instance, high concentrations of ROS and Zn^{2+} may be toxic to normal human cells [19], thereby endangering human health.

As shown in the growth curve of Figure 6, both nano ZnO and bulk ZnO are toxic to *P. subcapitata* even at very low concentrations (less than 0.1 mg/L), and complete inhibition of algal growth is observed at a zinc concentration of 0.16 mg/L (data not shown). However, when compared, nanoscale ZnO shows slightly weaker inhibition of cell activity (Figure 6) [41].

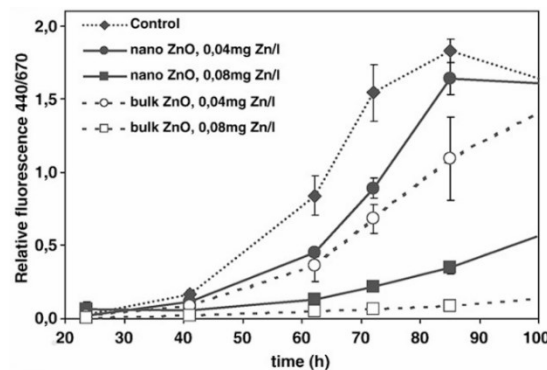


Figure 6: Growth kinetics of ZnO-treated *Pseudokirchneriella subcapitata*. 3 replicates' mean ± standard deviation is used to report values [41].

The growth inhibition of *Pseudokirchneriella subcapitata* by bulk and nano ZnO and ZnSO₄ was statistically plotted by Aruoja et al. [41] in Figure 7. It was found that the concentrations of the three substances that caused growth inhibition of 50% of the test algae at a given exposure time (i.e., EC₅₀) were approximately equal (0.037, 0.042 and 0.042 mg Zn/l, respectively), so it can be inferred that in this concentration range, the toxicity of ZnO is mainly due to dissolved Zn²⁺. In addition, we can find that at concentrations greater than this range, bulk ZnO inhibits algae to a greater extent than nano ZnO, and therefore the size of ZnO also affects its toxicity, with smaller sizes resulting in weaker toxicity.

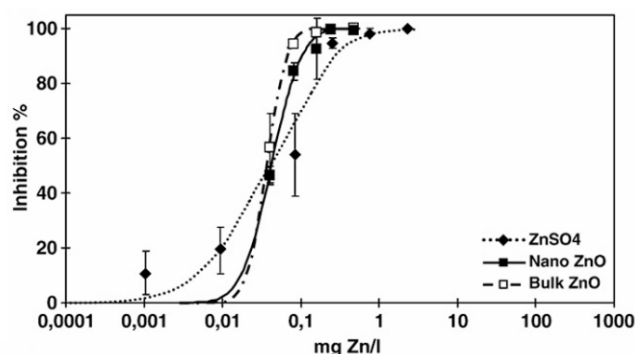


Figure 7: *Pseudokirchneriella subcapitata*'s reaction to ZnO-NPs and corresponding Zinc salts [41].

6. Conclusion

This article focuses on the mechanisms and influencing factors of the antibacterial, cytocompatible, osteogenic and cytotoxic properties of ZnO-NPs. ZnO-NPs carry a positive charge in water and accumulate on the surface of bacteria through electrostatic interaction with negatively charged bacteria, which ultimately causes the bacterial cell membrane to rupture and die. The ROS and Zn²⁺ released by ZnO-NPs can not only achieve the antibacterial effect by destroying the bacterial cell membrane, but also enable ZnO-NPs to have good biological functionality (promoting new blood vessel formation) and osteogenic properties by inducing cell expression of VEGF and activating the MAPK pathway, respectively. Although studies have shown that high concentrations of Zn²⁺ can be toxic to normal human cells, other studies have also shown that the amount of Zn²⁺ released by ZnO-NPs is very small. The concentration of Zn²⁺ released by 2 μ m high ZnO NR arrays cultured in a dark environment for 1 hour is only 13.2 μ g/mL/cm², and its contribution to the antibacterial effect is less than 3%. Therefore, the toxicity of nano ZnO used in orthopedic implant coatings to the human body may not need to be overly concerned.

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