Dubai Medical Journal
Volume 7, Issue no. 3, DOI 10.18502/dmj.v7i3.17731
Production and Hosting by KnE Publishing

KNE Publishing

Research Article

Evaluation of Antibacterial (Antibiofilm) Activity Potential of ZnONPs Coated on Wound Dressing Cloth

Mathiyazhagan Narayanan*

Center for Research and Innovation, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai-602 105, Tamil Nadu, India

Abstract

Introduction: In light of the emergence of antibiotic-resistant bacteria and the necessity for efficient wound treatment, zinc oxide nanoparticles (ZnONPs) have garnered interest for their potent antibacterial and antibiofilm characteristics. This study examines the incorporation of green synthesized ZnONPs into wound dressing fabric to inhibit bacterial colonization and biofilm development, and significant obstacles in wound healing. The present study aims to assess the antibacterial efficacy of plant-mediated and pre-synthesized as well as characterized ZnONPs against opportunistic bacterial pathogens to create more effective wound dressings that facilitate expedited, infection-free recovery.

Methods: The antibacterial efficiency of this green-mediated ZnONPs coated wound dressing material against the opportunistic Gram-positive and negative bacterial pathogens were checked. Various concentrations (0.20, 0.40, and 0.60%) of ZnONPs were used to coat the dressing material. This ZnONPs antibacterial activity was analyzed quantitatively by various time intervals (4-24 hr) and incubated as per the standard bacterial growth conditions.

Results: The findings show that 20 hr after incubation, Gram-negative bacterial growth was inhibited on dressing cloth coated with 0.60% ZnONPs, while Gram-positive bacteria inhibition was observed 24 hr after incubation on dressing cloth coated with 0.40% ZnONPs. These findings suggest that 0.40% and 0.60% ZnONPs significantly kill both groups of opportunistic pathogens.

Discussion: Bacterial infections as well as biofilm formation on wound surfaces significantly impede effective healing, resulting in chronic wounds and elevated healthcare expenses. Conventional wound dressings frequently exhibit inadequate antimicrobial efficacy, particularly against antibiotic-resistant bacteria. ZnONPs have attracted interest owing to their strong antibacterial, antibiofilm, and biocompatibility characteristics. This study assesses the efficacy of ZnONPs-coated wound dressings in suppressing bacterial proliferation and biofilm development, potentially providing a remedy for infection-associated complications in wound care. The results may facilitate the creation of more efficient wound dressings, thereby decreasing infection rates and enhancing patient outcomes in clinical environments.

Conclusion: Thus, these ZnONPs could be employed as an antibiofilm/antibacterial coating material in wound dressing cloths to prevent secondary opportunistic bacterial infections.

Corresponding Author:
Mathiyazhagan Narayanan
Email: mathimicro@gmail.com

Received: September 12, 2024
Accepted: October 2, 2024
Published: December 8, 2024

Production and Hosting by Knowledge E

OPEN ACCESS

Keywords: ZnONPs, anti-biofilm activity, opportunistic pathogens, quantitative analysis, various time intervals

1. Introduction

Bacterial biofilms substantially impede wound healing by shielding bacteria from the immune system as well as antibiotics, resulting in chronic or non-healing wounds [1]. They result in protracted healing, persistent infections (such as diabetic and pressure ulcers), antibiotic resistance, recurrent infections, and elevated healthcare expenses. Widespread bacteria that constitute biofilms in wounds comprise *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa, Enterococcus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Streptococcus pyogenes* [2]. Effective biofilm management necessitates sophisticated wound treatment, mechanical trimming, and specific antimicrobial treatments.

A nanoparticle (NP) is defined as a fundamental particle with dimensions ranging from 1 to 100 nanometers (nm). Nanomaterials are also referred to as "zero-dimensional" substances. ZnO is a biocompatible and sustainable material suitable for direct application in biomedical fields [3]. Due to the exceptional properties of nano-sized ZnO, significant efforts are being directed toward ZnO nanoparticles (ZnONPs) synthesis with intriguing morphologies and assemblies. The synthesis of ZnONPs using plant extracts is increasingly recognized as a sustainable and advantageous method for producing pharmaceutically significant ZnONPs [3]. Secondary microbial infections in the medical field pose significant challenges to patient treatment, particularly in wound cases, due to bacterial biofilm formation on dressing materials [4]. Bacterial biofilm is a type of bacterial life made up of bacteria and an extracellular polymer matrix (EPM), which contains complex biochemicals like carbohydrates, proteins, nucleic acids, lipids, as well as glycopeptides [5].

The ZnONPs are being studied to treat different skin medications [6]. While these ZnONPs may promote wound healing and reduce inflammation, their efficacy as an antibacterial or antibiofilm agent in wound-dressing-cloth coatings has not been documented. Recent research has revealed that the discharge of Zn^{2+} ions from ZnONPs is a significant mechanism underlying oligodynamic effects on both prokaryotic and eukaryotic organisms [7]. The excessive release of Zn^{2+} ions under certain conditions is considered a major obstacle in achieving satisfactory commercial formulation [8]. Consequently, an excessive quantity of NPs is necessary, which may undermine the therapeutic benefits of the formulation, in addition to increasing the likelihood of adverse reactions and preparation costs [9]. This analysis aimed to create a ZnONPs-coated wound dressing (polyester-nylon), previously synthesized from plant extract and thoroughly characterized, to evaluate its antibacterial efficacy against specific Gram-positive (*E. faecalis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) opportunistic bacterial species through quantitative assessment.

2. Methods

2.1. Materials Profile

In this study, we used the pre-synthesized and characterized ZnONPs [10]. The following steps were taken to create surface-modified wound healing dressings: The wound dressing's material (polyester-nylon) was sliced toward 10 mm sized specimens as well as submerged in 100 mL of NaOH (3%) solution (to facilitate the ZnONPs coating by altering the surface properties of cloth). The blend was constantly stirred at 72°C before being dropped into various amounts (0.20%, 0.40%, and 0.60%) of 100 mL of ZnONPs solution (dissolved in distilled water at 70°C. The ZnONPs-coated dressings were rinsed with double distilled water and evaporated at ambient temperatures. According to the concentration used for preparing the dressing material, the sample was labeled as 0.20%, 0.40%, and 0.60%.

2.2. Test Bacterial Culture

The test bacterial pathogens used in this study were opportunistic pathogens collected from the Department of Microbiology (medical), Government Medical College and Hospital in Krishnagiri district of Tamil Nadu. The Gram-negative pathogens namely *E. coli,* and *P. aeruginosa* as well as the Gram-positive species such as *E. faecalis* and *S. aureus* were used in this study. The obtained cultures were properly maintained as per the standard microbiological process [11].

2.3. Antibacterial/Antibiofilm/Bacterial Viability Analyses

The log phase opportunistic test bacterial pathogens (1.4 $\times 10^5$ CFU mL) both Gram-negative and Gram-positive were prepared in the sterilized 0.5 McFarland concentration in a solution of phosphate-buffered saline (PBS) [12]. Prior to the investigation, wound dressing materials were sterilized through UV irradiation for over 20 mins. They were subsequently inserted in sterilized 24 multiple-well plates that included 1 mL of nutritional value broth as well as 10 µL of the recently acquired suspension. The plates were allowed to incubate at 38°C for 4-24 hr. Following the duration of incubation, each dressing (ZnONPs-coated) sample was immersed in PBS (1 mL) and transferred to the Eppendorf tube in order to acquire viable bacterial cells. Uncoated dressing material was used as control. For 20 sec, these tubes were firmly agitated to release bacteria into the liquid suspension. The resulting suspensions were subsequently shifted in 25 µL increments to 96 well plates that included 275 µL of PBS. Each sample was serially diluted before being cultivated in nutrient agar media for a day at 37°C. Colonies that emerged were measured as well as the CFU mL^{-1} was determined. The penicillin antibiotic (10 µL) and sterile distilled water (10 µL) were used as positive and negative control respectively.

2.4. Statistical Analysis

Each analysis was performed in triplicates to ensure the reproducibility and accuracy of the results. The mean and standard error values were used for the figures. The one-way ANOVAs were performed to determine the statistical significance value of the test and control sample using SPSS.

3. Results

Figure **1** shows the ZnONPs (green mediated) coated and uncoated (control) wound wrapping cloth. Interestingly, the antibacterial activity of ZnONPs, which was coated on wound dressing cloth against the test opportunistic Gram-positive (*E. faecalis* and *S. aureus*) and Gram-negative bacterial (*E. coli* and *P. aeruginosa*) pathogens were effective in 20 and 24 hr of incubation (i.e., exposure). Figures **2a** and **2b** clearly state that Gram-negative bacterial species (*E. coli* and *P. aeruginosa*) growth at 0.60%, was inhibited on dressing cloth coated with 0.60% dosage ZnONPs.

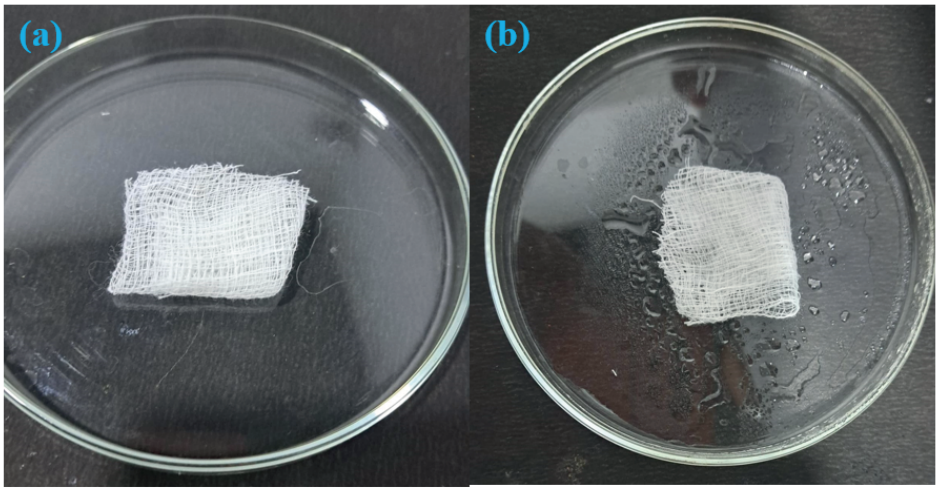

Figure 1: Visual observation of wound wrapping cloth material **(a)** Uncoated wound wrapping cloth, **(b)** ZnONPs coated wound wrapping cloth.

Similarly, the NPs coated wound dressing showed bactericidal activity upon the Gram-positive bacterial species (*E. faecalis* and *S. aureus*) showed inhibition at 0.40%, and at 0.60% showed considerable antibiofilm/antibacterial activity in 20 and 24 hr of exposure (Figures **3a** and **3b**). Notably, the obtained test values were statistically significant and ranged from * $p < 0.05$; ** $p < 0.001$ when compared with viability on control. These findings suggest that 0.40% and 0.60% ZnONPs significantly inhibit the growth of both Gram-positive and Gram-negative opportunistic selected pathogens, and they can be used to coat the wound and any other dressing materials to avoid the formation of secondary infections on patients who are already suffering a lot. ZnONPs interact with bacterial cell membranes, causing structural damage and cell lysis. They induce reactive oxygen species, damaging cellular components and DNA. Dissolution

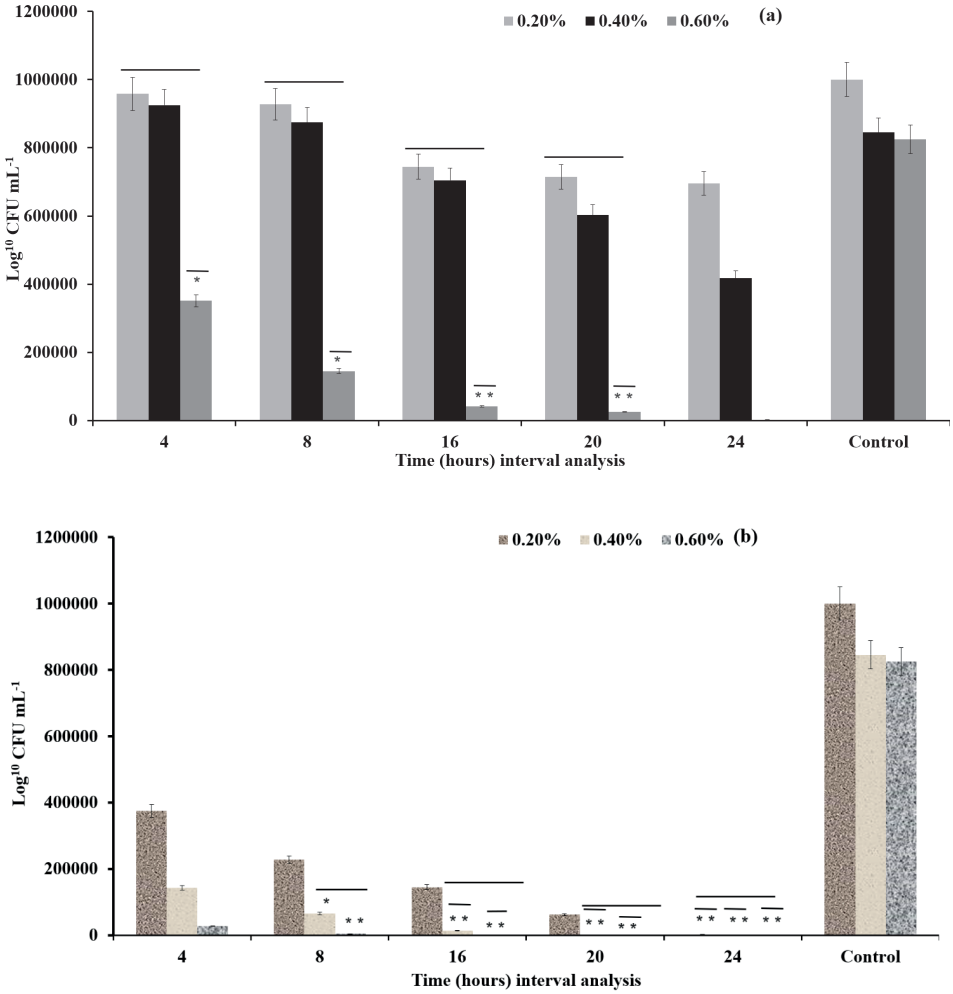

Figure 2: Bar graphs presents the log10 CFU mL^{-1} counts attained for test bacterial pathogens (a) *P. aeruginosa* (b) *E.coli* viability on ZnONPs-coated (0.20%, 0.40%, and 0.60%) wound dressings and analyzed at 4 hr, 8 hr, 12 hr, 20 hr, and 24 hr. (The * and **: indicates $p < 0.05$ and $p < 0.001$ values when compared with viability on control).

releases toxic zinc ions, disrupting enzymatic functions, and affecting biofilm formation, making them effective against various bacterial pathogens.

4. Discussion

The plant-mediated ZnONPs were effectively coated with the wound dressing material. Fortunately, at minimal dosages (0.40 and 0.60%) it effectively kills the selective opportunistic bacterial pathogens (Gram-positive: *E. faecalis* and *S. aureus* as well as Gram-negative: *E. coli* and *P. aeruginosa*) in a short duration of (20 and 24 hr) exposure. The bactericidal activity variations among this test bacterial pathogen in the condition of ZnONPs-coated wound dressing's materials might be attributed to an alteration in the cell walls of both bacterial groups [13, 14]. The primary bactericidal mechanism demonstrated through ZnONPs has been linked to permeation modifications as well as cellular integrity of membrane loss [15, 16]. Gram-negative bacteria could be vulnerable to cellular barrier damage through ZnONPs due to their

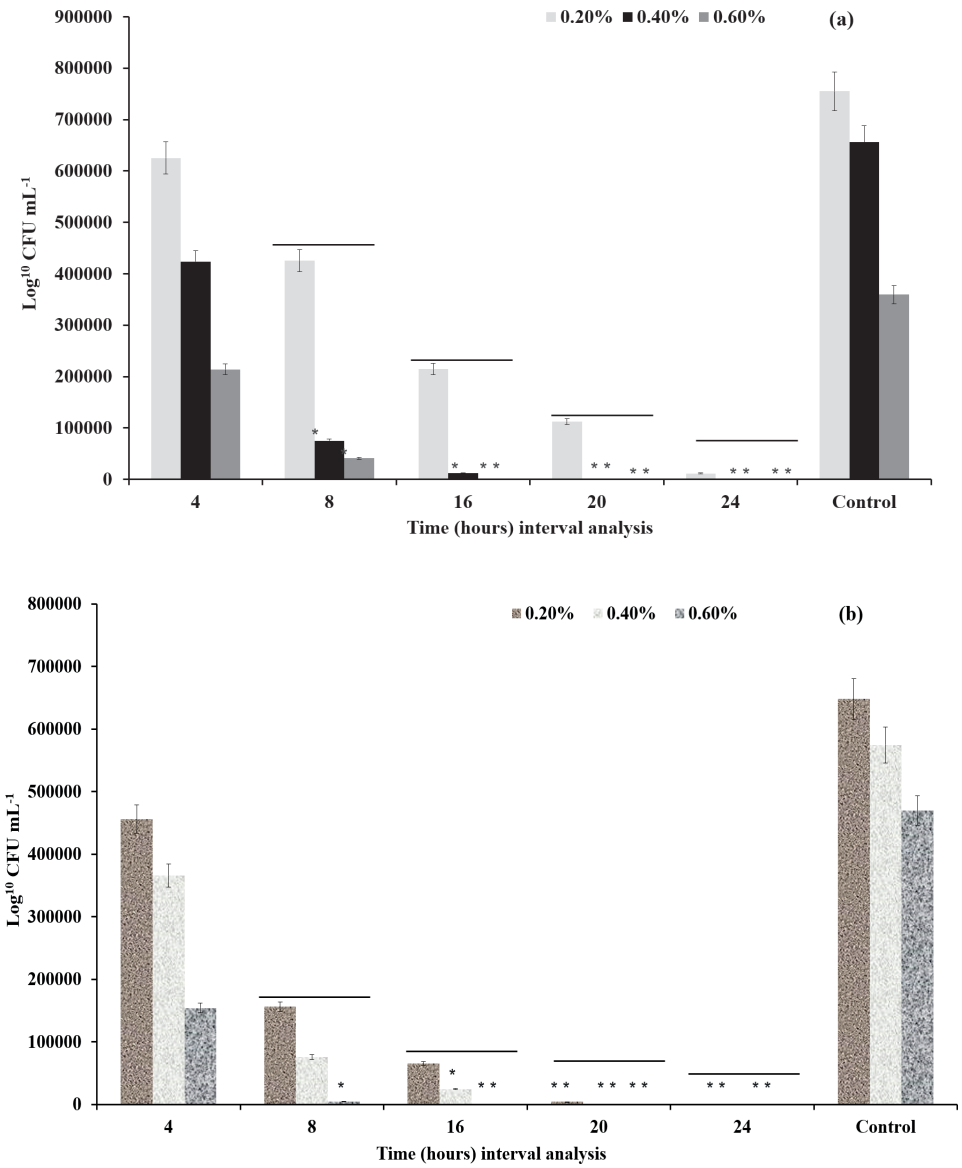

Figure 3: Bar graphs presents the $\log^{10}$ CFU mL^{-1} counts attained for test bacterial pathogens (a) *E. faecalis* (b) *S. aureus* viability on ZnONPs-coated (0.20%, 0.40%, and 0.60%) wound dressings and analyzed at 4 hr, 8 hr, 12 hr, 20 hr, and 24 hr. (The * and **: indicates $p < 0.05$ and $p < 0.001$ values when compared with viability on control).

inner cell framework (the occurrence of a further outer membrane as well as a fragile peptidoglycan layer) [17-19]. The free-floating microbial development was found to be critical for identification and treatment of infections associated with wounds [20]. In recent years, it has been proposed that a high level of bacterial replication is essential for an infection as well as prolonged wound progress [21]. As the viability of affecting opportunistic bacterial pathogens in persistent wounds, this component appears to be much more significant than bacterial virulence and biofilm development [22]. Our bacterial expansion results showed that the antibacterial properties of the examined ZnONPs-coated wound dressing's materials differed based on the quantity of ZnONPs. A group of researchers discovered that after 24 hr of incubation in nutritive broth with ZnONPs-coated wound dressing substance, every tested strain demonstrated substantially

decreased growth of bacteria when exposed to 0.60% as well as 0.9% ZnONPs [23, 24]. Nanoparticles, including ZnO as well as silver nanoparticles (AgNPs), exhibit considerable antimicrobial efficacy owing to their elevated surface area-to-volume proportion and capacity for the gradual release of antimicrobial agents. While integrated into wound dressings, such nanoparticles can consistently deliver antibacterial properties, thereby, diminishing the probability of secondary infections [25]. Their modes of action involve the production of reactive oxygen species as well as the resulting breakdown of bacterial cell membranes, effectively targeting a diverse array of pathogens [26]. Nanoparticles can impede biofilm formation by obstructing bacterial adhesion and disrupting established biofilms. ZnONPs have demonstrated efficacy in inhibiting biofilm formation by compromising bacterial cells and modifying biofilm matrix constituents [27]. This property is essential for sustaining a sterile wound environment and facilitating optimal healing. Well-designed nanoparticles ought to be harmless to human cells as well as tissues [28]. Investigating the biological compatibility of nanoparticles utilized in wound dressings guarantees the absence of negative effects while delivering essential antimicrobial properties. Nanoparticle stability is influenced by various factors such as size, shape, surface chemistry, solvent properties, concentration, ionic strength, temperature, steric stabilization, electrostatic interactions, aggregation, environmental conditions, time, and interactions with biological systems [29]. Smaller particles have higher surface energy, while larger particles have higher surface energy and interaction with solvents. Surface modifications and solvent properties also impact stability. Temperature fluctuations can affect kinetic energy and destabilization. Although green-synthesized ZnONPs present numerous advantages owing to their environmentally benign synthesis processes, it is imperative to comprehend their long-term toxicity and biocompatibility to guarantee safety in their utilization [30]. Ongoing study is essential to comprehensively assess their effects on health and the environment, facilitating safe and effective application across diverse domains.

5. Conclusion

Interestingly, these results conclude that at 0.40 and 0.60% of ZnONPs showed notable bactericidal activity against the selected test opportunistic bacterial pathogens in a short duration of (20 and 24 hr) exposure. The obtained test values were statistically significant and ranged from * $p < 0.05$; ** $p < 0.001$ when compared with viability on control. Thus, these ZnONPs could be employed as an antibiofilm/antibacterial coating material in wound dressing cloths to prevent secondary opportunistic bacterial infections. The stability and resilience of the ZnONPs were the limitations of this study. Since, the constancy of nanoparticles within the dressing's matrix and their resilience throughout the wound-healing process is a critical factor, as it is crucial to maintain the efficacy of nanoparticles during healing. Furthermore, the legal and production considerations about the integration of nanoparticles into wound dressings must comply with regulatory standards for medical products. Manufacturing procedures must guarantee a consistent and regulated dispersion of nanoparticles. Additional research is required to

optimize the concentration as well as dimensions of nanoparticles employed in wound dressings to achieve a balance between antimicrobial safety and efficacy. Furthermore, investigating the amalgamation of various nanoparticles or their integration with additional drugs may improve the efficacy of wound healing products.

Acknowledgment

The author would like to acknowledge the Center for Research and Innovations, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science (SIMATS), Chennai-602 105, Tamil Nadu, India for constant support in completing this work.

Ethical Statement

This study did not utilize human-derived materials, genetic modifications, or sensitive information. Additionally, all laboratory facilities and apparatus were utilized solely for this study, in compliance with institutional regulations. No ethical issues emerged during the research, as it complied with established protocols. A written statement was obtained from the institute's ethical committee.

Informed Consent Statement

Not applicable.

Conflict of Interest

The authors declare that there is no conflict of interest.

Artificial Intelligence (AI) Disclosure Statement

AI-unassisted work.

Funding

No funding.

Author Contribution

Mathiyazhagan Narayanan: Conceptualization, data analysis, writing, and reviewing the manuscript.

Data Sharing Statement

All data generated during this report have been included in the article.

References

[1] Cavallo I, Sivori F, Mastrofrancesco A, Abril E, Pontone M, Di Domenico EG, et al. Bacterial biofilm in chronic wounds and possible therapeutic approaches. Biology (Basel). 2024 Feb;13(2):109.

[2] Uberoi A, McCready-Vangi A, Grice EA. The wound microbiota: Microbial mechanisms of impaired wound healing and infection. Nat Rev Microbiol. 2024 Apr;22(8):1–5.

[3] Narayanan M, Vigneshwari P, Natarajan D, Kandasamy S, Alsehli M, Elfasakhany A, et al. Synthesis and characterization of TiO2 NPs by aqueous leaf extract of Coleus aromaticus and assess their antibacterial, larvicidal, and anticancer potential. Environ Res. 2021;200:111335.

[4] Mirhaj M, Labbaf S, Tavakoli M, Seifalian A. An overview on the recent advances in the treatment of infected wounds: Antibacterial wound dressings. Macromol Biosci. 2022;22(7):2200014.

[5] Costa OY, Raaijmakers JM, Kuramae EE. Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. Front Microbiol. 2018;9:1636.

[6] Mirzaei H, Darroudi M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceram Int. 2017;43(1):907–914.

[7] Tan LY, Sin LT, Bee ST, Ratnam CT, Woo KK, Tee TT, et al. A review of antimicrobial fabric containing nanostructures metal-based compound. J Vinyl Additive Technol. 2019;25 S1:E3–27.

[8] Liu Y, Zhang Y, Jiang H, Sun J, Feng Z, Hu T, et al. Synergistic engineering of oxygen-defect and heterojunction boosts Zn2+ (De) intercalation kinetics in vanadium oxide for high-performance zinc-ion batteries. Chem Eng J. 2022;435:134949.

[9] Manimaran V, Nivetha RP, Tamilanban T, Narayanan J, Vetriselvan S, Fuloria NK, et al. Nanogels as novel drug nanocarriers for CNS drug delivery. Front Mol Biosci. 2023 Aug;10:1232109.

[10] Chinnathambi A, Alharbi SA, Lavarti R, Jhanani G, On-Uma R, Jutamas K, et al. Larvicidal and pupicidal activity of phyto-synthesized zinc oxide nanoparticles against dengue vector *Aedes aegypti*. Environ Res. 2023;216:114574.

[11] Kirn T, Weinstein M. Update on blood cultures: How to obtain, process, report, and interpret. Clin Microbiol Infect. 2013;19(6):513–20.

[12] Pironti C, Dell'Annunziata F, Giugliano R, Folliero V, Galdiero M, Ricciardi M, et al. Comparative analysis of peracetic acid (PAA) and permaleic acid (PMA) in disinfection processes. Sci Total Environ. 2021;797:149206.

[13] Jebel FS, Almasi H. Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. Carbohydr Polym. 2016;149:8–19.

[14] Rather MA, Deori PJ, Gupta K, Daimary N, Deka D, Qureshi A, et al. Ecofriendly phytofabrication of silver nanoparticles using aqueous extract of Cuphea carthagenensis and their antioxidant potential and antibacterial activity against clinically important human pathogens. Chemosphere. 2022;300:134497.

[15] Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, Bakhori SK, et al. Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7(3):219–42.

[16] Guan G, Zhang L, Zhu J, Wu H, Li W, Sun Q. Antibacterial properties and mechanism of biopolymer-based films functionalized by CuO/ZnO nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. J Hazard Mater. 2021;402:123542.

[17] Hu X, Zhang H, Wang Y, Shiu BC, Lin JH, Zhang S, et al. Synergistic antibacterial strategy based on photodynamic therapy: Progress and perspectives. Chem Eng J. 2022;450:138129.

[18] Mahana A, Guliy OI, Mehta SK. Accumulation and cellular toxicity of engineered metallic nanoparticle in freshwater microalgae: Current status and future challenges. Ecotoxicol Environ Saf. 2021;208:111662.

[19] Aaditya Marthandan P, Geetha Sravanthy P, Snega R, Carmelin D, Surya M, Catakapatri Venugopal D, et al. Melothria maderaspatana mediated one-pot synthesis of cerium-doped Silymarin nanoparticles and their antibacterial and anticancer studies. Mater Technol. 2024 Dec;39(1):2315381.

[20] Schilcher K, Horswill AR. Staphylococcal biofilm development: Structure, regulation, and treatment strategies. Microbiol Mol Biol Rev. 2020;84(3):00026–00019.

[21] Kaufmann SH, Dorhoi A, Hotchkiss RS, Bartenschlager R. Host-directed therapies for bacterial and viral infections. Nat Rev Drug Discov. 2018;17(1):35–56.

[22] Pompilio A, De Nicola S, Crocetta V, Guarnieri S, Savini V, Carretto E, et al. New insights in *Staphylococcus* pseudintermedius pathogenicity: Antibiotic-resistant biofilm formation by a human wound-associated strain. BMC Microbiol. 2015;15(1):1–14.

[23] Mani VM, Nivetha S, Sabarathinam S, Barath S, Das MA, Basha S, et al. Multifunctionalities of mycosynthesized zinc oxide nanoparticles (ZnONPs) from Cladosporium tenuissimum FCBGr: Antimicrobial additives for paints coating, functionalized fabrics and biomedical properties. Prog Org Coat. 2022;163:106650.

[24] Saravanakumar K, SivaSantosh S, Sathiyaseelan A, Naveen KV, AfaanAhamed MA, Zhang X, et al. Unraveling the hazardous impact of diverse contaminants in the marine environment: Detection and remedial approach through nanomaterials and nano-biosensors. J Hazard Mater. 2022;433:128720.

[25] Xu C, Akakuru OU, Ma X, Zheng J, Zheng J, Wu A. Nanoparticle-based wound dressing: recent progress in the detection and therapy of bacterial infections. Bioconjug Chem. 2020;31(7):1708–723.

[26] Lam PL, Wong RM, Lam KH, Hung LK, Wong MM, Yung LH, et al. The role of reactive oxygen species in the biological activity of antimicrobial agents: An updated mini review. Chem Biol Interact. 2020;320:109023.

[27] Bianchini Fulindi R, Domingues Rodrigues J, Lemos Barbosa TW, Goncalves Garcia AD, De Almeida La Porta F, Pratavieira S, et al. Zinc-based nanoparticles reduce bacterial biofilm formation. Microbiol Spectr. 2023;11(2):e04831–04822.

[28] Hoseinzadeh A, Ghoddusi Johari H, Anbardar MH, Tayebi L, Vafa E, Abbasi M, et al. Effective treatment of intractable diseases using nanoparticles to interfere with vascular supply and angiogenic process. Eur J Med Res. 2022;27(1):232.

[29] Cavallo I, Sivori F, Mastrofrancesco A, Abril E, Pontone M, Di Domenico EG, et al. Bacterial biofilm in chronic wounds and possible therapeutic approaches. Biology (Basel). 2024 Feb;13(2):109.

[30] Yadav S, Raman AP, Singh MB, Massey I, Singh P, Verma C, et al. Green nanoparticles for advanced corrosion protection: Current perspectives and future prospects. Appl Surf Sci Adv. 2024 Jun;21:100605.