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An overview of Nanoparticles Approaches for Addressing Biofilms Formed by *Escherichia coli*: Mechanisms, Effectiveness, and Future Directions

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Abstract

Biofilms produced by *Escherichia coli* pose a significant obstacle due to their resilience against standard antibiotics, resulting in ongoing infections and contamination in both medical and industrial settings. These biofilms consist of bacterial populations surrounded by protective extracellular matrices that hınder the penetration of antibiotics. Given their minute size and reactive characteristics, nanoparticles (NPs) provide innovative methods to disrupt these biofilms. This review highlights existing NP-based methods targeting *E. coli* biofilms, focusing on their mechanisms, effectiveness, and future potential. A survey of the literature was performed utilizing databases such as PubMed and ScienceDirect, employing keywords including nanoparticles, *Escherichia coli*, and biofilms. The emphasis was on studies conducted in the last five years that analyzed metallic and metal oxide nanoparticles like silver, zinc oxide, and titanium dioxide, evaluating their abilities to combat biofilms and their modes of action.The primary mechanisms by which nanoparticles address *E. coli* biofilms include the disruption of the biofilm matrix, the generation of reactive oxygen species. and the disruption of bacterial communication systems like quorum sensing. Silver nanoparticles demonstrate potent antibacterial properties by releasing silver ions and inducing oxidative stress. Zinc oxide and titanium dioxide nanoparticles also possess biofilm elimination capabilities, particularly when exposed to light. The combination of nanoparticles with antibiotics can enhance effectiveness by aiding in deeper penetration into biofilms. However, issues such as nanoparticle toxicity, the development of bacterial resistance, and environmental concerns require consideration. Future strategies could include advanced nanoparticle systems featuring targeted and controlled release functionalities to optimize antibiofilm action and safety.Interventions utilizing nanoparticles offer a promising new approach to effectively tackle *E. coli* biofilms by attacking various biofilm elements and bacterial functions. Ongoing research is crucial to refine their design, reduce risks, and apply laboratory successes to practical medical and industrial contexts.

Key words: *Escherichia coli* , Nanoparticles , Biofilm , Antibiotics, Bacterial resistance

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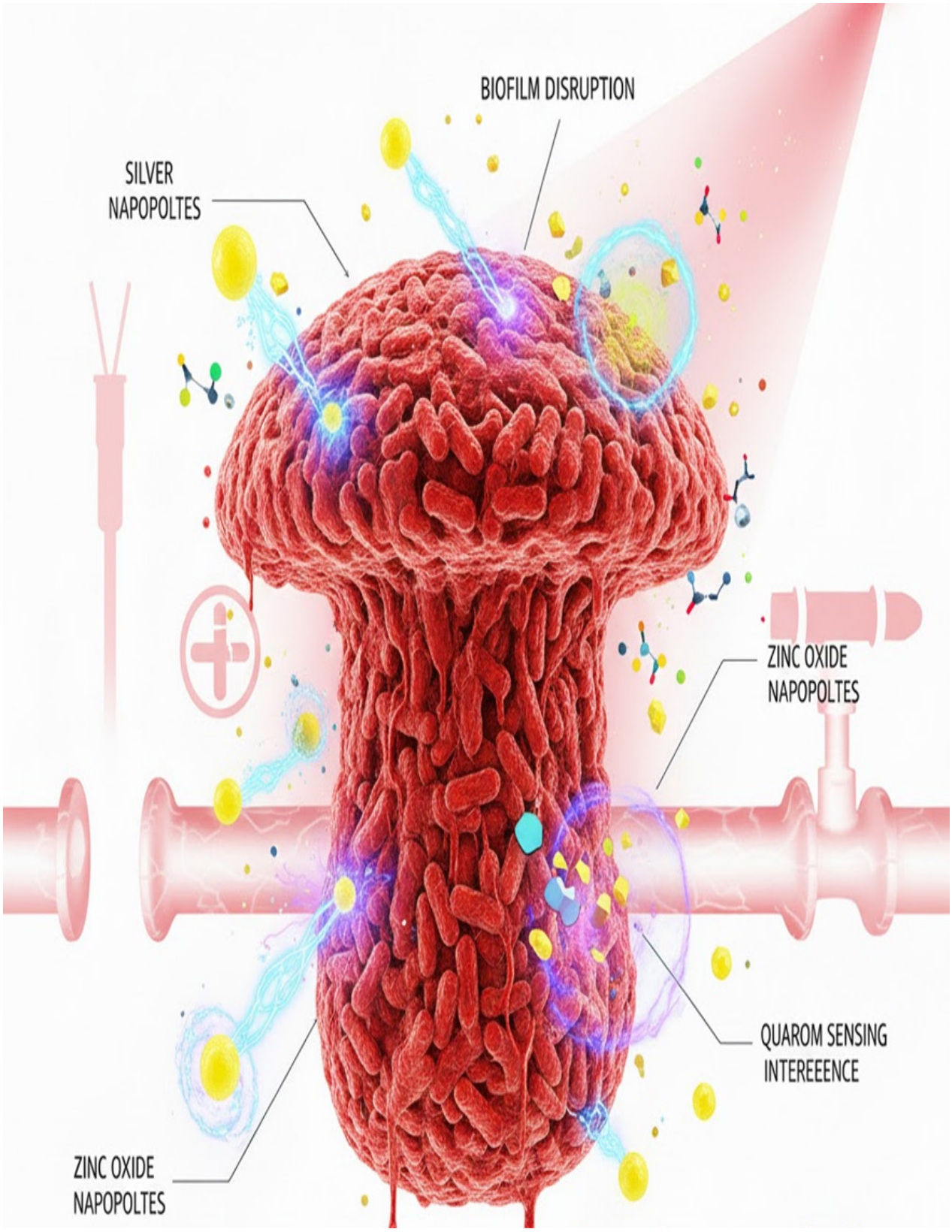
Graphical Abstract



Introduction

Biofilm formation by *Escherichia coli* represents a formidable challenge in clinical microbiology, biomedical engineering, and industrial biosafety. *E. coli* a ubiquitous Gram-negative bacterium can transition from a free-living planktonic state to a biofilm lifestyle as an adaptive survival strategy when exposed to environmental stressors, nutrient limitation, or surface contact. Biofilms are highly structured, multicellular microbial communities encased within an extracellular polymeric substance (EPS) composed of polysaccharides, proteins, lipids, and extracellular DNA. This matrix not only provides structural integrity but also creates a chemically heterogeneous microenvironment that shields bacterial cells from host immune responses, disinfectants, and conventional antibiotics(Sadeghzadeh et al., 2024). As a result, *E. coli* biofilms exhibit up to 1,000-fold increased tolerance to antimicrobial agents compared to planktonic cells, rendering many therapeutic approaches ineffective. In healthcare settings, *E. coli* biofilms are implicated in a wide range of persistent infections, including catheter-associated urinary tract infections (CAUTIs), prosthetic-device-related infections, chronic wound colonization, and gastrointestinal complications. Their capacity to colonize abiotic surfaces such as catheters, endotracheal tubes, and dialysis equipment significantly increases morbidity, prolongs hospitalization, and contributes to the global rise of antimicrobial resistance (AMR).). From an industrial perspective, *E. coli* biofilms contribute to biofouling, compromised water quality, product contamination in the food industry, and operational inefficiencies in bioprocessing systems. Their resilience against mechanical cleaning and chemical sanitizers has substantial economic and safety implications(Sharma et al., 2016). The growing crisis of antibiotic resistance further exacerbates the challenge. Traditional antibiotics often fail to penetrate the dense EPS matrix, and even when they do, the presence of slow-growing persister cells, efflux pumps, and stress-response regulators enables long-term bacterial survival.

These limitations have prompted an urgent search for alternative or complementary antimicrobial strategies capable of effectively disrupting biofilms while minimizing the development of resistance. Nanotechnology has emerged as one of the most promising frontiers in this context. Nanoparticles (NPs), particularly metal and metal-oxide nanoparticles such as silver (Ag-NPs), zinc oxide (ZnO-NPs), and titanium dioxide (TiO_2-NPs), offer unique physicochemical properties including high surface-area-to-volume ratio, tunable surface functionality, photoreactivity, and controlled ion release that enable multifaceted antibiofilm activity(Manisha et al., 2025). Unlike traditional antibiotics that rely primarily on single biochemical targets, nanoparticles exert their effects through multiple simultaneous mechanisms, such as generating reactive oxygen species (Sadeghzadeh et al.), disrupting membrane integrity, interfering with quorum sensing (QS), and degrading components of the EPS. This multimodal activity reduces the likelihood of resistance development and enhances the ability of NPs to eradicate mature, thick biofilms that are often completely refractory to standard treatments(Das et al., 2013). In recent years, substantial research has focused not only on understanding the fundamental interactions between nanoparticles and bacterial biofilms but also on engineering advanced nanomaterials with enhanced specificity, reduced cytotoxicity, and improved delivery efficiency. Strategies such as surface functionalization, green synthesis, hybrid nano-antibiotic systems, and stimuli-responsive nanoplatforms have shown encouraging outcomes in modulating nanoparticle behavior to achieve targeted biofilm disruption(Osman et al., 2022). Despite these advances, several challenges remain unresolved, including potential nanotoxicity to human cells, environmental persistence, scalability of synthesis, and the emerging though still limited evidence of bacterial adaptation to certain nanoparticles. Consequently, a comprehensive understanding of the mechanisms, strengths, limitations, and translational potential of nanoparticle-based antibiofilm strategies is essential.

This review therefore provides an in-depth examination of the current state of nanoparticle applications in combating *E. coli* biofilms. It synthesizes recent experimental findings, elucidates key mechanistic pathways, evaluates therapeutic efficiencies, and discusses critical safety, environmental, and regulatory considerations. Additionally, it highlights emerging trends and future research directions aimed at optimizing nanoparticle design for reliable clinical and industrial implementation.

Nanoparticles

Nanoparticles represent a versatile class of materials with dimensions typically below 100 nm, exhibiting unique physicochemical characteristics that enable advanced biomedical and antimicrobial applications. These nanoscale materials are broadly derived from metals, metal oxides, polymers, carbon-based structures, lipids, or hybrid systems, each offering distinct advantages for targeting bacterial pathogens such as *Escherichia coli.* Metallic nanoparticles, particularly silver, gold, and copper-based forms, are extensively studied due to their strong antimicrobial activity arising from metal-ion release, reactive oxygen species (Sadeghzadeh et al.) generation, membrane disruption, and interference with quorum-sensing pathways. Metal oxide nanoparticles such as zinc oxide, titanium dioxide, and iron oxides provide additional benefits through photocatalytic activity, high stability, and the ability to induce oxidative stress under light activation, making them effective candidates for biofilm destruction. Polymeric nanoparticles, synthesized from natural or synthetic polymers, offer biocompatibility and controlled release capabilities that enhance drug delivery into dense biofilm matrices(Ansari et al., 2014). Carbon-based nanomaterials, including graphene derivatives, carbon nanotubes, and fullerenes, exhibit exceptional mechanical and electronic properties that enable physical membrane disruption, high drug-loading efficiency, and photothermal or photodynamic antibacterial mechanisms(Alvandi et al., 2025).

Lipid-based nanoparticles, such as liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, further contribute through their high biocompatibility and capacity to encapsulate diverse antimicrobial agents, thereby improving penetration into biofilms. Hybrid nanoparticles, combining metallic, polymeric, or magnetic components, provide multifunctional platforms capable of targeted delivery, stimuli-responsive activity, and reduced cytotoxicity. Collectively, these diverse nanoparticle systems offer powerful and complementary strategies for combating *E. coli* biofilms, especially in environments where conventional antibiotics face limitations due to restricted penetration or microbial resistance(Karimi et al., 2016).

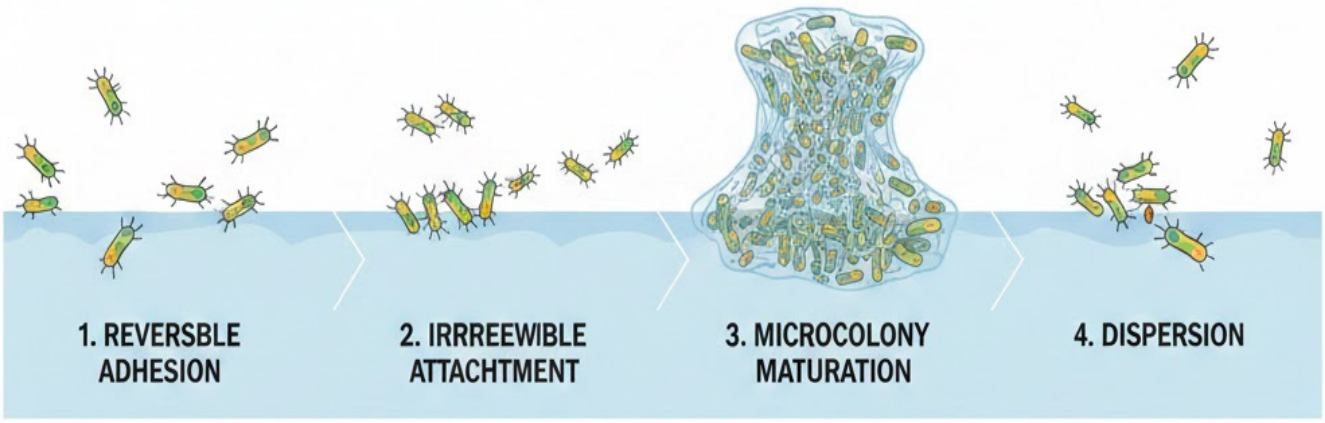
Biofilm and *E. coli* and Specific Pathways

Biofilm formation in *Escherichia coli* represents a complex, multistage biological process that enables the bacterium to survive under hostile environmental conditions and resist antimicrobial treatments. An *E. coli* biofilm consists of densely packed bacterial cells embedded within an extracellular polymeric substance (EPS) matrix composed primarily of polysaccharides, proteins, lipids, and extracellular DNA. This matrix not only provides mechanical stability but also acts as a barrier that restricts antibiotic penetration, limits immune system access, and facilitates horizontal gene transfer, thereby enhancing bacterial persistence and adaptability. The development of an *E. coli* biofilm proceeds through several well-defined stages, including initial reversible adhesion to a surface, irreversible attachment mediated by fimbriae and adhesins, microcolony formation, maturation into a three-dimensional architecture, and eventual dispersion of cells to colonize new sites(Sharma et al., 2016). Several molecular pathways regulate biofilm formation in *E. coli,* with quorum sensing (QS) functioning as one of the central communication systems. Through the synthesis and detection of autoinducer molecules such as AI-2, *E. coli* coordinates population-wide behaviors including EPS production, motility, and

virulence factor expression. Additionally, the intracellular messenger cyclic di-GMP plays a critical regulatory role by modulating the transition between motile and sessile states; high levels of c-di-GMP promote biofilm formation by increasing adhesin expression and enhancing polysaccharide synthesis(Cabuhat & Moron-Espiritu, 2022). The two-component regulatory systems, such as CpxAR and EnvZ/OmpR, further contribute to biofilm development by sensing environmental stressors and adjusting gene expression accordingly. Specific structural and functional components also underpin the robustness of *E. coli* biofilms. Curli fibers major amyloid structures facilitate irreversible adhesion and strengthen the biofilm matrix(Xiao et al., 2022). Cellulose, colanic acid, and other exopolysaccharides contribute to water retention, structural rigidity, and protection against desiccation and oxidative stress. Flagella-driven motility initially aids surface attachment but is subsequently downregulated during maturation through regulatory pathways involving c-di-GMP, reflecting a tightly controlled transition from motility to sessility. Furthermore, *E. coli* biofilms exhibit elevated resistance through multiple defense mechanisms, including reduced metabolic activity of deep-layer cells, overexpression of efflux pumps, enzymatic degradation of antimicrobials, and the emergence of persister cell populations. These factors collectively complicate eradication efforts and underscore the need for innovative therapeutic strategies(Suchanek, 2017). Understanding the intricate pathways governing E. coli biofilm formation is therefore essential for developing targeted interventions, including nanoparticle-based approaches capable of disrupting QS signaling, degrading EPS components, or altering regulatory networks that sustain biofilm integrity(Figure1).

Nanoparticles: A Novel Weapon Against *E. coli* Biofilms

Nanoparticles are powerful tools for combating *E. coli* biofilms, acting through several key mechanisms. One major mechanism is the production of reactive oxygen species (Sadeghzadeh et al.) These molecules induce oxidative stress, damage cell membranes, degrade DNA, and disrupt bacterial metabolism. For example, zinc oxide (ZnO) nanoparticles generate hydroxyl radicals, hydrogen peroxide, and singlet oxygen in the presence of light or moisture(Sirelkhatim et al., 2015). Green-synthesized titanium dioxide (TiO_2) nanoparticles can also inhibit biofilm formation and reduce EPS production. Another mechanism is the release of metal ions. Silver nanoparticles (AgNPs) release Ag^+ ions, which bind to proteins and nucleic acids, causing metabolic stress. This disrupts cellular respiration, decreases ATP production, and ultimately kills bacteria. Proteomic studies show that AgNPs alter bacterial metabolic pathways and reduce biofilm resistance. Disruption of quorum sensing (QS) is also important(Lahiri et al., 2021). QS is a bacterial communication system that regulates biofilm-related gene expression. ZnO nanoparticles and biosynthesized silver nanoparticles can interfere with QS, reducing bacterial adhesion and biofilm formation. Nanoparticles can also interact with the extracellular polymeric substance (EPS) matrix. Their small size allows them to penetrate deep into biofilms and interact with polysaccharides, proteins, and extracellular DNA(Hu et al., 2024). This weakens the biofilm structure and enhances antibacterial efficacy. Surface properties, such as charge and hydrogen-bonding capacity, influence nanoparticle distribution and penetration within EPS. Together, these mechanisms make nanoparticles an effective strategy against resistant *E. coli* biofilms. Combining metal ion release with ROS production can have synergistic effects, significantly reducing biofilm formation. Inhibiting quorum sensing further decreases EPS production and bacterial adhesion, improving nanoparticle penetration. These actions not only exert direct antibacterial effects but also reduce bacterial resistance, offering a promising approach for treating persistent infections(Table1).



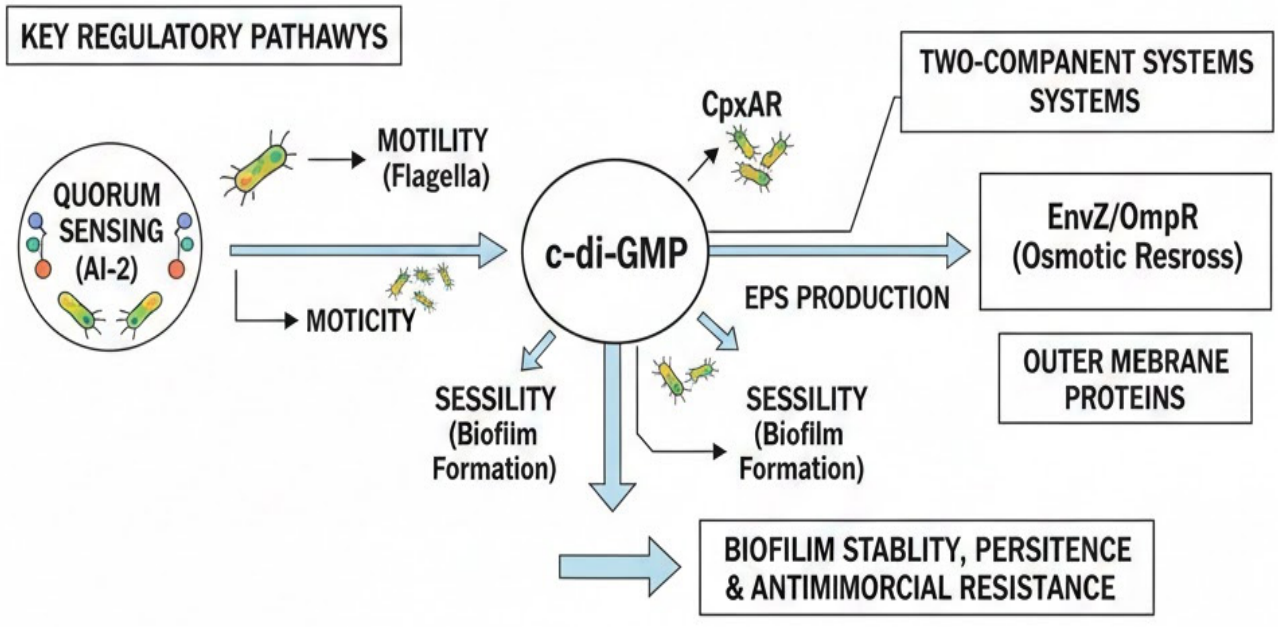


Figure1: Schematic representation of *Escherichia coli* biofilm development and the key regulatory pathways involved in its formation. The upper panel illustrates the sequential stages of biofilm formation, beginning with reversible adhesion, followed by irreversible attachment mediated by fimbriae and adhesins, microcolony expansion and maturation within an EPS-rich matrix, and finally the dispersion of planktonic cells. The lower panel highlights critical molecular pathways governing biofilm regulation, including quorum sensing via AI-2 signaling, c-di-GMP–mediated transitions between motility and sessility, and two-component systems such as CpxAR and EnvZ/OmpR that respond to environmental cues. Together, these interconnected mechanisms contribute to the structural stability, persistence, and antimicrobial resistance of biofilms.

Table1: Nanoparticles Strategies Against *E. coli* Biofilms: Mechanisms, Efficacy, and Future Perspectives

Mechanism	Nanoparticles Involved	Mode of Action	Effectiveness / Observations	Future Directions
ROS Production	ZnO, TiO_2	Induces oxidative stress → damages membranes, DNA, disrupts metabolism	Green-synthesized TiO_2 inhibits biofilm formation, reduces EPS	Targeted ROS delivery, combination therapies for resistant strains(Afrasiabi & Partoazar, 2024)
Metal Ion Release	AgNPs	Ag^+ binds proteins & nucleic acids → metabolic stress, disrupts respiration, reduces ATP → bacterial death	Reduces biofilm resistance, alters bacterial metabolic pathways	Optimize ion release kinetics, synergistic use with other antimicrobials(McNeilly, 2023)
Quorum Sensing Inhibition	ZnO, biosynthesized AgNPs	Interferes with bacterial communication → reduces expression of biofilm-related genes, decreases adhesion	Significant reduction in biofilm formation and EPS production	Design nanoparticles specifically targeting QS pathways for enhanced control(Owrang & Gholami, 2024)
EPS Matrix Interaction	AgNPs, AuNPs, ZnO	Penetrates biofilm → interacts with polysaccharides, proteins, extracellular DNA → weakens biofilm structure	Improves penetration and antibacterial efficacy, disrupts EPS integrity	Surface functionalization to enhance penetration, targeting multi-species biofilms(Souza et al., 2021)
Combined / Synergistic Approaches	Metal NPs + ROS-inducing NPs	Combines ROS stress + metal ion toxicity → enhanced biofilm disruption	Synergistic effects significantly reduce biofilm formation	Develop multi-modal nanoparticles with controlled release and targeted delivery(Balestri et al., 2023)

Conclusion

Nanoparticles are recognized as novel and effective tools to combat E. coli biofilms. They not only have direct antibacterial activity, but also reduce biofilm resistance by utilizing multiple mechanisms including reactive oxygen species generation, metal ion release, quorum sensing inhibition, and extracellular matrix interaction. Studies show that the combination of these mechanisms and the design of multimodal nanoparticles can produce synergistic effects and significantly enhance biofilm penetration and destruction. Given their high efficacy and the ability to tune surface and functional properties, nanoparticles offer a bright prospect as a novel therapeutic strategy to combat resistant infections. Future research can focus on the targeted optimization of these nanoparticles, reducing potential toxicity, and developing their clinical applications.

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