Metal Oxide Nanoparticles Against Bacterial Biofilms: Perspectives and Limitations

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Abstract: At present, there is an urgent need in medicine and industry to develop new approaches to eliminate bacterial biofilms. Considering the low efficiency of classical approaches to biofilm eradication and the growing problem of antibiotic resistance, the introduction of nanomaterials may be a promising solution. Outstanding antimicrobial properties have been demonstrated by nanoparticles (NPs) of metal oxides and their nanocomposites. The review presents a comparative analysis of antibiofilm properties of various metal oxide NPs (primarily, CuO, Fe3O4, TiO2, ZnO, MgO, and Al2O3 NPs) and nanocomposites, as well as mechanisms of their effect on plankton bacteria cells and biofilms. The potential mutagenicity of metal oxide NPs and safety problems of their wide application are also discussed.

Keywords: metal oxide nanoparticles; nanocomposites; biofilm; antibiofilm properties; antibacterial properties; mutagenicity

1. Introduction

Biofilm is a wide-range life form of bacteria that consists of the association of microorganisms cultures and extracellular polymer matrix (EPM), a complex biochemical mixture of polysaccharides, proteins, glycopeptides, nucleic acids, and lipids [1,2]. This slimy three-dimensional biopolymer is heterogeneous in different layers and contains structures similar to transport and water channels [3]. In biofilm formation, there are three main stages: adhesion, colonization, and maturation [4,5]. From the mature biofilm, the plankton microorganisms are isolated or dispersed into the environment. Thus, biofilm is a complex three-dimensional biological structure with a higher microbial life organization, in many respects, similar to a multicellular organism [6].

The primary function of biofilm is to protect the microorganism inside it from unfavorable physical, chemical, and biological factors in the environment, such as temperature, drying, ultraviolet radiation, biocides, humoral, and cellular factors of immunity [4]. Therefore, biofilms are stable, stress-resistant structures, which are difficult to destroy. They can cause many problems in various areas: water treatment (biofilm can disrupt the microbial community of active silt) [7]; food production (biofilm formation by pathogenic microorganisms and spoilage microorganisms) [8]; implantology (biofilms are capable of infecting medical devices: intravenous catheters, vascular prostheses, heart valve prostheses, urinary catheters, joint prostheses, pacemakers, and contact lenses) [9]; treatment of chronic diseases (according to the Center for Disease Control, biofilms cause 65% of chronic infections, and the National Institute of Health increases this index to 80%) [10,11]. All these problems have emerged since standard antibacterial therapy is practically ineffective against biofilms.
In most cases, the concentrations of antibiotics needed to kill the floating (planktonic) forms of bacteria can be 1000 times less than the required concentration for the complete removal of biofilms [10]. This tolerance to antibiotics causes the transition of infections caused by biofilms to a chronic form [7,12,13]. Initially, it was suggested that a unique biofilm structure could be a physical barrier to the inward penetration of antibiotics. However, more recent studies have shown that the diffusion of antibiotics is not hampered by EPM [14,15]. A further hypothesis is that after binding to polysaccharides, proteins, and DNA present in the biofilm, antibiotics may no longer be biologically active or cannot reach a necessary concentration for effective bacterial destruction [16].

Additionally, there is a gradient of oxygen concentration and nutrients inside the biofilm, which makes antibiotics, whose action is associated with metabolic disorders, much less effective against bacteria due to slowing or stopping metabolism [17]. Besides the high biofilm resistance to antibiotics, there has been an increased amount of information about biofilms formed by multidrug-resistant strains over the past decade. The latter further enhances the problem of combating biofilms in medicine and other areas.

The problems described above cause an urgent need to develop new ways of inhibiting biofilm growth and elimination. One of the potentially successful strategies may be transitioning from standard therapy to high-tech solutions based on nanomaterials.

Nanoparticles (NPs) are considered a promising tool for the treatment of bacterial biofilms. It is due to antibiotic resistance mechanisms not being effective against NPs [18]. Among many NPs, the most promised and widely studied are metal oxide nanoparticles, such as TiO₂, Fe₃O₄, ZnO, CuO [19], and some mixed metal oxides [20–22]. It has been shown that many metal oxide NPs exhibit biological properties much better than the NPs of the parent metals. That is why the metal oxide NPs provoked the highest interest in the scientific community [23,24].

In this review, we collected and systematized all of the information received in recent years on the effectiveness of using metal oxide NPs against biofilms. A comparative analysis of CuO, Fe₃O₄, TiO₂, ZnO, MgO, and Al₂O₃ NPs and nanocomposites of oxides was conducted. Potential mutagenicity and biosafety problems of these nanomaterials are also discussed.

2. The Mechanism of NPs Interaction with Biofilms

EPM of biofilms is heterogeneous from the point of physicochemical properties of a structure containing many polymer molecules carrying a charge [25]. Therefore, biofilm can be considered a three-dimensional filter capable of capturing organic molecules, ions, and NPs.

The interaction between NPs and biofilm can be considered as a three-stage process: (1) transfer of NPs in the vicinity of the biofilm; (2) attachment to the biofilm surface; and (3) migration in biofilms (Figure 1). The implementation of each stage is conducted by many factors: the physicochemical characteristics of NPs, EPM, and the environment.

![Figure 1. Interaction between metal oxides nanoparticles (NPs) and biofilm.](image-url)
Primarily, the interaction between NPs and biofilm is determined by their electrostatic characteristics. These features depend on the zeta potential of NPs and the charge of the biofilm matrix [26–29]. The majority of bacteria have a polyionic biofilm matrix due to the presence of uronic acid or metal-bound pyruvate with the functions of carboxylic acid and residual phosphate or rarely sulfate [30,31]. This negatively charged matrix can interact with positively charged metal ions and organic compounds through electrostatic forces [32,33].

Successfully associated NPs with EPM on the biofilm surface can penetrate deep into the biofilm at different rates. The penetration of NPs and movement within the biofilm is considered to be primarily due to diffusion [34]. In this case, the NPs’ diffusion inside the biofilm may depend on the size of its pores [28], water channels presence [35], the charge of NPs and EPM [34], hydrophobicity of the environment [36], the chemical gradient within the matrix. The EPM pore spaces containing water can have different ion concentrations. Ions and organic molecules diffuse and penetrate the biofilm, move, and are distributed through these pore spaces. This gives a plausible possibility that the interval between EPM pores will be especially crucial in this process. However, this variability on the scale of nanometers is not sufficiently characterized and understandable [37].

Thus, the penetration and migration of NPs inside the biofilm will be determined mainly by the charge and size of the particles and the composition and structure of EPM. However, many details of this interaction have yet to be determined.

3. Effects of Metal Oxide NPs on Plankton Cells and Biofilm

Three main mechanisms of antibacterial effects of NPs are well known: (1) mechanical damage to the cell wall as a result of electrostatic interaction, (2) oxidative stress as a result of the generation of reactive oxygen species (ROS), and (3) disruption of proteins functions and cell structures as a result of metal cations release [18] (Figure 2).

![Figure 2. Effects of metal oxide NPs on the bacterial cell. Brown line—cell surface (cell wall and cell membrane), blue line—DNA, arrow—electromagnetic irradiation.](image)

For negatively charged bacteria, adhesion on metal oxide NPs increases due to a positively charged surface of NPs [38]. Figure 3 shows how actively NPs interact with the cell wall on the example of magnetite. In this way, metal oxide NPs binds to the cell wall through electrostatic and Van der Waals interactions, in particular, to cell membrane proteins that disrupt bacteria functions. MgO NPs also show strong interactions not only with the cell surface but also with spores [39,40].
Figure 3. Electrostatic and Van der Waals interactions between magnetite (Fe₃O₄) NPs and bacterial cells: NPs adhere to the cell wall and form a magnetite shell. The images obtained by scanning electron microscopy (Tescan VEGA 3, Brno, Czech Republic).

The primary mechanism of bacterial cell destruction under the NPs’ influence is currently considered to be the induction of oxidative stress through the generation of ROS under electromagnetic irradiation of NPs [41]. The generation of ROS induces oxidative stress in the cell, as a result of which it dies. In most cases, ROS production is also directly related to the release of cations. In particular, Fe₃O₄ NPs release Fe²⁺ ions, which cause the generation of ROS after a reaction with hydrogen peroxide (Fenton reaction) [42]. Copper ions can also disrupt biochemical processes and significantly damage nucleic acids [43]. It is assumed that after the specific binding of copper to DNA, repeated cyclic redox reactions generate several OH⁻ radicals near the binding site, causing multiple damages to the nucleic acids, but in some microorganisms, copper oxidative damage to the genetic material may occur through the Fenton mechanism [44].

Calcium and magnesium oxides can generate superoxide radical O₂⁻, whereas zinc oxide NPs produce H₂O₂ and hydroxyl radical OH⁻ under ultraviolet and visible light, but not O₂ [45,46]. The molecules of OH⁻ and O₂ cannot penetrate the cell membrane due to their charges or reactivity [47], and probably remain on the cell surface, whereas H₂O₂ can penetrate bacterial cells, thereby inducing cell death [48,49]. Meanwhile, copper oxide NPs can produce all four types of reactive oxygen. Thus, CuO NPs are toxic enough for bacteria and have shown significant effects against biofilms.

TiO₂ NPs under irradiation can generate electron-hole pairs with photoexcitation, initiate cascade oxidation–reduction reactions on the surface of TiO₂, and, consequently, produce ROS for further reactions [50,51]. Thus, TiO₂ NPs inhibit bacterial growth by lipid peroxidation in the membranes, DNA damage, nucleotide and amino acid oxidation, or destruction of protein-catalytic sites by photocatalysis [52].

Despite the above, there is evidence that ROS generation does not always directly cause cell death. In particular, the analysis of gene expression has shown that ZnO NPs inhibit the expression of oxidative stress genes, despite the ROS generation. The antibacterial effect may be due to biomimetic action and other mechanisms [53].

The release of metal cations can also have a devastating effect on cells, regardless of ROS products. Different bacterial species have varying sensitivities to metal ions. Cations can interact with sulphydryl groups in enzymes, with amine and carboxyl groups on microbial cells [54–56]. In particular, Zn²⁺ ions influence peptides by changing their conformation [57]. Cations can cause the mismetallation of proteins in a cell. In particular, copper ions can mismetallate proteins that require a molybdenum or iron cofactor because of their affinity for thiol ligands [58,59]. This process may
affect cell metabolism due to improper assembly of biosynthetic enzymes [60]. All this leads to disruption in the functioning of cellular components and cell death in the end.

The NPs discussed in this review exhibit antibacterial properties to varying degrees by implementing the mechanisms mentioned above. Conditionally, NPs can be arranged in the following order of their decreasing antibacterial and antibiofilm properties: CuO–ZnO–MgO–TiO$_2$–Fe$_3$O$_4$–Al$_2$O$_3$. Physical and antibiofilm properties of some NPs oxides are presented in Table 1.

**Table 1.** Physical and antibiofilm properties of metal oxide nanoparticles (NPs) and nanocomposites.

| NP  | Synthesis                                      | Physical Properties | Antibiofilm Properties Against Species: | Reference |
|-----|------------------------------------------------|---------------------|----------------------------------------|-----------|
| Al$_2$O$_3$ | Purchased at the Sigma–Aldrich, no information about the synthesis method | 50 nm | *P. putida, Aeromonas hydrophila* | [61] |
| CuO   | Sonochemical method                            | 40 nm | *Candida sp.* | [62] |
| CuO   | Green synthesis                                | 50 nm | *E. coli, P. aeruginosa, K. pneumonia, P. vulgaris* | [63] |
| CuO   | Green synthesis                                | No information | *E. coli ATCC 25922, S. aureus ATCC 45500* | [64] |
| CuO   | Chemical reduction method                       | 20 ± 1.24 nm | *E. coli, P. aeruginosa, B. subtilis, S. aureus* | [65] |
| ZnO   | Wet chemical method                            | ~15 nm | *S. pneumoniae MTCC 2672* | [11] |
| ZnO   | Purchased at the Sigma–Aldrich, no information about the synthesis method | <50 nm | *P. aeruginosa PA01* | [66] |
| ZnO   | Green synthesis                                | 10–50 nm, triangles, hexagons, rods, and rectangles | *B. licheniformis, B. pumilus, E. coli, P. vulgaris* | [67] |
| ZnO   | Wet chemical method                            | 7–10 nm | Classical (O395) and ElTor (N16961) *V. cholerae* | [68] |
| ZnO   | No information                                 | 65 nm | *P. aeruginosa* | [69] |
| ZnO   | Green synthesis                                | 8–18 nm, spherical, oval, hexagonal | *E. coli, P. aeruginosa (ESBL), MRSA, MSSA* | [70] |
| ZnO   | Green synthesis                                | 20–50 nm, spherical and hexagonal | *MRSA* | [71] |
| ZnO   | Purchased at the Sigma–Aldrich, no information about the synthesis method | 50–500 nm, spherical | *M. smegmatis, M. bovis BCG, E. coli, P. aeruginosa, S. aureus, MRSA* | [72] |
| ZnO   | Green synthesis                                | 90–100 nm, triangles, hexagons, rods, and rectangle | *V. cholerae, E. coli (ETEC)* | [73] |
| Material | Synthesis/Method | Shape/Size | Species/Strains |
|----------|-----------------|------------|-----------------|
| ZnO      | Purchased at the Sigma–Aldrich, no information about the synthesis method | ~100 nm | MRSA [74] |
| ZnO      | No information | 20 nm, plates, spheres, pyramids | E. coli UTI89 and MG1655, K. pneumoniae LM21, MRSA SH1000, S. epidermidis RP62A [75] |
| ZnO      | No information | 20 nm | E. coli, S. aureus, S. sobrinus ATCC 27352, Enterobacter sp, Marinobacter sp. [76] |
| ZnO      | Green synthesis | 26 nm | C. violaceum 12472, C. violaceum CVO26, E. coli, L. monocytogenes, P. aeruginosa PAO1 [77] |
| ZnO      | Microwave radiation | No information | C. violaceum ATCC 12472, E. coli ATCC 25922, P. aeruginosa PAO1, K. pneumoniae ATCC 700603, S. marcescens ATCC 13880 [78] |
| ZnO      | Sol-gel method | No information | R. dentocariosa, K. mucilaginosa [79] |
| ZnO      | Purchased at US Research Nanomaterials Co, no information about the synthesis method | 10–30 nm | Uropathogenic E. coli [80] |
| ZnO      | Sonochemical method | No information | E. coli ATCC 25922, S. aureus ATCC 29213, P. mirabilis [81] |
| CeO₂     | Sonochemical method | ~100 nm | Periphytic biofilm [82] |
| MgO      | Chemical synthesis | No information | K. pneumoniae KT273996, E. coli KT273995 [83] |
| MgO      | Purchased at the Sigma–Aldrich, no information about the synthesis method | No information | R. solanacearum [84] |
| MgO      | Microwave radiation | No information | E. coli, S. aureus [85] |
| NiO      | Green synthesis, Eucalyptus globulus leaf extract | 19 nm | ESβL (+) E. coli, P. aeruginosa, methicillin-sensitive and resistant S. aureus [86] |
| Fe₂O₃    | No information | No information | B. subtilis (ATCC 6633) [87] |
| Fe₂O₃    | Wet chemical method | 10 nm | S. aureus, P. aeruginosa, E. coli [88] |
| Fe₂O₃    | Co-precipitation method | 10.64 ± 4.73 nm | E. coli BW 25113, E. hirae ATCC 9790 [89] |
| hematite (α-Fe₂O₃) | No information | 2 to 540 nm | P. aeruginosa (PA01) [90] |
| TiO₂     | Sol–gel method | No information | B. subtilis strain 168 [91] |
| TiO₂     | No information | <100 nm | MRSA biofilm [74] |
| WO₂      | Acid precipitation routes | No information | B. subtilis strain 168 [91] |
The minimum inhibitory concentration (MIC) for CuO NPs against a wide range of Gram-negative and Gram-positive bacteria is 15 µg/mL to 100 µg/mL on average. [63,64,98]. In particular, MIC of CuO NPs for *E. coli* is 22 µg/mL, and for *S. aureus* is 15 µg/mL [63,64].

CuO NPs inhibit the formation of biofilms and promote the eradication of already formed biofilms. It is mainly due to the toxicity of copper ions for plankton and biofilm cells. For example, CuO NPs efficiently reduced the biofilm formation by MRSA and *E. coli*. Almost all MRSA and *E. coli* biofilm cells died within four days of exposure to CuO NPs [99]. CuO NPs at a 50 µg/mL concentration significantly inhibited the growth of total oral bacteria, extracellular polysaccharide (EPS) production, and biofilm formation on the glass, acrylic dentures, and cultured human epithelial cells as models [100]. It has also been shown that CuO NPs’ inhibitory concentration on *Ralstonia solanacearum* biofilms is 125 and 250 µg/mL after an incubation period of 24 and 72 h [101]. Thus, it is evident that CuO NPs can be potentially effective against biofilms, as shown in different groups of microorganisms.

### 3.2. ZnO

For ZnO NPs, the effective antibacterial concentration increases to an average of 20–500 µg/mL [102–108]. For example, there was 50 µg/mL against *E. coli* [105]. However, the antibacterial properties of ZnO NPs can be enhanced by additional physical exposure. In particular, cell penetration by NPs can be amplified by ultrasound. The combination of ZnO NPs with ultrasound enhances the antibacterial effect on *S. aureus* by 76% due to generating more hydrogen peroxide [109]. Besides, ultrasonic treatment can physically promote the dissociation of cell membranes, thereby increasing the penetration of ZnO NPs into cells [18].

ZnO NPs can be considered a potential agent for the inhibition of microbial biofilms. The results of the inhibition of *S. pneumoniae* biofilm showed that the sub-MIC doses (3, 6, and 12 µg/mL) of ZnO NPs exhibited significant antibiofilm activity. The main mechanism preventing the formation of *S. pneumonia* biofilms in the presence of zinc oxide NPs is that it reduces the adhesion of cells to the surface [11]. Studies of biofilms on dentures revealed the ZnO NPs’ effectiveness in controlling the formation of biofilms *Rothia dentocariosa* and *R. mucilaginosa*. Zinc ion generation inhibits the
enzymatic activity of the DapE protein involved in the synthesis of peptidoglycans, which leads to the failure of biofilm formation in the initial stage [79]. The recent studies on the effect on biofilms formed by uropathogenic strains of *E. coli* have shown that MIC concentration of NPs fully inhibited biofilm formation in 20% of isolates, and 30% of isolates reduce the optical density of biofilm formation from a moderate to weak level [80]. In this way, ZnO NPs also have excellent potential for antibacterial materials’ development.

### 3.3. MgO

MgO NPs show activity against Gram-positive and Gram-negative bacteria, spores, and viruses [110–113] at sufficiently high particle concentrations (on average 100–1200 µg/mL). In particular, MIC of MgO NPs for *E. coli* were determined to be 1 mg/mL [114]. Recent studies have shown the efficiency of MgO NPs in the action against biofilms of *E. coli* (250 µg/mL), *K. pneumoniae* (125 µg/mL), and *S. aureus* (500 µg/mL). Bacterial adhesion to the plastic surface decreased markedly after 12 h incubation of *E. coli*, *S. aureus*, and *K. pneumonia* with MgO, thus preventing the biofilm formation. The effect of MgO on mature biofilms was also detected. Biofilm biomass was significantly reduced when treating biofilms with a subinhibitor concentration of 0.5 MIC. [83]. In another recent study, it was reported that 10 µg/mL significantly complicates the formation of *S. aureus* biofilms [115]. MgO NPs had a strong inhibitory effect on the formation of biofilms *E. coli* and *S. aureus* at a size of 8 nm [85]. MgO NPs reduced the biofilm growth of *R. solanacearum*, and the biofilm formation gradually decreased with the bulk MgO treatments. The 200 and 250 µg/mL treatments of MgO NPs exhibited high inhibitory effects on the *R. solanacearum* biofilm formation. The biofilm formation was reduced by 61% and 71% after 24 h and by 67% and 72% after 72 h, respectively [84].

Thus, MgO NPs have significant antibiofilm properties, but significant effects are achieved at sufficiently high particle concentrations (above 125 µg/mL).

### 3.4. TiO₂:

TiO₂ NPs are effective against bacteria, viruses, and even to purify specific odor molecules in the range of 20 µg/mL to 1400 µg/mL [116–120]. TiO₂ NPs show antibacterial properties against Gram-positive and Gram-negative, the latter being more sensitive [121,122]. It could be related to the fact that Gram-positive bacteria have a thick layer of peptidoglycan that facilitates the absorption of reactive radicals, thereby preventing cell damage from radical attack [123]. In addition, it shows that TiO₂ has a potential against bacteria through the reception of an electron from intracellular coenzyme A (CoA) after photocatalysis of TiO₂, followed by the formation of dimer CoA and subsequent inhibition of respiration [124].

TiO₂ NPs can reduce the adhesion of bacteria and inhibit biofilms. Exposure to titanium oxide leads to the destruction of bacteria inside the biofilm, primarily due to the generation of ROS and lipid oxidation on the cell wall membrane [92,120]. It has been shown that TiO₂ NPs are effective against biofilms of MRSA [92] and *S. mitis* [125]. TiO₂ NPs could control the growth and biofilm formation of *S. mitis* ATCC 6249 and Ora-20, and it can be used as a means for oral hygiene. TiO₂ NPs have a reduced impact on *Pseudomonas aeruginosa* biofilms at a 31.25 µg/mL concentration and disrupt previously established biofilms in the microtiter plate [125]. In the presence of TiO₂ NPs, biofilm formation of *E. coli* and *B. subtilis* was reduced by 40–50% [122]. However, NPs TiO₂ did not show significant bactericidal properties against certain types of drug-resistant bacteria (ex, *Cupriavidus metallidurans* CH34), which have a remarkable ability to withstand ROS membrane damage through overexpression of protective components and membrane repair elements [126].

### 3.5. Fe₃O₄

Fe₃O₄ NPs has slight antibacterial properties, and their effective concentrations reach 10–20 mg/mL [38,42,127,128]. Fe₃O₄ NPs against biofilms showed mostly insignificant effects. To obtain significant antibiofilm effects, Fe₃O₄ NPs have to be used in high concentrations. In this case, the
particles are able to destroy the cells inside the biofilm. It has been shown that iron-oxide NPs were able to reduce biofilm growth by *S. aureus*, *E. coli*, *P. aeruginosa* [88], *S. epidermidis* [38], and *Enterococcus hirae* [89].

In addition to the passive electrostatic effect on biofilm, NPs effectively penetrate deep into the biofilms in the presence of a magnetic field [129,130]. In this case, NPs can have a mechanical effect on the biofilm due to the destruction of the matrix structure and its whole architecture [87,131,132]. Due to these properties, the particles are mainly used as a carrier of biocides in biofilms. In particular, the NPs enhance the action of various antibiotics on biofilm. The effectiveness of conjugates with penicillin, streptomycin, erythromycin, kanamycin, cefotaxime against *S. aureus* biofilm [133,134] and amphotericin B, nystatin against *Candida* spp. biofilm has been shown [135]. In recent years, many biocide-conjugated particles have been developed against biofilms. In all cases, magnetite serves as an efficient matrix for delivering biocides inside the biofilms and has a synergetic effect due to its unique properties.

Thus, FeO NPs have a less significant antibacterial property but have a significant antibiofilm potential due to the delivery of different antimicrobial drugs into the biofilm via a magnetic field [87,129–136].

### 3.6. *Al*O3

AlO3 NPs are effective against bacteria only when in high concentrations (reach 10–20 mg/mL) [137,138]. AlO3 NPs weakly inhibit *E. coli* at high concentrations up to 1 mg/mL [137,138]. In the study of AlO3 NPs’ effect on *P. putida* and *A. hydrophila* in biofilms and planktonic forms, it has been shown that NPs are toxic to bacteria, but plankton cells are more susceptible to AlO3 NPs than biofilms [61]. The minimal inhibitory concentration of AlO3 NPs against the biofilm of *P. aeruginosa* was found to be 1.6–3.2 mg/mL. Treatment at a 2 mg/mL concentration resulted in complete growth inhibition of extended-spectrum b-lactamases and metallo-b-lactamases clinical isolates of *P. aeruginosa* [139]. Therefore, antimicrobial and antibiofilm properties of AlO3 are less than those of other oxides. Thus, it can only be effective in nanocomposites and conjugates with biocides.

It is clear from the above that metal oxide NPs can be promising materials against biofilms. Different particles exhibit antibiofilm properties to varying degrees, which depends directly on their antibacterial properties. These properties are determined mainly by the synthesis method, size, and shape of the particles (Table 1). Particles, such as FeO and AlO3, have weak antibiofilm properties, but they can be much more effective in nanocomposites.

### 4. Metal Oxide Nanocomposites Against Plankton Cells and Biofilms

It can be seen from the previous section that the antibiofilm properties of metal oxide NPs are demonstrated to varying degrees. Nanocomposites of mixed metal oxides are actively studied and developed to improve antimicrobial properties and reduce adverse cytotoxic effects and reactions of the immune system to monoxide NPs.

Nanocomposite CuO doped with Zn showed an increase in antibacterial activity of 10 times against *E. coli* and *S. aureus* bacteria compared to pure ZnO or CuO [140]. Moreover, CuO doped with TiO3 has a more significant antibacterial effect than pure TiO3. Li-doped MgO NPs are more efficient than pure MgO, whereas Zn and Ti-doped MgO exhibit lower antibacterial activity than MgO [22]. TiO3-ZnO-MgO mixed oxides nanomaterials have a strong antibacterial effect against Gram-negative and Gram-positive bacteria [141].

The combination of three metal oxides in the CuZnFe oxide NPs may also enhance the therapeutic abilities of the NPs against a wide range of microbial infections. Antibacterial activities of CuZnFe oxide NPs were tested against Gram-negative *E. coli* and Gram-positive *E. faecalis*. CuZnFe oxide NPs affected bacterial species by reducing their viability and their ability to synthesize biofilm. CuZnFe oxide NPs have more detrimental effects on *E. coli* than individual CuO and ZnO NPs. CuZnFe oxide NPs were also found to be more bactericidal than ZnO NPs against *E. faecalis*, but 7% lower than CuO NPs [94]. It was observed that this oxide could slowly release metal ions, which can penetrate through the membranes and disrupt cellular processes from within the cell [142]. CuZnFe
oxide NPs affected biofilm formation to a lesser degree than those of individual ZnO and CuO NPs [94].

The pro-oxidative and pro-inflammatory effects of highly toxic ZnO NP can be significantly reduced by iron doping [143,144]. A similar effect can also be achieved by adding magnesium to the nanocomposite: mixed ZnMgO NPs, are safe for mammalian cells with the non-toxic MgO monoxide NPs [20]. ZnO NPs doped with Mn and Fe ions exhibit even higher antibacterial activity on a wide range of bacterial species, including S. aureus, E. coli, K. pneumoniae, S. typhi, P. aeruginosa, B. subtilis, and Proteus mirabilis as compared to the ZnO monoxide [21,145]. In the study of FeOx-ZnO nanocomposite, it has shown more significant antibacterial activity on E. coli than S. aureus and B. subtilis [145].

The inclusion of Ag and Au in composites also significantly enhances their antimicrobial properties. Ag-ZnO nanocomposites showed a high antibacterial effect against antibiotic-resistant E. coli and S. aureus [146]. High antibacterial activity against E. coli and S. aureus was shown by Ag-SiO2 nanocomposite. This composite is perfect for the treatment and infectious control of superficial wounds [96]. Moreover, the deposition of Au particles on the surface of ZnO NPs, even at a low molar ratio of ZnO/Au (0.2%), significantly improves the photocatalytic antibacterial activity of ZnO [147]. Furthermore, Ag-TiO2 nanocomposite demonstrates antibacterial activity against S. aureus biofilms [93,148,149]. It was shown that the inclusion of a 2% composite significantly reduced the formation of biofilms on the surface of the composite resin [150]. Ag/FeOx NPs significantly improve antibacterial properties against E. coli [151]. CeO2-CdO nanocomposites also exhibit broad-spectrum antimicrobial activity against Gram-positive (S. aureus MTCC96 and C. pyogenes MTCC 1926) and Gram-negative (P. aeruginosa and K. pneumoniae) [95].

Additionally, the introduction of nanocomposites based on oxides induced by external physical factors can add additional functions and significantly increase antimicrobial action effectiveness. For example, compared to the monoxide TiO2 NPs, which exhibit photocatalytic activity in the UV spectrum, the doped form can significantly expand the active spectrum to the visible light region [152,153]. An example of a highly effective nanocomposite can be ZnOAu, which possesses photoactivity due to zinc, and thermal sensitivity due to gold [97].

Some nanocomposites have been studied in vivo. CuO ligated Zn shows a successful inhibition of biofilm formation in vitro and in vivo experiments on rabbits. At the same time, this composite is biocompatible [81].

As shown in the above, the antibacterial activity of initially low-efficiency oxides (such as FeOx NPs) in mixed oxides can be significantly increased. It becomes evident that oxide particles, and especially composites based on them, can become promising materials. Reducing their cytotoxicity and immunogenicity will also allow their introduction into biomedicine. Ultimately, the widespread application of nanomaterials also needs to be determined by their safety to the environment.

5. Potential Adverse Effects of the Broad Implementation of Metal Oxide NPs

Along with their numerous remarkable applications, NPs also have potential limitations. In particular, a large surface area and high reactivity can be considered as one of the NPs advantages, but at the same time cause side effects. Moreover, the non-specificity of the antimicrobial action on pathogenic and symbiotic microorganisms exhibited by NPs can be seen as an advantage or disadvantage [154]. Chemically synthesized NPs have toxicity problems since dangerous compounds are used in their synthesis. These toxic compounds remain within NP in trace amounts and cause undesirable effects [155]. A successful approach to solving these problems is developing methods to synthesize environmentally friendly and less toxic NPs—the so-called “green synthesis” [156].

The most important aspect of the safe use of nanostructures is their mutagenicity. Even though this issue remains the least studied, there are already some literature reports that raise concerns. As mentioned earlier, the NPs and ROS that they generate can themselves influence the DNA of bacteria. If the concentration of NPs is not sufficient to eradicate the biofilm, but enough to trigger mutations, which can lead to the appearance of “super mutants”.

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The study of NPs genotoxicity using tungsten oxide as an example has shown that direct interaction NPs with DNA can lead to damage by single-strand breaks. After the mix with NPs DNA was introduced into bacterial cells, the cells mostly died, and the surviving cells were almost all mutants. The results provide clear evidence that one of the mechanisms involved in nanomaterials toxicity directly damages DNA, which can then cause biological cell death and mutation [157].

The potential mutagenicity of certain oxides (Al$_2$O$_3$, CuO, TiO$_2$, and ZnO) was investigated using reverse mutation (Eames analysis). The results showed that the mutagenicity was negative for four nanoparticles (Al$_2$O$_3$, CuO, TiO$_2$, and ZnO) to 1000 µg/plate for all three strains tested (S. typhimurium TA97a, TA100, and E. coli WP2 trp+uvrA) without metabolic activation of S9. Using the pre-incubation procedure and high activation of S9 (9%), TiO2 and ZnO induced marginal mutagenesis for the E. coli strain WP2. The CuO exhibited a low mutagenic potential in S. typhimurium TA97a and TA100 at specific concentrations [158].

In addition to direct DNA damage under the influence of NPs, there are also data on increasing the efficiency of horizontal transfer of genetic material when exposed to NPs. It has been shown that Al$_2$O$_3$, AIOOH, TiO$_2$, SiO$_2$, and Fe$_3$O$_4$ NPs can contribute to the conjugating transfer of plasmid (up to 20–100 times) [159–161]. It is important to note that the transfer increased inside the species or genus of bacteria. The most significant effect was produced by Al$_2$O$_3$ NPs, which led not only to an increase in the number of conjugating cells but also to one bacterium being conjugated to several other bacteria [159].

Such manifestations of NPs mutagenicity in the biofilms of bacteria are especially important. Genetic components from lysed bacterial cells, such as plasmids, are stored inside the EPM, increasing the gene transfer frequency between bacterial cells [162]. These plasmids can contain genes useful for bacteria, such as genes for antibiotic resistance [163]. Thus, it can be assumed that metal oxide NPs can contribute to the formation of antibiotic-resistant bacteria. Further research on the issues described above is essential for the safe use of NPs.

An essential aspect of the widespread introduction of nanostructures is the ecological one. Currently, metal oxide NPs are already widely used in various fields. The presence of nanomaterials in the environment is not new. Nevertheless, the current growth in the production of anthropogenic nanomaterials will increase their level in the environment. The release of nanoparticles into the biosphere will occur from point sources (for example, production sites, landfills, treatment plants) and secondary sources (for example, release into the environment when using and consuming materials containing nanomaterials). For example, a global estimate for nanomaterial production goes up to 5000 tons/year for TiO$_2$ NPs [164].

There is a gradual increase in the concentration of these NPs in wastewater as contaminants. This can threaten ecological communities in treatment plants [165]. Recent studies have shown that significant accumulation of NPs is observed in biofilms of the river and marine sediments [166,167]. A high concentration of 20 nm TiO$_2$ is found in river biocenoses [166]. NP can pose a danger to the environment, causing the disappearance of some biocidal sensitive bacterial strains and expanding a set of insensitive strains to various influences [126].

The properties of NPs described in this section show that currently, available research data are insufficient to assess the safety of NPs use. More studies need to be carried out before these nanomaterials can enter the broad market.

6. Conclusion

In addition to the biofilms spread, which are resistant to antibiotics, the spread of biofilms produced by antibiotic-resistant bacteria grows. Using metal oxide NPs and their nanocomposites is one of the few methods that can soon enter into broad practice.

Among the NPs discussed in this review, CuO and ZnO NPs showed the most significant antibiofilm properties, which initially had high antibacterial characteristics. These NPs have proven effective against biofilms of a wide range of bacterial species. Fe$_3$O$_4$, TiO$_2$, and MgO NPs have a lower ability to eradicate biofilms. However, magnetite can be used with a magnetic field to deliver biocides and has shown high biofilm destruction efficiency. Al$_2$O$_3$ NPs have the weakest properties against
biofilms, and these particles can only be effective in high concentrations. However, the introduction of nanocomposites consisting of several oxides can significantly enhance the action of NPs against biofilms. Several of the composites described in this review have excellent antibacterial and antibiofilm properties.

However, the mechanisms of NPs action on biofilms are still weakly studied and controversial. A more thorough study of the mechanisms of NPs’ action on plankton cells and biofilms may allow more targeted use and increase their effectiveness. Another issue is the potential particle mutagenicity, resulting from which bacteria can acquire new dangerous properties by analogy with the development and spread of antibiotic-resistant strains. Under conditions of mixed cultures of bacteria, especially in biofilms, NPs can, hypothetically, accelerate bacterial evolution. Nevertheless, this aspect of the NP influence on prokaryotes is the weakest studied.

It can be concluded that, despite extensive research on NPs worldwide, many issues require more in-depth studies. Without them, nanostructured drugs introduction to therapy can have unpredictable consequences for both the evolution of bacteria and the human body. The use of NPs on the industrial level can also provoke severe environmental consequences.

Thus, the current review formulates specific issues that have to be addressed to push the research forward. Since it is essential to develop new approaches for combating biofilms of bacteria in various fields, new information on the effectiveness of the use of NPs against biofilms, their mechanisms of influence on cells, mutagenicity, and genotoxicity will be crucial.

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