Sohag University Sohag Medical Journal Faculty of Medicine

Copper Oxide Nanoparticles' Anti-biofilm Activity against MDR Gram negative bacilli

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Abstract:

Emerging pathogens known as multidrug-resistant (MDR) bacteria have resistance characteristics that have a major negative impact on human health. The emergence of microbes resistant to currently available medications has been influenced by the misuse of antibiotics. A number of methods, such as the change of antibiotics, the alteration of target sites, and the production of biofilms, are used by pathogenic bacteria to build resistance. Healthcare professionals are very concerned about bacterial colonization on surfaces in the form of biofilms because it can lead to chronic infections.

It is currently urgently needed in both medicine and industry to create novel defenses against the growth of biofilms and planktonic infections, particularly strains that are resistant to antibiotics. The use of nanoparticles could be a potential solution in case of ineffectiveness of conventional methods for eliminating biofilms and the rising issue of antibiotic resistance. Metal oxide nanoparticles, like copper oxide, are among the many nanoparticles (NPs) that have attracted the greatest attention and promise.

Existing biofilms are encouraged to be destroyed by copper oxide nanoparticles, which also inhibit formation of new ones. The primary cause is the toxicity of copper ions for plankton and biofilm cells. Many metal oxide nanoparticles have shown biological characteristics that are significantly more favorable than those of the parent metals NPs. For this reason, the metal oxide NPs sparked the most interest from scientific community.

Keywords: MDR bacteria, Biofilm, CuO NPs.

Introduction:

Over the past few decades, the prevalence of multidrug-resistant (MDR) bacteria, often known as superbugs, has increased to the point where one may argue that we are progressively moving into a "post-antibiotic era" in which common bacterial infections can cause patient morbidity and mortality. New therapy techniques are being created now to lessen this imminent hazard [(1)]

Multidrug-resistant organisms are those that exhibit in vitro resistance to at least one compound from three or more antimicrobial classes [(2).] Patients around the world are at risk from infections brought on by MDR Gram-negative bacilli (GNB). Some clinically significant MDR-GNBs include ca-

rbapenem-resistant (CR) *Acinetobacter* species, MDR-*Pseudomonas aeruginosa*, and MDR *Enterobacteriaceae* (such as *Klebsiella pneumoniae* and *Enterobacter* species) are particularly concerning because it has been revealed that over 50% of these gram-negative bacteria that led to infections in healthcare settings are MDR. MDR-GNB infections usually result in worse results than their antibiotic-susceptible GNB counterpart illnesses, including lengthier hospital admissions, greater mortality, and higher healthcare costs. It is generally recognized that the misuse of antibiotics has led to a selection pressure that has resulted in the emergence of MDR microorganisms [(3).]

Genetic alterations have typically been blamed for antibiotic resistance, but growing recognition of the importance of biofilms and their recalcitrance, which is mostly caused by growth state-dependent adaptive resistance [(4).]

Typically, a biofilm is defined as an accumulation of one or more bacterial strains encased in a matrix that clings to biological or non-biological surfaces and interacts by releasing chemicals [(5).]

The treatment of biofilm-associated acute and chronic infections, such as hospital acquired pneumonia, catheter-associated infections, surgical site infections, etc., is hampered by the global increase in antibiotic resistance. This is because bacteria living in biofilms demonstrate considerably higher pattern of adaptive resistance to antibiotics and other disinfectants than bacteria living in planktonic compartments. On the other hand, improper use of antibiotics also aided in the emergence of drug resistance, which could exacerbate bacterial infection-related illnesses. Therefore, there is a critical need for new compounds that directly inhibit and/or eliminate biofilms as an alternative to conventional antibiotics [(6).]

By disabling current drug resistance mechanisms such as destroying biofilm formation and preventing the creation of biomolecules, nanoparticles have an antibacterial nature. The ability of copper-containing NPs to function as antimicrobial coating agents, inhibit the growth of MDR biofilms, and be effective against bacteria has been demonstrated [(7).]

Since the 19th century, copper oxide (CuO) nanoparticles—along with other inorganic metal oxide nanomaterials—have been widely employed as a secure, straightforward, and efficient antimicrobial agent. Due to their less toxic, environmentally friendly makeup, the US Environmental Protection Agency has approved CuO nanoparticles for use on people [(8).]

The capacity of metals to target many areas in an organism makes them preferable to traditional antibiotics [(9).]

Bacterial Biofilms; Formation to Dissemination:

Most sessile communities arising from bacteria are now recognized to be able to produce irreversible biofilms on surfaces and interfaces by deeply enmeshing themselves in self-produced polymer matrices [(10)].

According to ***Neethirajan et al.*** environmental factors and a sequence of alterations in planktonic cells are key factors in the mechanism of biofilm development [(11)]. According to ***Kostakioti et al.*** there are five primary steps that are thought to occur during biofilm formation: (I) adhesion, (II) microcolony creation, (III) three-dimensional biofilm formation, (IV) maturation, and (V) dispersion [(12).]

The first stage of biofilm development entails surface preconditioning, the adhesion of macromolecules, and the creation of a conditioning layer within seconds of surface exposure. The unfolding of cell surface structures causes

the perfusion of a polysaccharide slime, which attracts cells and debris, and strong chemical bindings to the matrix polymer promote bacterial adhesion and co-adhesion during the second stage. In the third stage, a 3D biofilm with a much higher thickness develops as a result of the nutrient-rich biofilm environment promoting fast bacterial growth. When biofilm thickness increases, the fourth maturation stage, which is connected to antibiotic resistance, is accomplished. In the final stage, due to dynamic flow of the biofilm matrix, bacteria separate, either actively or passively, and enter the surrounding environment as planktonic cells on a routine basis. Separated biofilm clusters or fluid-driven cell groups are further ways that detached cells can spread to new surfaces. Additionally, bacteria from biofilm communities spread to form new sessile populations in new sites [10].

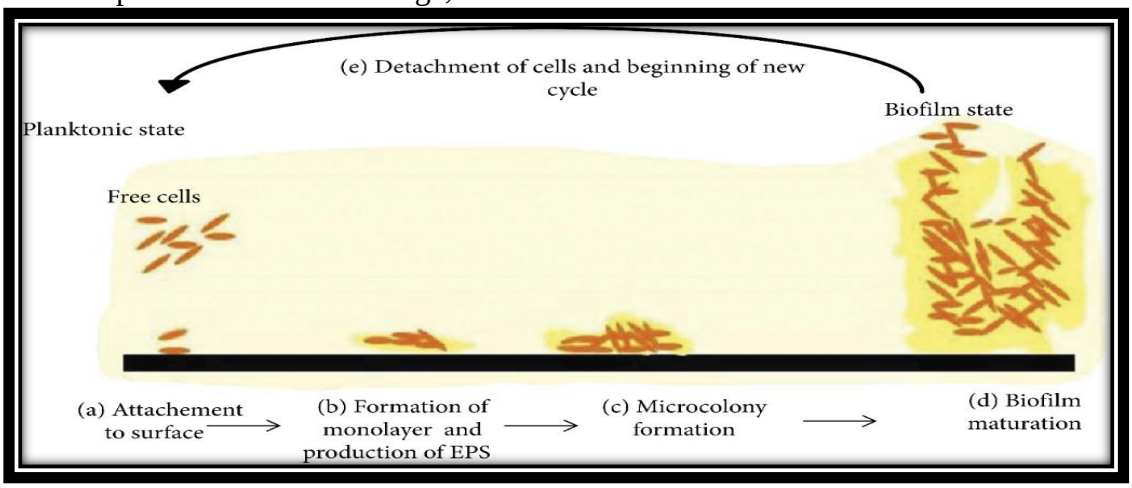

Fig. 1: Stages of biofilm formation (13)

Copper oxide nanoparticles (CuO NPs):

The ability to manipulate structures at the atomic scale allows for the creation of nanomaterials [14]. Nanometer size materials have been used for many biomedical applications due to the increased reactivities provided by their big surface to volume ratios and ability to modify their physical and chemical properties. In fact, the use of nanotechnology in medicine has given rise to a brand-new discipline called "nanomedicine," which has already developed cutting-edge cures for a variety of diseases. Currently, developing nanomaterials is seen to be a promising approach for managing or treating pathogenic biofilms [15] [16].

Today, it has been established that using antibiotics to treat biofilms is futile since many compounds miss the target cells that are deeply embedded in the biofilm matrix. To control the diseases caused by biofilms, a different strategy is required [17]. A number of approaches were considered to address the ineffectiveness of antibiotics, including the use of bacteriophages and the production of semi-synthetic analogues of natural items to inhibit bacterial biofilms. Contrary to the usual approaches previously shown, the nanotechnology-based strategy is one of the most effective ways to prevent the creation of biofilms. Due to their antimicrobial characteristics, nanoparticles have been suggested as a feasible strategy to combat infections. The versatile potential of nanoparticles as

antimicrobial agents is explained in a number of papers [9].

Nanoparticles are thought to be a viable strategy for treating bacterial biofilms. This is attributed to the fact that NPs are not targets of antibiotic resistance mechanisms [14]. The most promising and well-studied NPs are metal oxide nanoparticles, such CuO, TiO2, ZnO, and Fe3O4. It has been demonstrated that several metal oxide NPs display biological features that are far more favorable than those of the parent metals NPs'. The metal oxide NPs attracted the most attention from scientists as a result [18] [19].

The way that metal oxide nanoparticles affect plankton cells and biofilm is as follows:

Reactive oxygen species (ROS), structural damage to the cell wall caused by electrostatic interaction, oxidative stress brought on by the production of ROS, and disruption of protein functions and cell structures brought on by metal cation release are the three main mechanisms identified by ***Wang et al.*** as the means by which NPs exert their antibacterial effects [14] (fig 2).

Because metal oxide nanoparticles have a positively charged surface, negatively charged bacteria are more likely to stick to them. Through electrostatic and Van der Waals interactions, metal oxide nanoparticles bind to the cell wall, specifically to cell membrane proteins that impair bacterial activity [20].

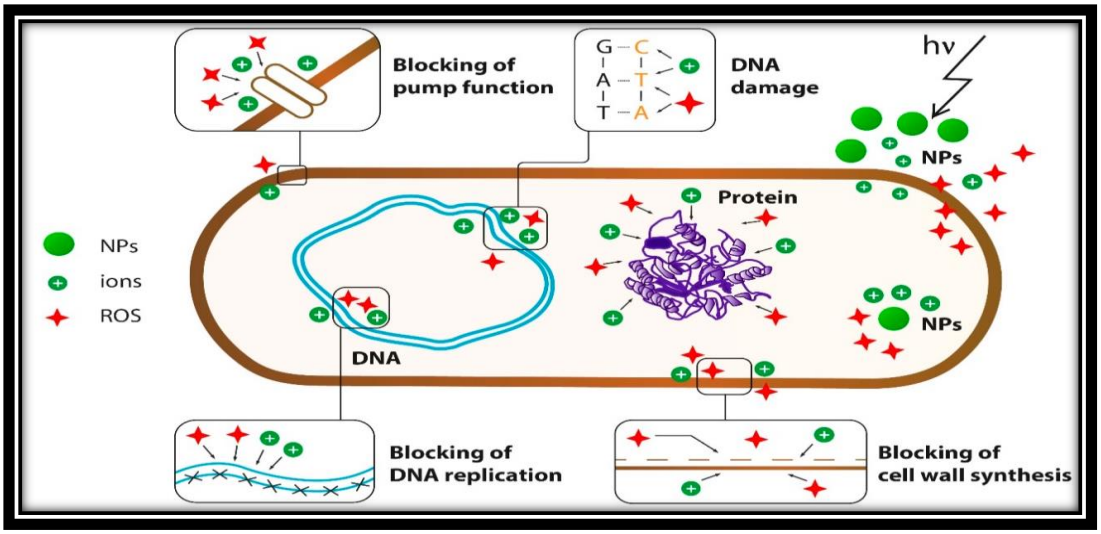

Fig. 2: Actions of metal oxide nanoparticles on bacterial cell.
Brown line: cell surface (cell wall and membrane), blue line: DNA, and arrow: electromagnetic irradiation [21]

An inorganic substance known as copper oxide can be found naturally as the crystalline mineral. It has a chemical formula of CuO and is one of the stable copper oxides. Its color is blackish. Researchers are particularly interested in copper oxide nanoparticles due to their accessible availability, low cost, and minimal toxicity [22].

Many researchers have given CuO-NPs a lot of attention due to their chemical and biological properties, which can be attributed to their morphology [23] [24]. CuO-NPs have been used in numerous biological applications as drug delivery and anticancer, antifungal, antioxidant, and antibacterial activities ([25] [26].

Copper-containing NPs have been demonstrated to have antibacterial activity, to inhibit the growth of MDR biofilms, and to function as antimicrobial coating agents. CuO NPs slit a hole in the cell membrane, making it easier for foreign substances to enter the cell [(7)]. According to ***Su et al 's*** investigation, when CuO NPs enter bacteria, metabolic processes like active transport, electron transfer, and nitrogen metabolism are disrupted [(27)]

CuO NPs successfully suppressed the growth of Methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* biofilms. According to ***Agarwala et al***. virtually all MRSA and *E. coli* biofilm cells perished four days after being exposed to CuO NPs [(28).] On glass, acrylic dentures, and cultured human epithelial cells as models, CuO NPs with 50 g/mL concentration greatly suppressed the development of total oral bacteria, extracellular polysaccharide synthesis, and biofilm creation. Additionally, it has been demonstrated that after 24 and 72 hours of incubation, CuO NPs have an inhibitory concentration of 125 and 250 g/mL on *Ralstonia solanacearum* biofilms. Therefore, it is evident that CuO NPs have the potential to be effective against biofilms, as demonstrated in a variety of bacterial groups [(29)]

The activity of the biosynthesized CuO NPs from *C. fistula* and *M. azadarech* against *K. pneumonia* biofilm formation was studied. The MIC, higher-MIC value, and sub-MIC values were used. The results revealed that the biosynthesized CuO NPs from *C. fistula* and *M. azadarech* were able to inhibit the biofilm formation by *K. pneumonia* at the MIC value (1 µg/ml) by 99.8% and 92.5% respectively. Nevertheless, once the concentration of the CuO NPs started to decrease, the inhibition percentage of the biofilm also decreased [(30).]

Agarwala et al. evaluated the efficiency of CuO nanoparticles to inhibit biofilm formation by *E. coli* at different concentrations (4MIC to 1/32-MIC). The results clearly revealed that inhibition of biofilm formation was concentration dependent. From 2MIC to 1/2MIC of CuO nanoparticles, biofilm was almost completely eradicated as the OD_{545nm} of biofilm was <0.1 [(28).]

A study by ***Shehabeldine et al***. investigated the effect of CuONPs on biofilm formation by *Escherichia coli*. The minimum biofilm inhibition concentration (MBIC) result was more evident, that the CuONPs have excellent anti-biofilm activity at sub-MIC levels reducing biofilm formation by 59% against *Escherichia coli* [(31).]

Desai et al. investigated the inhibitory effect of nanoscale CuO structures on *P. aeruginosa* biofilm formation by the samples S1, S2, S3 with various concentrations (500, 250, and 125 µg/-ml). The results revealed that S1 showed remarkable 80% antibiofilm activity at 125 µg/mL concentration as compared to control, while S2 and S3 showed inhibition in the biofilm formation only up to 40% at higher concentration (500 µg/mL) [(32)].

Conclusion:

Antibiotic resistance is now growing alarmingly quickly, necessitating immediate intervention**.** The alarming health crisis of antibiotic resistance is unquestionably caused by the indiscriminate and inappropriate use of antibiotics. Only the appropriate genotype may flourish in this circumstance due to selective pressures, which leads to the emergence of MDR strains and emphasizes a need for new therapeutic approaches.

Timely, during the past few years, the evolution of increasingly ferocious and resistant types of bacteria has overtaken the discovery of new antibiotics.

Of the several approaches being considered to address this issue, NPs appear to have the most promise. The antibacterial and biocidal characteristics of metal oxide nanoparticles, such copper oxide (CuO), have drawn attention, and they may be employed in numerous biological applications. CuO NPs promote the breakdown of existing biofilms and inhibit the development of new ones. The toxicity of copper ions for biofilm and plankton cells is the main cause of it.

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