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Antibiofilm Potential of Metal Based Nanoparticles: Synthesis and Mode of Action

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ABSTRACT

Biofilm refers to a group of microbes colonizing together and often adhered to a surface. The adherence is attributed to secretion of polymeric substances comprising of extracellular DNA, proteins, and polysaccharides thereby limiting the access and inhibitory activity of existing antimicrobial agents. Biofilm are a major cause of acute infections and pose immense clinical threat especially in conditions employing the use of invasive devices thus being a major source of mortality and morbidity. Hence there is a dire need to develop alternative treatment against biofilm related infections. Advances in nanotechnology has opened new horizons. Nanoparticles derived from various metal present promising candidates to ameliorate biofilms owing to their antioxidant potential.

1. Introduction

Biofilm is constituted by a colony of microbes that stick together and often attach to a surface. The attachment to biotic or abiotic surface is due to the production of the polymeric substance comprising of extracellular DNA, proteins, and polysaccharides (Donlan, 2002). Biofilm is initiated by adherence of free floating microorganisms to a substratum, if not removed early they anchor permanently by cell adhesion structures such as pili (Fux *et. al*., 2005). Hydrophobicity contributes immensely in formation of the biofilm by reducing the repulsion between the extracellular surface and microorganism. Cell-cell talk referred to as sensing has been known to be implicated in development of biofilms (Sakuragi and Kolter, 2007). A number of microorganisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia*

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coli, Candida albicans, Acinetobacter baumanii etc. produce biofilms. Biofilm formation occurs in stages including intial attachment, irreversible attachment, maturation and dispersal.

Biofilms provide a shield to the parent organisms thus limiting the penetration of antimicrobial compounds and in turn promoting resistance to the same. Biofilms pose a major hazard in clinical conditions involving the use of invasive devices like catheters, prosthetic joints, dental implants etc.that provide an ideal surface for their adherence and growth. The immune response of the affected person also faces a setback. Antibodies produced as a defence mechanism fail to penetrate owing to matrix binding catalase secreted by biofilm that protect colonized bacteria and inhibit the access to hydrogen peroxide into the film. Other methods of resistance encompass the secretion of enzymes by biofilm that alter or breakdown the antibiotics like lactamases and aminoglycosides, change of cell constituents including cell wall as observed in vancomycin resistance.

Impaired antibiotic permeation, nutrient limitation, decreased growth and birth of persister population constitutes the multipronged defense (Stewart, 2002). The presence of efflux pumps impart resistance to several antibiotics classes like tetracycline, betalactams and fluoroquinoles (Sara, 1999).

Various biofilm promoting factors are being researched upon to ameliorate the harmful effect of these clinical biofilms. Biofilm development is controlled by the intracellular adhesion molecule that aids in intercellular adhesion and is a product of icagene (Cramton et. al. 1999). N-acetyl glucosamine-1-phosphate acetyl transferase needed for peptidoglycan, lipopolysaccharide formation. Another target includes Dispersin B, a glycoside hydrolase that cleaves b1®6 N-acetyl glucosamine polymers and is effective against *S.aureus,S. epidermalis* (Sveltana *et. al.* 2012). Chelating agents such as sodium citrate, EDTA are useful to ameliorate *S.aureus and P.aeruginosa* biofilms (Robert and Rodney, 2011). The plant defensins, lytic peptide, anti-adhesion agents such as pillicides are known to play a role in biofilm inhibition.

Another promising strategy against sessile biofilm forming microbes is nanoprticles owing to their their smaller size, high surface area and hence increased penetration potential. Nanoparticles are microscopic particles with at least one dimension less than 100nm. This provides a tremendous driving force for diffusion across the calyx, a property that can be exploited for access through biofilms.The charge of nanoparticles appears to be an essential factor regulating the permeation of nanoparticles (Mahmood *et. al*., 2013). Among the various types of nanoparticles, metals derivatized into nanoparticulate forms are emerging as potential biofilm scavengers beacuse of high surface area, being stable at increased temperatures and translocation into the cells, etc.

Increased levels of metals ions in a microbial cell leads to oxidative stress and generate hydrogen peroxide, resulting in oxidative damage, decrease in the membrane integrity of microbes, causing leaking out of important cell nutrients, promoting desiccation and subsequent cell death. Metal nanoparticles can bind to protein, in biofilms causing function loss of the bacterial protein, its degradation into non functional moiety. Different metals have been exploited for the formation of nanoparticles, the prominent ones are listed below:

1.1 Zinc Nanoparticles

Zinc is a shiny bluish-white colored metal. It is important for all life on Earth, and helps in functioning of many enzymes. Zinc nanoparticles are gaining huge attention owing to their antimicrobial properties, anticorrosive nature, thermal and mechanical stability. *Pseudomonas aeruginosa bacterium* secretes numerous virulence factors, and hence develops biofilms that are resistant to antimicrobial agents as

compared to free floating cells. Thirty-six metal ions were evaluated to check the biofilm scavenging potential. ZnO nanoparticles showed inhibition biofilm formation and by limiting the realease of antivirulence factor pyrocyanin (Lee *et. al*., 2014). Silver and zinc nanoparticles exhibit actibacterial activity against *Vibrio cholera* and *E.coli* (Salem *et. al*., 2015). Biofilm disruption and antibacterial nature of zinc oxide nanoparticles was also confirmed against the oral biofilm forming microbes *Rothia dentocariosa* and *Rothia mucilaginosa* both in planktonic and sessile pathogens (Khan *et. al*., 2014). ZnO NPs can break bacterial cell membrane, decrease hydrophobicity and reduce the activity of the transcription genes responsible for oxidative stress-resistance in bacteria (Pati *et. al*., 2014).

1.2 Copper Nanoparticles

Copper is required by all living organisms as a trace **dietary mineral.** It is an important component of the respiratory enzyme complex, **cytochrome c oxidase.** The biofilm amelioration potential of copper nanoparticles have been evaluated for *P.aeruginosa* and *Listeria monocytogenes (*Ghasemian *et. al*., 2015). The biofilm disruption potential of copper nanoparticles (CuNPs) is dependent on the synthesis route and process parameters. The CuNPs can be employed as coating agents on invasive devices to disallow the devlopment of biofilms. The biofilm scavanging activity of copper nanoparticles (CuNPs) synthesized against *P. aeruginosa* showed that CuNP treatments resulted in inhibition in biofilm, cell surface hydrophobicity and decreased the content of exopolysaccharides respectively, without bactericidal activity (Lewis *et. al*., 2015). The Copper particles can also be fabricated into nanofibers which has the potential to inhibit *P.aeruginosa* PA01 and *S. aureus* to form biofilms (Ahire *et. al*., 2016).

1.3 Tellurium and Selenium Nanoparticles

Tellurium (Te) and Selenium (Se) belong chemically to the VIa group of elements. The selenium nanoparticles enhance efficacy of glutathione peroxidase and thioredoxin reductase (Wang *et. al*., 2007). Selenium oxide and Tellurium oxide nanoparticles produced by selenite- and tellurite-reducing bacterial strains, from polluted locations were shown to have antimicrobial and biofilm inhibition activity against *E.coli*, *P.aeruginosa* and *S.aureus* (Emanuele *et. al*., 2015). In particular, Se0 nanoparticles showed antimicrobial potential at quite low concentrations, compared to selenite. Antimicrobial activity of Selenium and Tellurium nanoparticles can be attributed to the formation of reactive oxygen species in biofilm forming bacteria (Zhang *et. al*., 2004). The activity was inversely proportional to the size of the nanoparticles.

1.4 Silver Nanoparticles

Silver is a soft white lusturous transistion metal. Silver is most extensively studied and is found to be effective against numerous biofilm forming microorganisms (Anna *et. al*., 2013). Bacterial biofilms pose a major obstcale to wound healing. The predominant species isolated in these infections are *S. epidermidis* and *S.aureus*. Biofilm forming staphylococci usually colonise catheters and medical devices and are a cuase of infections. Anti-biofilm potential of silver nanoparticles against isolated from wounds were shown to kill local bacteria, without causing damage to the host tissue. In nano size range, the properties of metals differ significantly as compared to parent metal of same amount, mainly due to high surface area and reactivity, causing enhanced bioavailability (Ansari *et. al*., 2015). Silver ions have been

depicted to interact with the thiol group in enzymes and deactivate them causing microbial cell death, they are known to bind with DNA to increase pyrimidine dimerization through photodynamic reaction and hence inhibit DNA replication.These nanoparticles hold the ability to counter multidrug resistance which is a big problem in chemotherapy. The silver nanoparticles have also been found to play a role in inhibition of adhesive compounds, thus inhibiting biofilm formation.

1.5 Methods for synthesis of Metal Nanoparticles

Methods employed for the formulation of nanoparticulate metals can be clssified as physical, chemical and biological processes respectively (Iravani *et. al*., 2014).

Physical methods: Physical methods employ energy like heat, light, ultrasonic waves etc. for the formulation of metal nanoparticles. "Thermolysis" is one of the popular methods for the synthesis accomplished by treating the metal precursors at elevated temperatures alongwith a stabilizing compound. The nanoparticles show an increase in size relating to the temperature rise owing to removal of stabilizing molecules, leading to a greater aggregation of the particles. The synthesis of nanoparticles can be executed by employing ultrasound. This method reduces the corresponding metal salts. By harnessing high frequency ultrasound, formulated nanoparticles are dispersed in a polymer matrix. Another physical method popularly used is the "photochemical" technique in which light pulses are used for the development of nanoparticles. These synthesis methods offer the benefit of being simple and fast, and nanoparticles are formed with a variety of shapes and chemical natures. Super paramagnetic iron oxide nanoparticles (SPION) having discrete surface properties have been explored for diverse *in vivo* applications like magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, hyperthermia, drug delivery and in cell separation, etc. Magnetic nanoparticles can interact with drugs, proteins, enzymes, antibodies, or nucleotides and can be mediated to an organ, tissue, or tumour using an extrinsic magnetic field or can be heated in alternating magnetic fields for application in hyperthermia (Kumar and Gupta, 2005) . Similarly Aluminimum nanoparticles can also be formulated by physical techniques like solid phase, liquid-phase and gas-phase processes and offer wide applications in various fields (Reza and Horbani, 2014).

Chemical methods: In chemical synthesis, the synthesis mechanism is due to the reduction of the metal salt to the respective metal atoms, these atoms then form a nucleation center guiding the development of atomic clusters, with stabilizing molecules around them that inhibit the aggregation of atoms. The advantages of chemical synthesis include reproducibility, availability of reactives and lesser monetary input, but these methods require preparation and long-times to set up the experimental conditions.The gold nanoparticles have been synthesized by by reduction by citrate and ascorbic acid (Kimling *et. al*., 2006). The silver nanoparticles with controlled sizes have been produced by reducation of $[Ag(NH_3)_2]^+$ complex cation by employing; sugars like glucose, galactose, maltose and lactose. These nanoparticles were synthesized at various ammonia concentrations and pH conditions producing a spectrum of particle sizes ranging from 25 to 450 nm (Panacek *et. al*., 2006).

Biological methods: There is an ever increasing requirement to develop eco-friendly methods, which minimize the application of non-biodegrable chemical compunds in the synthesis. Green synthesis approaches offer the benefits over traditional methods employing chemical agents posing a threat to the environment. The biologically synthesized nanoparticles synthesized biologically by employing metals

exhibit enhanced. Selection of eco-friendly nontoxic reducing and stabilizing agents are very important in green synthesis of nanoparticles.There are reports for the synthesis of metallic nanoparticles using plant extract (Ahmed *et. al*., 2016). Silver nanoparticles formulated using leaf extract of *Azadirchata indica* have been shown to exhibit biofilm inhibition of clinical isolate for *S. aureus*. Further it has also been shown that the selenium nanoparticles also have been produced by probiotic lactic bacteria (Verma, 2015). The advantage of this method is that it is cost effective and requires low maintainence. The biological methods offer low cost, environment compatible, low toxicity alternatives to synthesize nanoparticulate metals.

2. Conclusions

In recent past, the occurence of antibiotic resistance has emerged as a serious threat. The situation is significantly serious in treating the biofilm-associated infections, due to the fact that biofilm mode microbes are more resistant to antibiotics as compared to planktonic ones. Hence, its is all the more important to develop novel antimicrobial agents having bactericidal activity.

Presently the use of metal ions and metal nanoparticles has developed as a substitute to the organic compounds as antimicrobial agents. A strong antimicrobial activity is usually associated with nanomaterials, primarily because of the high surface to volume ratio of their constituent particles. This implies a newer application for these nanoparticles as coating agents in medical devices indewelling devices prevent bacterial infections. Moreover, they can find promising applications also in industrial settings as a potential tool to overcome biofouling. Presently the major drawback in the use of metal nanoparticles is the high cost associated with their synthesis. Consequently, a increasing interest has developed in using new eco-friendly processes for the synthesis of metal nanoparticles as biofilm scavangers offers dual benefits of higher penetration and antimicrobial potential.

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