Antimicrobial and antibiofilm activities of green synthesized silver nanoparticles for water treatment

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Abstract. Nanoparticles have found applications in wide areas due to their unique properties. Green synthesis of nanoparticles is an ecofriendly, safe and non-toxic approach that solve the problems associate with conventional methods. Waterborne pathogenic diseases have resulted in millions of deaths in humans especially children annually and infectious microorganism associated with water have all shown multiple resistance to conventional treatments. Bacteria biofilm accounts for majority of microbial infections and are more resistant to antimicrobial agents. The use of antibiotics is ineffective for treating biofilm-associated infections. Green synthesized silver nanoparticles (AgNps) have been reported to have important antimicrobial applications over a wide range of microorganisms, although many researchers have demonstrated in in vitro the antimicrobial activity of AgNps, few researches have been conducted on the exact mechanism of action of AgNps as antibiofilm, the possibility of infectious microbes to develop resistance and the toxicity limit of silver suitable for human consumption. This review discussed biocompatibility of green synthesis AgNps, the concept of biofilm formation, the impacts of AgNps water microbial environment, its mechanisms of action as antimicrobial agent in water treatment and the potential human toxicity.

Keyword: Silver nanoparticles (AgNps), Waterborne pathogenic diseases, Water treatment, Antimicrobial and Antibiofilm.

1. Introduction

The united nation (UN) under the 17 sustainable development goals aims to ensure fair access to safe, affordable water, and improved water quality by reducing contaminants. To achieve this, nanoparticles in...
water treatment have gained a lot of attention from researchers in the past few years as an alternative for the conventional methods used in water disinfection such as chlorination, ozonation, ultraviolet treatment, reverse osmosis and application of silver catalyst, etc. These conventional methods have varieties of unpleasant effects on treated water such as Carcinogenic by-products, ineffectiveness on highly resistant pathogens, high-cost commitment, reinfection in the distribution network, etc. [1-2]. Nanomaterials have found extensive application in water treatment and environmental remediation due to their ability to remove different contaminants such as heavy metals, nitrates, metal ions, organic pollutants, inorganic anions, and pathogens. With the increasing use of nanoparticles in the treatment of water, there is developing concern about the biological characteristics, environmental safety and health risks associated to their production. Many approaches have been developed to synthesize the particles such as different physical techniques such as evaporation-condensation, and chemical processes such as chemical reduction. These approaches require harmful chemicals that may adverse effects on the environment and living organisms, much cost requirements and large energy consuming. To overcome these disadvantages associated with physical and chemical processes, highly efficient, cost-effective, and eco-friendly biological approaches are established. In recent years, Green synthesized silver nanoparticles have been reported to have important antimicrobial applications over a wide range of microorganisms. However, while researchers are developing interest in the application of nanomaterials in water purification processes, the materials have been discovered to be potentially toxic to human. Regrettably, only a few researches have been carried out their human cytotoxicity to date [3], [4].

2. Methodology

2.1 Green synthesized silver nanoparticles and its mechanism

The major techniques utilized for synthesizing nanoparticles (Nps) are Top-down and Bottom-up. Top-down technique synthesizes nanoparticles using the various physical techniques available to reduce the bulk material into particles of nanoscale and Bottom-up technique synthesizes nanoparticles using chemical and biological methods to self-assembled atoms to new nuclei which grow into a particle of a nano size ($10^{-9}$). Green synthesis of silver nanoparticles involves using biological material to synthesize nano silver and different biological materials such as bacteria, fungi, yeast, plant phytochemicals, cobweb, honey, microbial enzymes to mention a few have been utilized by researches to synthesize metal nanoparticles and a number of parameters affect the properties of the synthesized nanoparticles which include the concentration of the reactants, mix ratio, pH value of the solution, reaction kinetics, solvent chemistry, incubation temperature, and time etc. Different sizes and structures of nanoparticles can be synthesized for various purposes when these parameters are varied and maneuvered. [5], [6].

Table 1: Represents most recent honey mediated AgNps.

| Sr. No | biological Species | Precursor | Reaction Temperature | Solvent | Size       | Morphology | Ref. |
|--------|-------------------|-----------|----------------------|---------|------------|------------|------|
| 1.     | Natural honey     | AgNO$_3$  | Room temperature     | distilled water | 200-900nm | -          | 7    |
| 2.     | Honey Z. spina-christi | AgNO$_3$  | -                    | distilled water | 51.3nm    | Spherical  | 8    |
| 3.     | Honey A. gerrardii | AgNO$_3$  | -                    | distilled water | 79.1nm    | Spherical  | 9    |
| 4.     | Black seed        | AgNO$_3$  | -                    | distilled     | 25-70nm    | Spherical  | 9    |
Researchers have documented detailed studies on the numerous methods of green synthesis using the aforementioned materials as presented in Table 1. In Green synthesis of silver nanoparticles, appropriate biological material which may be in the form of enzymes or phytochemicals, with the ability to acts as bioreducing and capping/stabilizing agents induced the reduction of metal salt solution to its metallic nanoparticles. The reaction usually occurs at ambient temperature and the mixing ratio is a small quantity of bioreducing agents to a large volume of the metal salt solution of minimum concentration which usually for the biosynthesis of AgNps is 1:40 or 1:10 [13]. The formation is a three stages process that involves the reduction of silver ion (metal ion) to their nanoparticles, growth phase (aggregation of nanoparticles), and stabilization of nanoparticles formed to control growth rate/size when stored. The biomanufactured nanoparticle can be monitored and characterized by UV–Visible absorption spectroscopy, FTIR, XRD, SEM, X-ray diffraction, and TEM analysis [14] as presented in figure 1. UV-visible spectroscopy monitors the bioreduction of Ag ion to Ag⁰ and confirms the manufacture of nanoparticles, FTIR investigates the functional groups, SEM analyses the morphology and size and TEM gives a confirmatory image of the shape of the nanoparticle. Consequently, the shape and size of nanoparticles in aqueous suspension can be assessed.
2.2 Pathogens in water and need for remediation

Plants and animals cannot live without fresh water because most organisms are made up mostly by water. Water is known as a “universal solvent” that can dissolve more substances than other solvents on earth and consequently it is easily polluted with toxic substances. There are hundreds of substances reported as pollutants in freshwater and major water pollutants classes includes inorganic pollutants (acids, salts, and toxic metals), nutrients pollutants (nitrates and phosphorus), organic pollutants (detergents, insecticides, oil, pesticides), radioactive pollutants and pathogens (bacteria, viruses, protozoa, and parasitic worms). Human and animals organic waste may be rich in pathogens, fecal contaminations of water can introduce a number of pathogens into waterways and common sources of fecal matters include inadequate on-site human waste disposal systems and animal wastes. Pathogens are the cause of water-borne diseases which have resulted in many outbreaks. This has led to millions of death in humans especially children and this threat has called for urgent remediation of water resources [15], [16].

2.3 Antimicrobial and antibiofilm agents

Biofilms consists of multiform microorganisms, which are embedded in the self-produced extracellular polymeric matrix. Bacteria in a biofilm can also survive severe conditions, resistant to antimicrobial agents, and survive the immune system of the host. Pathogenic microorganisms associated with water include bacteria such as Shigella dysenteritia, Escherichia coli, Vibrio cholera, Salmonella, Legionella spp., Aeromonas spp., Mycobacterium avium, viruses such as Norwalk virus and Rotavirus gastroenteritis and protozoan such as Entamoeba hystolitica, Giardia and cryptosporidium. It is reported that 10 to 20 million of total annual death worldwide is caused by waterborne pathogens which include diarrhea, gastrointestinal diseases, and systematic illness and of which most of these deaths are children, the trends in waterborne disease outbreaks in the USA and some other countries from 1920-2012 has been briefed [17], [18]. Biofilms are agglomerates of bacteria that attaches reversibly to a substrate and then produce polysaccharides, an extracellular polymeric binding molecules that lead to irreversible bond. After initiating colonization, the bacteria proliferate into colonies inside a matrix, resulting to the formation of a mature biofilm that is specialized by certain physiological changes like size, shape, division of labor, etc. the bacteria then become more resistant to antibacterial agents and the body’s immune system and also allow dispersal which enables bacteria to spread throughout the body and colonize new substratum, reinitiating the biofilm lifecycle. Biofilms have severe effects on health and account for over 80 percent of microbial diseases in the body. Moreover, biofilms do not respond well to common antibiotics and may develop antibiotic resistance, biofilm-associated bacteria can resist antibiotics in the range 10 to 1,000 times than planktonic (free-living) bacteria and consequently, a novel of antimicrobial agents with improved activities are required. The major groups of antibiotics, currently in use, generally affect three bacterial targets: cell wall formation, protein bio-assembly, and DNA reproduction. Regrettably, there may
be resistance against each one of these mechanisms of action. The classification, strategies of action, target bacteria, and the limitations of the different antimicrobial and antibiofilm agents manufactured were also reviewed. The side effect of the highlighted treatment includes highly expensive cost and increased antibiotic resistance. Various mechanisms that contribute to bacterial tolerance and resistance have been discussed [19], [20].

Despite widespread use and efficiency, water antimicrobial and antibiofilm agents still pose a danger to living organisms and the environment. Consequently, there is a need for the investigation and production of novel agents that are biocompatible, safe, eco-friendly, and nontoxic. Nanotechnology has represented a new approach to fighting and eradicating microbes and biofilm-forming microorganisms using a method by which nontoxic and eco-friendly nanoparticles are used as either microbicides on its own or coupled with existing treatments.

2.4 Biosynthesized silver nanoparticles as antimicrobial and antibiofilm agents

Applications of AgNPs has extended and found as a useful nanotechnology in water treatment and environment remediation where biosynthesized AgNps are utilized to control microbial infection in water systems. Some silver nanoparticles have been synthesized from different origin of biological materials and employed as both reducing and capping agents have shown the tendency to act as antimicrobial agents. Silver nanoparticles have enhanced the antimicrobial activity when impregnated with filter paper, cotton fabric, and cellulose gels. Silver and some other metals to preserve water is not a new technique but has been in use for an age, and the antimicrobial feature of these metals are well known and silver nanoparticles have been used previously in water filtration applications [21]. Silver nanoparticles synthesized using T. longibrachiatum fungi isolate exhibited highly efficient antifungal activity of 76-99% inhibition zone against A. alternata, F. verticillioides, F.moniiliforme, A. flavus, A. heteromorphus, P. glabrum, P. brevicompectum, P. grisea, and H. oryzae phytofungi [22]. The coating of Pencillium citreorignum and Scopulaiosos brumptii-AgNps with foam has powerful bactericidal action on both gram-positive and gram-negative bacteria, exhibited potent antibacterial property on E. coli (17 and 15 mm), S. aureus (25 and 17 mm) and P. aeruginosa (17 and 17mm) respectively and the filtrated water silver concentration did not exceed WHO guideline limits (<0.10 mg/l) as presented in Table 2[23]. Bacteria mediated silver nanoparticles using Aeromonas sp. revealed good antimicrobial activity against C. albicans (20+0.1mm), P. aeruginosa (16+0.1), V. parahaemolyticus (16+0.1), S. aureus (15.5+0.5mm), C. tropicalis (15+0.5mm), B. cereus (13.5+0.5mm), B. subtilis (13 +1.0mm), E. coli. (13+0.2mm), and S. enterica (11+0.2mm) and enhanced the performance of antibiotics when the antibiotics discs were impregnated with silver nanoparticles [24]. Atrocarpus atilis leaf extract mediated nanosilver showed higher sensitivity to E. coli and P. aeruginosa, reasonable sensitivity towards S. aureus towards the AgNPs and minimal level of sensitivity towards the fungus A. vesicolor [25]. The growth of a Gram-positive bacteria isolated from well water and Escherichia coli was inhibited by silver nanoparticles biomanufactured from Azadirachta indica leave extract in a dose sensitive manner [26]. A. vera, P. oleracea and C. dactylon mediated AgNps exhibited antibacterial activity against B. subtilis, B. cereus, S. aureus, Enterococcus faecalis, S. typhi, Shigella sp., E. coli, P. aeruginosa and A. baumannii at concentrations 50, 5 and 0.5µg/ml and the average zone of inhibition value varies among the bacterial species and Gram-positive bacteria was more sensitive to the bactericidal action as presented in Table 2[27]. It is clearly evident that silver nanoparticles influence the water microbial environment, silver nanoparticles have sound inhibitory effects on the growth of bacteria, fungi, virus, etc. because of their size and form. Moreover, it seems that bacteria have minimum tendency to develop resistance against Ag than against commonly used antibiotics. However, numerous opposing opinion have been raised on the possibility of microbes to develop resistant strains, whether silver is effective against biofilm and the
bactericidal mechanisms of AgNps, the harmful effects of Ag on humans, and the determination of the toxic level is not fully understood. However, regardless of the contrasting opinion, AgNps are definitely very promising as an antimicrobial Metal Nanoparticles. More so, AgNp antimicrobial property supersedes that of their bulk material.

Table 2: Inhibition zone of AgNps against three pathogenic bacteria via agar well diffusion method

| Biomaterial                  | Conc.     | Zone of inhibition(mm) | Ref. |
|------------------------------|-----------|-------------------------|------|
|                              |           | S. aureus | P. aeruginosa | E. coli |
| *Penicillium Citreonigum*    | 200 g/ml  | 13        | 13           | 13 | 23 |
|                              | 300 g/ml  | 15        | 15           | 15 | 23 |
|                              | 500 g/ml  | 25        | 17           | 17 | 23 |
| *Scopulaopsos brumptii Salvanet* | 200 g/ml | 12        | 13           | 13 | 23 |
|                              | 300 g/ml  | 15        | 15           | 14 | 23 |
|                              | 500 g/ml  | 17        | 17           | 15 | 23 |
| *Aloe vera*                  | 50µg/ml   | 18.1      | 6.4          | 6.0 | 27 |
|                              | 5µg/ml    | 16.3      | 6.0          | 3.8 | 27 |
|                              | 0.5µg/ml  | 13.4      | 3.3          | 3.4 | 27 |
| *Portulaca oleracea*         | 50µg/ml   | 17.7      | 5.0          | 4.9 | 27 |
|                              | 5µg/ml    | 14.8      | 4.8          | 3.2 | 27 |
|                              | 0.5µg/ml  | 12.8      | 3.0          | 2.8 | 27 |
| *Cynodon dactylon*           | 50µg/ml   | 16.5      | 6.0          | 6.0 | 27 |
|                              | 5µg/ml    | 13.3      | 5.6          | 4.5 | 27 |
|                              | 0.5µg/ml  | 13.0      | 3.0          | 3.0 | 27 |

2.5 Proposed mechanism of action for microbicidal by silver nanoparticles

Nanosilver may have the sufficient capacity to deform lipopolysaccharide molecules, aggregate inside the membrane creating hole, and cause abundant increment in membrane permeability as observed by [28] and consequently induce cell lysis; to date, many researchers have postulated several mechanisms for the antimicrobial action of AgNps. Two mechanisms were proposed: (1) Bactericidal effect in which AgNps directly acts on bacteria and releases Ag⁺ which adheres to its negatively charged cell wall, resulting to a change in the cell wall permeability and together with protein denaturation, induces cell lysis and death or (2) Bacteriostatic effect in which AgNps releases Ag⁺ which interferes with the bacterial growth by inhibiting the cells ability to replicate. In addition to the mechanisms, the Nps may be capable to prevent the activities of the bacteria by DNA modification and causing abnormalities to sensitive cells [29]. The occurrence of the two mechanisms simultaneously leads to pronounced antimicrobial activities as observed in most researches. However, the mechanism of action of AgNps as an antimicrobial agent in water treatment has received minimum interest from researchers.

2.6 Cytotoxicity of green silver nanoparticles based antimicrobial and antibiofilm agents

Green synthesized silver nanoparticles have important antimicrobial applications over a wide range of microorganisms. However, while researchers are developing interest in the application of nanomaterials in
water purification processes, the materials have been discovered to be potentially toxic to human. Regrettably, only a few researches have been conducted on their human cytotoxicity to date. [30] reported that AgNps cause mitochondrial damage, induce apoptosis, and cell death. In vitro study of AgNps in normal human epithelial cells showed size-dependent cytotoxicity where 10nM AgNps is cytotoxic for human lung cells, induce DNA damage, no cellular ROS increase upon exposure to AgNps, AgNps are readily taken up by human lung cells via active mechanisms and that small AgNps release more Ag in the biological medium, silver induced toxicity on bone cells have been investigated and showed that 50nm AgNps have strong cytotoxic effects on osteoblasts and osteoclasts, another study also reported that AgNps of less than 20nm increased cytotoxicity in human periodontal fibroblasts in a dose and time-dependent manner [31], [32], [33], [34]. Other related work on nanoparticles are linked to reference [35, 36].

3. Conclusion
The presence of pathogenic organisms in water is a major concern for environmental conservation and human health. The removal process of these microbes has not been fully delved into. Nanomaterials have found applications in wide areas due to their unique properties and the recent most explored approach is the biocompatible and cost effective green synthesis method. Green synthesized silver nanoparticles (AgNps) have important antimicrobial applications over a wide range of microorganisms, smaller AgNps is very effective against E. coli, S. aureus, P. aeruginosa and fungi at varied concentrations in a dose and time dependent manner showing potential as an effective antimicrobial agent, it has been found to inhibit bacteria biofilm formation signaling its antibiofilm activity. However, minimum work has been conducted on the exact mechanism of bactericide and despite the numerous potential effectiveness on water microbial environment, it is not completely nontoxic and the toxicity limit of silver suitable for human consumption may be compromised. Extensive research on the human cytotoxicity of nanosilver and the mechanism of action require urgent attention.

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Conflict of Interests
The authors declare that there is no conflict of interest.

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