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ORIGINAL ARTICLE

Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage

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Abstract The growing need of antimicrobial agent for novel therapies against multi-drug resistant bacteria has drawn researchers to green nanotechnology. Especially, eco-friendly biosynthesis of silver nanoparticles (Ag NPs) has shown its interesting impact against bacterial infection in laboratory research. In this study, a simple method was developed to form Ag NPs at room temperature, bioreduction of silver ions from silver nitrate salt by leaf extract from *Ocimum gratissimum*. The Ag NPs appear to be capped with plant proteins, but are otherwise highly crystalline and pure. The Ag NPs have a zeta potential of −15 mV, a hydrodynamic diameter of 31 nm with polydispersity index of 0.65, and dry sizes of 18 ± 3 nm and 16 ± 2 nm, based on scanning and transmission electron microscopy respectively. The minimum inhibitory concentration (MIC) of the Ag NPs against a multi-drug resistant Escherichia coli was 4 μg/mL and the minimum bactericidal concentration

Abbreviations: Ag NPs, Silver nanoparticles; DAD, Disk agar diffusion; DLS, Dynamic light scattering; EDX, Energy-dispersive X-ray; FTIR, Fourier transform infrared spectroscopy; MBC, Minimum bactericidal concentration; MIC, Minimum inhibitory concentration; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; PBS, Phosphate buffer saline; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; XRD, X-ray diffraction; Rh-B, Rhodamine B

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(MBC) was 8 μg/mL, while the MIC and MBC against a resistant strain of *Staphylococcus aureus* were slightly higher at 8 μg/mL and 16 μg/mL respectively. Further, the Ag NPs inhibited biofilm formation by both *Escherichia coli* and *S. aureus* at concentrations similar to the MIC for each strain. Treatment of *E. coli* and *S. aureus* with Ag NPs resulted in damage to the surface of the cells and the production of reactive oxygen species. Both mechanisms likely contribute to bacterial cell death. In summary, this new method appears promising for green biosynthesis of pure Ag NPs with potent antimicrobial activity.

1. Introduction

Different pathogenic bacteria and the antibiotic resistance by bacteria, have become a great challenge in current times. Several efforts are involved till now by the researchers in search of new antibacterial agents. In the present scenario, nano-scale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical complexities. Nanotechnology is a rapidly growing field for the purpose of manufacturing new materials at the nanoscale level. This area is expected to open a new platform to fight and prevent disease using nanomaterials. Among the most promising nanomaterials with antibacterial properties, metallic nanoparticles became the most interesting tool (Morones et al., 2005; Albrecht et al., 2006).

Silver nanoparticles have become the focus of extensive research due to its good antimicrobial efficacy against multidrug resistant bacteria, viruses and other eukaryotic microorganisms (Gong et al., 2007). Previous studies showed that Ag NPs are capable of performing effective antibacterial property against *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Morones et al., 2005; Moyer et al., 1965; Li et al., 2010). In medical treatment, silver nitrate is combined with sulfonamide to form silver sulfadiazine cream, which was used as a broad-spectrum antibacterial agent and was used for the treatment of burns. It is also highly effective against bacteria such as *E. coli*, *S. aureus*, *Klebsiella* sp., and *Pseudomonas* sp. and showed considerable antifungal and antiviral activities (Fox and Modak, 1974). These applications strongly depend on the physico-chemical properties of the produced Ag NPs such as particle size and shape, size distribution and the surface charge (Soukupov et al., 2008). Nanoparticles can be synthesized using various approaches including chemical, physical, and biological methods. Silver nanoparticles synthesized by chemical methods are toxic to different normal cells and lead to non-eco-friendly by-products which may disturb the normal cells. Ag NPs were found to exert strong acute toxic effects to various cultured cells. Only Ag NPs exposure, exhibited significant cytotoxicity at higher doses and induced abnormal cellular morphology, displaying cellular shrinkage and acquisition of an irregular shape (Kawata et al., 2009). So, an ever increasing need for environmentally friendly, non-toxic protocols for nanoparticle synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by-products. Different biological aspects for nanoparticles fabrications have been reported up to date which include bacteria (Dickson, 1999; Pum and Sleytr, 1999; Joerger et al., 2001; Nair and Pradeep, 2002), fungi (Mukherjee et al., 2001; Ahmad et al., 2003; Durán et al., 2005) and plants (Singhal et al., 2011; Huang et al., 2007; Leela and Vivekanandan, 2008; Singh et al., 2015). Due to the growing needs of eco-friendly nanoparticles, green methods are used for the synthesis of various metal nanoparticles. But recently, plant extract mediated nanoparticles fabrication proved as an advantageous way over other methods. Plant extracts mediated synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness (Awwad and Salem, 2012). The mechanisms of NPs inhibiting bacterial growth remain unclear. It has been reported that the size and shape of NPs could affect their antibacterial activity (Zhou et al., 2012). Studies suggested four mechanisms are hypothesized for antibacterial activity and these are firstly, accumulation and dissolution of nanoparticles in the bacterial membrane changing its permeability, with subsequent release of different intracellular biomolecules and dissipation of the proton motive force across the plasma membrane (Amro et al., 2000). Second is generation of reactive oxygen species (ROSs) in the cell by NPs, with subsequent oxidative damage to cellular structures (Applerot et al., 2012). Third is uptake of nanoparticles and/or metallic ions into cells, followed by depletion of intracellular ATP production, disruption of DNA replication and DNA damage and fourth is nanoparticles and its active ions which bind with different enzymes and inactivate them, resulting in arrest of cellular respiration (Morones et al., 2005; Raffi et al., 2008; Rai et al., 2009). The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria and form reactive oxygen species (ROS). The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds such as DNA. Ag NPs destabilize plasma membrane potential and depletion of levels of intracellular ATP by targeting bacterial membrane resulting in bacterial cell death (Raffi et al., 2008; Rai et al., 2009). So, recent studies suggested that generating reactive oxygen species, damaging cellular enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately lead to cell lysis and death.

The antibacterial activity of green synthesized Ag NPs against some drug-resistant bacteria has been established, but further investigation is needed to determine whether these particles could be an option for the treatment and prevention of drug-resistant microbial infections.

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