Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

Geno-toxic study of silver bio-nanoparticles toward Gram-positive and Gram-negative clinical isolates

*Lakshmipathy Muthukrishnan, Anima Nanda**

Department of Biomedical Engineering, Sathyabama University, Rajiv Gandhi Road, Chennai 600119, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 8 May 2013
Accepted 12 June 2013
Available online 30 July 2013

Keywords:
Antibacterial activity
Antimicrobial resistance
DNA
Geno-toxicity
SNPs

ABSTRACT

Background: In recent years, with the implication of green chemistry principles in the synthesis protocol, *nano* silver is gaining much attention in biomedical and clinical field. Alongside, with the increased incidence of antibiotic(s) resistance among various micro-organisms, there is also a need for an effective containment strategy to prevent their escape in the environment. The so called *elixir* (drug) proving its inability, an effective nano-biotechnological approach has ventured in to combat such pathogens.
Objective: To study the toxic effect of silver bio-nanoparticles synthesized using a soil bacterium, *Bacillus* sp. on genomic DNA isolated from pathogenic bacteria.
Methods: In this study, silver bio-nanoparticles (SNPs) were synthesized using extracellular bacterial filtrate. Characterization of SNPs was done using UV–vis spectrophotometer, X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and energy dispersive spectroscopy (EDX). Antibacterial study of SNPs toward the pathogenic bacteria was studied by Kirby Bauer's Disc diffusion method. The toxic effect of SNPs on the genomic DNA isolated from the pathogenic microorganisms was investigated by agarose gel electrophoresis.
Results: The time dependent toxic effect of SNPs on genomic DNA has been validated.
Conclusion: The SNPs exhibit potential antibacterial activity toward clinical isolates tested. DNA fragmentation by SNPs was observed in the form of shear may be due to the affinity and complex forming tendency with Reactive Oxygen Species (ROS) present as phosphates on nucleic acid resulting in fragmentation. This modus operandi toward DNA isolated from the clinical isolates might have a vigil over genetically altered organisms.

1. Introduction

The widespread application of silver nanoparticles (SNPs) in personal care products, food production and medical instruments has encouraged its use in biomedical applications due to broad-spectrum antimicrobial properties.[1] Despite innumerous metal nanoparticles, silver is being engineered extensively for use in sensing, catalysis, transport

* *Corresponding author*. Tel.: +91 44 24503804; fax: +91 44 24502344.
E-mail addresses: biju_nandu@yahoo.co.in, animananda@gmail.com, animananda72@gmail.com (A. Nanda).
0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.06.024

and in emerging medical applications such as drug delivery, biosensors and imaging. This is accomplished either by direct ingestion or injection of nanomaterials into the biological system. The crucial point lies in assessing the level of 'toxicity' as far biological systems and biomedical purpose is concerned.[2] Almost all forms of silver possess antimicrobial potential through release of silver ions whereas SNPs might exhibit additional biocidal activity against bacteria, fungi, virus and even humans not exerted by its bulk counterpart. The exploitation of SNPs upon beneficial implication may get released to the environment impacting the lowest trophic levels i.e. bacteria. Studies on induction of apoptosis or necrosis in higher cell lines like zebra fish, clams, rats and humans by SNPs have also been reported.[3,4] This could pose a major threat globally with increased rates of morbidity and mortality preceded by antimicrobial resistance prevailing in bacterial community. It is noteworthy to say that such bacteria becoming resistant to toxic metal or antimicrobials have the tendency to transfer that DNA fragment(s) via horizontal gene transfer/transduction.[5] This has been a long term goal in containing the drug resistance and metal tolerance relying upon various approaches: the inhibition of induced mutation during therapy, inhibition of horizontal DNA transfer to prevent the spread of pre-existing antibiotic resistance and inhibition of antibiotic/metal tolerance in bacteria that are not heritably resistant. In order to make both the ends meet, a study on the toxic effects of unmodified SNPs at bio-molecular level appending the bacterial genetic material and characterizations of the physico-chemical properties, a prerequisite for assessing the toxicity potential is investigated.

2. Materials and methods

2.1. Materials

Silver nitrate ($AgNO_3$) was purchased from Qualigens, India. Nutrient Agar (NA), Luria Bertani (LB) and Mueller–Hinton Agar (MHA) medium were supplied by HiMedia, India. Agarose low EEO was supplied by HiMedia, India. Proteinase-K and 1 kb DNA marker were supplied by Medox Biotech. All the other reagents which were of analytical grade were obtained from Fisher Scientific, India and used without further purification. Sterile discs of size 6 mm used in this study were supplied by HiMedia, India.

2.2. Bacterial strains

Bacillus sp. used in this study was isolated from polluted soil environment in the outskirts of Chennai city and identified as *Bacillus subtilis* A1.[6] Screening for reducing potential of nitrate was performed and the isolate that formed a clear zone was designated A1 and retained for further experimental.[7] Common human pathogenic bacterial strains such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Serratia marcescens* were used for assessing the antimicrobial potential and geno-toxic nature of SNPs synthesized in the laboratory. The strains were obtained from SRM Medical College, Chennai and were cultured at 35 °C on Mueller–Hinton agar.

2.3. Silver bio-nanoparticle synthesis

The SNPs were prepared according to the procedure described in the literature.[7,8] In brief, 24 h old culture of *B. subtilis* A1 was used as inoculum and grown in LB broth. Cultivations were performed and incubated at 30 °C for 18 to 20 h on a rotatory shaker at 150 r min^{-1} and the cells harvested by centrifugation and the supernatant was used for the synthesis of SNPs using 1 mM $AgNO_3$ prepared using Milli-Q water (Milli-Q Integra 3, Millipore, MA). The experiment was run along with control and the flasks incubated on a rotatory shaker at 150 rpm in dark condition at 30 °C.

2.4. Characterization

Shimadzu UV-1800 UV–visible spectrophotometer was used to monitor the optical measurements by random sampling of 2 mL aliquot of the reaction mixture in the range 200–800 nm at a resolution of 1 nm. The X-ray diffraction patterns were recorded on a Rigaku multiflex diffractometer using Cu-Kβ radiation ($\lambda = 0.1542$ nm) operated at 40 kV and 100 mA. The experiments were performed in the diffraction angle range of $2\theta = 20–80°$. The morphology and elemental composition of the SNPs were analysed by field emission scanning electron microscopy (FESEM) and energy dispersive spectroscopy (EDX) using a 10 KeV Hitachi S-3000H microscope.

2.5. Antimicrobial susceptibility testing (Disc diffusion method)

The bactericidal activity of SNPs was determined by performing Kirby Bauer's disc diffusion method. Log phase bacterial inoculums (10^8 cfu/mL) were standardized using McFarland's standard and were uniformly spread over MHA plate using a sterile swab (HiMedia, India). SNPs of various concentrations (5 μg, 10 μg, 15 μg, 20 μg/mL) were prepared and adsorbed onto sterile discs. The discs were then carefully placed on the MHA plates and incubated at 37 °C for 24 h. Control discs were run using culture filtrate and aqueous silver nitrate.

2.6. Geno-toxic study

The geno-toxic study was performed on the genomic DNA extracted from the clinical strains by alkali lysis method.[9] The DNA extracted was made in aliquots of 10 μg/mL tris acetate buffer (pH 8.0) and stored at −20 °C. The aliquots of SNPs were added separately to the purified DNA samples and incubated at 37 °C for 6 h and 12 h respectively. Gel Electrophoresis was carried out using 1% agarose prepared in tris acetate buffer and stained with 0.5 μg/mL ethidium bromide. The set up was run at 100 Amp for 30 min after which the gel was visualized in a Gel documentation system.

3. Results

The extracellular synthesis of SNPs using the culture supernatant of *B. subtilis* A1 was observed. The color of the challenged solution changed to yellowish brown after 24 h which further deepened to brown in 72 h confirming the reduction of