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Biofabrication of Ag nanoparticles using *Sterculia foetida* L. seed extract and their toxic potential against mosquito vectors and HeLa cancer cells

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ABSTRACT

A one-step and eco-friendly process for the synthesis of silver-(protein-lipid) nanoparticles (Ag-PL NPs) (core–shell) has been developed using the seed extract from wild Indian Almond tree, *Sterculia foetida* (L.) (Sterculiaceae). The reaction temperature played a major role in controlling the size and shell formation of NPs. The amount of NPs synthesized and qualitative characterization was done by UV–vis spectroscopy and transmission electron microscopy (TEM), respectively. TEM studies exhibited controlled dispersity of spherical shaped NPs with an average size of 6.9 ± 0.2 nm. Selected area electron diffraction (SAED) and X-ray diffraction (XRD) revealed 'fcc' phase and crystallinity of the particles. X-ray photoelectron spectroscopy (XPS) was used to identify the protein–lipid (PL) bilayer that appears as a shell around the Ag core particles. The thermal stability of the Ag-PL NPs was examined using thermogravimetric analysis (TGA). Further analysis was carried out by using Fourier transform infrared spectroscopy (FTIR), where the spectra provided evidence for the presence of proteins and lipid moieties ((2n-octyl*cyclo*prop-1-enyl)-octanoic acid (I)), and their role in synthesis and stabilization of Ag NPs. This is the first report of plant seed assisted synthesis of PL conjugated Ag NPs. These formed Ag-PL NPs showed potential mosquito larvicidal activity against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say. These Ag-PL NPs can also act as promising agents in cancer therapy. They exhibited anti-proliferative activity against HeLa cancer cell lines and a promising toxicity was observed in a dose dependent manner. Toxicity studies were further supported by the cellular DNA fragmentation in the Ag-PL NPs treated HeLa cells.

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1. Introduction

With advent of nanotechnology, many laboratories around the world have investigated Ag NP synthesis in various approaches. An important area of nanotechnology is to achieve a controlled synthesis of NPs in different compositions (core–shell) and sizes. Especially applications of core–shell nanomaterials have attracted increasing research interest due to their unique structural features. These particles are made of inner core and an external shell of different chemical compositions aiding in their improved physical, chemical and biological properties. So far a large number of core–shell nanomaterials have been successfully fabricated using approaches ranging from laser ablation techniques, chemical precipitation, high-temperature evaporation and hydrothermal methods [1]. Some of these methods are simple and provide control over crystallite size by restoring the reaction conditions. But problems still exist with the general stability of the product and in achieving uniform sized NPs. These chemical methods are cumbersome and are not eco-friendly [2]. Synthesis of NPs through green processes is a good alternative over the chemical and physical methods as they produce highly stable NPs which are environment friendly and economic. Hence attempts were made to synthesize core–shell NPs in a much simpler method using biological approaches.

The Ag NPs were also found to have potential applications, as optical receptors [3], as catalysts in chemical reactions for biolabelling [4] and as antimicrobials [5,6]. In recent times the research on silver nanoparticles for biomedical applications has increased and especially emphasised being laid in the areas of biogenic production. In this aspect it has become prerequisite to establish the biocompatibility of NPs towards the biological systems. In recent studies, potential mosquito larvicidal activities of synthesized Ag NPs from chemical and biological routes are reported [7–9]. They can offer remarkable interactions with both the biomolecules on the surface and inside of the body cells [10,11], which have potential to bring advances in diagnosis and treatment of many life-threatening diseases [12]. In particular, Ag NPs are becoming more useful in cancer therapy by showing various actions on cancer cells such as reducing ATP content, damaging mitochondria, increasing the production of reactive oxygen species and disturbing other useful metabolic pathways for cell survival [13]. In other reports potential anti-proliferative activity of Ag NPs synthesized by certain plant extracts is well documented [14–16]. However, the stability and surface functionalization of such Ag NPs are still challenging factors in many

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situations. An ideal solution is to encapsulate the NPs with a protective layer [17,18] and the benefit of protein coating is very obvious in biological applications [19]. Hence in this study we have tried to synthesize the protein–lipid conjugated Ag NPs and observed their differential effects on mosquito vectors and HeLa cell lines (a cancerous cell line).

Biomolecules such as glycolipids, phospholipids and lipopeptides are amphiphilic in nature with polar and non-polar moieties. They aid in reducing the surface tension and increased interaction with other molecules at interfaces [20,21]. Mukherjee et al. [19] reported that protein coated gold NPs were used as doxorubicin loaded efficient drug delivery systems on breast cancer cell lines. In our earlier study, we had successfully synthesized the protein coated (thickness of 4.8 nm) Ag-protein (core–shell) NPs using cell free aqueous extract of the plant *Piper betle* L., under direct sunlight exposure conditions which showed non toxic effects on *Daphnia magna* [22]. This has motivated us to further explore the chemical moieties linked with the synthesized Ag NPs. Hence, an attempt is made, for the first time, to synthesize biologically active Ag-PL NPs using *Sterculia foetida* seed extract.

The *S. foetida* (L.) tree is useful for its medicinal properties associated with the plant and has attracted attention of many researchers [23]. The *S. foetida* plant seeds are rich in fat (30–36%) and contain protein content to an extent of 11.4% [24]. Earlier literature survey on this plant revealed that the cyclopropene fatty acid, (2n-octyl*cyclo*prop-1-enyl)-octanoic acid (I) isolated from the *S. foetida* seeds have associated with a large spectrum of biological properties ranging from insecticidal, antifungal, antibiotic, antiviral, hormonal, antitumoral activities, enzyme and gluconeogenesis inhibitions of neurochemical activity [25,26]. In this study we have utilized a one pot green chemistry approach for the synthesis of Ag-PL NPs by in vitro method using an industrially and medicinally important edible plant, *S. foetida*. Thus synthesized Ag-PL NPs were tested against mosquito larvae *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say and antiproliferative effects on human cervical carcinoma cells (HeLa).

2. Materials and methods

2.1. Chemicals

Silver nitrate ($AgNO_3$) was purchased from Sisco Research Laboratories Pvt. Ltd. India, and used without further purification. Silver nanoparticles (original nanoparticles) were purchased from Sigma Aldrich Inc., USA, and were <100 nm from the size given by the manufacturers. These nanoparticles were dispersed in the water (Millipore water) and sonicated to prevent aggregation and used for experimental purposes.

2.2. Test organisms

A. aegypti (L.), *A. stephensi* Liston and *C. quinquefasciatus* Say colonies were cultured in our laboratory in large enamel basins (45 × 45 × 40 cm) and were maintained at 28 ± 2 °C temperature, 68 ± 5% relative humidity (RH) and photo regime of 16:8 h light and dark period as per the procedure of Sharma and Saxena [27]. The egg strips were obtained from mosquito control board, Osmania University, Hyderabad, India to start the colony. The strips were immersed in dechlorinated tap water for hatching. Larvae were fed on a diet of finely ground brewer yeast and dog biscuits (3:1). The emerged adults were fed on rabbit blood supplemented with 10% glucose solution. Small porcelain dishes having 50 mL of tap water lined with filter paper were kept inside the cage for oviposition.

2.3. Cell culture

Cervical cancer cell lines (HeLa) were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 5% L-glutamine, and 1% penicillin and streptomycin. Cells were maintained in a humidified 5% CO_2 incubator at 37 °C.

2.4. Preparation of S. foetida seed extract

S. foetida L. plant seeds used in this study were obtained from the trees growing in the campus of Indian Institute of Chemical Technology (IICT) at Hyderabad, India (17°21′58″ N, 78°28′34″ E). The plant was authenticated at Department of Botany, Osmania University, Hyderabad, India. A voucher specimen was deposited at the herbarium of IICT, Hyderabad, India. The dried seeds from the plants were collected and washed with hexane to ensure that the seeds are dust free and clean. The seeds (10 g) were finely powdered, extracted twice with hexane (50 mL) at room temperature and filtered.

2.5. Synthesis of Ag-PL NPs

For the synthesis of Ag-PL NPs, silver nitrate ($AgNO_3$) was purchased from Sisco Research Laboratories Pvt. Ltd. India, and used without further purification. Ag-PL NP synthesis was carried out by taking 10 mL of hexanic extract of *S. foetida* seeds and added to 150 mL of 1×10^{-3} M aqueous $AgNO_3$ solution at room temperature. The reaction mixture was exposed to different temperatures ranging from 35 to 80 °C for a period of 15 min. Appearance of a yellowish brown color was a characteristic signature of Ag in the spectrum. The Ag-PL NP colloidal solution thus obtained was purified by centrifugation (by using REMI ultramicrocentrifuge) at 5000 rpm for 10 min followed by redispersion of the pellet of Ag-PL NPs into 20 mL of double-distilled water. The centrifugation and redispersing proceses were repeated for three times. The rate of reduction of the Ag^+ ions in solution was monitored by periodic sampling of aliquots of the reaction mixture and measuring the UV–visible spectrum of the solution.

2.6. Characterization of Ag-PL NPs

The UV–vis spectra were recorded in a Perkin Elmer model UV–vis double beam spectrophotometer from 300 to 800 nm, at the resolution of 1 nm. Samples for transmission electron microscopy (TEM) analysis were prepared by placing a drop of the nanoparticle solution on carbon-coated copper grids, allowing the solvent to evaporate. TEM analysis was performed on a FEI Tecnai F12 (Philips Electron Optics, Holland) instrument operated at 100 kV. From the TEM images, the sizes of the synthesized Ag-PL NPs were measured by using SIS imaging software (Munster, Germany). TEM was also used for the study of selected area electron diffraction (SAED) patterns. The biogenic NPs were freeze-dried and the dried powder was used for X-ray diffraction (XRD) analysis. The spectra were recorded in Siemens/D-5000 X-ray diffractometer using CuKα radiation of wavelength 1.54 Å and continuous speed of 0.045° min^{-1}. X-ray photoelectron spectra were recorded on a KRATOS AXIS 165 with a dual anode (Mg and Al) apparatus using the Mg Kα anode. The pressure in the spectrometer was about 10^{-7} Pa. For energy calibration the carbon 1s photoelectron line was used. The carbon 1s binding energy was taken to be 284.6 eV. Spectra were deconvoluted using Sun Solaris based Vision 2 curve resolver. The location and the full width at half maximum (FWHM) for a species were first determined using the spectrum of a pure sample. The location and FWHM of products, which were not obtained as pure species, were adjusted until the best fit was obtained. Symmetric Gaussian shapes were used in all the cases. Binding energies for identical samples were, in general, reproducible within ±0.1 eV. The TGA measurements were carried out on a TGA/SDTA Mettler Toledo 851e thermal system (Zurich, Switzerland) with open alumina crucibles containing samples weighing about 8–10 mg nanopowder. The Fourier transformed infrared (FTIR) spectra were recorded using thermo Nicolet Nexus 670 spectrometer in the diffuse reflectance mode at a resolution of 4 cm^{-1}

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