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Evaluation of cell toxicity and DNA and protein binding of green synthesized silver nanoparticles

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ABSTRACT

Silver nanoparticles (AgNPs) were prepared by GREEN chemistry relying on the reduction of $AgNO_3$ by phytochemicals present in black tea extract. AgNPs were fully characterized by transmission electron microscopy (TEM), ultraviolet-visible spectroscopy ((UV–vis)), X-ray diffraction (XRD) and energy dispersive absorption spectroscopy (EDS). The synthesized AgNPs induced a decrease of the cell viability in a dose-dependent manner with a low IC_{50} (0.5 ± 0.1 μM) for an ovarian carcinoma cell line (A2780) compared to primary human fibroblasts (IC_{50} 5.0 ± 0.1 μM). The DNA binding capability of CT (calf thymus) DNA was investigated using electronic absorption and fluorescence spectroscopies, circular dichroism and viscosity titration methods. Additionally, the AgNPs strongly quench the intrinsic fluorescence of BSA, as determined by synchronous fluorescence spectra.

1. Introduction

Silver and gold nanoparticles (NPs) have shown their importance in catalysis [1], optics [2,3], biosensing [4] and in various biomedical applications [5]. Transition-metal NPs are often synthesized via chemical reduction by organic and inorganic reducing agents, such as hydrazine, sodium borohydride ($NaBH_4$) or *N*,*N*-dimethylformamide, which can also act as stabilizing agents to avoid the coalescence of NPs [6].

There has been a growing interest to eliminate the use of toxic chemicals and organic solvents as reducing agents since they show considerable deleterious effects to the environment and biological systems. Biosynthetic sustainable methods of NPs (based on top-down and bottom-up approaches), comprising either microorganism [7–9] or plant extracts [10–14], have arisen as an alternative to traditional methods and been applied to the preparation of a variety of metallic NPs [15]. Such approaches present two unequivocal advantages: i) the high diversity and abundance of plant extracts of renewable sources; and ii) the simplicity and cost-effectiveness of the methods [15,16]. “Green” synthesis has been focused on silver nanoparticles (AgNPs) [17–19], whose interest is related to the easy reduction of silver(I) salts to form zerovalent silver and its antibacterial properties. Due to its health benefits and antioxidant properties, black tea [18b] has been used to produce biocompatible nanoparticles for application in health and energy. The phytochemicals present in tea, specifically phenols, flavonoids and terpenoids [20], show a dual role: i) as reducing agents to reduce silver, and ii) as stabilizers to provide a robust coating on the AgNPs in a one-pot process. Recently, microwave [21–23] and sonochemical [24,25] methods have been used for the rapid generation of nanostructures [26,27].

The study of the interaction of NPs with nucleic acids is of interest to understand their possible effects on the synthesis, replication and structural integrity of DNA and RNA [28]. In fact, AgNPs derived from plant extracts have shown superior antioxidant and anticancer properties [29,30]. However, despite the extensive usage of AgNPs, very few reports [31] on the interaction of AgNPs with DNA are available, and to our knowledge, this is the first report to show that tea extract derived AgNPs act as DNA intercalator.

BSA is the most abundant protein in blood plasma and has an extensive range of physiological functions like binding, carriage of fatty

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acids, nutrients, transport, etc [32]. Thus, for downstream *in vivo* applications (*e.g.* drug delivery, receptor targeting, etc.), it is of utmost importance to study the interaction mechanism between BSA and foreign molecules. In this context, we investigated the biophysical mechanisms of AgNPs-BSA interactions using (UV–vis) and fluorescence spectroscopies.

In this work, we have prepared AgNPs via a green synthetic pathway using tea extracts. The AgNPs were fully characterized and their cytotoxicity in human ovarium carcinoma cell assessed. Interaction of AgNPs with DNA and BSA was also investigated. All these studies highlight the relevance of "green" synthetic pathways for AgNPs for a plethora of downstream applications. The simple low-cost process, relying on renewable sources, may pave the way for additional usages, some of which relying on the properties herein reported.

2. Materials and methods

2.1. Materials

All reagents and solvents were obtained from commercial sources and used as received, *i.e.*, without further purification or drying. Black tea from Tetley, England, and $AgNO_3$ (BDH), Sulfuric acid (97%, Aldrich), ferric chloride (Aldrich), were used as received. All synthetic work was performed in air.

2.2. Ag nanoparticles synthesis using black tea extracts

AgNPs synthesis using black tea extracts was carried out as previously described [33]. A 1% tea extract solution was prepared by vigorous mixing of black tea leaves in distilled water for 15 min at room temperature. After filtration, 0.1 mL of an $AgNO_3$ solution (0.1 M) was added to 6 mL of the prepared tea extract solution, with stirring for 3 h at room temperature. The solution changed from pale yellow to brownish color, indicating the formation of AgNPs. UV/Vis spectroscopy was used to confirm the formation of the AgNPs due to the occurrence of the characteristic surface plasmon resonance (SPR) band at 432 nm of these particles.

2.3. Test for phenolic and flavonoid compounds

For determining the presence of polyphenols in the tea, several drops of a 5% ferric chloride aqueous solution were added to 2 mL of the tea extract. The appearance of a dark green color indicates the presence of polyphenolic compounds [34].

For flavonoids detection, 5 mL of diluted ammonium solution were mixed with 2 mL of tea extract and then several drops of concentrated sulfuric acid were added. The appearance of a yellowish color indicated the presence of flavonoids [34].

2.4. Nanoparticle characterization

The synthesized AgNPs were characterized using UV–vis spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive Spectroscopy (EDS) techniques. (UV–vis)ible spectroscopic measurements of the synthesized AgNPs were carried out on a PerkinElmer Lambda 750 (UV–vis)ible spectrophotometer. TEM measurements were performed on a Transmission Electron Microscope Hitachi 8100 with ThermoNoran light elements EDS detector and digital image acquisition. Morphology and distribution of AgNPs were characterized using SEM (JEOL 7001F with Oxford light elements EDS detector and EBSD detector). The phase purity of the prepared AgNPs was determined by X-ray diffraction (XRD) performed at room temperature on a X'pert PRO of PANalytical diffractometer, Cu-Kα X-rays of wavelength (λ) = 1.54056 Å and data were taken for the 2θ range of 10° to 90° with a step of 0.02°.

2.5. Stability of AgNPs in biological media

For stability studies over time, AgNPs were incubated in phosphate buffered saline 1X (PBS) (Invitrogen), Dulbecco's Modified Eagle Medium (DMEM), DMEM without (w/o) phenol red and Roswell Park Memorial Institute (RPMI) (ThermoFisher) for 24 h at 37 °C, and visible spectra were recorded in 400–800 nm range.

2.6. Cell culture

Human ovarian (A2780) and colorectal (HCT116) carcinoma cell lines were grown in DMEM (Invitrogen Corp., Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Invitrogen Corp.) and maintained at 37 °C in a humidified atmosphere of 5% (v/v) CO_2. Primary Dermal Normal Human Fibroblasts (Neonatal) (American Type Culture Collection (ATCC) - PCS-201-010™) were grown as previously described [34]. All cell lines were purchased from ATCC (www.atcc.org).

2.7. AgNPs exposure for dose-response curves

Cells were plated at 5000 cells/well in 96-well plates. Media was removed 24 h after platting and replaced with fresh media containing: 0.1–10 μM of AgNPs solution or water (vehicle control). For comparison purposes, a 1% tea extract solution was used as a control.

2.8. Viability assays

After 24 h of cell incubation in the presence or absence of the AgNPs, cell viability was evaluated with CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA), using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) as previously described [35,36]. In brief, this is a homogeneous, colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The CellTiter 96® AQueous Assay is composed of solutions of MTS and an electron coupling reagent (phenazine methosulfate, PMS). MTS is reduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan product at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product was measured in a Bio-Rad microplate reader Model 680 (Bio-Rad, Hercules, *ca*, USA) at 490 nm, as absorbance is directly proportional to the number of viable cells in culture.

2.9. DNA interaction experiments

2.9.1. Absorption spectral studies

The DNA binding capability of the AgNPs was determined by (UV–vis) spectral titration method in 5 mM Tris HCl/50 mM NaCl buffer at pH 7.5. The concentration of CT-DNA was determined from its known extinction coefficient ε value [37] (6600 M^{-1}) at 260 nm. Absorption titration experiments were made using concentration of AgNPs as constant with increasing concentrations of CT-DNA. The absorbance ratio of about 1.7-1.8:1 at 260 and 280 nm, indicating that the CT-DNA was sufficiently free of protein. The binding constant K_b was determined from the spectral titration data using the McGhee von Hippel equation [38]:

$$\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - \ (b^2 - 2K_b{}^2C_t\ [DNA]/s)^{1/2}/2K_bC_t\ (\quad (1a)$$

$$b = 1 + K_bC_t + K_b\ [DNA]/2s \quad (1b)$$

where [DNA] is the concentration of CT-DNA in base pairs, ε_a is the apparent extinction coefficient of the AgNPs at a given concentration of