Journal of Photochemistry & Photobiology, B: Biology 169 (2017) 21–26

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Synthesis and characterization of core-shell bimetallic nanoparticles for synergistic antimicrobial effect studies in combination with doxycycline on burn specific pathogens

Ali Fakhri [a,*], Shiva Tahami [b], Mahsa Naji [c]

[a] Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran
[b] Department of Biology, Karaj Branch, Islamic Azad University, Karaj, Iran
[c] Department of Materials Engineering, Karaj Branch, Islamic Azad University, Karaj, Iran

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 11 January 2017
Accepted 21 February 2017
Available online 27 February 2017

Keywords:
Bimetallic nanoparticles
Nanotechnology
Burn healing
Synergistic effect

ABSTRACT

Nano-medicine is a breakthrough discovery in the healthcare sector. Doxycycline is a new generation antibiotic which is proved to be a boon in the treatment of patients with complicated skin infections. We have tried to explore the benefits of synthesized bimetallic silver-gold nanoparticles in combination with new generation antibiotic for burn infections. The bimetallic nanoparticles synthesized by core-shell method were characterized using scanning electron microscopy equipped with an energy dispersive spectrometer, transmission electron microscopy, X-ray diffraction and UV–Vis spectroscopy. The calculated average particle sizes of the Ag-Au NPs were found to be 27.5 nm. The Ag-Au core-shell BNPs show a characteristic Plasmon peak at 525 nm which is broad and red shifted. The synergistic antimicrobial activity of doxycycline conjugated bimetallic nanoparticles was investigated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus luteus*. This combined therapeutic agent showed greater bactericidal activity. Synergy of antibiotic with bimetallic nanoparticles is quite promising for significant application in burn healing therapy. The mechanism of the antibacterial activity was studied through the formation of reactive oxygen species (ROS) that was later suppressed with antioxidant to establish correlation with the Ag-Au NPs antimicrobial activity. Ag-Au NPs showed effective antiproliferative activity toward A549 human lung cancer (CCL-185) and MCF-7 human breast cancer (HTB-22) cell lines.

1. Introduction

Metal nanoparticles have gained prominence in therapeutic and diagnostic applications [1]. Several conventional antimicrobials are rendered obsolete due to drug resistant pathogenic bacteria. Researchers are now focused on alternative medicines and conjugated drug therapy to combat this problem [2–3]. Several studies have reported that metal nanoparticles can be conjugated with different drugs to enhance their activities. The drug dosage often prescribed for a particular therapy is much higher leading to harmful effects. To keep up good health, the antibiotic dose should be reduced and the stability should be increased to make the usage economical. Literatures support the fact that metal (gold and silver) nanoparticles have the potential to enhance the antimicrobial effects of the drug, reduce the dosages of the drug, combat its side effects and target the drug to the appropriate site of action. Generally, the combinatorial therapy involves functionalized metal nanoparticles, where chemical functionalizing agents are used [4–5]. Bimetallic nanoparticle synthesis is a quite promising health solution which involves simultaneous or successive reduction of two metals resulting into either core–shell or alloy form of nano-arrangement. Sequential reduction refers to reduction of shell metal over pre-formed seed of core metal. The formation of bimetallic nanoparticles depends on purity, non-reactivity and reducibility of the metal salts. Nucleation and transition phases of ions to nanoparticles need to be controlled especially in chemical synthesis [6]. Previous reports have focused on physical and chemical synthesis of bimetallic nanoparticles like sol-gel method, sono-chemical method [7], micro-emulsion technique [8], aerosol technology [9]. Core–shell nanoparticles (CSNs) are a class of nanostructured materials that have recently received increased attention owing to their interesting properties and broad range of applications in catalysis, biology, materials chemistry and sensors. Gold and silver in nano form possess antimicrobial properties which are enhanced when they are combined to form bimetallic nanoparticles. The silver nanoparticles play an important role in the current increase of nanoparticle usage [10–12]. Burn infections caused by multidrug-resistant nosocomial pathogens especially, *Pseudomonas aeruginosa*, *E.coli* and *Staphylococcus aureus* are lethal [13]. The significance of metal

* Corresponding author.
E-mail address: ali.fakhri88@yahoo.com (A. Fakhri).

http://dx.doi.org/10.1016/j.jphotobiol.2017.02.014
1011-1344/

nanoparticles has been explored in different spheres [14]. The gold nanoparticles conjugated antibiotics were seen to be more stable than their respective free forms. Gold nanoparticles conjugation can stabilize β-lactam antibiotics by preventing ring disruption at elevated temperatures. Therefore, gold nanoparticles conjugated antibiotics have shown more stability and thereby enhanced antibacterial activity [15,16]. In the present study, doxycycline, an antibacterial antibiotic which treats acne, urinary tract infections, skin infections, eye infections, gonorrhoea, chlamydia, and periodontitis (gum disease) was combined with non-functionalized bimetallic nanoparticles are non-toxic. Therefore, the antimicrobial activity of doxycycline combined with gold nanoparticles was emphasized in the present work.

2. Materials and Methods

2.1. Chemicals

Silver nitrate, sodium acrylate, gold tetrachloroaurate trihydrate and common solvents were obtained from Aldrich. Water was purified with a Millipore Direct-Q system (18.2MU). Dialysis membranes with molecular weight pore size of 10,000 Da were obtained from Spectra/Por and were rinsed in pure water before use.

2.2. Preparation of Nanoparticles

2.2.1. Synthesis of Ag NPs

Ag NPs were synthesized by first mixing 50 milliliter of water with 0.0125 mM of silver nitrate, and then adding 0.00675 mM of sodium hydroxide, which results in a dilute yellow-colored solution of silver hydroxide. This solution is purged with argon and is then brought to reflux. At reflux, 0.255 mM of sodium acrylate is added, causing the solution to turn completely clear. The solution is refluxed for 1 h, during which time the solution color changes from clear to green-yellow to yellow-orange.

2.2.2. Purification of As-synthesized Ag NPs.

Prior to deposition of Au on the Ag NP seeds, the as-synthesized particles are purified to remove excess acrylate, silver, sodium and other ions from the solution. Purification is performed by enveloping the particle solution inside a cellulose dialysis membrane with pore size of 10,000 Da and soaking in a distilled water bath. After sonication in an ultrasonic homogenizer (APU500, 40 kHz, 100W, Adeeco Co., Iran), the water was changed every 12 h for 48 h.

2.2.3. Deposition of Au on the Ag Cores to Form Ag-Au NPs

Fifty millilitres of the dialysed Ag particles are brought to reflux and 10 ml of a gold tetrachloroaurate trihydrate solution (0.0625 mM according to the thickness of the Au shell desired) and 10 ml of a sodium acrylate solution (0.00510 mM) is added dropwise simultaneously. The solution color changes depending on the amount of Au added. In general, as Au and sodium acrylate are added to the Ag particles, the color changes from yellow-amber to dark amber to grey to grey-purple and finally to purple.

2.3. Characterizations

A scanning electron microscopy (SEM-Hitachi SU8000) and X-ray diffractometer (XRD) Philips X'Pert were used to examine the morphology of the adsorbent synthesized here. The particle size of the Ag-Au BNPs was measured using Transmission Electron Microscope (TEM) (Zeiss EM-900). UV–Vis studies were performed using TEC Avaspec 2048 Spectrophotometer (excitation source = Xenon arc lamp 450 W).

2.4. Antimicrobial Assay

In order to determine antibacterial activity of synthesized bimetallic nanoparticles, standard well agar diffusion method was carried out against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. The agar plates having suitable nutrient media was prepared, sterilised and allowed to solidify. After solidification, the agar plates were inoculated with bacterial cultures. Wells were bored in petri-plates containing suitable nutrient agar medium seeded with 120 μL of 36 h of each pathogen. 5 μl of doxycycline antibiotic and a mixture of bimetallic solution with antibiotic (equal amount) was added into each of the wells and incubated at 28 °C for 2 days. The diameter of zones of inhibition was measured using a ruler and mean value was recorded for each pathogen and expressed in millimetre (mm). All the above experiments were carried out in duplicate.

2.5. Effect of Oxygen and Antioxidants on the Antibacterial Activity of Ag-Au NPs

To confirm the effect of oxygen on the antibacterial activity of Ag-Au NPs against bacteria, a comparative study was performed. Fresh bacteria culture at approximately 10^6 cfu/mL was inoculated in fresh LB medium. Ag-Au NPs were added to these mixtures at final concentrations from 3 to 7 mg/L. One set was incubated under aerobic conditions while another was incubated anaerobically.

The antioxidant *N*-acetylcysteine (NAC) was used to investigate the effect of reactive oxygen species (ROS) on the antibacterial activity of Ag-Au NPs. To do this, two sets of culture, one with Ag-Au NPs alone and another with Ag-Au NPs and NAC (10 mM) were prepared containing 5 and 10 mg/L Ag-Au NPs. A control containing 10 mM NAC in the presence of bacteria alone under similar conditions was used to establish the effect of NAC in the absence of Ag-Au NPs.

2.6. Cell Culture

The A549 human lung cancer cell line (CCL-185) and MCF-7 human breast cancer cell line (HTB-22 cell line (CRL-5822) were purchased from ATCC (American Type Culture Collection, VA, USA), and cultured in RPMI-1640 (Gibco BRL, Grand Island, NY, USA) or Eagle's minimal essential medium (EMEM, Gobco BRL) supplemented with 10–20% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 °C in a humidified incubator under 5% CO_2.

2.7. Uptake of Ag-Au NPs

The uptake of Ag-Au NPs to human lung and breast cancer cell was examined by inductively coupled plasma mass spectrometry (ICPMS). The cells were seeded in six-well culture plates (at a density of 2×10^6 cells per ml growth medium/well) and exposed. Control cells were incubated with ultrapure water (added in the amount which corresponds to volumes of Ag-Au NPs stock suspensions added to the cell medium) and in normal cell medium (RPMI supplemented with 10% FBS and antibiotics). After a treatment period of 24 h, the medium was removed and the adherent cells were washed several times with phosphate-buffered saline (PBS) solution, and then detached from the culture plates by adding 1 ml of 0.25% enzyme trypsin for 3 min at 37 °C. Subsequently, the cells were washed with PBS (pH 7.4) and collected for ICPMS analyzes. The harvested cells were digested in closed-vessels with the Ultra CLAVE IV Milestone digestion device (MLS GmbH Mikrowellen-Laborsysteme, Leutkirch, Germany) with a modified US EPA method 3052. Briefly, 5-ml concentrated HNO3 was added into the obtained buffer phase and the mixture was irradiated at 120 °C (800 W) for 10 min. After digestion, samples were diluted with water to 50 ml and stored at 4 °C for further analysis. The uptake of Ag-Au NPs by cells was quantified by measuring the total Ag-Au