Journal of Colloid and Interface Science 482 (2016) 151–158

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

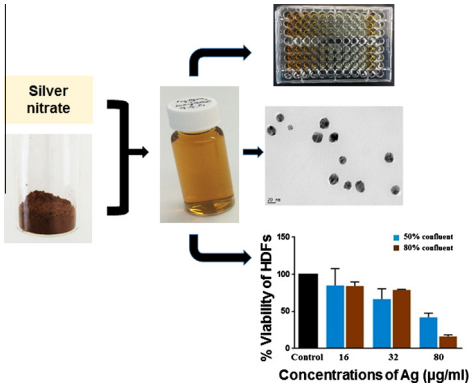
journal homepage: www.elsevier.com/locate/jcis

‘Chocolate’ silver nanoparticles: Synthesis, antibacterial activity and cytotoxicity

Neelika Roy Chowdhury [a], Melanie MacGregor-Ramiasa [b], Peter Zilm [c], Peter Majewski [a], Krasimir Vasilev [a,*]

[a] School of Engineering, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[c] Microbiology Laboratory, The School of Dentistry, The University of Adelaide, Adelaide, SA 5005, Australia

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 June 2016
Revised 31 July 2016
Accepted 1 August 2016
Available online 2 August 2016

Keywords:
Antibiotic resistance
Antimicrobial
Cacao
Cytotoxicity
Pathogens
Silver nanoparticles

ABSTRACT

Hypothesis: Silver nanoparticles (AgNPs) have emerged as a powerful weapon against antibiotic resistant microorganisms. However, most conventional AgNPs syntheses require the use of hazardous chemicals and generate toxic organic waste. Hence, in recent year’s, plant derived and biomolecule based synthetics have has gained much attention. Cacao has been used for years for its medicinal benefits and contains a powerful reducing agent - oxalic acid. We hypothesized that, due to the presence of oxalic acid, cacao extract is capable of reducing silver nitrate ($AgNO_3$) to produce AgNPs.
Experiments: In this study, AgNPs were synthesized by using natural cacao extract as a reducing and stabilizing agent. The reaction temperature, time and reactant molarity were varied to optimize the synthesis yield.
Findings: UV–visible spectroscopy (UV–vis), dynamic light scattering (DLS) and transmission electron microscopy (TEM) characterization demonstrated that the synthesized AgNPs were spherical particles ranging in size from 35 to 42.5 nm. The synthesized AgNPs showed significant antibacterial activity against clinically relevant pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Importantly, these green AgNPs are not cytotoxic to human dermal fibroblasts (HDFs) at concentrations below 32 µg/ml. We conclude that cacao-based synthesis

* Corresponding author.
E-mail addresses: neelika.roy_chowdhury@mymail.unisa.edu.au (N.R. Chowdhury), Melanie.Ramiasa@unisa.edu.au (M. MacGregor-Ramiasa), peter.zilm@adelaide.edu.au (P. Zilm), Peter.Majewski@unisa.edu.au (P. Majewski), Krasimir.vasilev@unisa.edu.au (K. Vasilev).

http://dx.doi.org/10.1016/j.jcis.2016.08.003
0021-9797/

is a reproducible and sustainable method for the generation of stable antimicrobial silver nanoparticles with low cytotoxicity to human cells. The AgNPs synthesized in this work have promising properties for applications in the biomedical field.

1. Introduction

Noble metals such as gold and silver have been appreciated for millennia not only for their beauty but also for their ability to fight diseases [1]. Silver and its nano-particulate forms are well known for their powerful antimicrobial activity towards a large number of pathogens including bacteria, viruses and even protozoa. This inspired numerous applications such as in wound care, dentistry, medical equipment and filtration systems where pathogenic invasion is a health hazard [1–4]. The strength of silver and AgNPs is in their multifaceted mechanism of antimicrobial activity. Silver ions and nanoparticles can bind to sulphur groups of proteins in the bacterial cell membrane, thus inducing changes in membrane permeability and integrity leading to cell death [5]. The AgNPs also interfere with adenosine triphosphate (ATP) production, enzyme synthesis and cell replication by binding to bacterial DNA [4,6,7]. Because of its complex mode of action against bacteria, it is difficult for microorganisms to build effective resistance against silver. For this reason, AgNPs have become the object of much research in the growing field of bio-nanotechnology.

To date, a number of physical and chemical procedures have been used to synthesize silver nanoparticles; however, many of these techniques utilize hazardous and sometimes flammable chemicals [8–10]. More recently, alternative eco-friendly and inexpensive synthetic routes have been proposed in which microorganisms, enzymes, plants or plant extracts are used as potential substitutes to conventional organic chemicals [11]. Pioneering studies demonstrating that nanoparticles synthesized in a speedy manner using plant derived biomolecules can be more stable than their conventional counterparts have encouraged researchers to pursue further investigations in this area [12]. In spite of innumerable attempts of synthesizing nanoparticles using phytochemicals, the potential of plant extracts as biosynthesis reagent has not yet been fully explored. Furthermore, little is known about the interaction between human cells and nanoparticles synthesized utilizing plant extracts [11,13–15].

Here, we report on the synthesis of 'green' silver nanoparticles using cacao (*Theobroma cacao*) extract and evaluate their toxic effects towards microorganisms and human cells. For centuries cacao has been known to exhibit substantial health-beneficial properties including anti-inflammatory, antidepressant, anti-atherogenic, antiaging, aphrodisiac, anti-ulcer, anti-thrombotic, anti-cancer, immune-modulating, antimicrobial, vasodilatory, analgesic, antioxidant and energy stimulator [16–24]. In addition, cacao contains oxalic acid which is a powerful natural reducing agent [25]. We hypothesize that this naturally occurring molecule can trigger the reduction of silver salt to silver nanoparticles. To the best of our knowledge, there has been no report on the synthesis of silver nanoparticles using the extract of cacao. Here, for the first time, we probed the potential of cacao extract as a biosynthesis agent for the production of antimicrobial silver nanoparticles. The reaction conditions were varied in a systematic manner, and the resulting silver nanoparticles were characterized with UV–vis spectroscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM). The antimicrobial effects of the cacao AgNPs suspensions were then evaluated against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 35984, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Finally, the cytotoxicity of the cacao AgNPs suspensions was tested against primary human dermal fibroblast (HDFs) cells.

2. Experimental

2.1. Preparation of cacao extract and silver nitrate solution

Cold pressed cacao powder was purchased from Forest Foods, Australia and stored in an air tight light-proof container. The aqueous extract was prepared by mixing varying amount of cacao powder in 10 ml of ultrapure water (MilliQ system, Millipore Corp.) at room temperature. The extract obtained after filtration (0.45 μm-sterile EO, Sartorius Stedim Australia Pty. Ltd.) of the suspension was stored for the synthesis of AgNPs. Silver nitrate (ProSciTech, Australia) solution was prepared by dissolving varying amounts of silver nitrate in 40 ml of MilliQ water and stored for further use.

2.2. Synthesis of silver nanoparticles

In order to determine the optimum parameters for AgNPs synthesis; a range of experimental conditions were investigated. The synthesis was carried out at different temperatures (25 °C and 100 °C) and for increasing reaction times (30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 24 h, 7 days and 15 days), using varying amounts of silver nitrate (0.25 mg/ml and 0.5 mg/ml in MilliQ water) and cacao extract (25 mg/ml and 50 mg/ml in of MilliQ water). In a typical synthesis procedure 10 ml of the cacao extract was mixed with 40 ml of silver nitrate ($AgNO_3$) solution. The reaction mixtures were either incubated at room temperature (25 °C) or heated to 100 °C under reflux. In both cases, the reaction mixtures were stirred for 30 min and then allowed to cool to room temperature. The originally transparent light yellow reaction solution became increasingly darker and browner as the reaction progressed. This color change was indicative of the formation of silver nanoparticles, as discussed below. The samples, S1L, S2L, S3L, and S4L refer to the AgNPs suspensions synthesized at 25 °C where L signifies lower temperature. The samples synthesized at 100 °C, are denoted as S1H, S2H, S3H and S4H, and H refers to higher temperature. Table 1 summarizes the concentrations of silver nitrate and cacao extract used for the synthesis of the different AgNPs samples.

2.3. Characterization

2.3.1. UV–vis spectroscopy

UV–vis spectroscopy was used to monitor the progress of the reaction, the UV–vis absorbance spectra of the cacao-based AgNPs

Table 1
Synthesized green AgNPs with different amount of cacao and $AgNO_3$.

AgNPs sample	Cacao extract (mg/ml)	Silver nitrate (mg/ml)
S1H, S1L	50	0.25
S2H, S2L	25	0.25
S3H, S3L	50	0.5
S4H, S4L	25	0.5