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A caffeic acid mediated facile synthesis of silver nanoparticles with powerful anti-cancer activity

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ABSTRACT

Green synthesis, especially in biological processes, has gained more attention with increasing application of silver nanoparticles (AgNPs) in biomedical fields. However, the biologically synthesized AgNPs have been to be anomalous in size and shape in most cases, as well as exhibiting certain difficulties when used in therapy. We used caffeic acid, a naturally plant polyphenol, to prepare the AgNPs in the current study and also evaluated their anti-cancer activity against the human hepatoma HepG2 cells. Results showed that the AgNPs could rapidly and simply be synthesized using caffeic acid as both a reducing agent and stabilizer. The synthesized AgNPs possessed characteristics of having small size, narrow distribution and high surface negative charge, as well as being stable in aqueous solution. Furthermore, the AgNPs could enter cells and effectively inhibit viability of tumor cells via induction of apoptosis. In conclusion, a caffeic acid mediated facile method was successfully developed to prepare the AgNPs as a potential alternative agent for human hepatoma therapy.

1. Introduction

Much time and resources have been devoted to cancer research in the hope of discovering the most cost-effective and efficacious strategy for cancer diagnosis, monitoring and treatment. Nanotechnology is envisaged to be the next frontier in the ongoing development of cancer therapy [1,2], as researchers in the biomedical and material engineering fields are nowadays eagerly working together to explore the possibility of using nanomaterials as novel tools for cancer therapy. Silver nanoparticles (AgNPs) have recently been proved to have great potential in the tumor theranostics fields, such as their use as nanoprobes for detection and imaging of tumors, vectors for drug delivery, and inhibitors for suppression of angiogenesis and tumor growth [3–5].

Biological synthesis of AgNPs is currently a highly focused research area [6], considered to be more eco-friendly and cost-effective compared to other chemical and physical methods [7,8] due to reduced use of hazardous reagents and solvents, improved material and energy efficiency from the chemical process, and enhanced design of non-toxic products. Fungi, bacteria, and plants are commonly used to biologically synthesize nanomaterials [9,10]. The use of plant extracts for synthesis of nanoparticles has been proved to be advantageous when compared to microbial processes, because pathogenic bacteria may contaminate the nanoparticles when used in biomedical fields [11]. Furthermore, the plant-based materials seem to be best candidates for large-scale production of nanoparticles [12,13]. The size and shape of nanomaterials provide efficient control over physical, chemical and biological properties of nanomaterials, however, the biologically synthesized nanoparticles have been found to be anomalous in size and shape in most cases, and they also display certain difficulties when used for therapy [14].

Polyphenols are key active ingredients found in plant leaves, roots, latex, seeds and stems, and they are widely used for the green synthesis of metal nanomaterials due to their influence on the nanoparticles' reduction process and stability [15–17]. It has been reported that the green synthesis of silver and palladium nanoparticles, with sizes ranging between 20 and 60 nm, was performed at room temperature using coffee and tea extracts [18], and the main constituent of the coffee extracts was caffeic acid (Fig. 1). This is a natural plant polyphenol which is widely distributed in many agricultural products, such as fruits, vegetables, wine, olive

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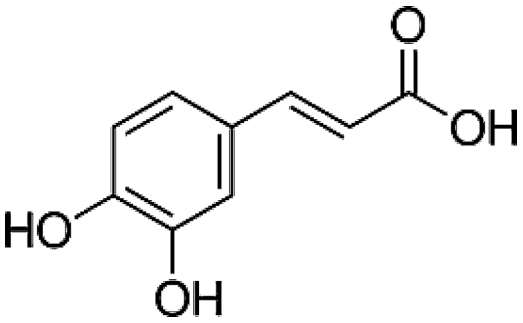

Fig. 1. Chemical structure of caffeic acid.

oil, and coffee [19]. The caffeic acid has been reported to have a wide variety of biological properties, including antioxidants, antithrombosis, antihypertensive, antifibrosis, and antiviral activities [20]. Several studies have also demonstrated that the caffeic acid exerts anti carcinogenic effects against various cancer cell lines [21–25]. In addition, the combination of plant polyphenols and anti-cancer molecules is considered to be a novel treatment strategy for cancer chemotherapy due to their synergistic effect [26,27].

The current work therefore mainly focused on the facile preparation of uniformly sized AgNPs using the caffeic acid as both a reducing agent and stabilizer under ambient conditions without additional reagents. The anti-cancer activity of the as-synthesized AgNPs was subsequently evaluated against human hepatoma HepG2 cells to explore their potential application in cancer therapy.

2. Materials and methods

2.1. Synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized in the current study using a polyphenol reduction method. The caffeic acid was utilized as both a reducing agent and stabilizer. A classical synthesis process is described as follows (Fig. 2); 4 mM (10 μL) of caffeic acid solution was firstly mixed with 1 mL of de-ionized water under magnetic stirring. The pH value of the solution was adjusted to 10.5 using 0.1 M of sodium hydroxide solution, followed by addition of 8 mM (20 μL) silver nitrate solution. The reaction mixture was subsequently kept under magnetic stirring at room temperature for about 30 min until it turned yellow. The freeze-dried AgNPs powders were finally obtained after filtration, centrifugation and lyophilization.

2.2. Characterization of AgNPs

The as-synthesized nanoparticles were primarily characterized by UV–vis spectroscopy (Hitachi U-2000, Tokyo, Japan) and transmission electron microscopy (TEM, JEM-2000EX, JEOL, Japan). The TEM samples were prepared by placing few drops of aqueous dispersions on carbon coated copper grids followed by drying at room temperature. The nanoparticles' mean size was then calculated from a random field of TEM images that displayed general morphology of the nanoparticles. The hydrodynamic diameter and *zeta* potential of the AgNPs were measured by dynamic light scattering (DLS), using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The final silver concentration in the aqueous solution was determined using a graphite furnace atomic absorption spectroscopy (AAS, Z2000, Hitachi, Tokyo).

2.3. Cell culture

Human hepatoma (HepG2) cell line was maintained at 37 °C in an incubator with a 5% CO_2 humidified atmosphere in RPMI 1640 (Hyclone), supplied with 10% FBS and penicillin/streptomycin mix (100 U/mL and 100 mg/mL, respectively). The cells were detached by trypsinization when they reached 80% confluency during subculture.

2.4. Intracellular localization of AgNPs

The HepG2 cells were washed with PBS, detached by trypsinization and centrifuged at 2000 rpm for 5 min after 24 h incubation with 5 μg/mL AgNPs. The medium without nanoparticles was used as control in the experiment. The supernatants were removed and cell pellets fixed with 2.5% glutaraldehyde in PBS for 24 h, followed by post-fixation in 1% osmium tetroxide (Agar Scientific, Stansted Essex, England, UK) for 1.5 h. The cell pellets were then dehydrated through a series of ethanol concentrations (20%, 30%, 40%, 50%, 60%, 70%, and 90%). This was followed by treatment with 2% uranyl acetate in 95% ethanol (Enblock stain) for 1 h and further dehydration with 100% ethanol for 1 h. The cell pellets were twice treated with propylene oxide for 15 min each, followed by 1:1 propylene oxide: araldite resin overnight, and infiltration with fresh araldite resin (3 changes with a gap of 3 to 4 h). This was finally followed with subsequent embedding in araldite resin at 60 °C for 48 h. The ultra-thin sections were then cut with glass knives in an ultra microtome (LEICA EM UC6, Netherlands). The sections were then mounted on copper grids and stained with 1% uranyl acetate and 0.2% aqueous lead citrate. The stained sections were then scanned with TEM (JEM-2000EX, JEOL) for ultra structural observations at 80 kV.

2.5. Effect of AgNPs on viability of HepG2 cells

MTT (dimethyl thiazolyltetrazolium bromide) assay was performed to determine the AgNPs' cytotoxic effect at various concentrations against the human hepatoma HepG2 cells. The

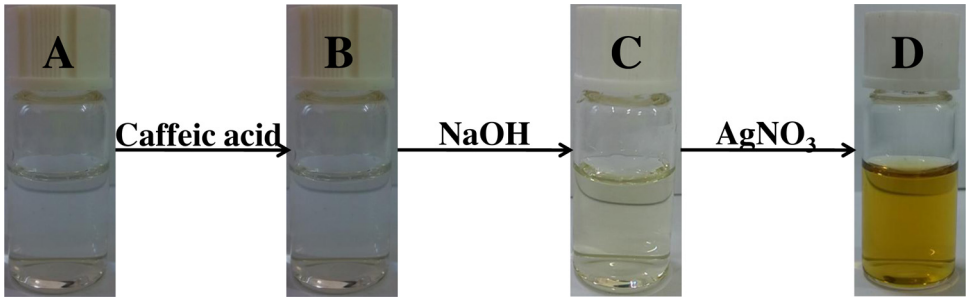

Fig. 2. Visual observations of reaction mixtures in preparation process for silver nanoparticles.
(A) deionized water, (B) aqueous solution containing caffeic acid, (C) alkaline solution containing caffeic acid, (D) nanoparticles solution.