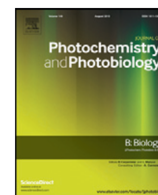
Journal of Photochemistry & Photobiology, B: Biology 156 (2016) 29–34

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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



In vitro anticancer potential of $BaCO_3$ nanoparticles synthesized via green route

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ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 9 January 2016
Accepted 11 January 2016
Available online 13 January 2016

Keywords:
Green synthesis
Mangifera indica
$BaCO_3$ nanoparticles
Caspase-3
Cytotoxicity

ABSTRACT

Green synthesis of nanoparticles is a growing research area because of their potential applications in nanomedicine. Barium carbonate nanoparticles ($BaCO_3$ NPs) were synthesized using an aqueous extract of *Mangifera indica* seed as a reducing agent. These particles were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), Transmission electron microscopy (TEM), selected area electron diffraction (SAED), Energy-dispersive-X-ray (EDX) and X-ray photoelectron spectroscopy (XPS) analysis. HR-TEM images are confirmed that green synthesized $BaCO_3$ NPs have spherical, triangular and uneven shapes. EDX analysis confirmed the presence of Ba, C and O. The peaks at 2θ of 19.45, 23.90, 24.29, 27.72, 33.71, 34.08, 34.60, 41.98, 42.95, 44.18, 44.85, and 46.78 corresponding to (110), (111), (021), (002), (200), (112), (130), (221), (041), (202), (132) and (113) showed that $BaCO_3$ NPs average size was ~18.3 nm. SAED pattern confirmed that $BaCO_3$ NPs are crystalline nature. $BaCO_3$ NPs significantly inhibited cervical carcinoma cells, as evidenced by cytotoxicity assay. Immunofluorescence and fluorescence assays showed that $BaCO_3$ NPs increased the expression and activity of caspase-3, an autocatalytic enzyme that promotes apoptosis. According to the results, green synthesis route has great potential for easy, rapid, inexpensive, eco-friendly and efficient development of novel multifunctional nanoparticles for the treatment of cancer.

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1. Introduction

Barium carbonate ($BaCO_3$), also known as witherite, is an important mineral as a more thermodynamically stable crystal modification among heavy metal carbonates. $BaCO_3$ has attracted attention due to its needle-like subunit, which has a close relationship with aragonite [1–4]. In addition, $BaCO_3$ has important applications in many industries, such as ceramics, pigment, electric condensers, optical glass, piezoelectrics, and as a precursor in the synthesis of magnetic ferrites and ferroelectric materials [5].

Various chemical and physical methods are available for the synthesis of barium nanoparticles ($BaCO_3$ NPs), including solid state decomposition [6], reversed micelles [7], chemical co-precipitation [8], spray pyrolysis [9], ultrasonic irradiation [10], sol–gel [11], microemulsion-mediated solvothermal [12], sonochemical [13], microwave-assisted [14], semi-batch crystallizer [15], microemulsion-based [16], electrodeposition [17], and self-organized formation methods [18]. Most of these methods are expensive and involve hazardous chemicals such as stabilizers, which may pose potential environmental and biological risks. However, green synthesis of NPs is an eco-friendly method and does not involve expensive, toxic, or hazardous chemicals. Green synthesized NPs are suitable for use in the medical field because the particle surface does not contain toxic reducing agents.

Nanomaterials have been widely investigated for their use in the field of medicine, biology, and life science research [19]. Nanomaterials also have been used in the drug designing, tracer and drug carriers [20]. It has been reported as toxic to animal cells, and it can induce the apoptosis in tumor cells [21]. It is known to elicit a significant biological response than microparticles [22]. Nanoparticles significantly affect cellular and biological activity [23]. Nanoparticle's cytotoxicity is due to size, mass, surface area and production of intracellular ROS [24]. Cancer is a cellular disease characterized by loss of normal cells, resulting in aberrant tissue growth, lack of differentiation, ability to invade local tissues, and metastasis [25]. Cervical cancer is the second most common cancer among females worldwide [26]. Over the past years, some drugs and treatments for this disease have been developed. The main problems with these treatments are toxicity to the normal cells, drug resistance, and expensiveness [27]. Therefore, there is a constant demand to develop new, safe, effective, and inexpensive anticancer drugs with lesser side effects on the immune system.

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http://dx.doi.org/10.1016/j.jphotobiol.2016.01.008
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Mangifera indica (family: Anacardiaceae) is found all over tropical regions of the world. *M. indica* is used medicinally to treat a cough, diarrhea, jaundice, asthma, malaria, and pains [28]. The fruits contain fat, carbohydrates, minerals, proteins, amino acids, and vitamins A, B and C [29]. In the present study, we report synthesis and characterization of $BaCO_3$ NPs using an aqueous extract of *Mangifera* seed and their in vitro anticancer activity.

2. Materials and Methods

2.1. Chemicals

High purity barium chloride ($BaCl_2 \cdot 2H_2O$) and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (Seoul, South Korea). *Mangifera* fruits were purchased from a supermarket in Gyeongsan, South Korea. Dimethyl sulphoxide (DMSO) and sulforhodamine B (SRB) were purchased from Sigma-Aldrich (St. Louis, MO 63178 USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, and trypsin–EDTA were obtained from Welgene (Daegu, South Korea). Rabbit polyclonal caspase −3 antibodies (C1815) were purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA). Donkey anti-rabbit IgG H&L (FITC) conjugated secondary antibody (GR200554-2) was purchased from Abcam (330 Cambridge Science Park, Cambridge).

2.2. Synthesis of $BaCO_3$ NPs

The *M. indica* seeds were rinsed with running tap water and then sun-dried for 2 weeks. *M. indica* seeds were ground into a fine powder, and 5 g was mixed with 100 mL of deionized water in a beaker, stirred at 60 °C for 30 min, and filtered through Whatman No. 42 filter paper and makeup up to 100 ml. The required quantities of $BaCl_2 \cdot 2H_2O$ and Na_2CO_3 were dissolved in 100 mL of the *Mangifera* seed extract, and the final concertation was 0.2 M. The resulting solution was stirred at 120 °C for 6 h and allowed to cool naturally to room temperature. A white precipitate was formed, and the supernatant was discarded. Finally, the precipitate was calcined at 400 °C for 2 h to obtain pure $BaCO_3 \cdot$NPs [30].

2.3. Characterization of $BaCO_3$ NPs

A dried sample of $BaCO_3$ NPs was pelletized with KBr and examined by Fourier transform infrared spectroscopy (FT-IR, Perkin-Elmer-Spectrum Two). Energy dispersive X-ray spectroscopy (EDAX) was performed using FE-SEM (S4200, Hitachi, Ltd.). For more information about the size and shape of the NPs, they were observed by high-resolution transmission electron microscopy (HR-TEM, Tecnai G2F20 S-Twin, USA) operating at an accelerating voltage of 200 kV with a point resolution of 0.24 nm and Cs of 1.2 mm.

To prepare a sample, a small amount of powder was dispersed in acetone, sonicated for 25 min, and drop-coated on a commercially available carbon-coated copper TEM grid. This was dried under a visible-light lamp for 15 min. The length scales are indicated systematically as a black bar on the bottom corner of the images. The NPs were examined by X-ray diffraction (XRD, PANalytical X'Pert^3 PRO, USA) using Cu K_α radiation ($\lambda = 1.54$ Å) at 40 kV and 30 mA. The chemical state and composition of the elements present in the $BaCO_3$ NPs were examined by X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha) using an Al K_α X-ray source (1486.6 eV). The source energies of 200 eV and 30 eV were used for the high and low-resolution scans, respectively.

2.4. Cell Culture

MDCK and HeLa cells were obtained from ATCC (10801 University Boulevard Manassas, VA 20110 USA). Cells were maintained in growth medium supplemented with 10% FBS and 1% antibiotics (penicillin–streptomycin). The cells were grown in a CO_2 incubator at 37 °C in 5% CO_2.

2.5. SRB Assay

MDCK and HeLa cells were seeded at a density of 2×10^4 cells/well into 96-well plates and allowed to adhere for 24 h at 37 °C. The cells were then treated with $BaCO_3$ NPs at different concentrations (0.001, 0.01, 0.1, 1, and 2 mg/mL) for 24 h. The cytotoxic effect on MDCK and HeLa cells was determined by SRB assay [31].

2.6. Caspase-3 Fluorescence Assay

HeLa and MDCK cells were cultured (cell density: 2×10^4 cells/well). Cells were treated with $BaCO_3$ NPs (0.001, 0.01, 0.1 and 1 mg/ml) for 24 h. At the end of 24 h, cells were centrifuged, and the supernatant was removed. Then, 200 μl of caspase-3 assay buffer was added to each tube, and then, 100 μl of cell-based assay lysis buffer was added. All the tubes were incubated on a shaker for 30 min at room temperature. All the tubes were centrifuged at 800 ×g for 10 min, and 90 μl of supernatant was transferred to the black 96-well plate. Then, 10 μl of caspase-3 assay buffers and 100 μl of caspase-3 substrate solution were added to each tube. The fluorescent intensity of caspase-3 was measured with excitation at 485 nm and emission at 530 nm (Caspase-3 Fluorescence Assay Kit, Item No-10009135, Cayman Chemical, 1180 East Ellsworth Road Ann Arbor, Michigan 48108 USA).

2.7. Immunofluorescence for Caspase-3

HeLa cells were cultured (cell density: 2×10^4 cells/well). Cells were treated with $BaCO_3$ NPs (0.1 and 1 mg/mL) for 24 h. At the end of 24 h, cells were fixed and permeabilized in 0.1% Triton X-100 in PBS for 20 min and then washed twice with PBS. Blocking was carried out with 3% bovine serum albumin (BSA) in PBS for 30 min, and the cells were incubated with rabbit polyclonal caspase-3 antibody (1:500 dilutions) (Santa Cruz Biotechnology, Inc.) for 12 h at 4 °C in PBS-1% BSA. Cells were washed with PBS, incubated with donkey anti-rabbit IgG H&L (FITC) conjugated secondary antibody (1:300 dilutions), and washed three times with PBS. The coverslips were mounted with a fluorescent mounting medium and viewed under a Confocal Laser Scanning Microscope (CLSM) (1×81^R Motorized Inverted Microscope, Olympus) [32].

3. Results and Discussion

3.1. Characterization of $BaCO_3$ NPs

Fig. 1a shows the FTIR spectra of the $BaCO_3$ NPs. The strong (1417, 858, 692 and 610 cm^{-1}) and low (1078 cm^{-1}) frequency absorption bands correspond to stretching vibrations [8]. The strong narrow absorption peaks at 692, 610, and 858 cm^{-1} can be assigned to the bending out-of-plane vibrations and in-plane vibrations of CO_3^{-2} [8]. The strong absorption peak centered at about 1417 cm^{-1} is related to the asymmetric stretching vibration [8,33] and the weak absorption band at about 1078 cm^{-1} for $BaCO_3$ is due to the symmetric stretching vibrations [34].

Fig. 1b presents the XRD pattern of the green synthesized $BaCO_3$ NPs. All the reflections obtained for the $BaCO_3$ NPs matched well with the standard crystallographic data from the Joint Committee on Powder Diffraction Standards (JCPDS). The peaks at 2 θ of 19.45, 23.90, 24.29, 27.72, 33.71, 34.08, 34.60, 41.98, 42.95, 44.18, 44.85, and 46.78 were assigned to the (110), (111), (021), (002), (200), (112), (130), (221), (041), (202), (132) and (113), planes, respectively. The standard XRD peaks confirmed the common orthorhombic structure (JCPDS No: 00-005-0378). The mean crystallite size D_{hkl} was calculated using the Scherrer's formula:

$$D_{hkl} = k\lambda/\beta\cos\theta \tag{1}$$

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