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Thiolated-2-methacryloyloxyethyl phosphorylcholine protected silver nanoparticles as novel photo-induced cell-killing agents

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ABSTRACT

Silver nanoparticles (AgNPs) have several medical applications as antimicrobial agents such as in drug delivery and cancer therapy. However, AgNPs are of limited use because of their toxicity, which may damage the surrounding healthy tissue. In this study, thiolated-2-methacryloyloxyethyl phosphorylcholine (MPC-SH) protected silver nanoparticles (MPC-AgNPs) are prepared as cell-killing agents under UV irradiation. MPC-AgNPs are characterized by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and UV–visible spectrophotometry. The surface plasmon resonance (SPR) band of MPC-AgNPs is observed at 404 nm, and the average diameter of the particles is determined at 13.4 ± 2.2 nm through transmission electron microscopy (TEM) and at 18.4 nm (PDI = 0.18) through dynamic light scattering (DLS). Cell viability in contact with MPC-AgNPs is relatively high, and MPC-AgNPs also exhibit a cell-killing effect under UV irradiation.

1. Introduction

Synthesized silver nanoparticles (AgNPs) with predictable and well-defined structures have many medical applications [1], such as in drug delivery [2], biosensing [3,4], and bioimaging [5]. Moreover, AgNPs are important cancer-treating drugs [6–8], photosensitizing agents for photodynamic therapy, and antimicrobial agents [9].

The mechanisms causing cell death by AgNPs are associated with the toxicity of the substance itself, the release of silver ions, and the generation of reactive oxygen species (ROS) [10] on the surface of AgNPs. Miura and Shinohara observed the cytotoxicity of AgNPs and $AgNO_3$ by testing their influence on the expression levels of several stress-response genes in a HeLa cell [11]. Their results showed that AgNPs toxicity was lower than toxicity induced by $AgNO_3$. In addition, Guo et al. studied AgNPs cytotoxicity on acute myeloid leukemia (AML) cells [7], and their results supported the model that both the release of silver ions and the generation of ROS induces a cytotoxic effect on cells.

However, the use of AgNPs is limited so far, because they may also damage the surrounding healthy tissue. Recently, interest arose on the potential surface modification of AgNPs using polymers; these studies show that toxicity is decreased and the stability of the AgNPs particles increases [9,12,13]. Ahn et al. modified AgNPs with polyvinylpyrrolidone (PVP) [12]; when the LC_{50} of the modified AgNPs was compared with the LC_{50} of $AgNO_3$ (LC_{50} = 0.046 μg/mL) and with bare AgNPs (LC_{50} = 0.041 μg/mL), the AgNPs toxicity was found to be decreased. In addition, the toxicity of PVP-coated AgNPs was compared using small particle sizes (8-nm-sized particles, LC_{50} = 0.607 μg/mL) and large particles sizes (38-nm-sized particles, LC_{50} = 3.262 μg/mL). The smaller particles were more toxic than the larger particles. Ag toxicity is considered to cause oxidative stress-related mitochondrial and DNA damage. In addition, Roh et al. studied the dispersion stability of citrate and PVP-stabilized AgNPs with and without photo-irradiation in various biological media such as fetal bovine serum (FBS), phosphate-buffered saline (PBS), and deionized water at various pHs (2, 7, and 9) [13]. They found that the steric hindrance of PVP-AgNPs resulted in good dispersion stability in biological media, when compared with the electrostatic repulsion of citrate-AgNPs. Furthermore, Lee et al. synthesized polyethylenimine (PEI)-capped

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silver nanoparticles (PEI-AgNPs) in the presence of sodium borohydride ($NaBH_4$) for an antimicrobial application [9]. PEI can act as a stability agent via agglomeration, and $NaBH_4$ was added to decrease the amount of cytotoxicity. Lee et al. found that the particles have excellent colloidal stability and antimicrobial activity.

Nowadays, photodynamic therapy (PDT), which uses certain drugs or photosensitizing agents and a photosensitizer, is widely used in cancer therapy. PDT works only after photosensitizing agents are activated by light of a specific wavelength, and it selectively targets diseased cells. Boca et al. synthesized chitosan-coated silver nanotriangles (Chit-AgNTs) as effective photothermal transducers (hyperthermia therapy) for in vitro cancer therapy [6]. These particles exhibit good biocompatibility and can act as effective photothermal transducers under laser irradiation. However, few studies involving PDT using AgNPs, including AgNTs [6,8], exist, and no research using PDT with modified AgNPs as photosensitizing agents for cancer therapy has been conducted. Moreover, the photosensitizer's performance with capping agents requires further development to deliver minimal or no toxicity, stability, and high efficiency of PDT, as this could potentially be one of the best future medical treatments.

In the present study, we synthesize reduced-toxicity thiolated-2-methacryloyloxyethyl phosphorylcholine (MPC-SH) protected AgNPs (MPC-AgNPs). The modified AgNPs are not only biocompatible particles, thus avoiding damage to healthy tissue, but also exhibit improved dispersion stability. Furthermore, MPC-AgNPs act as cell-killing agents under UV irradiation.

2. Materials and methods

2.1. Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC) was kindly donated by the NOF CORPORATION, Tokyo, Japan, and the other chemicals of extra-pure grades were used in polymer synthesis and were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan; these were employed without further purification. Water was purified using the Millipore Milli-Q system, which involves UV irradiation, ion exchange, and filtration (18.2 $M\Omega\,cm^{-1}$).

2.2. MPC-SH synthesis

MPC-SH was synthesized using a previously described method [14]. Briefly, MPC (14.76 g, 50 mmol) and 1,6-hexanedithiol (15.03 g, 100 mmol) were dissolved into a 200-mL round-bottom flask containing 100 mL of degassed chloroform. Diisopropylamine (278.8 μL, 2.0 mmol) was added to the mixture and stirred for 22 h at room temperature. The mixture was precipitated into acetone and was subsequently dried in a vacuum desiccator for 2 h to eliminate the residual acetone. Finally, the product was dissolved in water and lyophilized.

2.3. MPC-AgNPs synthesis

MPC-SH (32.5 mg, 0.08 mmol), silver nitrate (39.95 mg, 0.24 mmol), and formic acid (8.45 μL, 0.45 mmol) were all respectively dissolved in 20 mL of deionized water. After preparing each reagent solution, the silver nitrate solution and the MPC-SH solution were mixed with magnetic stirring at 70 °C in an oil bath. After five minutes, a freshly prepared formic acid solution was added to the mixture and stirred for 4 h at 70 °C in the oil bath. The solution mixture was then dialyzed using a dialysis membrane with a molecular weight cut off at 14 kDa for three days to eliminate any unreacted MPC-SH and $AgNO_3$. Finally, the mixture was centrifuged at 6000 rpm for 15 min, and the supernatant was lyophilized and kept in a refrigerator.

2.4. MPC-AgNPs characterization

MPC-AgNPs were characterized using a UV–visible spectrophotometer (V-650 spectrophotometer, Jasco, Tokyo, Japan), X-ray diffraction (XRD; D2 Phaser, Bruker, Billerica, USA), X-ray photoelectron spectroscopy (XPS; ESCA-3400, Shimadzu, Kyoto, Japan) with Al Kα X-rays at a take-off angle of 10°, and thermogravimetric analysis (TGA; TG-8120, Rigaku, Tokyo, Japan). A platinum (Pt) crucible was used for heating, and measurements were carried out in a nitrogen atmosphere at a heating rate of 5 °C/min.

The morphology and size of the particles were then analyzed by transmission electron microscopy (TEM; JEM-1400, JEOL, Tokyo, Japan) operating at 200 kV. TEM samples were prepared by dropping particles on a TEM grid before being dried in a desiccator for one day. The average diameters of the observed particles were reported from measurements of 200 random particles using the Semaphore software. Dynamic light scattering (DLS; ZETASIZER NANO-ZS, Malvern Instruments Ltd., Worcestershire, UK) was also used to analyze the average diameter of the particles and the polydispersity index (PDI). The FT-IR spectra of the modified particles were recorded in a frequency range of 500–4000 cm^{-1} by an FT-IR spectrometer (FT/IR-6300, Jasco, Tokyo, Japan) with 64 scans using a TGS detector.

2.5. Cell cultures

HeLa cells were grown in Dulbecco's modified Eagle's Medium (D-MEM; Gibco, Life Technologies, New York, USA), high glucose medium supplemented with 10% (v/v), fetal bovine serum (FBS; Biowest, Perth, Australia), and 1% antibiotic–antimycotic (Gibco, Life Technologies, New York, USA). Cells were cultured in a cell culture flask at 37 °C in a humidified atmosphere of air and 5% CO_2. A routine subculture was made every four days during the experiment by detaching cells with trypsin solution (0.25% trypsin containing 0.01% EDTA), and changing and diluting the medium at 2.4×10^5 cells/mL.

2.6. Cell viability test

The viability of the cells in contact with MPC-AgNPs was determined using a Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan). HeLa cells (1.0×10^5 cells/mL, 100 μL) were seeded in a culture medium on 96-well plates and were then incubated for 24 h at 37 °C in a 5% CO_2 incubator. After incubation, the cells were treated with MPC-AgNPs at various concentrations (0–90 μg/mL), and the particles in contact with cells were exposed to UV irradiation (365 nm) for 1 h. The cells were incubated for 24 h. At the same time, Control A (cells and a culture medium), Control B (culture medium only), and Blank (particles in a culture medium) were monitored using the same conditions. After incubation, 10 μL CCK-8 reagent were added into each well, and the cells were incubated in the dark for 4 h at 37 °C. Finally, absorbance was measured at 450 nm using a microplate reader. To ensure the reproducibility of the results, the experiment was conducted thrice. The percentage cell viability was calculated using the following Eq. (1):

Cell viability $< MML : MOSTRETCHY = "FALSE" > (< MML : MO > \% < MM$

The LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, Eugene, Oregon, USA) was used to test the viability of cells in contact with MPC-AgNPs. The HeLa cells (2.0×10^5 cells/mL, 1000 μL) were seeded in the culture medium on a 22-mm covered glass-bottom dish and, then, incubated for 4 h at 37 °C in a 5% CO_2 incubator. Afterward, the culture medium in each of the cell dishes