Biomaterials 34 (2013) 5833–5842

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Platinum nanoparticles for the photothermal treatment of Neuro 2A cancer cells

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ARTICLE INFO

Article history:
Received 31 January 2013
Accepted 23 March 2013
Available online 30 April 2013

Keywords:
Neuro 2A cancer cells
Pt NPs
Photothermal treatment
Compatibility
Toxicity

ABSTRACT

This study demonstrates the effective synthesis of five different sized/shaped Pt NPs, within a narrow size regime of 1–21 nm using a modified methodology and the toxicity/biocompatibility of Pt NPs on Neuro 2A cancer cells was investigated elaborately by using light microscopic observations, tryphan blue exclusion assay, MTT assay and ICP-MS. The Pt NPs-C with sizes 5–6 nm showed superior non-cytotoxic property compared to the other four Pt NPs. These non-cytotoxic Pt NPs were employed for successful photothermal treatment of Neuro 2A cell lines using near-IR 1064 nm of laser irradiation. The Pt NPs-C could generate a 9 °C increase in temperature leading to effective photothermal killing of cancer cells. The MALDI-MS was used to prove the possibility of apoptosis related triggering of cell death in the presence of the Pt NPs. The results confirm that the current approach is an effective platform for *in vivo* treatment of neuro cancer cells.

1. Introduction

Cancer is a disease of multiple complexities and affects millions of people irrespective of age and sex. It has been estimated that human mortality of one in four is due to cancer [1]. Cancer arises from multifactorial causes in which genetic and environmental factors are the major concerns [2–4]. Dissection of the genetic networks and molecular and cellular interactions of the cancer models generated a rich knowledge about new targets and created new platforms for therapies [5]. However, the resistant nature of cancer cells against traditional anticancer drugs [6], the activity of cancer stem cells [7] and insufficient bioavailability of the anti-cancer drugs to exert pharmacological effects [8] have become potent challenges during cancer therapies. Although the current cancer therapies offer a notable fight against cancer, they could eventually trigger numerous deleterious side effects. Therefore, there is an absolute need for novel cancer treatment methods to overcome the above-said bottle necks of the present cancer therapies which would obviously destroy the diseased tissues without harming the normal tissues.

Scaling down the materials to nanometer size offers particles with unique physicochemical properties such as, a large surface to mass ratio as well as high reactivity [9]. Nanotechnology has created an excelling platform for cancer diagnostics, drug delivery and therapeutics. NPs with the fluorescence emitting properties have been used in cancer diagnostics. Different nanomaterials such as quantum dots (QDs) [10,11], gold nanoparticles [12], silica nanoparticles [13] and nanocomposites [14,15] have been employed for various biomedical applications. In addition to that, nanomaterials from carbon origin such as carbon nanotubes [16], graphene sheets [17] and nanodiamond [18] have also become promising candidates in cancer diagnostics and therapies. Non-cytotoxic nanoparticles which do not show fluorescence have been used for the detection of cancer by functionalizing fluorescent emitting molecules on their surfaces [19]. Nanomaterials are being used as carrier vehicles for selectively transporting the anticancer drugs and RNAi to selectively induce some pathways which can destruct the diseased cells [20,21].

Some nanomaterials are capable of absorbing the laser light and transforming it into localized heat energy, are being exploited for cancer cell/tumor destruction (photothermal therapy (PTT)) in order to replace surgery or chemotherapy. NIR radiation around 700–1000 nm can pass through the biological systems without causing

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http://dx.doi.org/10.1016/j.biomaterials.2013.03.077

any harm, but could effectively create local heating around the NPs thereby resulting in cell death during photothermal treatment [22,23]. Noble metal NPs have received a great deal of attention for their excellent photothermal property in response to laser and thus have been extensively investigated as photothermal agents for *in vivo* cancer therapy [24].

Of the noble nanomaterials, Au NPs with different nanostructures such as Au nanorods [25,26], Au nanoshells [27,28], Au nanocages and spherical Au NPs have been extensively used in photothermal treatments for cancer cells [24]. Moreover, Au NPs in combination with other noble metals like Ag (bimetallic) nanomaterials [29] and Au/Ag nanostructures with dendrite morphology and hollow interior [30] have shown prominent *in vivo* cancer cell destruction potency. Au ornamented Si nanowires [12], magnetic or paramagnetic materials [31,32] and carbon based nanomaterials [33] have been reported to have the capability to enhance the photothermal effect on cancer cells.

Although the nanomaterials from noble metals were used in photothermal treatment, Au based nanomaterials were employed predominantly because of its non-cytotoxic property. Platinum NPs, one among these noble metals exhibits dual functionality, generates strand breaks in DNA when it was used in soluble forms [34] and on the other hand, its insoluble forms possess notable antioxidative capacity [35,36]. Hence, the pure metallic Pt NPs have been recommended for novel anti-cancer approaches [37]. However, the Pt NPs render toxicity to normal cells too, when it circulates in the blood stream. This has to a large extent discouraged researchers from experimenting on Pt NPs for cancer treatment. In the present study, we report the size/shape dependant cell-compatibility of Pt NPs. For the first time, we have screened the optimal size of Pt NPs for successful photothermal treatment of Neuro 2A cancer cells.

2. Materials and methods

2.1. Synthesis of Pt nanoparticles

Different sized Pt nanoparticles stabilized with PVP were prepared with moderate procedural modification from previous research [38]. For the synthesis of different size Pt NPs, the precursor Pt complex, dihydrogen hexachloroplatinate (IV) hexahydrate, was taken in a 50 mL round bottom flask in varying quantities such as 5.0, 20.0, 200.0 and 500.0 mg and 10 mL PVP solution (90 mM in ethylene glycol, based on polymer monomer) was added and stirred till the precursor dissolved. Reaction temperature was maintained 120 °C and the platinum nucleation–reduction reaction was initiated by adding NaOH (1 M, 50 μL) drop wise. Slowly, a brown color appeared which finally turned to a tinted shade indicating the formation of Pt nanoparticles stabilized with PVP. The reaction mixture was stirred for 30 min more and cooled to room temperature. The Pt NPs stabilized by PVP were precipitated using acetone (1:3). Later, the mixture was centrifuged at 18,000 rpm for 5 min and the supernatant was discarded. Pt NPs were dispersed in deionized water and also in MEM medium for further use and stored at 4 °C. A double-beam spectrophotometer (U3501, Hitachi, Tokyo, Japan) was used for the characterization of the Pt NPs. The samples were prepared by vacuum drying. 0.5 μL of the aqueous solution of Pt NPs was deposited on a copper grid separately with the help of a micropipette, and then kept overnight in a vacuum drying oven (Hipoint Jih Her Tyan Scientific, Taiwan). After drying, the sample was analyzed using a high resolution-transmission electron microscope (TEM) (JEOL TEM-3010, Tokyo, Japan) at 75 keV.

2.2. Cell culture

Neuro 2A cell lines were purchased from Bioresource Collection and Research Center (BCRC), Taiwan. The cells were grown in the Minimum Essential Medium (MEM) medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FCS) and 1% (v/v) penicillin/streptomycin (PS). Cells were incubated at 37 °C in a humidified incubator supplied with 5% CO_2 (Sanyo Inc, Japan) and sub-cultured regularly at 80–90% confluence.

2.3. Size and shape effect of Pt NPs on the Neuro 2A cells

Neuro 2A cancer cells (1×10^5) were grown in the tissue culture dishes (3.5 cm diameter) for 24 h at 37 °C in a humidified incubator supplied with 5% CO_2. Three different concentrations of Pt NPs (with different size and shape) were prepared in fresh MEM medium by filter sterilization of the medium and the Pt NPs mixture using a membrane filter (0.2 μm). This was used to replace the aspirated medium of the cultured cells. The cells were allowed to grow for 12 h. Then the dead cells were observed under light microscope in a floating planktonic state. Further, the dead cells were also enumerated by tryphan blue exclusion assay for which the total cells were collected and washed with PBS and stained with 0.4% Tryphan blue solution and counted under the light microscope (ESPA FI40 (NIB-100F, ESPA systems Co. Ltd., Taiwan)). The cell viability was expressed as the percentage of living cells.

2.4. Pt NPs heating model

Initially, all Pt NPs were screened for the temperature raise irrespective of the size/shape, by laser irradiation. Each Pt NPs at 50 μg/mL were resuspended in complete MEM medium and transferred into the quartz cuvette. The nanoparticle suspension was irradiated with 1064 nm near-infrared pulse wave and the Nd YAG laser (Minilite, Continuum Inc., USA) set at 3 W for 480 s. Pre and post-exposure temperature measurements were determined using a thermocouple. The photothermal conversion efficiency was calculated by the method described by Lambert et al. [39], using the following equation

$$Q\text{abs}/Q\text{tot} \times 100\% \quad (1)$$

where, *Q*abs represents the total energy absorbed by the aqueous solution containing the Pt NPs and *Q*tot represents the total laser energy supplied to the samples over time. *Q*tot was calculated using the multiple of the following parameters such as, laser power, laser beam spot and irradiation time. The resulted energy of *Q*tot was expressed in Joules. To measure the *Q*abs the following equation was used

$$Q\text{abs} = [(M_{\text{Pt}} \times Cp_{\text{Pt}}) + (M_{\text{w}} \times Cp_{\text{W}})] \times (\Delta T) \quad (2)$$

where M_{Pt}, Cp_{Pt}, represent moles of Pt in Pt NPs and the heat capacity of Pt respectively. M_{w}, Cp_{W} are moles and heat capcity of water and ΔT is the temperature change after 8 min of laser irradiation.

The cells were cultured in 96-well plates with different dilutions of cells/well for 24 h. For the Pt NPs treatment of the cells, different concentration such as 25, 50, 75 and 100 μg was prepared as before in MEM medium and were replaced with aspirated medium followed by an incubation for 8 h at 37 °C with 5% CO_2 concentration. After the Pt NPs treatment, the plates were irradiated with 1064 nm near-infrared pulse wave Nd YAG laser (Minilite, Continuum Inc., USA) for 480 s and the cells were aseptically transferred to a fresh 96 welled plate and allowed to grow for further 24 h. Cell viability was assessed using MTT assay.

2.5. Evaluating cell viability using MTT assay

After the cells were treated using Pt NPs and laser irradiation, the cells attached to the surface were gently washed using PBS and incubated with 0.5 mg/ml of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) (Sigma Chemical Co., St. Louis, MO) in complete MEM medium for 3 h. The live cells converted MTT to formazan (blue-purple color) when dissolved in dimethyl sulfoxide (DMSO). The intensity of formazan was measured at 570 nm using a plate reader (Multiskan GO-Thermo Scientific Inc., USA) for enzyme-linked immunosorbent assays. Cell viability was calculated by dividing the absorbance of the cells treated with Pt NPs/laser by that of untreated cells.

2.6. ICP-MS measurements

For the determination of the platinum content, Neuro 2A cells with and without Pt NP treatment were analyzed by the inductively coupled plasma mass spectrometer (ICP-MS) in peak hopping gas mode. (Perkin–Elmer SCIEX ELAN 6100 DRC plus). The instrument was set with the nebulizer microflow at 0.98 L/min. The RF power was adjusted to 1300 W, and the flow rate of the plasma gas and auxiliary gas flow were 15.0 and 1.325 L/min respectively. The instrument was equipped with Rhodium (1.0 ppb, m/z 103) as the internal standard and the Pt content was monitored in its two isotopic forms at m/z 194, m/z 195. The cellular uptake of Pt NPs was measured by subtracting the quantity recovered from within the cells from the total quantity of Pt NPs incubated with the cells.

2.7. MALDI-MS based identification of apoptosis

Neuro 2A cells (1×10^4) were grown in 3 mm cell culture dishes for 24 h in MEM medium. The medium contained different concentrations of Hydroxy tetraindole (HTI), an apoptosis drug (Li et al., 2012) ranging from 1 to 5 μM with 1 μM increase was replaced and incubated for further 24 h. The cells were collected and washed in PBS and then subjected for MALDI MS analysis for detecting the apoptosis marker peaks against the non drug treated control cells. Similarly the Pt NPs (Pt NPs-A, Pt NPs-B, Pt NPs-C, Pt NPs-D and Pt NPs-E) treated cells were also processed for MALDI-MS analysis for detecting the apoptosis marker peaks.