Biomaterials 34 (2013) 1128–1134

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Photothermal cancer therapy via femtosecond-laser-excited FePt nanoparticles

Cheng-Lung Chen [a], Ling-Ru Kuo [a], Shin-Yu Lee [a], Yeu-Kuang Hwu [a], Shang-Wei Chou [a,b], Chia-Chun Chen [b,c], Fu-Hsiung Chang [d], Kung-Hsuan Lin [a], Dzung-Han Tsai [a], Yang-Yuan Chen [a,e,*]

[a] Institute of Physics, Academia Sinica, Taipei 11529, Taiwan
[b] Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan
[c] Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, Taiwan
[d] Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Taipei, Taiwan
[e] Graduate Institute of Applied Physics, National Chengchi University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 4 September 2012
Accepted 17 October 2012
Available online 5 November 2012

Keywords:
Photothermal therapy
FePt
Nanoparticle
Cancer therapy
Thermal lens
Laser

ABSTRACT

FePt nanoparticles (NPs) have recently been revealed to be significant multifunctional materials for the applications of biomedical imaging, drug delivery and magnetic hyperthermia due to their novel magnetic properties. In this study, a newly discovered photothermal effect activated by the near infrared (NIR) femtosecond laser for FePt NPs was demonstrated. The threshold laser energy to destroy cancer cells was found to be comparable to that of gold nanorods (Au NRs) previously reported. Through the thermal lens technique, it was concluded that the temperature of the FePt NPs can be heated up to a couple of hundreds degree C in picoseconds under laser irradiation due to the excellent photothermal transduction efficiency of FePt NPs. This finding boosts FePt NPs versatility in multifunctional targeted cancer therapy.

1. Introduction

The application of thermal heat to eliminate or restrain specific cancer cells is a widely acknowledged approach in optimizing cancer therapy [1]. This non-invasive technique to eradicate tumor cells is generally referred to as hyperthermia or thermotherapy, in which the elevated temperature can promote the denaturation of intracellular protein and/or the disruption of membrane, leading to cell death. The performance of thermotherapy is mainly influenced by the power density of the applicators, and the energy absorption and thermal conductance of the biological environment. Thermotherapy has far fewer restrictive side effects than conventional chemotherapy and radiotherapy [2]. It also has greater potential in evading any developed intracellular resistance mechanism, and is beneficial in dealing with some types of malignant tumor cells.

Thermotherapy has been under investigation for some time now, the main challenge in its development is in achieving highly localized thermal effect on tumor cells with defined lesion boundaries within a short period of time. Recently, thermotherapies based on NIR irradiation–activated nanomaterials have received significant attention due to their efficacy in destroying cancer cells. In particular, because of their unique optical and thermal properties, gold and carbon based nanomaterials gained significant interest [3–8]. Nonetheless, the approach is by no means perfect, and the investigation into exploring superior materials and methodology is still imperative. In this study, we present the possibility of using magnetic FePt NPs excited by NIR femtosecond laser for aforementioned tumor-targeted therapy. FePt NPs have recently gained recognition as a superior material in cancer diagnosis and therapy [9–11]. The excellent superparamagnetic property and high X-ray absorption of FePt NPs make it a potential dual modality contrast agent for computed tomography (CT) and magnetic resonance imaging (MRI). The transport and/or immobilization of magnetic nanoparticles to a targeted region of a tumor can be easily manipulated by an external magnetic field gradient. Similarly, the magnetic FePt NPs can respond resonantly to an external AC magnetic field and result in a controllable heating effect, serving as hyperthermia agents delivering thermal energy to targeted tumors. Thus, FePt NPs present an opportunity in realizing CT, MRI, magnetic–thermal, and photothermal treatments all within a single agent. The benefits of such versatility should make FePt NPs, or similar materials, competitive candidates in future cancer therapies.

* Corresponding author. Institute of Physics, Academia Sinica, Taipei 11529, Taiwan. Tel.: +886 2 27896725; fax: +886 2 27834187.
E-mail address: cheny2@phys.sinica.edu.tw (Y.-Y. Chen).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.10.044

FePt NPs were chemically synthesized and functionalized with folates to target breast cancer cells EMT-6. When subjected to activation by NIR femtosecond laser, we discovered that FePt NPs triggered considerable intracellular explosions, which resulted in the perforation and/or sudden rupture of the plasma membrane. The distinctive thermal–optical property of FePt NPs was further investigated using thermal lens spectroscopy. The energy fluence threshold for cancer cells destruction and the photon-to-heat conversion efficiency of FePt NPs were compared with that of Au NRs.

2. Materials and methods

2.1. Preparations of FePt NPs

The FePt NPs were prepared through confined decomposition of $Fe(CO)_5$ (iron pentacarbonyl, Fluka, 99%) and reduction of $Pt(acac)_2$ (platinum acetylacetonate, ACROS, 97%) in the presence of oleic acid (Aldrich, 90%) and oleyl amine (Aldrich, 70%) [9,12]. Briefly describing, $Pt(acac)_2$, 1,2-hexadecandiol (Aldrich, 90%), dioctyl ether (ACROS, 90%), $Fe(CO)_5$, oleyl amine and oleic acid were mixed and heated to 240° C. After several minutes, the reaction mixture was cooled to room temperature. The black product was separated by adding ethanol and centrifugation. The final product was stored in hexane or toluene, and the water-soluble NPs were obtained by the following ligand-exchange procedures. The dry FePt NPs were then redispersed in a mixture of Dimethylsulfoxide (DMSO, Sigma) and 3-mercaptopropionic acid. Then the nanoparticles were separated by centrifugation, and the precipitate was dispersed in water (pH ~12). The UV–visible absorption spectra of nanoparticles were obtained by the spectrophotometer (Spectronic, GENESYS-8), and the size and morphology of nanoparticles were analyzed on a Philips/FEI Tecnai 20 G2 S-Twin transmission electron microscope.

2.2. Preparation of the folate-conjugated FePt NPs

The folate-conjugated FePt NPs were prepared by conjugating the nanoparticles with carboxyl group with folic acid. These nanoparticles were first activated with *N*-ethyl-*N* 8-(dimethylaminopropyl)-carbodiimide (EDC), and then folic acid was added and stirred for at least 12 h at room temperature. The folate-conjugated nanoparticles were then separated by centrifugation and washed several times.

2.3. Cell culture and cell viability assay (MTT)

EMT-6 cells were cultured in folic acid free medium to ensure overexpression of folate receptors on the surface of the cells. To determine the cytotoxicity of nanoparticles, the cell viability is generally quantified by colorimetric assay. Briefly, the number of living cells is directly proportional to the absorbance of formazan which was produced from the mitochondrial oxidation of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) in living cell. EMT-6 cells were harvested after passage and plated at a density of 1.25×10^5 cells/mL in 96-well plates with 300 μL medium (100 μL/well), and incubated at 37° C under a 5% CO_2 atmosphere for 24 h. After that, these cells were further incubated for 24 and 48 h with 3-mercaptopropionic acid-modified FePt at varying concentrations; these were then treated with a freshly prepared 12 mM MTT solution (10 μL) and incubated for an additional 3 h. Next, MTT solution was removed and 50 μL of DMSO was added to each well. The wells were left for 30 min in the dark, and then assayed with an automated reader, the absorbance was fixed at 570 nm. The acquired cell viability was expressed as a percentage relative to cells incubated with medium only.

2.4. Femtosecond laser triggered photothermolysis of cancer cells

The photothermolysis of cancer cells was performed on an inverted scanning microscope (LSM510, META/Observer, Z1, Zeiss). A femtosecond (fs) Ti:Sapphire laser (Spectra-physics MaiTai HP) with a duration time of 100 fs, linearly polarized and with a repetition rate of 80 MHz was equipped in the microscope as the excitation source. The wavelength and average power of the laser beam were tunable, and a water-immersion objective lens (NA = 1.4) was used. For photothermolysis studies, the centered wavelength can be tuned from 700 to 990 nm, an area of $90 \times 90\ \mu m^2$ (512×512 pixels; each pixel area = $176 \times 176\ nm^2$) was scanned at a relatively slow exposure time of 164 μs per pixel per scan to activate FePt NPs. The exposure time of nanoparticles was calculated following the established method [13]. In this work, one scan of two different excited power densities (148 and 19 W/cm^2) were applied to the objectives. The calculated energy fluences were about 70 and 10 mJ/cm^2, respectively.

2.5. Preparation of cell specimens for electron microscopy

The EMT-6 cells were cultured in a Petri dish and treated by trypsins. Next, the cells were fixative and pelleted by a series of standard protocol. Then, cell pellets were post-fixed in OsO_4 solution, washed and finally dehydrated in ethanol series and acetone. The samples were further hardened under the procedure of infiltrating in Spurr's embedding mediums. The ultrathin sections were cut by using an ultramicrotome (LEICA, EM, UC6), and stained with uranyl acetate and lead citrate for observation by a transmission electron microscope (Hitachi, H-600).

2.6. Thermal lens spectroscopy

100 fs pump pulses (800 nm, 80 MHz) were focused to the samples with a 30 cm lens. The incidence power was ~30 mW, and the diameter of the focused spot was ~0.22 mm. The pump beam was modulated with a chopper, which controls the exposure time. The chopping frequency varies between 10 Hz and 400 Hz in the experiment. Another 638 nm continuous-wave lasers (probe) were focused with a 20 cm lens, and the incidence power was attenuated to 0.2 mW. The focal point was 2 cm in front of the samples, and the diameter of the spot was ~0.13 mm. The diverging probe beam (after the focal point) was carefully aligned to overlap with the pump spot at the sample. A photodiode (with active area 0.8 mm^2) was placed ~2 m behind the sample to measure the intensity at the center of the diverged spot. The electrical signal from the photodiode was demodulated with a lock-in amplifier.

3. Results and discussion

3.1. Characterization of FePt nanoparticles

Cubic-like shaped FePt NPs with a face-centered cubic structure were prepared by the thermolysis of metal-organic precursors and surfactant-assisted methods [9]. The average size of the prepared FePt NPs is 12 ± 1.0 nm in diameter, and its alloying composition is found to be $Fe_{34}Pt_{66}$ (Fig. 1). To characterize the magnetic properties of FePt NPs, a superconducting quantum interference device (SQUID) was employed to measure their magnetic properties. In the inset of Fig. 2a, the superparamagnetic behaviors of FePt NPs with blocking temperature (T_B) ~ 100 K is shown, and the magnetic field dependence of magnetization below and above T_B is revealed (Fig. 2a). To have FePt NPs suitable for biological applications, a ligand-exchange procedure was performed on FePt NPs to displace the original surfactants on the nanoparticles' surface. Here, 3-mercaptopropionic acid (MPA) was chosen to produce COOH-terminated water-dispersible FePt NPs [14]. The surfaces of MPA-FePt NPs were then modified with folic acid to specifically target folate receptors that are often overexpressed in breast, lung, colon

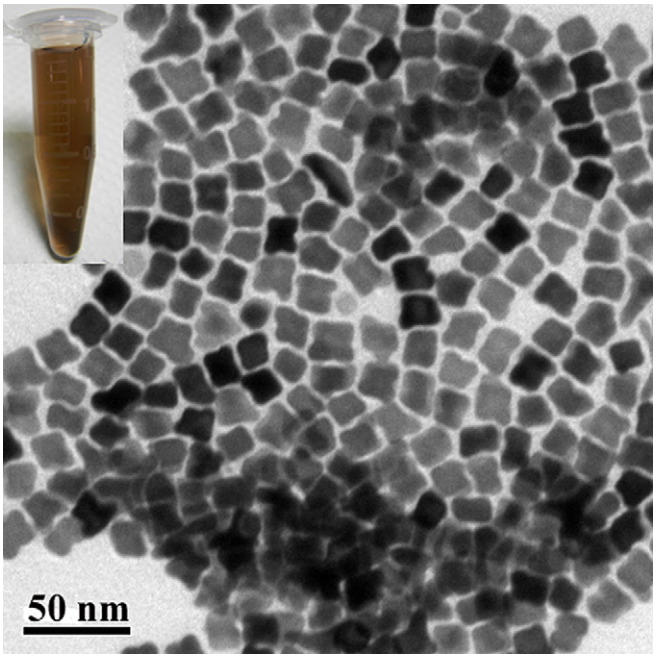

Fig. 1. The TEM image and solution color feature of FePt NPs.