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Magnetic nanoparticle clusters for photothermal therapy with near-infrared irradiation

Shun Shen [a, 1], Sheng Wang [a, c, 1], Rui Zheng [b], Xiaoyan Zhu [d], Xinguo Jiang [a], Deliang Fu [c], Wuli Yang [b, *]

[a] School of Pharmacy & Key Laboratory of Smart Drug Delivery, Fudan University, Shanghai 201203, China
[b] State Key Laboratory of Molecular Engineering of Polymers & Department of Macromolecular Science, Fudan University, Shanghai 200433, China
[c] Pancreatic Disease Institute, Department of Pancreatic Surgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai 200040, China
[d] Department of Integrative Oncology, Shanghai Cancer Center, Department of Oncology, Shanghai Medical University, Fudan University, Shanghai 200032, China

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ABSTRACT

In this study, the photothermal effect of magnetic nanoparticle clusters was firstly reported for the photothermal ablation of tumors both *in vitro* in cellular systems but also *in vivo* study. Compared with individual magnetic Fe_3O_4 nanoparticles (NPs), clustered Fe_3O_4 NPs can result in a significant increase in the near-infrared (NIR) absorption. Upon NIR irradiation at 808 nm, clustered Fe_3O_4 NPs inducing higher temperature were more cytotoxic against A549 cells than individual Fe_3O_4 NPs. We then performed *in vivo* photothermal therapy (PTT) studies and observed a promising tumor treatment. Compared with PBS and individual magnetic Fe_3O_4 NPs by NIR irradiation, the clustered Fe_3O_4 NPs treatment showed a higher therapeutic efficacy. The treatment effects of clustered Fe_3O_4 NPs with different time of NIR illumination were also evaluated. The result indicated that a sustained high temperature generated by NIR laser with long irradiation time was more effective in killing tumor cells. Furthermore, histological analysis of H&E staining and TUNEL immunohistological assay were further employed for antitumor efficacy assessment of PTT against A549 tumors.

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1. Introduction

Nanomaterials have been extensively used in biomedical research during the past twenty years [1–4]. Especially in recent years, more and more studies focused on photo-absorbing nano-agents, and photothermal therapy (PTT) employing photoabsorbers can convert optical energy into thermal energy to kill cancer cells without affecting healthy tissues [5,6]. Compared with radio-therapy, chemotherapy and surgical management, PTT is less invasive, controllable and highly efficient. Our and other research groups have developed a large number of nanomaterials as PTT agents, such as gold-based nanomaterials [5,7–9], carbon nano-tubes and graphene [10,11], all of which show strong optical absorbance in the near-infrared (NIR) tissue optical transparency window. The previous results demonstrated that photoabsorber-based therapy might be a promising approach for cancer therapy, which could effectively reduce tumor growth and enhance survival [12–15]. Even so, the potential toxicity induced by photothermal agents (especially for carbon nanotubes or graphene, and so on), is still an unresolved debate [16,17], which will inevitably limit future clinic applications of PTT. These non-degradable or slowly degradable nanoparticles easily accumulate in bodily organs, resulting in increased oxidative stress, inflammatory cytokine production and cell death [18]. Therefore, it is significant to explore a bio-safety and biodegradable photoabsorber for the photothermal ablation of cancer with NIR irradiation.

Magnetic iron oxides nanoparticles (namely Fe_3O_4 NPs) have received tremendous attention for their excellent magnetic, biocompatible and potentially non-toxic properties [19,20]. Fe_3O_4 NPs, which can be manipulated and controlled by an external magnetic field, are additional important materials and have been employed in many areas, including biology, pharmaceuticals and diagnostics. Moreover, iron is a nutrient and readily metabolized by cellular regulation using the transferrin pathway. Thus, Fe_3O_4 NPs are easily degradable and passes in and out of cells across the plasma membrane [21]. For the acknowledged advantages of Fe_3O_4 NPs, they are extremely suitable for *in vivo* applications. In addition,

* Corresponding author. Tel.: +86 21 65642385.
E-mail address: wlyang@fudan.edu.cn (W. Yang).
[1] Both authors contributed equally to this work.

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Fe_3O_4 NPs are exceptional materials for hyperthermia treatment of tumors [22,23]. Under an alternating magnetic field (AMF), magnetic hyperthermia of Fe_3O_4 NPs is produced via dipole relaxation, which can be employed to destroy tumor cells because these cells are more sensitive to temperatures in excess of ca. 41 °C than their normal counterparts [24]. Although magnetic hyperthermia has been clinically used [25–28], the technique requires high current and voltage due to large air volume within the applied field in which energy cannot be easily focused [29].

Very recently, NIR light induced photothermal effect for Fe_3O_4 NPs has been studied and it exhibits good photothermal converting efficiency. For example, Chu et al. applied individual Fe_3O_4 NPs with high concentration for the photothermal ablation of cancer by NIR laser irradiation [29]. Considering extra-high-dose magnetite might generate potential toxicity to the body, the number of usable elements should also be severely limited. Thus, Fe_3O_4 NPs used for PTT must be improved, mainly involving two routes. One is to modify NIR light-absorbing materials onto the surface of magnetic NPs, another is to concentrate the magnetic NPs. Newly, it has been shown that the clustering of magnetic NPs induces a significant increase in the magnetic moment, and consequently, the clustered magnetic NPs have a much higher relatively high saturation magnetization and specific absorption rate (SAR) than individual magnetic NPs [30–33]. Hayashi et al. have utilized the unique merit of magnetite clusters for the magnetic hyperthermia therapy of tumors by AMF [32,33]. More significantly, previous studies have also indicated that metallic NPs clusters can induce a red-shift in the light absorption spectra [34,35], which enhances the light absorbance in the NIR region, expanding new application fields for metallic NPs clusters to be utilized as photosensitizers in the NIR PTT. Therefore, numerous papers have been reported the photothermal ablation of tumors by utilizing aggregation-induced enhanced photothermo (AIEP) of metallic NPs clusters [36,37], such as gold NPs aggregation. Until now, to the best of our knowledge, the papers on the study of photothermal effect of magnetite clusters were rarely reported, let alone for the use of magnetite clusters with NIR irradiation to destroy tumor *in vivo*.

Herein, we present the synthesis of magnetic colloidal nanocrystal clusters that effectively produce heat in response to harmless NIR irradiation. The biomedical parameters were not only assessed *in vitro* in cellular systems but also *in vivo* study. The results proved that the photothermal effects of clustered magnetic NPs for the treatment of A549 (Human lung adenocarcinoma epithelial cell line) tumor were better than those obtained by single crystal magnetic NPs.

2. Materials and methods

2.1. Materials

Iron (III) chloride ($FeCl_3 \cdot 6H_2O$), iron (II) chloride ($FeCl_2 \cdot 4H_2O$), trisodium citrate dehydrate ($C_8H_5Na_3O_7 \cdot 2H_2O$), sodium acetate anhydrous (NaOAc), ethylene glycol (EG), acetone, sodium hydroxide (NaOH), and nitric acid (HNO_3) were purchased from Shanghai Chemical Reagents Company (China). MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and other biological reagents were purchased from Invitrogen Corp. Dulbecco's modified Eagle medium (DMEM), Fetal bovine serum (FBS), Penicillin-Streptomycin solution and Trypsin-EDTA solution were purchased from Gibco (Tulsa, OK, USA). All the other chemicals were of analytical grade, and purified water was produced by a Millipore water purification system.

2.2. Synthesis of individual magnetic NPs

The individual magnetic NPs were prepared by chemical coprecipitation method [38]. In a typical recipe, $FeCl_3 \cdot 6H_2O$ (10.81 g, 0.04 mol) was dissolved in 50 mL deionized water. The resulting solution was transferred into a three-necked flask (250 mL), which was equipped with a mechanical stirrer, a dropping funnel, and a nitrogen inlet. Then 3.98 g of $FeCl_2 \cdot 4H_2O$ (0.02 mol) was also dissolved in another 50 mL deionized water, and the resulting solution was transferred into the above-mentioned three-necked flask. The as-prepared mixture was then stirred at room temperature in nitrogen atmosphere, and NaOH solution (10 M, 50 mL) was then slowly dropped into the flask from the dropping funnel in 1 h. After that, the mixture was continuously stirred at room temperature for 1 h, which was then stirred at 90 °C for another 2 h and cooled to room temperature under continuous stirring. The whole process was in the nitrogen atmosphere. The products were washed with deionized water three times, collected with the help of a magnet. After that, nitric acid solution (2 M, 100 mL) was added into the above products, which were then washed with deionized water several times and collected with the magnet, until the pH value of the supernatant is neutral. Then 100 mL (0.3 M) of trisodium citrate was added into the resulting products, the mixture was stirred for 30 min at 90 °C in the nitrogen atmosphere. Then it was cooled to room temperature, transferred into a beaker and collected with the magnet, and 20 mL of deionized water and a lot of acetone were added into the beaker to wash the products twice. Finally, the obtained products were dispersed in 50 mL of deionized water, and stirred at 80 °C for a long time to remove acetone.

2.3. Synthesis of clustered magnetic NPs

The clustered magnetic NPs (magnetic particles) were prepared by a modified solvothermal reaction [39]. Briefly, $FeCl_3 \cdot 6H_2O$ (1.028 g), $C_8H_5Na_3O_7 \cdot 2H_2O$ (0.24 g), and NaOAc (1.2 g) were first dissolved in 20 mL of EG under vigorous stirring for 0.5 h. The resulting solution was then transferred into a Teflon-lined stainless-steel autoclave with a capacity of 50 mL. The autoclave was sealed and heated at 200 °C for 10 h. Then it was cooled to room temperature. The as-prepared black products were washed with ethanol and deionized water several times, and collected with a magnet. The final products were dispersed in 10 mL of ethanol for the further use.

2.4. Characterization

Ultraviolet–visible (UV–vis) spectra were performed using a Perkin–Elmer Lambda 750 spectrophotometer. Transmission electron microscopy (TEM) images were obtained on a Tecnai G2 20 TWIN transmission electron microscope. Magnetic characterization was carried out with a vibrating sample magnetometer (VSM) on a Model 6000 physical property measurement system (Quantum, USA) at 300 K. X-ray diffraction (XRD) measurements were recorded on a X'pert PRO diffractometer to determine the composition of Fe_3O_4 particles. All the diffraction peaks in the XRD patterns were indexed and assigned to the typical cubic structure of Fe_3O_4 (JCPDS 75-1609).

2.5. Cell lines and culture conditions

A549 cells obtained from Chinese Academy of Sciences Cells Bank, Shanghai, China, were routinely cultured in RPMI-1640 cell medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in 5% CO_2 and 95% air atmosphere with >95% humidity. All experiments were performed on cells in the logarithmic phase of growth.

2.6. NIR-heating effect of magnetite NPs in solution

The individual magnetic NPs or clustering of magnetic NPs stock solution at 1 mg/mL was diluted to the different concentrations (10–80 μg/mL) and 200 μL of aliquots were deposited into wells of a 48-well cell culture plate. Wells were illuminated by an 808-nm continuous-wave NIR laser (Changchun New Industries Optoelectronics Technology, Changchun, China; fluence: 5 W/cm^2, spot size: 5 mm) with the different exposure time from 60 to 180 s. Pre- and postillumination temperatures were taken by thermocouple.

2.7. Cell viability assay

A549 cells were seeded in 96-well plates with a density of 10^4 cells/well, and allowed to adhere for 24 h prior to assay. The cells were exposed to the clustered magnetic NPs or individual magnetic NPs with the same concentration of 50 μg/mL, respectively. The cells were or were not irradiated by NIR laser light at a power density of 5 W/cm^2 with different illumination time from 60 to 180 s. After the cells were incubated at 37 °C for another 24 h, they were incubated with 0.5 mg/mL MTT in DMEM for 4 h in dark and then mixed with dimethyl sulfoxide after the supernatant was removed. The OD value at 570 nm was read using the microplate reader (Synergy TM2, BIO-TEK Instruments Inc. USA). Cell viability was determined by the percentage of OD value of the study group over the control group. Afterward, cells were co-stained by a mixture of Calcein AM and PI solution for 20 min. The samples were subjected to observe using a ZEISS LSM710 live cell confocal laser imaging System (Carl Zeiss, German).

2.8. Flow cytometry analysis

To investigate the photothermal ablation of A549 cells by clustered Fe_3O_4 NPs without or with the 808 nm laser irradiation using flow cytometry, clustered Fe_3O_4 NPs dispersion (100 μg/mL in PBS solution) was added to a 12-well cell culture plate containing A549 cells with a density of 5×10^5 cells/well in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin, then the A549 cells were incubated for 4 h at 37 °C in 5% CO_2 and 95% air atmosphere

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