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Multifunctional Fe_3O_4@C@Ag hybrid nanoparticles as dual modal imaging probes and near-infrared light-responsive drug delivery platform

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ABSTRACT

Multifunctional nanocarriers based on Fe_3O_4@C@Ag hybrid nanoparticles with a diameter of 200 nm were fabricated by a facile method. Silver (Ag) nanoparticles were deposited onto the surface of Fe_3O_4@C nanospheres in dimethyl formamide (DMF) solution by reducing silver nitrate ($AgNO_3$) with glucose. The nanocarriers of doxorubicin (DOX) with a high loading content of 997 mg/g and near-infrared (NIR) light-responsive drug delivery based on Ag nanoparticles were realized. Strong fluorescence can be observed in cell nucleus due to the presence of DOX after irradiated by NIR, and most cells were in the state of apoptosis, which indicates NIR-regulated drug release was realized. Moreover, measurements show that the nanocarriers could also be used as magnetic resonance imaging (MRI) contrast agents and fluorescent probes. The combination of synergistic NIR controlled drug release and dual modal imaging of MRI and two-photon fluorescence (TPF) imaging could lead to a potential multifunctional system for biomedical diagnosis and therapy.

1. Introduction

Nanocarriers which can simultaneously perform multiple functions, such as drug delivery, MRI and optical imaging, photothermal therapy etc, have become more and more popular in the domain of cancer study [1–6]. To date, the technique of applying different individual functions into the cancer diagnosis and treatment is prevalent and mature. For instance, superparamagnetic nanoparticles have been used as contrast-enhancing agents for MRI [7], and magnetically assisted cell sorting and separation [8]. Noble metals are used for photothermal therapy [9], as well as contrast agents for TPF imaging [6,10]. And stimulation caused by various mechanisms such as temperature, magnetic field, pH and light has been applied for controlling drug release on the basis of quantity, location, and time [1,11–13]. In order to integrate various functions into a single structure for facilitating the treatment process, many efforts have been adopted to develop hybrid nanosystems. Such as the gold particles incorporated into thermally sensitized liposome membranes can make the conversion of the absorbed energy into heat and generate localized hyperthermia under irradiation, and then leads to transient defects in the lipid membrane that enable quick release of contents [14]. And formulations containing silica nanocapsules and magnetic iron oxide nanoparticles core, conjugated by a functionalized iridium complex provide a method to perform simultaneous magnetic resonance, luminescence imaging, and photodynamic therapy [15]. Besides, nano-pearl-necklaces based on Fe_3O_4 nanoparticles decorating Au nanorods have been used for MRI diagnosis, fluorescence imaging, and the NIR photothermal ablation of cancer cells [16].

Although there have been many types of hybrid nanoparticles that combining multiple functionalities into a single system, the problems that the multifunctional system can reduce the efficacy of the individual functions are still unresolved. Such as the loading content of the multifunctional drug delivery is still low because the traditional methods to fabricate the multifunctional nanoparticles by coating them with thin polymer layer couldn't provide enough space for the loading of drug [17,18], and the value is always below 900 mg/g [3,6], even many kinds of drug loading mechanisms, such as embedding, surface absorption, and hydrogen bonding have been used. Meanwhile, the development of an effective drug

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release system also faces several challenges. Nanoparticles with size beyond 70–200 nm tend to accumulate in reticuloendothelial sites such as the liver, spleen [19], leading to the insufficient uptake in tumor sites and decreasing the therapeutic effect of the administered drug dose [20]. To achieve high targeting efficiency at the tumor site and reduce serious side effects, cancer-targeting ligand is always conjugated on the surface of the nanocarriers. However, the release rate is still low after the drug-loaded nanocarriers have entered the cytoplasm of the cells for being lack of effective methods to regulate drug release and it limits the drug's treating effects [2]. Though experiments have proved that the DOX releasing rate increases with the pH reducing [21], the fact that the pH of the cancer cells cannot be lower than the value (pH = 2) reported in the vitro tests will limit the application of pH triggered drug delivery in clinical treatment [12]. The method of photo-regulated release of drug has been reported widely, however complicated preparation for drug carriers and the traditional utilization of ultraviolet radiation limits its applications [13]. In spite of the widespread use of fluorescent-dye and quantum dots (QDs) modified nanoparticles in optical imaging [1,3,22], the former is susceptible to photobleaching and the latter is difficult to functionalize in a controlled manner and long-term environmental concerns have hindered in vivo applications [16,23]. Meanwhile, the conventional materials of fluorescent probes have no ability to convert the absorbed energy into heat for thermal therapy and other applications compared with noble metals. Therefore, developing new multifunctional materials for high drug loading content, controlled drug release, multifunctional imaging and good biocompatibility is challengeable and highly desirable.

Herein, we demonstrated a mild synthetic method for making a highly integrated nanoparticle system (Fe_3O_4@C@Ag core–shell nanoparticles) capable of controlling the amount and rate of drug release upon NIR light radiation and dual-modal imaging. In such a system, drug could be incorporated within the carbon shell, while drug release can be externally triggered due to localized surface plasmon resonances of Ag nanoparticles. Dual-modal imaging of MRI and TPF could also be realized due to the Fe_3O_4 core and Ag nanoparticles formed on the Fe_3O_4@C surface, which would help us achieve real-time monitoring of drug delivery.

2. Experimental Section

2.1. Materials

Ferrocene ($Fe(C_5H_5)_2$, ≥98%), acetone (C_3H_6O) (≥99%), hydrogen peroxide (H_2O_2, 30%), silver nitrate ($AgNO_3$, ≥99%), glucose ($C_6H_{12}O_6$, ≥98%) were of analytic grade from the Shanghai Chemical Factory, China. All chemicals were used as received without further purification.

2.2. Synthesis of Fe_3O_4@C@Ag nanoparticles

Fe_3O_4@C@Ag nanoparticles were prepared by reducing Ag^+ ions on the surface of Fe_3O_4@C nanospheres according to the glucose reducing method with some modifications. The carboxyl and negative charge-functionalized superparamagnetic Fe_3O_4@C nanospheres with diameters of 200 nm were obtained following the method reported [24]. Then, 0.023 mg Fe_3O_4@C nanospheres were dispersed into 15 ml of DMF solution to form a colloidal suspension. And then 10 ml of DMF solution contained glucose (0.05 g/ml) was slowly added into the above suspension with mechanical stirring. After 1 h, 2 ml of DMF solution with $AgNO_3$ (0.01 M) was added dropwise into the above mixture every 30 min (the total volume of the needed DMF solution with $AgNO_3$ is 10 ml). The resultant solution was still kept stirring for 3 h after finishing adding the DMF solution with $AgNO_3$. The temperature was kept at 70 °C during the whole procedure. Finally, the products were collected through magnetic separation and washed with ethanol and distilled water several times and dried at room temperature for measurements.

2.3. Preparation of DOX-loaded hybrid nanoparticles

Drug-loaded nanoparticles were prepared by adding Fe_3O_4@C@Ag nanoparticles into phosphate buffered saline (PBS) (pH = 7.6) containing DOX (1 mg/ml) with magnetic stirring at 37 °C for 24 h under NIR radiation. Then the mixture was separated by a magnet with 0.20 T and the supernatant was used for the measurements of the encapsulation efficiency and drug loading content.

The free-DOX content in the supernatant was quantified by a UV–Vis spectrophotometer at 480 nm. The encapsulation efficiency and drug loading content were calculated by Equations (1) and (2):

$$\text{Encapsulation efficiency}\left(100\%\right) = \left(m_{\text{total drug}} - m_{\text{drug in supernatant}}\right) \Big/ m_{\text{total drug}} \times 100\% \quad (1)$$

$$\text{Drug loading content} = m_{\text{drug in supernatant}} / m_{\text{nanoparticles}} \times 1000 \quad (2)$$

In order to understand the mechanism of drug loading, another experiment of DOX-loaded hybrid nanoparticles was carried out in the dark without NIR radiation. And the variation of loading content with time was measured as follows: 1 ml of DOX aqueous solution (1 mg/ml) was mixed with Fe_3O_4@C@Ag nanoparticles under NIR radiation (M1) and in the dark (M2) respectively. A certain amount of supernatant was withdrawn repeatedly for measurement of DOX, simultaneously the same amount of fresh DOX solution was added into the system. Meanwhile, the concentration of DOX in the system was calculated and regarded as the initial concentration for the next recycle.

2.4. DOX release from Fe_3O_4@C@Ag nanoparticles

The drug release experiment was performed at 37 °C. Two equal quantity of DOX-loaded Fe_3O_4@C@Ag nanoparticles were separately dispersed in 5 ml PBS (pH = 7.6) in two 10 ml glass bottles (S1, S2). With magnetic stirring, S1 was irradiated with NIR laser centered at 808 nm at an output power of 2.0 W/cm^2 meanwhile S2 was kept in the dark. Periodically, at predetermined time intervals, S1 and S2 were separated by a magnet with 0.20 T and the supernatant was withdrawn for analysis of drug release, at the same time 5 ml fresh PBS was added for the later drug release experiments.

2.5. Magnetic resonance imaging (MRI) measurement

Relaxation properties of Fe_3O_4@C@Ag nanoparticles in aqueous solution were measured at 25 °C with a clinical magnetic resonance (MR) scanner (GE HDxt, 3.0 T). Tubes containing Fe_3O_4@C@Ag nanoparticles suspension with different iron concentrations at 0.009, 0.027, 0.036, 0.045, 0.09, 0.18 mM were placed into the MR scanner. The T_2^* relaxation times were determined from a multislice gradient echo sequence (repetition time (TR): 120 ms; echo times (TE): 2–22 ms).

2.6. Cell culture and viability tests

HeLa cells, from American Type Culture Collection (Rockville, MD) were used to determine cytotoxicity of Fe_3O_4@C@Ag nanoparticles by a tetrazolium dye (MTT) method. Cells were seeded in a 96-well plate and maintained as subconfluent monolayers in Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal bovine serum (Hyclone, Logan, UT) and 100 units/ml penicillin plus 100 g/ml streptomycin (Invitrogen) at 37 °C with 8% CO_2. After cultured for 24 or 48 h, the medium was replaced with those containing Fe_3O_4@C@Ag nanoparticles of different concentrations. For contrast, medium containing different concentrations of DOX-loaded Fe_3O_4@C@Ag nanoparticles was also employed. After incubation, MTT solution was put into each well for another 4 h incubation, which allowed the viable cells to reduce the yellow MTT into dark blue formazan crystals. Then an ELISA reader was used to measure the absorbance of each well.

2.7. Two-photon fluorescence microscopy measurment

MCF-7 cells were employed to evaluate the property of TPF imaging of Fe_3O_4@C@Ag nanoparticles that endocytosed by the cells. The MCF-7 cells were seeded onto sterile, acid-treated 12-mm coverslips in 24-well plates (Corning Glass Works, Corning, NY, USA). In general, 24–36 h after incubation with nanoparticles, MCF-7 cells were rinsed three times in PBS and fixed in freshly prepared 4% paraformaldehyde in PBS and then rinsed three times in PBS. Cells on the coverslips were blocked with 0.05% Tween 20 in PBS (TPBS) with 1% bovine serum albumin (Sigma). These cells were incubated with various primary antibodies in a humidified chamber for 1 h and then washed three times in TPBS. Filamentous actin was labeled with rhodamine-conjugated phalloidin for outlining general cytology of the plasma membrane while DNA was stained with DAPI (Sigma) for verification of internalization. Coverslips were supported on slides by grease pencil markings and mounted in Vectashield (Vector Laboratories). Images were taken with a laser scanning microscope (Zeiss LSM 710) using a 63 × 1.3 numerical aperture PlanApo objective. Figures were constructed using Adobe Photoshop. In the test for assessing the NIR improving the drug release rate, the cells were seeded and cultured in the same manner except that the Fe_3O_4@C@Ag nanoparticles were replaced by DOX-loaded nanocarriers.