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Near-infrared light triggered photo-therapy, in combination with chemotherapy using magnetofluorescent carbon quantum dots for effective cancer treating

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ABSTRACT

Magnetofluorescent carbon quantum dots (MCQDs) have attracted significant attention in biomedical studies due to their major role in cancer photothermal therapeutics. We synthesized the FeN@CQDs with intrinsic photoluminescent and magnetic properties with a green, hydrothermal method. These magnetofluorescent FeN@CQDs were conjugated with a folic acid and riboflavin (Rf-FA-FeN@CQDs) as the light-triggered theranostics for simultaneous photothermal therapy (PTT) and photodynamic therapy (PDT). In order to reduce Rf-FA-FeN@CQDs biological toxicity, we used a highly efficient cross-linking reaction to incorporate Rf-FA-FeN@CQDs nanostructures into polymer nanospheres. Doxorubicin, an anticancer drug, was further incorporated into the GP-Rf-FA-FeN@CQDs to form GP-Rf-FA-FeN@CQDs-DOX to enable targeted drug delivery. The uptake into cancer cells and the intracellular location of the GP-Rf-FA-FeN@CQDs-DOX were observed by confocal laser scanning microscopy. The results of both *in vitro* and *in vivo* experiments reveal that the developed can deliver anti-cancer drugs to target cells, release them intracellular upon NIR irradiation, and effectively eliminate tumors through chemo-photo synergistic therapeutic effect.

1. Introduction

With the rapid development of science and technology, miniaturization is the goal of equipment and instrument [1,2]. Thus nanomaterials are paid much attention, duo to their special structural characteristics and optical characteristics [3,4]. Quantum dots with excellent emission property, nanometal with surface plasmons resonance (SPR) properties, magnetic nanoparticles with superparamagnetism are three important nanomaterials [5–7]. Compared to traditional fluorescent dyes, quantum dots have many advantages, such as continuously adjustable emission spectrum, good resistance to bleach, good stability and so on [8,9]. Due to the localized surface plasmons, metal nanoparticles can enhance the fluorescence intensity of luminescent materials, which distribute around the metal particles [10]. Metal nanoparticle with special structure can work as light absorbers and produce localized high temperatures to kill tumor cells [11]. Photothermal therapy is a non-interventive, non-complaints care method [12]. Magnetic nanoparticles with superparamagnetism have many applications such as drug delivery magnetic separation and hyperthermia. Recently, increasing attention has been diverted to the fabrication of bifunctional nanostructures consisting two kinds of materials with different properties, such as the nanoparticles possessing magnetic and luminescent property [13,14].

Photodynamic therapy (PDT) is an extraordinary theranostic modality for a number of malignant and nonmalignant diseases. Its principle is photosensitizer selectively accumulates and retention

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in tissue, under the illumination of special wavelength, tumor tissue can be killed by photochemical or photobiological reaction, thus to achieve the purpose of partly treating tumor [15–17]. At the same time, the heat generated by the penetrating NIR radiation can be envisaged to kill the targeted cancer cells, which is the principle for photothermal therapy (PTT). PTT has been increasingly regarded as a highly selective and minimally invasive approach for cancer treatment [18,19]. However, in the practices of many photosensitizers used in PDT have several deficiencies such as short tissue penetration depth, limited tumor specificity and phototoxicity under sunlight, especially when photosensitizers inevitably distribute in skin and produce $^1O_2$ under sunshine, which may result in serious skin photosensitivity [20–22]. However, the efficiency of the cancer therapy using PTT or PDT alone is limited. Combining methods of treatment is a more promising efficient strategy for eradicating cancer. Therefore, the development of an effective method to produce theranostic nanoparticles (TNPs) as PDT, PTT and chemotherapy for cancer diagnosis and treatment may open a new path for the biomedical uses of magnetic quantum dots (MQDs) [23,24]. For example, photothermal therapy using gold nanostructures combined with chemotherapy or photodynamic therapy appears to be a more aggressive approach compared to any individual method of treatment as described [25,26]. Several *in vitro* and *in vivo* studies indicate that some magnetic CQDs are intrinsically cytotoxic because they release free radicals of heavy metals from the CQDs core into the blood stream [27]. Up to now, the reported metal based, carbon-based QDs/PTT can effectively produce high contrast intensity of cell imaging and PTT efficiency [28,29], but they suffer from complex multicomponent system and poor biocompatibility and biodegradability. Consequently, it has become increasingly urgent to address these existing issues for the clinical translation of QDs/PTT. Thus, it is a challenge to develop an economical, simple, biocompatible, and facile method to synthesize nano-theranostics materials with high magnetic and fluorescent property.

The general procedure of PDT involves the systemic, local, or topical administration of a non-toxic drug or dye known as a photosensitizer (PS) followed by selective illumination with appropriate wavelength and power of light [30]. In the presence of oxygen, PS can transfer the absorbed photon energy to surrounding oxygen molecules, generating reactive oxygen species (ROS) including singlet oxygen (SO) or free radicals and consequently causing cell death and tissue destruction [31]. Riboflavin (Rf) is a component of the B2 vitamin complex and a precursor of the redox coenzymes flavin mononucleotide and flavin adenine dinucleotide. Due to its photosensitive property, this vitamin has broad biological applications, such as reducing pathogens and inactivating white blood cells in donated blood products, as well as presenting antitumoral effects [32]. Therefore, Rf enables online imaging of drug for the detection of disease, image-guided drug delivery and treatments, guidance of surgical resection, and monitoring of treatment response [33]. Unfortunately, Rf generally was excited by blue or UV light that shows very limited penetration depth about a few hundred micrometers. For the Rf photosensitisation, one needs to devise means of conversion NIR to UV-blue light at the subcentimeter depth. With this in mind, Rf was grafted onto the surface of magnetic CQDs to form composite nanoparticles, which is complemented by a depth-resolved structural imaging modality. CQDs with the unique upconversion photoluminescence could serve as an energy donor useful in triggering PDT under NIR light, which offers greatly improved tissue penetration [34].

In this work, we report the preparation of fluorescent FeN@CQDs by a facile, green, and low-cost hydrothermal method using iron crosslinked chitosan complexes (Ch-Fe-CL) as the precursor [35]. To prepare Ch-Fe-CL conjugates, the iron crosslinked chitosan complexes were first synthesized the same as reported in the literature. Our strategy involves the one-pot hydrothermal assisted fabrication of magnetofluorescent FeN@CQDs. This step is followed by functionalizing the FeN@CQDs with a targeting ligand (folic acid, FA) and conjugation of photosensitizer (riboflavin, Rf). In order to reduce FeN@CQDs biological toxicity, we then used a highly efficient cross-linking reaction to incorporate FeN@CQDs nanostructures into polymer nanospheres. Further, chemotherapeutic molecules, doxorubicin (DOX), were effectively loaded through π-π tacking and hydrogen bonding interactions with GP-Rf-FA-FeN@CQDs (Fig. S1). The GP-Rf-FA-FeN@CQDs-DOX demonstrated strong NIR absorption and high photothermal conversion efficiency. The release of DOX could be selectively stimulated by NIR-light, enabling intracellular drug accumulation and thereby enhancing the efficiency to kill cancer cell.

2. Experimental

2.1. Materials

Chitosan, folic acid, riboflavin, and genipin was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Doxorubicin (DOX), Hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (Sulfo-NHS, 97%) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%) was purchased from shanghai Aladdin Company (Shanghai, China). $FeCl_3 \cdot 6H_2O$, sodium acrylate ($CH2{=}CHCOONa$), ethylene glycol (EG) and diethylene glycol (DEG) was purchased from Macklin Biochemical Company (Shanghai, China). Double-distilled water (18.2 MΩ) was obtained from Milli-Q system (Millipore, Bedford, MA, USA) and was used in the experiments. Adult whole blood was provided by Jiangsu blood centre. Dulbecco's modified eagle medium (DMEM) were purchased from Thermo Fisher HyClone (USA). Fetal bovine serum (FBS) was obtained from SunShine Biotechnology Co., Ltd. (China). Methyl thiazolyl tetrazolium (MTT) was purchased from Amresco (USA). Hoechst 33258 was purchased from Gibco Co., Ltd. (China). The HeLa, HepG2, and A549 cells were purchased from the Cell Bank of Culture Collection of Chinese Academy of Sciences (Shanghai, China).

2.2. Instruments and characterization

The photoluminescent (PL) spectra were recorded using molecular fluorescence spectrometer (Cary Eclipse, varian, USA). Nexus 670 FTIR type (Nicolet) infrared spectrometer was used to analyze the infrared spectrum of the sample. The X-ray diffraction (XRD) analysis was performed using a D/Max 2500V/PC diffractometer (Rigaku Corporation, Japan). The thermogravimetric (TG) measurements were performed on a Perkin-Elmer TG 7 instrument using a heating rate of 10 °C/min up to 800 °C in nitrogen atmosphere. Ultraviolet–visible (UV–Vis) absorption spectra were recorded using UV absorption spectrophotometer (Cary-50, varian, USA). The surface composition and element analysis of the samples were recorded using X-ray photoelectron spectroscopy (XPS, EscaLab-250, Thermo, USA). The Malvern ZEN 3600 Zetasizer (Malvern Instruments, UK) was used to study the prepared hydrodynamic size and Zeta potential. MR images were acquired by a 7 T BioSpec 70/30 experimental scanner (70/30 Bruker BioSpin; Ettlingen, Germany). The morphologies of the samples were characterized using the transmission electron microscope (TEM, H-7650, Hitachi, Japan) and scanning electron microscope (SEM, JSM-5610LV, Japan). A Siemens Trio TIM 3 T M.R.I. was used to measure the T2 relaxation times. *In vitro* bright field and fluorescence images were collected with a confocal laser scanning microscope (TI-E-A1R, Nikon, Japan). To monitor the temperature changes at the