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A supramolecular material for dual-modal imaging and targeted cancer therapy

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ABSTRACT

Recently, how to design a formulation system with simultaneous diagnosis and therapy toward cancer has attracted tremendous attention. Herein, a supramolecular material was prepared *via* a facile method by the co-intercalation of folic acid (FA) and doxorubicin (DOX) into the gallery of Gd^{3+}-doped layered double hydroxides (LDHs), followed by surface adsorption of fluorescein isothiocyanate (FITC). This supramolecular agent was proved to exhibit excellent magnetic resonance imaging (MRI) and fluorescence imaging (FI) behavior, as well as chemotherapy toward cancer (KB cell). The co-intercalated FA enables an efficient and selective drug delivery with good specificity. This work provides a facile approach for the fabrication of a drug formulation with dual-modal imaging and targeted therapy, which could be potentially used in the practical chemotherapy and medical imaging.

1. Introduction

A theranostic platform for simultaneous diagnosis and therapy has been explored over the decades [1,2]. The common diagnosis methods include magnetic resonance imaging (MRI) [3,4], computed tomography (CT) [5,6], positron emission tomography (PET) [7,8], photoacoustic imaging (PAT) [9,10] and fluorescence imaging [11]. However, owing to the limit of each individual imaging modality, a single methodology is rarely sufficient to provide high resolution and sensitivity [12]. For instance, fluorescence imaging is an essential technique for the biomedical investigations owing to its excellent sensitivity, but it lacks spatial and anatomical resolution. MRI presents high spatial resolution, but suffers from limited sensitivity [13,14]. Thus, the integration of multimodal imaging strategies for the purpose of achieving a synergistic imaging modality has attracted considerable research interest [15].

Chemotherapy is one of the most widely applied cancer treatments [16], but traditional chemotherapeutic agents lack of specificity to target at diseased regions and thus lead to undesired side effects to normal tissues [17,18]. Therefore, modeling chemotherapeutic drugs by virtue of a biocompatible and targeted drug delivery vehicle is highly necessary, so as to decrease side effects and enhance the tumor accumulation [19]. More interestingly, a combination of imaging agents and chemotherapeutic drugs into one platform with simultaneous diagnosis and therapy has drawn tremendous attention [20,21]. A various of nanocarriers such as Fe_3O_4 nanoparticles (NPs) [22,23], Au NPs [24,25], Upconversion NPs [6,26,27], graphene oxide NPs [28], were synthesized for the simultaneous bioimaging and cancer therapy. Nevertheless, these nanocarriers normally suffer from laborious preparation, limited loading capacity, or weak biocompatibility. Therefore, it is a major challenge to explore highly efficient drug formulation systems for simultaneous multimodal imaging and cancer therapy.

Layered double hydroxides (LDHs) are a typical class of layered materials represented by the general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2](A^{n-})_{x/n}\cdot mH_2O$ [29], which contains positively charged brucite-like layers and charge balancing anions between the host layers [30,31]. By virtue of its unique structure, LDHs have been explored as drug delivery and controlled release vehicle with enhanced cellular uptake and good biodegradation [32,33]. Moreover, the biocompatibility of LDHs has been demonstrated, which is almost nontoxic for normal cells as high as 1 mg/mL [34,35]. This motivates us to develop a dual-modal imaging and targeted cancer therapy based on LDHs supramolecular assembly: (1) doping Gd^{3+} in LDHs host layers and stabilizing fluorescein isothiocyanate (FITC) on LDHs surface for MRI and fluorescence dual-modal imaging; (2) co-intercalating chemotherapy drug (doxorubicin, DOX) and targeting agent (folic acid, FA) into the interlayer gallery of LDHs for targeted cancer therapy. This supramo-

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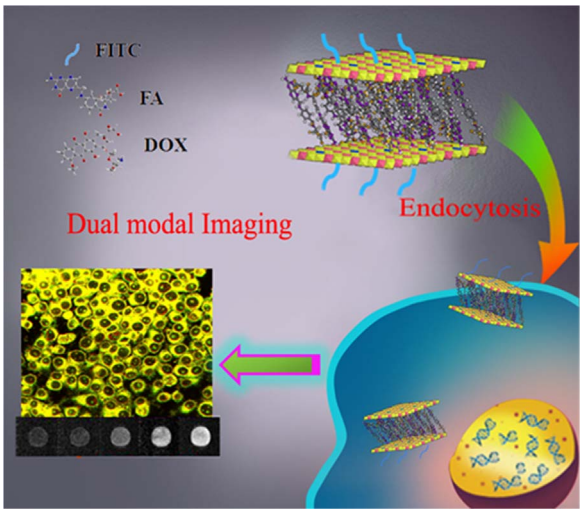
lecular drug formulation would show the following advantages: (i) the host layer provides dual docking sites for chemically-coordinated Gd^{3+} and electrostatically-adsorbed FITC, which endows good MRI and enhanced fluorescence imaging; (ii) the host-guest interactions could improve the stability and hydrophilicity of DOX, enhancing the drug permeability. In addition, the over-expression of FA toward cancer cells would increase the drug uptake.

In this work, a layered FITC/FA-DOX/Gd-LDHs material was synthesized through co-intercalation of FA and DOX into the interlayer gallery of Gd^{3+}-doped LDH, followed by surface adsorption of FITC, which shows satisfactory dual-modal imaging and anticancer behavior with targeted ability. XRD and UV–vis spectroscopy confirm that DOX and FA molecules are co-intercalated in the interlayer region of Gd-LDHs matrix. FT-IR and photoluminescence (PL) spectra testify the incorporation of FITC. The high-spin Gd^{3+} in LDHs host layers is conducive to trigger the longitudinal relaxivity, resulting in the positive contrast enhancement for T_1-MRI. Both satisfactory dual-modal imaging and anticancer behavior are demonstrated for the FITC/FA-DOX/Gd-LDHs over *in vitro* tests performed with KB cells, with the half maximal inhibitory concentration (IC_{50}) as low as 5.00 μg/mL at 48 h. In addition, this drug displays a high storage stability, good biocompatibility and targeting capability, which would be favorable for its potential practical application.

2. Results and discussion

2.1. Structural and morphological characterization

DOX and FA were co-intercalated into the interlayer region of Gd-LDH *via* separate nucleation and aging steps (SNAS) method developed by our group [36], in which Gd^{3+} as a MRI agent was doped into the host layers of LDH; FITC was further adsorbed by the host layers through electrostatic interaction. Scheme 1 illustrates the structural model of resulting DOX/FITC-FA/Gd-LDH. Since the doping amount of Gd (denoted as Gd(*x*%)-LDH) influences the LDH structure while the loading amount of FITC (denoted as FITC(*y*%)/LDH) determines its aggregation state and fluorescence property, these two parameters were firstly investigated. A series of Gd(*x*%)-LDHs are prepared and their XRD and FT-IR patterns are shown in Fig. S1a and b, respectively; their chemical compositions determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) are listed in Table S1. The Gd(2.5%)/LDHs displays series sharp (00*l*) reflections and the strong (003) reflection appears at 2θ 12.13°, indicating a typical CO_3^{2-}-LDHs phase with good crystallinity. As *x*% value rises from 5% to 20%, the (003) reflection of LDHs decreases obviously; new peaks in XRD assigned to $Gd(OH)_3$ are observed (Fig. S1a), and FT-IR spectra also show the absorption peak at 3616 cm^{-1} ascribed to $Gd(OH)_3$ (Fig. S1b) [37]. The increase of doped Gd^{3+} in Gd(*x*%)-LDH induces the increment of Zeta potential from +20.4 to +53.0 mV (Fig. S2 and Table S2). Taking into account the phase purity and crystallization, Gd(2.5%)-LDHs is chosen for the following study.



Scheme 1. Schematic illustration of FITC/FA-DOX/Gd-LDHs agent for dual-modal imaging and targeted chemotherapy.

We further optimized the loading amount of FITC, since its aggregation will result in fluorescence quenching and decrease the imaging ability. FITC is a derivative of fluorescein with an isothiocyanate group (–N=C=S), which takes the advantage of superior imaging performance without the deactivation of proteins compared with fluorescein. The FITC(*y*%)/Gd-LDHs samples are prepared and their fluorescence emission spectra are shown in Fig. S3c–e. The fluorescence intensity increases gradually from FITC(1%)/Gd-LDHs to FITC(5%)/Gd-LDHs, and then decreases from FITC(5%)/Gd-LDHs to FITC(25%)/Gd-LDHs owing to the aggregation of FITC. The sample of FITC(5%)/Gd-LDHs displays the maximum fluorescence intensity within the concentration range 15–400 μg/mL, and the fluorescence quantum yield of FITC(5%)/Gd-LDHs is 1.63 folds to protonated FITC and 31.1 folds to FITC at the concentration of 50 μg/mL (Fig. S3f). Therefore, FITC(5%)/Gd(2.5%)-LDHs is chosen as the optimum dual-modal imaging agent.

Monodispersed FITC/FA-DOX/Gd-LDH material was synthesized *via* SNAS method by incorporating DOX and FA into the Gd-LDH gallery. XRD pattern shows that the as-prepared FITC/Gd-LDH presents typical (00*l*) characteristic reflections with the (003) reflection at 2θ 10.18°, indicating FITC is mainly loaded on the surface rather than in the gallery (Fig. S4). After the co-incorporation of DOX and FA into the Gd-LDHs gallery, the (003) reflection of FITC/FA-DOX/Gd-LDH moves from 2θ 10.18° to 4.19° (Fig. 1a), with a corresponding basal spacing expansion from 0.86 nm to 2.21 nm, indicating FA and DOX are intercalated into the Gd-LDHs gallery. However, due to the high ratio of FA to LDHs (FA: LDHs=0.8), it is inevitable that part of FA is absorbed on the Gd-LDHs surface and results in the targeted drugs uptake. The incorporation of FITC, DOX and FA with the Gd-LDHs was studied by the FT-IR spectra (Fig. 1b). For the pristine Gd-LDHs, the band at 1375 cm^{-1} indicates a carbonate-type LDHs phase. As shown in Fig. 1b, the peak at 1606 cm^{-1} and 1401 cm^{-1} are attributed to the aromatic ring stretching of pteridine ring and *p*-amino benzoic acid of folic acid [26]. The vibrations at 1534 cm^{-1} and 1211 cm^{-1} are due to the C–O (phenolic) band and C–O–C band, respectively [38,39], demonstrating the presence of FITC. Moreover, the band at 1184 cm^{-1} corresponds to the $\delta(CH_3O–)$ stretching vibration of DOX [40]. The chemical composition of FITC/FA-DOX/Gd-LDHs is listed in Table S1. The energy dispersive spectrometer (EDS) (Fig. S5) proves the presence of Mg, Al, Gd, and S element, with a homogeneous dispersion confirmed by mapping image. SEM and HRTEM image (Fig. 1c and d) show hexagonal-like platelets with relatively uniform particle size. Dynamic light scattering (DLS) measurements give an average particle size of ~178 nm for FITC/FA-DOX/Gd-LDHs (Fig. S6f), which is suitable for permeability and retention (EPR) effect in the cell endocytosis [40,41]. As reference samples, Gd-LDHs and FITC/Gd-LDHs also illustrate uniform hexagonal platelets with particle size of ~98 nm and 107 nm, respectively (Fig. S6a–d).

The optical property of FITC/FA-DOX/Gd-LDHs was further investigated by the UV–vis absorption spectrometry (Fig. 2a). The pristine FITC shows a characteristic absorption peak at 490 nm, but its bandwidth broadens after incorporated with Gd-LDHs. After DOX is further intercalated into Gd-LDHs gallery, two typical peaks at 543 and 582 nm appear. For the FITC/FA-DOX/Gd-LDHs sample, an additional peak at 285 nm is observed compared with FITC/DOX/Gd-LDHs, originating from the absorption of FA. Fig. 2b displays the photoluminescence (PL) spectra of pristine FITC, FITC/Gd-LDHs, FITC/DOX/Gd-LDHs and FITC/FA-DOX/Gd-LDHs with the same DOX loading. Compared with pristine FITC in ethanol solution, a