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Intercalation of methotrexatum into layered double hydroxides via exfoliation-reassembly process

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ABSTRACT

In this paper, the intercalation of methotrexatum (MTX) into layered double hydroxides (LDHs) via an exfoliation-reassembly process was reported and the resulting hybrids were then characterized by X-ray diffraction (XRD) patterns, Fourier transform infrared (FTIR) spectroscopy and atomic force microscopy (AFM) patterns etc. In the synthesis procedure, LDHs particles were firstly delaminated into well-dispersed 2D nanosheets in formamide by ultrasonic treatment at room temperature, and then the resulting LDH nanosheets were reassembled in MTX solution to form MTX intercalated LDH (MTX/LDHs) hybrids. AFM images showed that during the exfoliation process a large part of LDHs particles were delaminated into single and double brucite layers. XRD patterns and FTIR investigations manifested the successful intercalation of MTX anions into LDHs interlayers for the final samples. It was also found out that the drug-loading capacity of the hybrids increased with the concentrations of MTX solutions, while the morphology became even aggregated. At last, the cell cytotoxicity of the hybrids was estimated by MTT assays on the human lung cancer cells (A549), and the results stated that MTX/LDHs hybrids had effective suppress role on the proliferation of cancer cells.

1. Introduction

In recent years, increasing attention has been paid to nanomaterial-based drug delivery systems in order to improve the pharmacological and therapeutic efficacy of the drugs. And inorganic nanoparticles are alternatively obtaining more attention nowadays because of their high drug loading, pronounced stability and biocompatibility. Thus, various inorganic nanocarriers including magnetite, calcium phosphate, carbon, gold, silica oxide and layered double hydroxides (LDHs) have been evaluated for delivering cytotoxic drugs [1–6].

Layered double hydroxides (LDHs) are a large family of anionic clays with the general formula of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}[A^{n-}]_{x/n}\cdot mH_2O$, where M^{2+} and M^{3+} are divalent and trivalent cations, and A^{n-} is interlayer anions [7]. Actually, the positive charge in brucite-like layers of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}$ is attributed to the isomorphous substitution of M^{3+} for M^{2+} and the layered structure is stabilized by hydrogen bonds among interlayer water molecules (mH_2O), anions and as well as by electrostatic interactions between the layer and the interlayer anions [8]. Due to the unique structural properties and their potential applications, LDHs have been widely applied in fields of catalysis, catalysis supporter, adsorption/separation agent, biomedicine carriers and so on [9]. LDH materials, especially MgAl-LDHs, can be quickly absorbed by various cell lines based on the conjugation of specific antibody and biodegraded in the cytoplasm [10]. Consequently, a series of pharmaceutically active compounds like ibuprofen, fenbufen, diclofenac, camptothecin, prednisone, vitamin etc., were intercalated into LDHs and demonstrated the feasibility of LDHs-based drug delivery systems [11,12].

Here, methotrexate (MTX for short, the structural formula is shown in Fig. 1) was chosen as the guest drug. MTX, one of the antifolate drugs, can effectively deactivate the metabolism of diseased cells through programmed cell death or apoptosis, and has been applied to certain human cancers such as osteosarcoma (bone cancer) and leukemia etc. [13]. Unfortunately, serious side-effect, short plasma half-life and high efflux rate of MTX restricted its application heavily [13]. Therefore, studies on how to improve the efficacy have attracted considerable attention recently, and synthesis of MTX/LDHs is one of the most well-established ways. Choy et al. reported that intercalation of MTX into LDHs interlayer may result in the controlled release in cells to maintain the drug efficacy and also protect MTX from deterioration during transportation. Furthermore, it also turned out that MTX/LDHs intercalation

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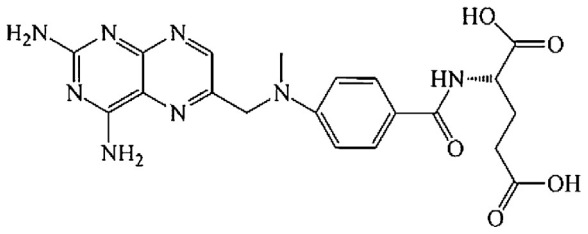

Fig. 1. Structural formula of the MTX molecule ($C_{20}H_{22}N_8O_5$).

hybrids were much more efficient in tumor suppression compared to MTX itself [13–16].

Owing to its property, exfoliation of LDHs yields positively charged nanosheets [17], and the nanosheets can be reassembled with anions to construct functional hybrids, which have received increasing attention lately [18]. Delamination of LDHs can be accomplished by different solvents and the most commonly used one is formamide [19]. Utilizing exfoliation-reassembly route, many biomolecules, such as carboxymethyl cellulose [20], polyacrylamide [21], bovine serum albumin [22], ibuprofen [23] and camptothecin [24] were intercalated into LDH galleries. In this work, positively charged LDH nanosheets and MTX anions were reassembled successfully. It was also found out that the MTX/LDHs nanohybrids could be easily synthesized via this route. Compared with the other conventional methods, this method showed many advantages, such as simple and short reaction time, enhanced thermal stability of the final hybrids.

2. Experimental

2.1. Chemicals

All chemicals – $Mg(NO_3)_2{\cdot}6H_2O$ (Guangdong Xilong Chemical Co.), $Al(NO_3)_3{\cdot}9H_2O$ (ShangHai Sinpeuo Fine Chemical Co.) and NaOH and formamide (Guangdong Xilong Chemical Co.) – were of analytical grade and used as received without further purification. Methotrexate (MTX) was purchased from Zhejiang province Huzhou prospect pharmaceutical Co., analytical grade. Human lung adenocarcinoma cells (A549) were purchased from Shanghai cell bank (Shanghai, CN).

2.2. Exfoliation of Mg_3–Al–NO_3-LDHs and synthesis of MTX/LDHs hybrids

Pristine Mg_3–Al–NO_3-LDHs were prepared by the modified coprecipitation method [25–27]. The exfoliation of LDHs was carried out according to the reference of Wu et al. [28]. Briefly, 0.4 g of LDHs sample was mixed with 20 mL of formamide in a flask, which was tightly sealed after purging with N_2. The mixture was sonicated to be transparent at room temperature and then the dispersion of delaminated LDHs nanosheets was obtained.

Straightly after that, MTX/LDHs hybrids were fabricated by the reassembly process. The process was as follows: a certain amount of MTX was dissolved into 20 mL 2.5% $NH_3{\cdot}H_2O$ to get 0.02 g/mL, 0.03 g/mL, 0.04 g/mL and 0.06 g/mL solutions, respectively. Under slow magnetic stirring, 20 mL of the delaminated LDHs nanosheets dispersion was dropped into MTX solution. Following a 1 h period of standing at 40 °C, the resulting dispersion was centrifuged and washed with deionized water and ethanol for several times. At last, the obtained samples were dried at 60 °C and the final samples were named as samples *a*, *b*, *c* and *d* in the order from low to high concentrations of MTX solutions.

2.3. Characterization

X-ray diffraction (XRD) patterns were obtained with a *D*/max-2500PC rotating anode X-ray powder diffractometer (Rigaku Co.), using Cu Kα radiation (λ = 1.5406 Å) from 2° to 70° at a scanning rate of 1°/min. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Bruker Tensor 27 spectrometer in the wavenumber region between 400 and 4000 cm^{-1}, using KBr pellets (with a weight ratio of sample to KBr being 1:100), and the resolution of the instrument is 4 cm^{-1}. Transmission electron microscope (TEM) images were obtained using a H-7650-HITACHI (Hitachi Medical Co.) machine operating at 200 kV. Samples for TEM were prepared by depositing a drop of sample solution onto a carbon-coated copper grid and the grid was dried in air. Thermogravimetric/differential scanning caborimetry (TG-DSC) analyses of the powders were carried out at a heating rate of 10 °C/min in N_2 atmosphere with the flow of 80 mL/min from ambient to 1000 °C in Al_2O_3 crucible by STA-449 C (Netzsch Co.) machine. Size and thickness were characterized by a Molecular Imaging PicoPlus AFM machine (MI Co.) in an AC mode and AFM samples were prepared by dropping diluted alcohol dispersion on a freshly cleaved mica substrate and drying at ambient temperature. A Cary 50 UV–vis (Varian Co., USA) spectrophotometer was used to determine the drug-loading capacity and release property.

2.4. Drug-loading capacity

To determine the amount of MTX loaded into LDHs, 0.01 g MTX/LDHs were dissolved in HCl solution (pH = 1.2). Under this circumstance, it can be assumed that 100% of MTX was released from MTX/LDHs. Then the concentration of MTX was determined by monitoring the absorbance at λ_{max} = 306 nm with UV–vis spectroscopy, it must be mentioned that the concentration was calculated by regression analysis according to the standard curve obtained from a series of standard solution of MTX in HCl solution [29]. At last, the intercalated amount of MTX into the LDHs was calculated. The data were collected in triplicate and presented in Table 1.

2.5. In vitro drug release

To measure the amount of MTX released from MTX/LDHs nanohybrids, the in vitro drug release test was performed as follow: 0.02 g MTX/LDHs were added into 500 mL phosphate buffer solution (pH = 7.4) in a closed glass bottle at a constant temperature of 37 °C. At selected time after addition of the nanohybrids, 4 mL solution was withdrawn and centrifuged, part of the supernatant was used for the measurement, and then the concentration of MTX was measured by UV–vis spectroscopy at λ_{max} = 306 nm, at last the release profiles were plotted as the relative release percentages of MTX against time. Dissolution medium was maintained at constant volume by replacing the samples with a fresh dissolution medium [30]. The data were collected in triplicate and presented in Fig. 8.

2.6. Cell culture conditions and cellular uptake evaluation

In vitro bioassay, human lung adenocarcinoma cells (A549) were routinely cultured at 37 °C in a humidified atmosphere with 5% CO_2 in 75 cm^2 flasks containing 10 mL of Dubecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 100 U/mL penicillin and 100 mg/mL streptomycin. When the cells were grown up to 80–90% of cellular confluence, the fault culture cells were differentiated with trypsin–EDTA and then washed twice with PBS (pH = 7.4) which was prior prepared. Then the cells were diluted with a volume of DMEM containing 10% FBS. For cell