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Magnetic lamellar nanohydroxyapatite as a novel nanocarrier for controlled delivery of 5-fluorouracil

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ABSTRACT

Magnetic nanoparticles are attractive carriers for drug delivery and layered materials intercalated by drug molecules exhibit improved safety and effectiveness of drug delivery. In this work, we report the loading of a model anticancer drug, 5-fluorouracil (5FU), into a magnetic layered nanohydroxyapatite (ML-HAP) by intercalation technique. The as-prepared ML-HAP nanoparticles with loaded 5FU were characterized using transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and vibrating sample magnetometer. We find that, within a certain drug concentration, the drug molecules can be intercalated into the gallery of ML-HAP without breaking its lamellar structure. The drug loading capacity of ML-HAP is extremely large. The drug release profiles display pH-dependent behavior and the drug release mechanisms are a combination of drug diffusion and HAP dissolution. Furthermore, ML-HAP/5FU shows strong superparamagnetism and good biocompatibility. The ML-HAP can be an efficient platform for targeted anticancer drug delivery.

1. Introduction

Hydroxyapatite (HAP) is one of the most studied biomaterials in the medical field due to its well-known biocompatibility and bioactivity. The combination of HAP and magnetic nanoparticles (such as Fe_2O_3 and Fe_3O_4) can extend the applications of HAP to adsorbents [1], catalysts [2], and hyperthermia agents [3]. Many magnetic nanoparticles with high magnetic moment such as $CoFe_2O_4$, $NiFe_2O_4$, and $MnFe_2O_4$ have been prepared, but their toxicity to cells limits their use in biomedical applications [4,5]. Unlike these magnetic nanoparticles, iron oxides such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), not only are relatively safe, but also possess high magnetic moments. They are widely used clinically as MRI contrast agents [5,6], magnetic scaffolds in bone tissue engineering [7,8], and carriers in targeted drug delivery [9]. The combination of hydroxyapatite (HAP) with iron oxide, especially Fe_3O_4, is expected to possess the advantages of both components. Thus the hybrid nanoparticles of Fe_3O_4-HAP have attracted much attention, particularly in the field of drug delivery. Therefore, preparation of Fe_3O_4-HAP nanohybrids has become a hot topic and numerous methods have been reported. The conventional method of mechanical mixing was used by Murakami and co-workers to prepare a porous Fe_3O_4-HAP composite which contained 30% Fe_3O_4 and the HAP particles are in rod shape [10]. Recently, Sneha and Sundaram prepared nanocomposites of Fe_3O_4 and HAP by ball milling Fe_3O_4 nanoparticles (prepared by alkaline coprecipitation of ferric and ferrous chloride in aqueous solution) and HAP particles (prepared by sol-gel method) [11]. Unlike these existing studies, we prepared Fe_3O_4-HAP nanohybrids via a template-assisted self assembly process [12,13]. Of particular importance is that the individual HAP is in nanoplate-like shape, which, to some extent, resembles the morphology of natural apatite in human bones [14,15]. Furthermore, the carrier is an assembly of HAP, i.e., lamellar HAP (or called layered HAP), which is self-assembled by several individual HAP nanoplates. The structure of lamellar HAP is similar to the well-known layered materials such as montmorillonite (MMT) and layered double hydroxide (LDH). Both MMT [16–20] and LDH [21–23] have demonstrated controlled drug release, enhanced thermal stability, and effective protection of drug molecules. Many biologically important molecules including gene and drugs can be incorporated into the interlayer of layered materials to achieve controlled release [24]. However, although magnetic MMT [25–27] and LDH [28–30] have been prepared for use in water treatment, no application in drug delivery can be found in literature.

In this work, we continue our effort to further explore the feasibility of ML-HAP as a drug delivery system by using 5-fluorouracil (5FU) as

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the model drug. The ML-HAP was prepared by the template method and the drug was loaded by intercalation technique. The as-prepared ML-HAP/5FU was analyzed by Transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), vibrating sample magnetometry (VSM), and cytotoxicity tests.

2. Materials and methods

2.1. Materials

The materials used in this work included calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), ammonium hydrogen phosphate ($(NH_4)_2HPO_4$), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), sodium hydroxide (NaOH), and sodium dodecyl sulphonate (SDS, $C_{12}H_{25}SO_3Na$). All analytical reagents were provided by Acros Organics.

2.2. Preparation of ML-HAP

The ML-HAP was prepared according to the procedure reported previously [31]. Briefly, 1.0g of SDS was mixed with 15 mL of deionized water and 30 mL of ethanol. The mixture was then heated to 60 °C, followed by addition of 15 mL of 3.3 M $Ca(NO_3)_2 \cdot 4H_2O$, 30 mL of 1 M $(NH_4)_2HPO4$ and 30 mL of ethanol. Afterward, 20 mL of 2.5 M NaOH and 20 mL of ethanol were added. After stirring for 0.5 h, 7.5 mL of 2.4 M $FeCl_3 \cdot 6H_2O$, 7.5 mL of 1.2 M $FeCl_2 \cdot 4H_2O$, 30 mL of 3 M NaOH and 45 mL of ethanol were added to the mixture. After the mixture was refluxed at 83 °C for 14 h, the precipitate was deposited at room temperature for 21 days. The precipitate was centrifuged and immersed in deionized water for 3 days, then centrifuged again and dried at 60 °C, giving rise to ML-HAP powder.

2.3. Drug loading experiment

50 mL of NaOH solution of 5FU (with varying drug concentration of 6, 10, and 12 mg mL^{-1}) was mixed with 0.1g of ML-HAP powder in a 250 mL round-bottomed flask. After one minute of intermittent ultrasonic dispersion, the mixture was stirred at 150 rpm for 8 h at 60 °C. The resultant mixture was filtered and the concentration of free 5FU in the filtrate was quantified by UV–vis spectroscopy (Beijing Purkinje General Instrument, UV-1810, China) at λ_{max}=266 nm. The samples prepared at 5FU concentrations of 6, 10, and 12 mg mL^{-1} were named as ML-HAP/5FU-1, ML-HAP/5FU-2, and ML-HAP/5FU-3, respectively. The 5FU loading capacity expressed by weight gain, wt%, was defined as the weight of loaded 5FU per unit weight of the initial ML-HAP.

2.4. Characterization

TEM images were recorded on a FEI Tecnai G2 F2 TEM operating at an accelerating voltage of 200 kV. XRD analysis was conducted using a Rigaku D/Max 2500 v/pc diffractometer (Rigaku, Japan) with Cu Kα radiation generated at 40 kV and 200 mA. The scanning rate was 1° min^{-1} over a range of 2θ=1–10° and 4° min^{-1} over a range of 2θ=10–60°. FTIR analysis (BIO-RAD FTS6000, KBr pellet 1:50–100, 4000–400 cm^{-1}) was applied to investigate the ion exchange during the intercalation process. The thermal stability and drug loading content were determined by TGA. TGA analysis was performed using a Netzsch STA 449C simultaneous thermal analyzer from 30 to 800 °C with a heating rate of 10 °C min^{-1} under air flow. Magnetic properties of ML-HAP and ML-HAP/5FU were examined by a vibrating sample magnetometer (VSM, Riken Denshi, BHV-525) at room temperature.

2.5. Cytotoxicity assay

Cytotoxicity of ML-HAP was evaluated using a HepG2 cell line by cell counting kit-8 (CCK8) assay. Briefly, cells were seeded in 96-well cell culture plates at a density of 5000 cells/well and incubated in the Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. Afterward, cells were moved to ML-HAP-containing media with various ML-HAP concentrations (0, 100, 200, 400, and 800 μg/mL) for another 24 h and 72 h. After the culture media was removed, cells were rinsed with Hank's balanced salt solution (HBSS) twice to remove excess materials, followed by the addition of 90 μl DMEM and 10 μl CCK8 solution for incubation for another 3 h. Finally, the media were transferred to new 96-well cell culture plates and the optical density (O.D.) of each well was read at 450 nm using microplate reader.

2.6. In vitro drug release

The *in vitro* release studies were performed in phosphate buffer saline (PBS, pH 7.4) and simulated gastric fluid (SGF, pH 1.2) by dialysis bag technique. Accurately weighed ML-HAP/5FU samples were placed in dialysis bags containing 20 mL of the release medium. The dialysis bags were then immersed in a beaker containing 500 mL of release medium. The samples were incubated at 37 ± 0.5 °C and the rotation frequency of basket was 50 rpm. Aliquots (4 mL) were withdrawn at predetermined intervals and the same volume was replaced with fresh release medium. The 5FU concentration was analyzed by the same aforementioned UV–vis spectrophotometer. These experiments were repeated at least three times for each sample and the average values are reported.

2.7. Drug release kinetics

In order to understand the drug release mechanisms, the data obtained from release experiments were fitted by various kinetic models including first-order (Eq. (1)) [32,33], Higuchi (Eq. (2)) [34], and Rigter-Pappas (Eq. (3)) [35].

$$M_t/M_\infty = 1 - e^{-k(t-\alpha)} \quad (1)$$

$$M_t/M_\infty = k(t-\alpha)^{1/2} \quad (2)$$

$$M_t/M_\infty = k(t-\alpha)^n \quad (3)$$

where M_t/M_∞ denotes the fractional drug release, k represents a kinetic constant, t and α are release time and modified parameter, respectively, and n is the diffusion exponent. The n value reflects the drug transport mechanism. The value of $n < 0.45$ is an indication of the drug diffusion-controlled drug transport, $n > 0.89$ indicates the dissolution of the carriers, and $0.45 < n < 0.89$ suggests the dual mechanisms of drug diffusion and carrier dissolution [32].

3. Results and discussion

3.1. Morphology and structure

As shown in Fig. 1a, ML-HAP nanoparticles can be well dispersed in water by shaking or sonication, resulting in a rust-colored suspension. Very fast aggregation of ML-HAP nanoparticles from their homogeneous dispersion was observed once an external magnetic field is applied and the solution became clear within minutes. This suggests that ML-HAP possesses excellent magnetic property. The magnetic property was further characterized at 300 K with a vibrating sample magnetometer. Fig. 1b reveals that the saturation magnetization values of ML-HAP and ML-HAP/5FU are 16.3 and 9.5 emu g^{-1}, respectively, suggesting the superparamagnetism of ML-HAP/5FU, although it is