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Folate attached, curcumin loaded Fe_3O_4 nanoparticles: A novel multifunctional drug delivery system for cancer treatment

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HIGHLIGHTS

- Folate attached, curcumin loaded Fe3O4 nanoparticles were prepared and characterized.
- The NPs have high curcumin loading capacity and good ability for hyperthermia.
- Folate shows its bioactivity of effectively targeting the NPs to tumor tissues.
- Chemotherapy, hyperthermia and targeting factor are all well combined in the NPs.

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ABSTRACT

Study and development of drug delivery nanosystem for cancer treatment are attracting great attention in recent years. In this work, we studied the role of folic acid as a targeting factor on magnetic nanoparticle Fe_3O_4 based curcumin loading nanosystem. Characteristics of the nanosystems were investigated by Fourier transform infrared spectroscopy (FTIR) and field-emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), thermal gravimetric analysis (TGA) and vibrating sample magnetometer (VSM), while targeting role of folic was accessed in vivo on tumor bearing mice. The results showed that folate attached Fe_3O_4 based curcumin loading nanosystem has very small size and exhibits better targeting effect compared to the counterpart without folate. In addition, magnetic induction heating of this nanosystem evidenced its potential for cancer hyperthermia.

1. Introduction

Cancer is one of the most dangerous diseases that has attracted great concern of scientists for many decades. A lot of methods have been applied for treating cancer including chemotherapy, radiation therapy, surgery, hyperthermia, biological therapies. In which chemotherapy is the most common method used to treat cancer. However almost anti-cancer drugs are poor water soluble, non-specific biodistribution and targeting leading to low therapeutic efficacy and serious side effects. The development of nanotechnology brings big advantages for chemotherapy. Compared to conventional drug delivery systems, drug delivery nanosystems possess unique properties. Their small size and modified surface enable them passing the biological barrier, thus, improve the biodistribution and circulation time in the body [1]. Further more because of the difference in structure between tumor and normal tissue, drug delivery nano system with the size below 200 nm will accumulate much more at the tumor than at the normal tissue known as the Enhanced Permeability and Retention (EPR) effect [2].

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This phenomenon is called “passive targeting”. In addition to this passive targeting mechanism, active targeting strategies utilize the specific interaction between targeting ligands attached on the drug delivery nanosystem and the receptors which are unique for each kind of tumor (including Human epidermal receptor, Transferrin receptor, folate receptors) [3]. Targeting ligands may be antibodies, aptamer, peptides or small molecules. Among them, folic acid, a small molecule is required for essential cell function, has high binding affinity to the folate receptor which is overexpressed on the cell surface of many human tumors providing a distinguishable marker from normal cells. Besides, folic acid has received promising consideration due to its nonimmunogenicity, high stability, low cost and its faster internalization kinetics through cellular membrane [4–6]. Conjugation of folate to nanocarriers facilitated their cellular uptake by folate receptor – mediated endocytosis which can avoid their non-specific attacks to normal tissues as well as increase their cellular uptake within target cells [7–9]. The passive and active targeting not only helps increasing the drug concentration at the tumor sites but also enhances the cellular uptake of drug leading to improving therapeutic efficacy [10].

Curcumin, a natural compound isolated from rhizomes of the herb *Curcuma longa*, has received considerable attentions thanks to its multiple biological activities including anti-inflammatory, antioxidant, wound healing, anti-microbial and anti-cancer activities [11]. However, like many other potent therapeutic agents, the biggest limitation of Curcumin is the poor water solubility resulting to low bioavailability. To address this issue, our group successfully prepared a nanosystem which has high curcumin loading capacity based on magnetic nanoparticles encapsulated by *O*-Carboxyl methylchitosan (Fe_3O_4/OCMCS/Cur NPs). The system was proved having much stronger internalization and cytotoxicity on HT29 cells (human colorectal adenocarcinoma cell line) compared to that of free Curcumin [12]. In addition, magnetic nanoparticle based nanosystems have been studied for cancer hyperthermia which is a treating method based on killing cancer cell by temperature. Normal cells are able to withstand temperatures of 42–46 °C, in contrast to cancer cells which undergo apoptosis at those temperatures. In hyperthermia, magnetic nanoparticle based nanosystems will be located at the tumors and under an external magnetic field they will induce heat which increases the temperature in the tumors. The higher amount of magnetic nanoparticle located at the tumor is, the better efficiency will be achieved [13].

In this study, we continue to develop the Fe_3O_4/OCMCs/Cur NPs by attaching folic acid with the aim of increasing the targeting efficacy to cancer cell. Magnetic properties of the nanoparticles were also studied. The results showed that folate attached Fe_3O_4/OCMCs/Cur NPs have potential for hyperthermia based on its targeting ability and heating induction.

2. Experiment

2.1. Synthesis of folate attached-OCMCS

Folic acid was attached to the OCMCS polymer chain through the formation of amide linkage between the carboxyl group of folic acid and the amine group of OCMCS. Firstly, folic acid was activated by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Folic acid was dissolved into 10 ml of distilled water adjusted to pH 8.5 by triethylamine (TEA). EDC and NHS were added to the solution and stirred for 24 h in dark at room temperature. The activated folic acid solution was filtered to remove the precipitate (Dicyclohexylurea - DCU). Next, OCMCS was dissolved into 30 ml of distilled water and then filtered to remove un-soluble OCMCS. The pH of OCMCS solution was adjusted to 8.5 by TEA and the activated folic acid was added slowly to this solution. After 24 h stirring, the solution was filtered and dialysed against distilled water to remove TEA and excess EDC, NHS.

2.2. Preparation of magnetic nanoparticles

Magnetic nanoparticles (Fe_3O_4) were synthesized by co-precipitation of Fe^{2+} and Fe^{3+} according the literature procedure [14]. Briefly, a mixture of iron (III) chloride hexahydrate and iron (II) chloride tetrahydrate (molecular ratio 2:1) was dissolved by a dilute HCl solution in a three necked round bottom flask. The solution was stirred at 500 rpm and heated to 70 °C under nitrogen atmosphere by a magnetic stirred. Then a dilute ammonium hydroxide solution was added dropwise until getting a total black solution. The reaction was continued for 30 min. Fe_3O_4 nanoparticles were collected by a magnet and washed three times by double distilled water.

Two systems which are Curcumin loaded magnetic nanoparticles encapsulated by OCMCs (Fe_3O_4/OCMCs/Cur NPs) and Curcumin loaded magnetic nanoparticles encapsulated by folate attached OCMCs (Fe_3O_4/OCMCs/Cur/Fol NPs) were prepared in a same procedure. In brief, for the preparation of the NPs, 30 ml of Fe_3O_4 fluid (2 mg/ml) was added dropwise to 30 ml of OCMCS solution or folate-attached OCMCS solution. The mixture then was ultrasonically vibrated for 1 h and stirred for 24 h. Next, 60 mg of Curcumin dissolved in 30 ml of ethanol was added dropwise to the mixture. The resulting mixture was ultrasonically vibrated for 1 h and stirred for 48 h. Ethanol was evaporated under vacuum. The solution was then dialysed against distilled water to remove unloaded curcumin.

2.3. Characterization methods

Phase structure of materials was determined by X-ray diffraction (SIEMENS-D5000). Magnetic property was measured in a vibrating sample magnetometer VSM (homemade). Molecular structure of materials was characterized by Fourier transform infrared spectroscopy (FTIR, SHIMADZU spectrophotometer) using KBr pellets in the wave number region of 400–4000 cm^{-1}. Surface morphology of materials was investigated by field emission scanning electron microscopy (FE-SEM) on a Hitachi S-4800 system. Thermal gravimetric analysis (TGA) method was used to determine curcumin loading capacity of Fe_3O_4 nanoparticles.

Biodistribution of nanoparticles was investigated in vivo on Sarcoma-180 solid tumor-bearing mice.

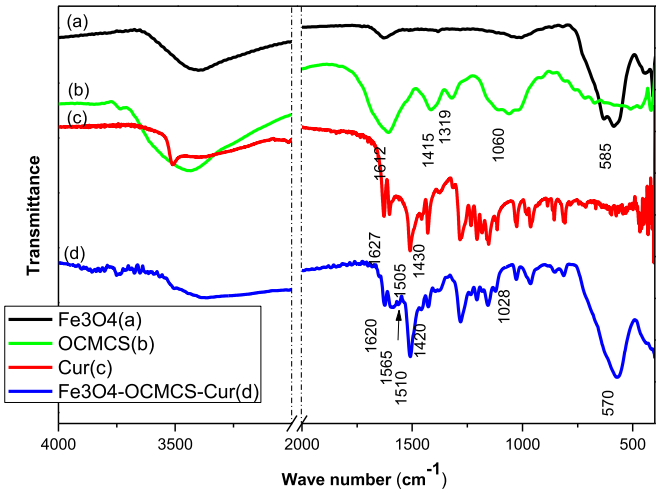

Fig. 1. IR spectra of (a) Fe_3O_4, (b) OCMCS, (c) Curcumin, (d) Fe_3O_4-OCMCS-Cur.