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Microfluidic assembly of a nano-in-micro dual drug delivery platform composed of halloysite nanotubes and a pH-responsive polymer for colon cancer therapy

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ABSTRACT

Harsh conditions of the gastrointestinal tract hinder the oral delivery of many drugs. Developing oral drug delivery systems based on commercially available materials is becoming more challenging due to the demand for simultaneously delivering physicochemically different drugs for treating complex diseases. A novel architecture, namely nanotube-in-microsphere, was developed as a drug delivery platform by encapsulating halloysite nanotubes (HNTs) in a pH-responsive hydroxypropyl methylcellulose acetate succinate polymer using microfluidics. HNTs were selected as orally acceptable clay mineral and their lumen was enlarged by selective acid etching. Model drugs (atorvastatin and celecoxib) with different physicochemical properties and synergistic effect on colon cancer prevention and inhibition were simultaneously incorporated into the microspheres at a precise ratio, with atorvastatin and celecoxib being loaded in the HNTs and polymer matrix, respectively. The microspheres showed spherical shape, narrow particle size distribution and pH-responsive dissolution behavior. This nanotube/pH-responsive polymer composite protected the loaded drugs from premature release at pH $\leqslant$ 6.5, but allowed their fast release and enhanced the drug permeability, and the inhibition of colon cancer cell proliferation at pH 7.4. Overall, the nano-in-micro drug delivery composite fabricated by microfluidics is a promising and flexible platform for the delivery of multiple drugs for combination therapy.

Statement of Significance

Halloysite nanotubes (HNTs) are attracting increasing attention for drug delivery applications. However, conventional HNTs-based oral drug delivery systems are lack of the capability to precisely control the drug release at a desired site in the gastrointestinal tract. In this study, a nanotube-in-microsphere drug delivery platform is developed by encapsulating HNTs in a pH-responsive polymer using microfluidics. Drugs with different physicochemical properties and synergistic effect on colon cancer therapy were simultaneously incorporated in the microspheres. The prepared microspheres prevented the premature release of the loaded drugs after exposure to the harsh conditions of the gastrointestinal tract, but allowed their simultaneously fast release, and enhanced the drug permeability and the inhibition of colon cancer cell proliferation in response to the colon pH.

1. Introduction

Oral drug delivery is the most widely used and most readily accepted administration route, with the main advantages being greatest safety, convenience and patient compliance [1]. However, it is challenging to achieve therapeutic levels of drugs via oral drug delivery due to the harsh conditions of the gastrointestinal (GI) tract and the poor solubility, stability and bioavailability of many drugs [2–4]. An ideal oral drug delivery system (DDS) should be able to protect the drugs from premature release and degradation in the GI tract and precisely release the drugs at the desired site. In addition, oral DDS should have high drug loading capacity and the possibility to load multiple drugs with different physicochemical properties to synergistically improve their therapeutic efficacy in the treatment of complex diseases [5]. All these requirements

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make it challenging to develop a satisfied oral DDS [1,2,6], especially when they are limited to be designed by commercially available materials.

Halloysite nanotubes (HNTs) are a naturally occurring aluminosilicate clay with an external diameter of ~50 nm, an inner lumen of ~15 nm and a length of ~500–1000 nm [7]. HNTs are abundantly and commercially available in cheap price, and they are attracting rapidly increasing interest beyond the ceramic society. For example, HNTs have been employed in environmental [8,9] and biomedical applications, including wound healing, tissue engineering and drug delivery [10–19], due to their good biocompatibility that has been assessed both *in vitro* and *in vivo* [20–28]. The lumen of HNTs makes them a particularly interesting nanocarrier for the loading and release of various biologically active molecules, including small molecules, enzymes and proteins [10,29–32]. Moreover, the loading capacity of HNTs can be further increased by selectively chemical etching to enlarge the lumen [33].

HNTs are being used for oral drug delivery [32,34], exhibiting a high degree of biocompatibility to the intestinal cells [22]. Also, HNTs have been shown to be a good candidate as a tablet compression excipient [35]. Current HNTs-based oral DDS is lack of the capability to precisely control the release of loaded drugs at a desired site in the GI tract. Therefore, there is a strong demand to develop advanced HNTs-based oral DDS to overcome these limitations. To prevent premature drug release and to obtain controlled drug release profiles, drug or drug loaded nanoparticles have been encapsulated in polymer matrices to generate composite particles [5,36–38]. Conventional emulsion methods have been frequently used in nano-in-micro encapsulation [39,40]; however, they usually produce microparticles with wide particle size distribution, low encapsulation efficiency and uncontrolled release kinetics.

Droplet microfluidics is a robust technique to manipulate nanoliter of immiscible solutions in the networks of microscale channels and to enable precisely generation of highly monodisperse droplet emulsions in microsized range, which subsequently can be solidified to form monodisperse microparticles [5,36,41–44]. Furthermore, the microfluidic technique has the advantage to achieve an encapsulation efficiency of almost 100% for different cargos [41].

In the present study, we used the microfluidic technique to encapsulate HNTs in a pH-responsive hydroxypropyl methylcellulose acetate succinate (HPMCAS) polymer in order to develop an oral drug delivery platform. HPMCAS, insoluble in acidic conditions, but highly soluble in neutral or alkaline conditions, is approved by FDA for oral administration. Atorvastatin (ATV) and celecoxib (CEL), which act synergistically on colon cancer prevention and inhibition [45–47], were chosen as the model drugs in this study to be co-loaded into the composite microspheres. Moreover, ATV and CEL have different physicochemical properties (such as solubility and hydrophobicity), which represent the typical drugs that are difficult to be simultaneously loaded into and released from a single carrier in a precise manner. The size, morphology, stability in GI conditions and pH-responsive drug release profiles of the prepared microspheres were characterized. Moreover, their effects on the viability and proliferation of colon cancer cells and on the drug permeation across the intestinal cell monolayers were also studied. To the best of our knowledge, this is the first time that HNTs are formulated with pH-responsive polymer for oral drug delivery.

2. Materials and methods

2.1. Etching and characterization of HNTs

1 g of HNTs (Sigma-Aldrich, USA) was sonicated in 50 mL of Milli-Q water for 30 min. Afterwards, the HNTs were collected by centrifugation and then ultrasonically suspended in 50 mL of 1 M hydrochloride acid (BDH Prolabo®, France). The suspension was magnetically stirred at 50 °C for 2 days. Subsequently, the HNTs were collected by centrifugation and washed with water for 5 times. Finally, the collected HNTs were dried in vacuum drying oven (Heraeus Vacutherm VT 6025, Thermo Electron GmbH, Germany) at 50 °C. The surface morphology and internal structure of HNTs were analyzed using scanning electron microscopy (SEM, Quanta 250 FEG, FEI, USA) and transmission electron microscopy (TEM, JEOL 1400, JEOL, Japan), respectively. Nitrogen adsorption–desorption isotherms of HNTs were measured at 77 K on a gas adsorption analyzer (Micromeritics TriStar 3000, USA). The specific surface area was determined from the adsorption branch of the nitrogen isotherm using the Brunauer-Emmett-Teller (BET) theory [48], and the pore volume was determined as the total adsorbed amount at a relative pressure (p/p_0) of 0.97.

2.2. Fabrication of the flow-focusing device for microfluidics

The microfluidic flow-focusing device was assembled with two borosilicate glass cylindrical capillaries (World Precision Instruments, USA) and a glass slide [5,36,49]. The inner capillary had an inner and outer diameter of 580 and 1000 μm, respectively. Firstly, the inner capillary was tapered to a diameter of 20 μm using a micropipette puller (P-97, Sutter Instrument, USA), and then enlarged to 100 μm. Afterwards, the tapered capillary was inserted into the right end of the outer capillary with an inner diameter of 1120 μm. Two syringes attached with polyethylene tubes were linked to the microfluidic device to allow the independent injection of the inner and out fluid at constant rates controlled by pumps (PHD 2000 pumps, Harvard Apparatus, USA). A transparent epoxy resin (5 min® Epoxi, Devcon, USA) was used to seal the microfluidic device where required.

2.3. Fabrication of pH-responsive microspheres

The microspheres were prepared by microfluidics with flow-focusing oil-in-water (O/W) emulsion (Fig. 1a). HF grade of HPMCAS (ShinEtsu, Japan) was dissolved in ethyl acetate (EA) at a concentration of 20 mg mL^{-1} and served as the inner oil fluid. 2% w/v Poloxamer 407 (P-407) (BASF, Germany) aqueous solution (pH 5.5) was used as the outer continuous fluid to obtain a stable O/W emulsion. The inner fluid (2 mL h^{-1}) was focused by the outer fluid (10 mL h^{-1}), and the formed droplets were collected in 1% w/v P-407 aqueous solution (pH 5.5) and solidified via the diffusion of EA to the external aqueous solution. Afterwards, the produced HF microspheres were collected by centrifugation and dried in a vacuum drying oven at 50 °C for 24 h. At last, the HF microspheres were obtained as white powder. For the preparation of HNTs encapsulated HF microspheres (HNT@HF), HNTs were dispersed in the inner fluid at a concentration of 4 mg mL^{-1}, and the following preparation procedure was as the same as the aforementioned (bare) HF microspheres.

2.4. Characterization of the pH-responsive microspheres

The surface morphology and internal structure of bare HF microspheres and HNT@HF microspheres were observed using SEM. For the internal structural study, the microspheres were embedded in paraffin and sections were cut on a microtome (HM 355S, Thermo Fisher Scientific, USA). Both the intact microspheres and the sectioned ones were mounted onto stubs using carbon tapes, and platinum sputtered before imaging. The particle size of the microspheres was determined using Image J (NIH, USA), and at least 100 particles were analyzed. To investigate the pH-responsive dissolution behavior of the prepared microspheres, they