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Diatom silica microparticles for sustained release and permeation enhancement following oral delivery of prednisone and mesalamine

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ABSTRACT

Diatoms are porous silica-based materials obtained from single cell photosynthetic algae. Despite low cost, easy purification process, environmentally safe properties, and rapidly increasing potentials for medical applications, the cytotoxicity of diatoms and the effect on drug permeation of oral formulations have not been studied so far. Herein, we have evaluated the potential of diatom silica microparticles (DSMs) for the delivery of mesalamine and prednisone, which are two commonly prescribed drugs for gastrointestinal (GI) diseases. Transmission electron microscopy analysis of the morphological surface changes of Caco-2/HT-29 monolayers and the cell viability data in colon cancer cells (Caco-2, HT-29 and HCT-116) showed very low toxicity of diatoms at concentrations up to 1000 μg/mL. The mesalamine and prednisone release under simulated GI conditions indicated prolonged release of both drugs from the diatoms. Furthermore, drug permeation across Caco-2/HT-29 co-culture monolayers demonstrated that diatoms are capable to enhance the drug permeability. Overall, this study evaluated DSMs' cytotoxicity in colon cancer cells and the effect of DSMs on drug permeability across Caco-2/HT-29 monolayers. Our results demonstrate that DSMs can be considered as a non-cytotoxic biomaterial with high potential to improve the mesalamine and prednisone bioavailability by sustaining the drug release and enhancing drug permeability.

1. Introduction

The development of micro- and nano-scale drug delivery systems has impacted tremendously the current pharmaceutical industry and research by attenuating the many traditional hurdles of drug delivery, including the low efficiency and poor safety [1]. The oral drug delivery has been the most popular and favorite route administration, because of the low expenses on medication and high compliance of the patients [2]. However, the harsh conditions of the gastrointestinal (GI) tract, including the variable pH-conditions, possible enzymatic degradation and food effects dramatically increase the complexity of oral drug administration [2,3]. Moreover, the poor water solubility, negligible permeation across biological barriers and extensive first-pass metabolism hinder many drugs to be dosed via the oral route [2,3].

In the past decade, porous silica-based nano- and micro-particles have attracted a lot of attention owing to their astonishing biomedical applications [4–9]. The biodegradability, low toxicity, large surface area and flexibility for surface modifications make them potential materials for imaging, biosensing, improved physicochemical properties of poorly water-soluble drugs, targeted drug delivery, tailored controllable drug release, as well as good candidates for cancer diagnostic and triggered cancer therapy [10,11].

Nature has provided elegant biologically based materials with advanced properties, including the ones that can be found in aquatic organisms, from both marine [12] and fresh water origin. For example, diatom silica-based materials are ornate art of design, with amorphous, clear silica glass shell and highly-ordered three-dimensional (3-d) pores [13]. Diatoms are also attractive due to their unique functionalities in photonics, filtration, water purification, microfluidics and biosensing [13–16]. Diatoms have probably

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more than 100,000 different species, which can be divided into two major types: centric and pennate diatoms [17]. The micro- and nano-size diatoms can be produced by cultivation. Moreover, the diatomaceous earth (DE), or diatomite silica microparticles, which are consisted of fossilized remains of diatoms [18], can be easily obtained from mining industry in millions of tons, and purified diatoms can be extracted from raw DE powder by a simple sedimentation process [19]. The production of diatoms is highly environmentally friendly compared to synthetic porous silica-based materials, because there is no toxic waste production and low energy consumption is required during the process. Moreover, diatoms are considered to be harmless due to the amorphous silica structure [20]. Food grade DE has been approved in USA to feed animals and there are already several human grade DE products in the market in Europe and Australia.

The purified diatoms have distinct 3-d pillbox structures with highly-ordered nano-size pores [19]. Generally, they are 4–6 μm in diameter and 10–20 μm in length. Recently, it has been recognized that diatom microcapsules can be used as micro-vehicles for oral drug delivery applications [18,19,21]. Surface modified diatoms are able to control the drug release of both hydrophilic and hydrophobic drugs [19,21]. However, critical issues in drug delivery such as the cytotoxicity [6] and the effect of diatoms on drug permeation are not fully understood, therefore, there is a high demand for further investigation. Moreover, just a limited number of drugs have recently been studied with diatom silica-based materials [18,19,21], showing the necessity of more investigations for clarifying the potential properties of this new group of biomaterials in drug delivery applications.

In this study, we have tested two commonly prescribed orally administrated drugs for GI diseases, mesalamine and prednisone. Mesalamine is an anti-inflammatory drug often used for the therapy of mild to moderate inflammatory bowel disease. Recently, it has been demonstrated that mesalamine has also potential in cancer prevention [22]. Mesalamine has poor systemic bioavailability due to the low permeability and extensive drug metabolism [23]. Prednisone is a well-known glucocorticosteroid drug for immunosuppressant treatment of inflammatory diseases [24,25]; at higher doses it can also be used for cancer therapy [26]. Like other corticosteroid drugs, prednisone is prescribed very carefully because of the risk of severe side effects such as immunosuppression related infections, osteoporosis, Cushing's syndrome and diabetes mellitus [27]. Therefore, the development of proper drug delivery systems, which can hinder the unfavorable pharmacokinetics of mesalamine and prednisone would significantly improve the therapeutic efficiency of these drugs.

In the present study, we have characterized the physicochemical properties and drug delivery behavior of diatom silica microparticles (DSMs), namely chemical composition, crystallinity, thermal behavior and surface properties, including area, morphology and chemistry. In particular, we have evaluated the cytotoxicity of DSMs by conducting a cell viability assay and monitoring the morphological surface changes using transmission electron microscopy (TEM). Drug loading and release behavior of the DSMs were investigated using two drugs, mesalamine and prednisone. Finally, the role of DSM on the drug permeation across Caco-2/HT-29 co-cultured monolayers was evaluated.

2. Material and methods

2.1. Purification of DSMs

DE was obtained from Perma-Guard, USA (Fossil Shell Flour®). The DE powder was suspended with Milli-Q water at the concentration of 50 mg/mL. Afterwards, it was sonicated in water bath sonicator for 10 min and then sedimented by leaving it in static condition for 30 min. Subsequently, the washing and sedimentation processes (without sonication) were repeated for 5 times. The purified DSMs were then dried at room temperature.

2.2. Physicochemical characterization of DSMs

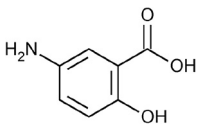
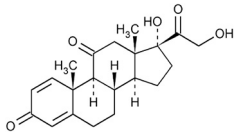
The structural properties of unloaded DSMs were determined by N_2 sorption measurements using a TriStar 3000 instrument (Micromeritics Inc., GA, USA) at −196 °C. The specific surface areas of the DSMs were determined from the adsorption branch of the N_2 isotherm using Brunauer–Emmett–Teller (BET) theory. The mesopore volume was determined as the total adsorbed amount at a relative pressure (p/p_0) of 0.97.

The surface morphologies of the unloaded and loaded DSMs were studied by scanning electron microscope (SEM; Quanta 250, FEG, USA). The samples were sputter coated with platinum prior to imaging.

The surface chemistry of mesalamine-loaded diatoms (MesaDia) and prednisone-loaded diatoms (PredDia) were studied with Fourier transformed infrared spectroscopy (FTIR) using a Vertex 70 Spectrometer (Bruker, USA) equipped with a horizontal attenuated total reflectance (ATR) accessory (MIRacle, PIKE Technologies, USA). The measurements were performed at room temperature and at wavenumbers between 4000 and 650 cm^{-1}.

Differential scanning calorimetry (DSC) analyses were carried out with DSC823e instrument (Mettler Toledo, USA) to investigate the crystal states of mesalamine and prednisone after loading into the DSMs. The DSM, bulk drugs and drug-loaded DSMs were put in 40 μL aluminum sample pans with holes and heated up at rate of 10 °C/min under N_2 gas purge of 50 mL/min. The heating range was 25–325 °C and 25–285 °C for mesalamine and prednisone, respectively, depending on the melting point of the bulk drug (Table 1).

Table 1
Physicochemical properties of mesalamine and prednisone.

	Mesalamine	Prednisone
Chemical formula	$C_7H_7NO_3$	$C_{21}H_{26}O_5$
Molecular weight (g/mol)	153.14	360.44
Chemical structure		
Melting point (°C)	282	243
Biopharmaceutical Classification System (BCS) class	III	I
Water solubility, pH 7.4 (mg/mL)	0.84	0.31
pKa value	2.09; 5.26; 13.64	12.58
HPLC detecting UV (nm)	303	243
Loading degree in diatoms (%)	11.5	9.9