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Highly fluorescent and morphology-controllable graphene quantum dots-chitosan hybrid xerogels for *in vivo* imaging and pH-sensitive drug carrier

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ABSTRACT

Highly fluorescent graphene quantum dots (GQDs)-chitosan (CS) hybrid xerogels (GQDs-CS) were facilely synthesized, and the morphology of GQDs-CS was controllable by varying the content of GQDs in the xerogel. The GQDs-CS exhibited a porous and three-dimensional (3D) network structure when the content of GQDs reached 43% (wt%) in the xerogel, which was beneficial for drug loading and sustained release. The as-prepared GQDs-CS could also be applied for *in vivo* imaging since it showed strong blue, green and red luminescence under excitation of varying wavelengths. Moreover, the pH-induced protonation/deprotonation of the $-NH_2$ groups on CS chains can result in a pH-dependent drug delivery behavior of the GQDs-CS hybrid xerogel.

1. Introduction

The past years have witnessed the development of graphene quantum dots (GQDs) because of their strong quantum confinement and edge effects [1,2]. However, tremendous efforts were devoted to developing approaches for GQDs synthesis (bottom-up and top-down methods) and exploring their properties [3–5], and their applications in biomedical field have not been extensively explored. Recently, GQDs have become the focus for fluorescent probes because GQDs can emit fluorescence of various wavelengths including near-infrared fluorescence (NIR) [6]. The size- and composition-tunable luminescence, high photostability and low toxicity make GQDs a promising material for drug carrier and *in vivo* imaging [7–12].

Chitosan (CS) is a natural cationic biopolymer composed of 2-amino-2-deoxy(1.4)-β-D-glucopyranose units, which has been widely used in biomedical and tissue engineering field due to its good biocompatibility, biodegradability, non-toxicity and antibacterial properties [13–16]. Of particular interest, CS exhibits intriguing pH-responsive swelling/deswelling transitions induced by protonation and deprotonation of the $-NH_2$ groups at the interpenetrated CS chains [17,18], and thus CS based pH-regulated drug delivery has been realized [19–21].

In the current study, CS was incorporated into GQDs through electrostatic interactions and H-bonding, and then GQDs-CS xerogel was prepared *via* a simple freeze-drying technique. The influence of GQDs content on the microstructure and fluorescent property of the xerogel was fully addressed. The feasibility of using the GQDs-CS as a fluorescent biomarker was investigated through *in vitro* and *in vivo* fluorescence imaging through subcutaneous injection in an animal model. Finally, the GQDs-CS was applied as a drug carrier, and it exhibited pH-sensitive drug delivery behavior, which opens a new avenue in the design of pH-triggered delivery at specific sites and in the protection of drugs from degradation when passing through different tissues and organs in the human body [22]. Here sodium salicylate (SS) is chosen as the model drug because it is widely used as prescribed agents for the treatment of inflammation and for curing diseases including wound healing [23], diabetes [24], arthritis [25] and cancer treatment [26].

2. Experimental

2.1. Reagents and apparatus

Citric acid monohydrate, chitosan (with a deacetylation degree of 85%), sodium salicylate (SS) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All other chemicals were of analytical grade and used as received without further purification. BALB/c nude mice

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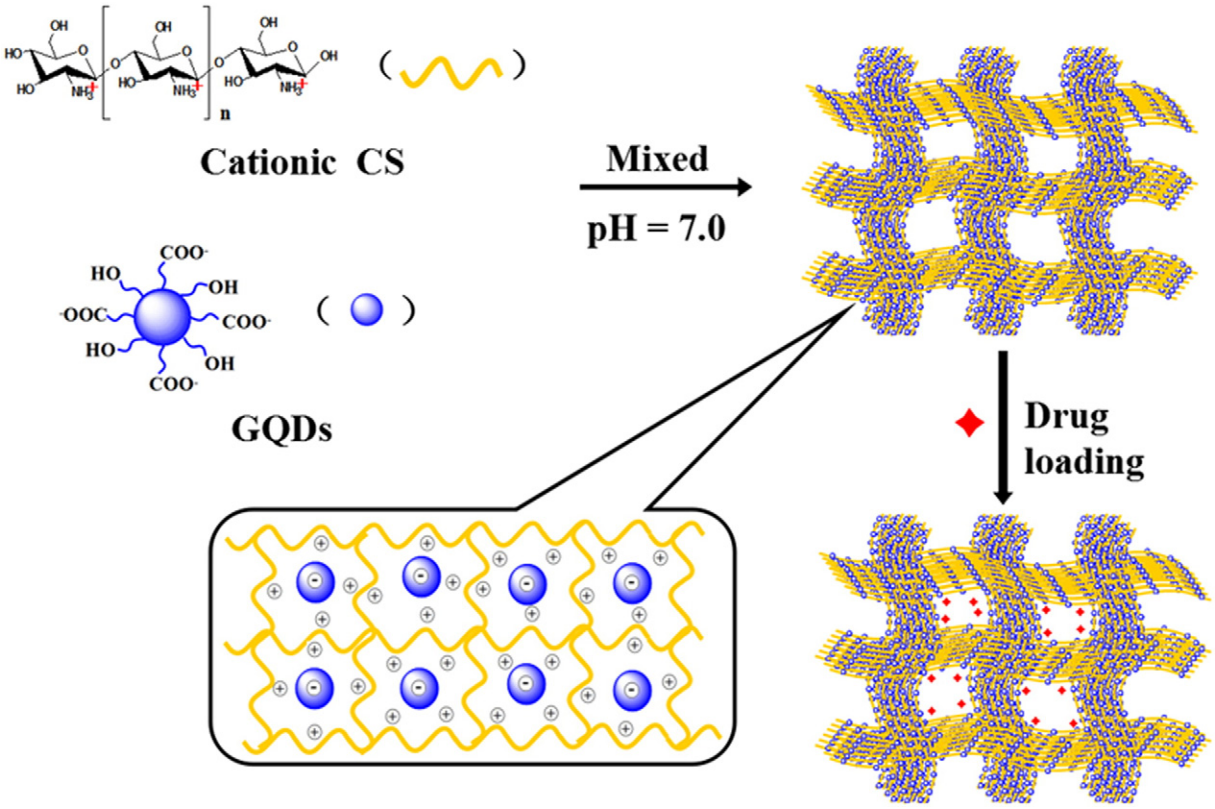

Fig. 1. Schematic illustration showing the formation of the drug-loaded GQDs-CS hybrid xerogel.

were supplied by Department of Experimental Animals, Fudan University (China). All solutions were prepared with ultrapure water with a resistivity of 18.2 MΩ.

The zeta potentials of GQDs and CS were determined by a ZEN3600 zeta potential analyzer (Malvern). The morphology and size of GQDs, CS and GQDs-CS with different GQDs contents were observed on a Supra55 field-emission scanning electron microscope (Zeiss, Germany) and a JEM-2100 transmission electron microscope (JEOL, Japan), respectively. The FT-IR spectra of different samples were measured by a FTIR-8400S spectrometer (Shimadzu, Japan). The N_2 adsorption/desorption isotherms were recorded at 77 K with an ASAP 2010 specific surface area and pore size analyzer (Micromeritics, USA). The *in vitro* fluorescence imaging of GQDs-CS and GQDs was conducted at different excitation wavelengths (Zeiss LSM-710 Confocal Microsope), and the *in vivo* fluorescence imaging of nude mice with subcutaneously injected GQDs-CS was carried out by an IVIS imaging system (Caliper).

2.2. Synthesis of drug-loaded GQDs-CS xerogel

GQDs were prepared according to the method previously reported [27]. In a typical synthesis, 2 g citric acid monohydrate was put into a ceramic crucible and heated to 200 °C in a muffle furnace. After 30 min, the muffle furnace was gradually cooled down to room temperature and brown GQDs powders were obtained. Next, 20 mg GQDs were dispersed in 10 mL ultrapure water by sonification to obtain a 2 mg mL^{-1} yellow GQDs dispersion.

Different amounts of CS powders were dissolved in 10 mL 0.1 M acetic acid solutions, and then the CS solutions of different concentrations (2, 4 and 6 mg mL^{-1}) were respectively transferred into the GQDs dispersion (2 mg mL^{-1}). The mixtures were vigorously stirred for 10 min, followed by neutralizing with diluted NaOH solution, and yellow solid (GQDs-CS) was precipitated. The products were separated and washed with ultrapure water several times to remove unreacted GQDs and CS, and subsequently freeze-dried in a lyophilizer under −45 °C for 24 h to obtain the GQDs-CS hybrid xerogel. For control experiment, pure CS xerogel was prepared by the same procedure without adding GQDs.

Each xerogel sample with the same mass (100 mg) was separately dispersed in 100 mL ultrapure water under stirring, and then 50 mg SS was added into the hybrid xerogel dispersion. After a continuous stirring for 24 h in the dark (SS is stable in the dark), the drug-loaded hybrids were then separated from the dispersion by filtering, washed thoroughly to remove unloaded drug, and freeze-dried to yield drug-loaded GQDs-CS xerogel. The schematic illustration showing the formation of the drug-loaded hybrid xerogel is shown in Fig. 1. The drug loading capacity and entrapment efficiency were calculated by the following two equations.

Drug loading capacity (%) = 100% × (weight of drug in the substrate) / (weight of the substrate) [28] Entrapment efficiency (%) = 100% × (weight of drug in the substrate) / (weight of the feeding drug) [29].

2.3. In vitro and in vivo fluorescence imaging

The *in vitro* fluorescence imaging of 1 mg mL^{-1} dispersion of CS, GQDs and GQDs-CS with various contents GQDs was conducted at different excitation wavelengths of 405, 430 and 535 nm. For *in vivo* fluorescence

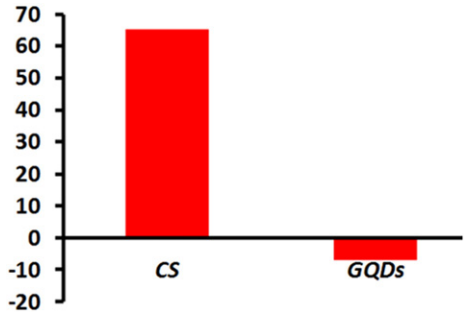

Fig. 2. Determined zeta potentials of CS and GQDs.