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Cationic carbon quantum dots derived from alginate for gene delivery: One-step synthesis and cellular uptake

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ABSTRACT

Carbon quantum dots (CQDs), unlike semiconductor quantum dots, possess fine biocompatibility, excellent upconversion properties, high photostability and low toxicity. Here, we report multifunctional CQDs which were developed using alginate, 3% hydrogen peroxide and double distilled water through a facile, eco-friendly and inexpensive one-step hydrothermal carbonization route. In this reaction, the alginate served as both the carbon source and the cationization agent. The resulting CQDs exhibited strong and stable fluorescence with water-dispersible and positively-charged properties which could serve as an excellent DNA condensation. As non-viral gene vector being used for the first time, the CQDs showed considerably high transfection efficiency (comparable to Lipofectamine2000 and significantly higher than PEI, $p < 0.05$) and negligible toxicity. The photoluminescence properties of CQDs also permitted easy tracking of the cellular-uptake. The findings showed that both caveolae- and clathrin-mediated endocytosis pathways were involved in the internalization process of CQDs/pDNA complexes. Taken together, the alginate-derived photoluminescent CQDs hold great potential in biomedical applications due to their dual role as efficient non-viral gene vectors and bioimaging probes.

Statement of Significance

This manuscript describes a facile and simple one-step hydrothermal carbonization route for preparing optically tunable photoluminescent carbon quantum dots (CQDs) from a novel raw material, alginate. These CQDs enjoy low cytotoxicity, positive zeta potential, excellent ability to condense macromolecular DNA, and most importantly, notably high transfection efficiency. The interesting finding is that the negatively-charged alginate can convert into positively charged CQDs without adding any cationic reagents. The significance of this study is that the cationic carbon quantum dots play dual roles as both non-viral gene vectors and bioimaging probes at the same time, which are most desirable in many fields of applications such as gene therapy, drug delivery, and bioimaging.

1. Introduction

Over the past few decades, luminescent quantum dots (QDs) have triggered intensive interest owing to their great potential for biological application [1–3]. QDs have compelling advantages over conventional fluorescent dyes; for example, they possess excellent photostability, high photoluminescence quantum yield, wide range of excitation spectrum and long lifetime of fluorescence [4]. To date, the QDs have been most frequently used as fluorescent probes for bioimaging [5–9] but seldom been employed for drug/gene delivery. This discrepancy is most likely due to the fact that traditional QDs are produced from heavy-metal elements such as PbSe, CdS and CdSe, which suffers from poor biocompatibility and high toxicity [10–13]. On the other hand, most of the current non-viral gene vectors (such as calcium phosphate nanoparticles [14] and cationic polymers [15,16]) do not possess the property of self-imaging. Therefore, the fabrication of a new type of benign and visible emitter that allows co-operative drug/gene delivery and bioimaging [17] could be an interesting challenge.

Photoluminescent carbon quantum dots (CQDs), as a newcomer from the carbon nonmaterials family, have been gathering considerable attention in the nanotechnology field due to their low

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toxicity, excellent biocompatibility, strong fluorescence, stable photoluminescence and broad excitation spectra [10,18]. CQDs may therefore be a promising alternative to the traditional toxic heavy metal-based semiconductor QDs in a wide range of applications. They can also be developed into positive surface charges through appropriate modifications, which can act as a self-tracking drug/gene carrier.

To date, a considerable number of carbon source materials, including glycerol [19], polyvinyl pyrrolidone [12], candle soot [20], carbon fibers [21], citric acid [22], and various carbohydrate-based food caramels (e.g., bread, jaggery, sugar caramel, corn flakes, and biscuits) have been employed to construct CQDs [23]. Additionally, various approaches to synthesizing CQD have also been developed, such as the top-down methods of laser ablation [24], high-energy ion beam irradiation [25], and electrochemical oxidation [26] and the bottom-up methods of microwave-assisted synthesis [27–29], hydrothermal synthesis [30], and combustion thermal oxidation [20]. Among these methods, the hydrothermal synthetic route has been widely applied because of its simple procedure, low cost and green properties [31,32].

Herein, we report on CQDs prepared by a one-step, green and low-cost process by relying on the hydrothermal carbonization of a new CQDs carbon source, namely, sodium alginate. The CQDs were designed to serve two purposes: as a gene vector to deliver plasmid TGF-β1 into 3T6 cells and as a fluorescent probe to directly monitor the gene delivery process so as to shed light on the cellular uptake mechanisms of the CQDs/pDNA complexes. Sodium alginate, a natural polysaccharide extracted from seaweed, has been widely used in food, medicine, textile, printing and dyeing, paper making, and daily chemical products [33,34]. Recently, it has been employed as an important pharmaceutic adjuvant and biomedical material [35–37]. Previous studies used alginate to prepare gene/-drug delivery formulations to achieve selective cell targeting [38,39]. However, it has never been used as a carbon source to produce CQDs. The alginate could also serve as a prototype for the development of natural polysaccharide into multi-functional materials of bioimaging and targeted delivery of gene/drugs. Therefore, it is prudent to explore the potential of alginate as a desirable material for producing CQDs with dual functions (gene delivery and bioimaging).

Based on the self-tracking ability, the cellular uptake pathways of CQDs have been investigated. Previous reports have revealed several mechanisms associated with cellular uptake of nanoparticles, including clathrin/caveolae-mediated endocytosis, clathrin/caveolae-independent endocytosis, lipid raft-mediated endocytosis, cell surface ruffling-mediated endocytosis and specialized endocytosis via cell membrane receptors [40–42]. Generally, nanoparticles may be taken up by cells via size and shape selectivity, surface coating and charge, and cell lines [43]. Upon entering the cells, nanoparticles are transported via typical cellular processes from early endosomes to late endosomes or lysosomes to endosomal escape and dissociation of vector/gene complexes, which is often considered a rate-limiting step for the efficient transport of genes to the nucleus [44–46]. The CQDs prepared in the present study is expected to serve as an efficient gene carrier. Therefore, it is crucial to explore the pathways responsible for their cellular uptake.

The CQDs were also fully characterized in terms of morphology, size, zeta potential, Fourier transform infrared (FT-IR) spectra, fluorescence emission spectra and absorbance spectra. Enzyme-linked immuno sorbent assay (ELISA) and confocal laser scanning microscopy were further used to evaluate gene transfection and effects of cellular uptake inhibition, respectively. Compared with previous reports in which QDs were mostly investigated using their luminescent properties and applications in bioimaging [9,10,18], sodium alginate-derived CQDs in this study not only show low toxicity and excellent biocompatibility, but also act as both an effective gene vector and a fluorescent probe without additional modifications. Altogether, the findings will provide a new source for CQDs generation and solid evidence for the application of CQDs as an effective non-viral gene vector and bioimaging probe.

2. Materials and methods

2.1. Materials

Two different batches of sodium alginate (CAS number: 9005-38-3, analytical reagent and biological reagent) were obtained from Aladdin Reagent Corporation (Shanghai, China). Hydrogen peroxide (3%), glycerin (ultrapure, molecular biology grade), sodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), quinine, sulfuric acid solution (H_2SO_4), ammonium chloride (NH_4Cl), sodium orthovanadate (SOV), glucose, and colchicine were purchased from Chemical Reagent Co., Ltd. of China National Pharmaceutical Group (Shanghai, China) and used without further purification. Dulbecco's modified Eagle's medium (DMEM), trypsin and fetal bovine serum (FBS) were obtained from Gibco BRL (Invitrogen Co., Carlsbad, CA, USA). The TGF-β1 ELISA kit was provided by Yantai Science and Biotechnology Co., Ltd., (Shandong, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma (Sigma, USA). Chlorpromazine hydrochloride (CPZ), 5-(N,N-dimethyl)-amiloride (DMA), and genistein were obtained from ChromaDex (ChromaDex, USA). YOYO-1 was purchased from Invitrogen (Invitrogen, USA). Semiconductor quantum dots (cadmium telluride quantum dots, CdTe QDs), were kindly provided by Doctor Shanshan Tong at School of Pharmacy, Jiangsu University. The structure of the CdTe QDs is as shown in Fig. S1.

All animal experimental protocols were in accordance with the Helsinki Convention, which were duly approved by the Jiangsu University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals.

2.2. Synthesis of CQDs

Sodium alginate, as a carbon source, together with DDW and hydrogen peroxide, was employed to produce CQDs via a simple hydrothermal carbonization route. The optimal formulation and reaction conditions were obtained based on preliminary experiments (data not shown). Specifically, 0.25 g of sodium alginate and 2.5 mL of 3% hydrogen peroxide were mixed together in double distilled water (10 mL) in a stainless steel autoclave with a Teflon liner with a 100-mL capacity, followed by ultrasonication to form a homogeneous solution. The autoclave was sealed and heated to 220 °C for 12 h. The reactor was then allowed to cool at room temperature, and the resulting solution diluted with double distilled water (DDW, 10 mL) and dialyzed (MW cut-off 1000 Da, Shanghai Green Bird Science and Technology Development Co., Ltd., Shanghai, China) against frequently replenished (every 2 h) DDW for 48 h. After dialysis, the sample was filtered through a 0.22-μm filter followed, by freeze-drying which led to the production of CQDs with excellent fluorescence performance.

2.3. Preparation of CQDs/pDNA complexes

The CQD powder was dissolved in DDW to form a 2 mg/mL solution and filtered through a 0.22-μm filter to become sterilized. Plasmid TGF-β1 (pTGF-β1), as a model gene, was diluted with