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Folate conjugated trimethyl chitosan/graphene oxide nanocomplexes as potential carriers for drug and gene delivery

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ABSTRACT

Folate conjugated trimethyl chitosan (FTMC)/graphene oxide (GO) nanocomplexes (FG NCs) prepared via electrostatic self-assembly were here developed as a targeted delivery vehicle for both doxorubicin (DOX) and plasmid DNA (pDNA). FG NCs exhibited the diameter of 112.0 nm and the thickness of 3.038 nm. Negligible cytotoxicity of FG NCs was observed in Hela and A549 cells. The higher uptake level in Hela cells with folate-receptor proved the targeting ability of FG NCs. Both DOX and pDNA could be loaded into FG NCs, wherein the loading capacity of DOX reached 30.9% and migration of pDNA could be completely retarded. Therefore, FG NCs could be served as a promising candidate for targeted delivery of both anticancer drugs and genes.

1. Introduction

Graphene oxide (GO), an oxidative derivative of graphene, has attracted extensive interests in drug delivery because of its water solubility and ultra-high surface area available for efficient drug loading [1,2]. However, GO suffers from aggregation and is negatively charged [3], which severely compromises its application in loading anticancer drugs and genes. Further modification of GO is therefore needed. Natural hydrophilic polymers including dextran, cyclodextrin, and chitosan have been widely used to modify GO to decrease its aggregation [4]. In our previous study, trimethyl chitosan (TMC) has been demonstrated as an efficient delivery vector with desired solubility and fixed positive charges [5,6]. Therefore, complexation of TMC with GO was anticipated to be capable of reducing the aggregation of GO, as well as providing the opportunity for gene delivery. To further enhance the delivery performance, folic acid (FA) was adopted as the targeting moiety to promote specific endocytosis by cancer cells [7].

Herein, for the first time, we combined GO, TMC, and FA in a single system through synthesis of FA modified TMC (FTMC) and subsequent self-assembly of FTMC with GO. The cytotoxicity and targeting ability of the resultant FTMC/GO nanocomplexes (FG NCs) in Hela cells (FA-receptor positive) and A549 cells (FA-receptor negative) were evaluated by water-soluble tetrazolium salt-8 (WST-8) assay and confocal laser scanning microscope (CLSM), respectively. FG NCs were also tested for the efficiency of loading doxorubicin (DOX) and plasmid DNA (pDNA).

2. Experimental section

Materials: Graphite (8000 mesh) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Chitosan (deacetylation degree of 85% and molecular weight of 37.6 kDa) was purchased from Golden-shell Biochemical Co., Ltd. (China). The target sequence of Survivin shRNA-expressing pDNA (4.7 kb) was GAATTAACCCTTGGTGAAT. Other reagents were of analytical grade.

Preparation and characterization of FG NCs: FG NCs were prepared as illustrated in Scheme 1. TMC was prepared according to our previous study [6]. FTMC was synthesized via the amidation reaction between TMC and FA (Supporting Information (SI)). GO was prepared according to Hummers' method [8] with slight modification. The gelatinous hydrated product obtained after oxidation was diluted and sonicated for 120 min (600 w, 6 s/2 s, on/off), followed by centrifugation at 13,300 rpm for 30 min. The relatively small GO sheets in the supernatant were collected and lyophilized. GO lyophilized powder was allowed to continue sonication for 200 min (600 w, 6 s/2 s, on/off) and centrifugation at 13,300 rpm for 1 h, and thereafter GO suspension with single-layered was obtained. The concentration of GO suspension was determined by the absorption at 230 nm [9]. GO suspension was added into FTMC solution under sonication at the weight ratio of 1:1, which was incubated for 1 h to obtain FG NCs. The detailed characterization of FG NCs was described in SI.

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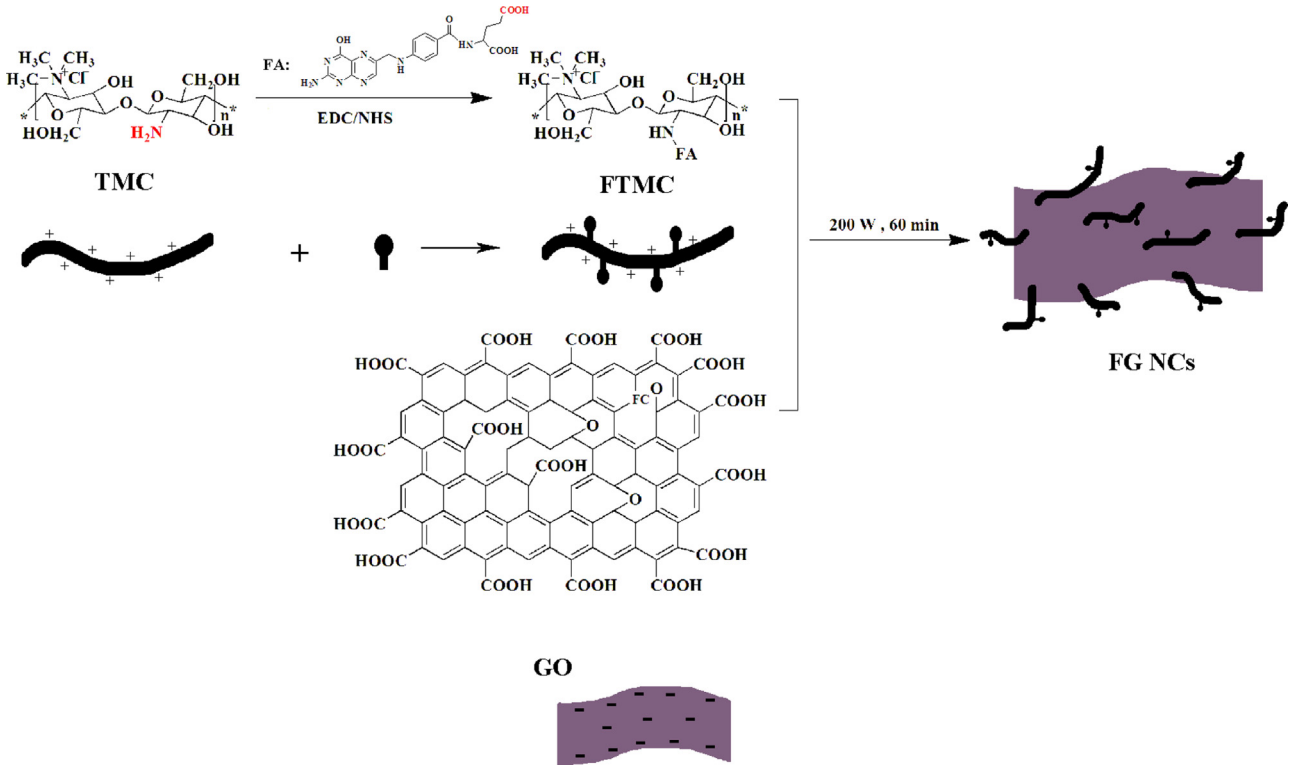

Scheme 1. Synthesis of FG NCs.

Cytotoxicity and cellular uptake: The cytotoxicity of FG NCs in A549 and Hela cells was determined by standard WST-8 assay. For cellular uptake, FG NCs were labeled with rhodamine B (RhB) (RhB-FG NCs, SI) and then incubated with A549 and Hela cells at its concentration of 10 μg/mL for 2 h, followed by observation with CLSM.

DOX loading and release: DOX solution (2 mg/mL) was added into FTMC solution (1 mg/mL) at the weight ratio of 2:1 and sonicated for 20 min. Then GO suspension was added at the FTMC/GO weight ratio of 1:1. The resultant mixture was sonicated for another 1 h to obtain DOX loaded FG NCs. Free DOX was removed via dialysis. in vitro DOX release was monitored in phosphate buffered saline (PBS, pH 7.4) and the whole media was replaced with fresh PBS at predetermined time intervals. The amount of released DOX was detected by fluorescence spectrometry (λ_{ex}=488 nm, λ_{em}=598 nm).

pDNA loading and release; GO suspension was mixed with pDNA at the weight ratio of 5:2. Different amount of FTMC was then added as listed in Table S1 and allowed for 30-min incubation. Eight kinds of pDNA loaded FG NCs (D-FG NCs), namely D-FG NCs1–8, were obtained and then analyzed by agarose gel electrophoresis (1% (w/v), 120 V, 50 min). The release of pDNA from D-FG NCs4 was conducted as described in "DOX loading and release" and the amount of released pDNA was examined by ethidium bromide intercalation (λ_{ex}=518 nm, λ_{em}=605 nm) [10].

3. Results and discussion

Preparation and characterization of FG NCs: Compared with Raman spectrum of graphite (Fig. 1A), both G and D bands were broadened in GO while the 2 D band decreased. Moreover, the ratio of G and D band intensity (G/D) of GO decreased to 0.76. These changes implied the reduction in size of in-plane sp^2 domains, possibly due to the extensive oxidation and ultrasonic exfoliation [11]. G band, D band, and G/D of FG NCs were similar to those of GO, indicating a negligible influence of FTMC on sp^2 domains of GO. Compared with FTIR spectrum of graphite (Fig. 1B), peaks at 1708, 1185, and 1048 cm^{-1} of GO indicated the existence of C=O and C–O bond, which was ascribed to the oxidation [4]. In FTIR spectrum of FTMC, peaks at 1607 and 1506 cm^{-1} proved the linkage of FA with TMC, consistent with the results of ^{1}H NMR spectra (Fig. S1). Compared with FTMC, the strong peak at 1746 cm^{-1} in FG NCs was assigned to C=O stretching, implying the complexation of FTMC with GO. AFM image of GO (Fig. 1C) visualized a height of 0.838 nm, suggesting a single-layer graphene sheet. The height of FG NCs increased to 3.038 nm (Fig. 1D), probably due to the attachment of FTMC on GO sheet [3]. In addition, some protuberances could be observed on the surface of FG NCs, indicating a considerable amount of FTMC immobilized on GO sheet [12]. As shown in Table S1, GO exhibited the particle size of 87.7 nm and the zeta potential of −32.9 mV while the size of FG NCs increased to 112.0 nm and the zeta potential reversed to 30.9 mV, also suggesting the complexation of FTMC with GO.

Cell viability and cellular uptake: The positively charged FG NCs might facilitate their cellular uptake via electrostatic interaction with negatively charged cell membrane. As shown in Fig. 1E and F, FG NCs did not undermine the cell viability even at the concentration of 80 μg/mL after incubation for 48 h, confirming their biocompatibility. As illustrated in Fig. 1G, RhB-FG NCs could be internalized by cells and mainly distributed in the cytoplasm. Much stronger fluorescence of RhB-FG NCs was seen in Hela cells than in A549 cells, suggesting that FG NCs might be internalized through FA-receptor mediated endocytosis [13,14]. Such targeting efficacy of FG NCs might decrease the side effect of loaded anticancer drugs.

DOX loading and release: As shown in Fig. 2A, the mixing of DOX and GO triggered the aggregation of GO. Following the addition of FTMC, GO precipitation was redissolved, possibly due to the surface hydrophilic modification of FTMC. Raman spectrum of DOX loaded FG NCs (Fig. S2) showed the G/D of 0.90, indicating an