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Quantum dots encapsulated glycopolymer vesicles: Synthesis, lectin recognition and photoluminescent properties

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ABSTRACT

Biomimetic star-shaped glycopolymer poly(ε-caprolactone)-*b*-poly(2-aminoethyl methacrylate-*b*-poly(gluconamidoethylmethacrylate) (SPCL–PAMA–PGAMA) was synthesized by the combination of ring opening polymerization (ROP) and reversible addition-fragmentation chain transfer (RAFT) polymerization. The glycopolymer self-assembled into vesicles with low critical aggregation concentration (CAC) (0.0075 mg/mL). Then, the carboxylic capped CdTe QDs were encapsulated within the glycopolymer vesicles. The QDs encapsulated glycopolymer vesicles (Gly@QDs vesicles) could specifically bind Concanavalin A (Con A) without changing the photoluminescent properties of the Gly@QDs vesicles. Cell viability studies revealed that the cytotoxicity of the Gly@QDs vesicles was remarkably improved as compared to that of the original QDs. The Gly@QDs vesicles were internalized by Hep G2 cells and then emitted green fluorescence in the cells. Consequently, these Gly@QDs vesicles provided a multifunctional platform for targeted delivery and imaging.

1. Introduction

In recent years, amphiphilic block copolymers self-assembled vesicles (also referred to as polymersomes) have attracted rapidly growing interest due to their potential applicability in life science and biomedicine [1–4]. The vesicles can encapsulate various agents within the vesicles core or in the hydrophobic bilayer due to the hollow and spherical morphology [5–8]. However, in spite of the successful application of polymeric vesicles for delivery both *in vitro* and *in vivo*, one of the intractable problems is the lack of active targeting to specific tissues or organs, which leads to the decreased therapeutic efficacy. Moreover, it is important to identify the location of the vesicles after their uptake by cells, which can be achieved by using the imaging techniques, thereby providing precise evaluation of therapeutic efficacy. In this case, multifunctional vesicles for targeted delivery and imaging become important.

Targeted delivery can be achieved by using a variety of targeting ligands to functionalize the polymeric vesicles. The targeting ligands include small organic molecules, carbohydrates, aptamers, peptides and antibodies [9–11]. Among these targeting ligands, carbohydrates show highly specific interactions with endogenous lectin, a carbohydrate binding glycoprotein which is expressed on mammalian cell surfaces [12]. Synthetic glycopolymers containing carbohydrate moieties exhibit similar functionality to natural oligosaccharides [13–15]. However, only few reports are available on glycopolymer vesicles [16–18].

The fluorescence-based imaging technique has aroused significant interest in the area of biological applications [19,20]. QDs have received enormous applications in biomedical fields [21,22], owing to their high luminescence, single excitation narrow emission and low photo bleaching properties [23]. However, the cytotoxicity of QDs is still the major obstacle for their applications nowadays. Carbohydrates have been reported to improve the biocompatibility and bioactivity of QDs by covalent conjugation [24]. Thus, the glycopolymer vesicles are expected to reduce the toxicity of the QDs. Moreover, the embedded QDs may turn the glycopolymer vesicles into a potential fluorescent probe for identifying the location of the glycopolymer vesicles after their uptake by cells.

Herein, biomimetic glycopolymer vesicles embedded with QDs were designed. On the one hand, the embedded QDs turned the

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glycopolymer vesicles into a potential fluorescent probe; on the other hand, the glycopolymer vesicles were expected to improve the biocompatibility of the QDs. To archive this goal, the biomimetic star-shaped SPCL–PAMA–PGAMA glycopolymer was synthesized by the combination of ROP and RAFT polymerization. The glycopolymer self-assembled into vesicles in phosphate buffered saline (PBS) and were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Then, the glycopolymer vesicles were decorated with QDs. The photoluminescent properties of the Gly@QDs vesicles were characterized by the luminescence spectrometer. The recognition properties of the Gly@QDs vesicles with Con A were investigated at room temperature by measuring the turbidity. Cell internalization and cytotoxicity of the Gly@QDs vesicles were studied by fluorescence microscopy and MTT assay, respectively.

2. Experimental

2.1. Materials

Stannous octoate ($SnOct_2$), 4-(dimethylamino) pyridine (DMAP), *N*,*N*-dicyclohexylcarboiimide (DCC), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CTP), D-gluconolactone, ethanolamine hydrochloride, dimethylsulfoxide (DMSO), mercaptoacetic acid (TGA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), Dubelcco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) were all purchased from Sigma-Aldrich and used as received. Methacryloyl chloride, cadmium chloride, sodium borohydride and tellurium powder were all purchased from Aladdin and used as received. ε-Caprolactone (Sigma-Aldrich), toluene (Beijing Chemical Reagent Co., Ltd.) and dichloromethane (DCM) (Beijing Chemical Reagent Co., Ltd.) were dried over CaH_2 and distilled prior to use. Pentaerythritol from Sinopharm chemical reagent company was recrystallized from water. 2-Aminoethyl methacrylate hydrochloride (AMA·HCl) was synthesized from ethanolamine hydrochloride and methacryloyl chloride according to the literature procedure (72.0% yield) [25]. D-Gluconamidoethyl methacrylate glycomonomer (GAMA) was synthesized from D-gluconolactone and 2-aminoethyl methacrylate hydrochloride according to the literature procedure (70.0% yield) [25]. TGA capped CdTe QDs (1 μM in water, emission maxima wavelength and full-width at half-maximum (FWHM) are 554 nm and 75 nm at 360 nm of excitation, respectively) were prepared according to the literature procedure [26].

2.2. Synthesis of biomimetic star-shaped SPCL–PAMA–PGAMA glycopolymer by the combination of ROP and RAFT polymerization

The biomimetic star-shaped SPCL–PAMA–PGAMA glycopolymer was prepared as shown in Scheme 1. According to the literature [27], four arms star-shaped PCL with four hydroxyl end groups (SPCL-OH) was synthesized by ROP of ε-caprolactone monomer using pentaerythritol as an initiator and stannous octoate as a catalyst at 110 °C for 24 h. After cooling to room temperature, the crude product was dissolved in DCM and precipitated into methanol to give white solid. The solid was dried in vacuum to constant weight in a yield of 95.0%. ^{1}H NMR (400 MHz, $CDCl_3$, δ) of $SPCL_{27}$-OH sample: 1.38 (m, 54*H*, —$COCH_2CH_2C\mathbf{H_2}CH_2CH_2O$—), 1.63 (m, 108*H*, —$COCH_2C\mathbf{H_2}CH_2C\mathbf{H_2}CH_2O$—), 2.28 (m, 54*H*, —$COC\mathbf{H_2}CH_2$ $CH_2CH_2CH_2O$—), 3.65 (t, 2*H*, —$C\mathbf{H_2}OH$), 4.05 (m, 54*H*, —$COCH_2CH_2CH_2CH_2C\mathbf{H_2}O$—).

Subsequently, the SPCL-OH was converted into macro-RAFT agent (SPCL-CTP) *via* esterification with CTP. $SPCL_{27}$-OH precursor (1.10 g, 0.1 mmol), CTP (279.38 mg, 1 mmol) and DMAP (12.22 mg, 0.1 mmol) were dissolved in DCM (5 mL) and cooled to 0 °C Then, DCC (247.60 mg, 1.2 mmol) was dissolved in DCM (1 mL) and added dropwise into the reaction mixture. The mixture was stirred in the dark at room temperature for 60 h. Dicyclohexylurea was removed by filtration, the filtrate was concentrated and precipitated into methanol under vigorous stirring. The pink solid was filtered and dried under reduced pressure (92.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ) of $SPCL_{27}$-CTP sample: 1.38 (m, 54*H*, —$COCH_2CH_2C\mathbf{H_2}CH_2CH_2O$—), 1.63 (m, 108*H*, —$COCH_2C\mathbf{H_2}CH_2C\mathbf{H_2}CH_2O$—), 1.86 (s, 3*H*, —$C(CN)(C\mathbf{H_3})$—), 2.28 (m, 54*H*, —$COC\mathbf{H_2}CH_2CH_2CH_2CH_2O$—), 2.31–2.64 (t, 4*H*, —$C\mathbf{H_2}C\mathbf{H_2}COO$—), 4.05 (m, 54*H*, —$COCH_2CH_2CH_2CH_2C\mathbf{H_2}O$—), 7.3–7.8 (t, 5*H*, Ar**H**).

Finally, the SPCL–PAMA–PGAMA glycopolymer was synthesized by the RAFT polymerization of AMA·HCl and GAMA using SPCL-CTP as the macro-RAFT agent in one pot. $SPCL_{27}$-CTP (216.00 mg, 0.02 mmol), AMA·HCl (135.00 mg, 0.8 mmol) and AIBN (2.624 mg, 0.016 mmol) were dissolved in DMSO (1 mL). The solution was degassed by three freeze-evacuate-thaw cycles and polymerized at 60 °C for 5 h. Then a degassed solution of GAMA (370.00 mg, 1.2 mmol) in DMSO (1 mL) was transferred into the stirred reaction mixture directly, without adding further AIBN initiator. The polymerization was typically terminated after 3 h by exposure to air. The pink crude product was precipitated into 2-propanol, washed three times with warm methanol, and then dried under reduced pressure. The yield was 69.4%. ^{1}H NMR (400 MHz, DMSO-d_6, δ) of $SPCL_{27}$–$PAMA_{10}$–$PGAMA_{11}$ sample: 0.6–1.0 (m, 63*H*, —$C(C\mathbf{H_3})$ —), 1.6–2.0 (m, 42*H*, —$C(C\mathbf{H_2})$—), 1.38 (m, 54*H*, —$COCH_2CH_2C\mathbf{H_2}CH_2CH_2O$—), 1. 63 (m, 108*H*, —$COCH_2C\mathbf{H_2}CH_2C\mathbf{H_2}CH_2O$—), 1.86 (s, 3*H*, —$C(CN)(C\mathbf{H_3})$—), 2.28 (m, 54*H*, —$COC\mathbf{H_2}CH_2CH_2CH_2CH_2O$—), 2.31–2.64 (t, 4*H*, —$C\mathbf{H_2}C\mathbf{H_2}COO$—), 4.05 (m, 54*H*, —$COCH_2CH_2CH_2CH_2C\mathbf{H_2}O$—), 3.00–3.75 and 4.02–4.63 (glucose residue).

2.3. Formation of SPCL–PAMA–PGAMA glycopolymer vesicles

Typically, $SPCL_{27}$–$PAMA_{10}$–$PGAMA_{11}$ (10 mg) was dissolved in DMSO (1 mL). Then PBS (pH = 7.4) was added dropwise at a rate of 1 mL/min under stirring until the appearance of a blue tint. Then, the solution was dialyzed against PBS for 48 h (MWCO = 12000). The morphology and the size of the formed glycopolymer vesicles were characterized by TEM and DLS.

The critical aggregation concentration (CAC) was determined by using pyrene as a fluorescence probe. Steady state fluorescence spectra were obtained by a Shimadzu RF5301 luminescence spectrometer.

2.4. Preparation of Gly@QDs vesicles and their lectin recognition properties

QDs solution (2 mL) was added to PBS solution (pH = 7.4, 1 mL) of EDC (2.6 mg) and NHS (1.5 mg). The reaction mixture was stirred for 30 min at room temperature. Then the $SPCL_{27}$–$PAMA_{10}$–$PGAMA_{11}$ glycopolymer solution (10.0 mg in 10 mL of PBS) was added to the above PBS solution [24]. After stirring the reaction mixture overnight at room temperature, the mixture was purified by centrifuge for 30 min at 6000 rpm twice and redispersed in PBS at a concentration of 1 mg/mL.

The recognition properties of the Gly@QDs vesicles with Con A were investigated at room temperature and measured by UV–vis spectrophotometer at 360 nm to determine the turbidity [27].

2.5. Cellular uptake

Fluorescence microscopy was performed to determine the uptake of Gly@QDs vesicles by Hep G2 cells. The cells were seeded