Biomaterials 31 (2010) 3087–3094

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

ZnO QD@PMAA-co-PDMAEMA nonviral vector for plasmid DNA delivery and bioimaging

Peng Zhang, Wenguang Liu*

School of Materials Science and Engineering, Tianjin Key Laboratory of Composite and Functional Materials, Tianjin University, Tianjin 30072, PR China

ARTICLE INFO

Article history:
Received 6 November 2009
Accepted 3 January 2010
Available online 21 January 2010

Keywords:
DNA condensation
Gene transfection
Plasmid DNA
Quantum dots
ZnO

ABSTRACT

Low cytotoxic ZnO quantum dot-based nonviral vectors with the dual functions of delivering plasmid DNA and labeling cells were fabricated by capping the surface of ZnO QD with poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), which was synthesized in situ by radical polymerization. The polycation-modified ZnO QDs were capable of condensing plasmid DNA into nanocomplexes (Qdotplexes) loaded with ZnO QDs emitting strong yellow luminescence under UV light. The Qdotplexes could mediate an efficient transfer of plasmid DNA into COS-7 cells with much lower cytotoxicity, meanwhile allowing real-time imaging of gene transfection.

1. Introduction

Semiconductor quantum dots (QDs) have attracted tremendous interest in biolabeling and bioimaging applications due to their considerable advantages over conventional organic dyes, such as high quantum yield, size-tunable emission, photostability and improved signal brightness [1–3]. Recently surface modification on QDs has led to the development of new generation of probes with integrated functionalities of labeling and drug/gene delivery [4–6]. Gao and his coworkers [7] reported a novel CdSe QD-amphipol nanocomplex which was shown to successfully delivery siRNA into cancer cells with significantly reduced cytotoxicity, and offer real-time imaging of siRNA delivery in live cells. 2-Vinylpyridine functionalized silicon QDs [8] and QD-peptide conjugates [9] were also reportedly developed for siRNA transfection and therapeutic imaging. Cysteamine capped-CdTe QD vectors were constructed lately by Li and his coworkers [10]. These water soluble QDs were able to complex with DNA via electrostatic interaction, and the QD–DNA complexes formed were found to be disrupted by intracellular glutathione, allowing controllable release of DNA and gene expression in HEK293 cells in visible mode.

Apart from imaging and gene transport functionalities, a QD-based nonviral vector should not affect the function of tagged plasmid DNA. Srinivasan et al proposed to conjugate plasmid DNA to phospholipid/polyethylene oxide-encapsulated CdSe/ZnS QDs by PNA–SPDP linker. The QD-tagged DNA could transfect CHO-K1 cells with high efficiency and that the cellular internalization of plasmid DNA could be tracked through time [11].

However, CdSe and CdSe/ZnS QD-based vectors pose a potential risk to biological systems in spite of surface shielding or coating because of the unavoidable leakage of Cd ions originated from shell defect and degradation of modifiers [12].

To address the toxicity problem of CdSe QDs, the innate less toxic nanodiamond was modified with 800 Da polyethyleneimine (PEI800). The low cytotoxic PEI800-modified nanodiamonds could mediate the transfection of Luciferase plasmid (pLuc) and GFP plasmid in Hela cells though the efficiency was 2–3 fold lower than that of PEI25K [13].

ZnO QD is noncytotoxic and its blue fluorescence, which is unfit for bioimaging, has been well documented [14,15]. The latest work on synthesis of ZnO QD emitting yellow or green luminescence by sol–gel method may extend ZnO QD to biological application [16]. Nevertheless, since these ZnO@polymer core–shell nanoparticles were capped with polymethacrylate-co-poly(ethylene glycol) methyl ether methacrylate (PMMA–PEGMEMA), they cannot be used as nonviral vectors for gene delivery due to the inability of the external neutral PEGMEMA and internal negative polymethacrylate to condense DNA.

To design low cytotoxic ZnO QD-based nonviral vectors with the capabilities to transfer plasmid DNA and label live cells, we have synthesized poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA)-co-PMAA capped ZnO QDs by similar sol–gel method

* Corresponding author. Tel./fax: 86 22 27402487.
E-mail address: wgliu@tju.edu.cn (W. Liu).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.01.007

reported. It is expected that the outer cationic PDMAEMA will be able to mediate the transfection of plasmid DNA, and the entrapped ZnO QD emitting discernible luminescence from the background of cells can be used as a real-time imaging probe.

2. Materials and methods

2.1. Materials and chemicals

Zinc methacrylate ($Zn(MAA)_2$, 99%) and branched polyethylenimine (PEI, 25 kDa) were purchased from Sigma–Aldrich. 2-(dimethylamino)ethyl methacrylate (DMAEMA, 97%) and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) were supplied by Alfa Aesar. Rhodamine 6G (99%) was obtained from Acros Organics. Fluorescein (Standard Fluka) was purchased from Fluka. Plasmid pGL3-control with SV40 promoter and enhancer sequences encoding luciferase (5262 bp) was obtained from Promega. All other reagents were of analytical grades and used without further purification.

2.2. Instrumentation and characterization

The FTIR spectra of the samples were measured on a Perkin–Elmer spectrum 100 spectrometer (Perkin–Elmer, America) by ATR method. The absorption and fluorescence spectra were recorded on a TU-1810 UV–Vis Spectrophotometer (Pgeneral, China) and FLS920 fluorometer (Edinburgh Instruments, Britain), respectively. Thermogravimetry (TG) curves for the samples were obtained by a Perkin–Elmer Pyris TG/DTA Thermal Analyzer (Perkin–Elmer, America), in air atmosphere. The morphology and microstructure of the QD and QD/DNA complex were examined by high-resolution transmission electron microscopy (HRTEM) on a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. The samples for HRTEM were made by dropping an aqueous solution onto a 300-mesh copper grid coated with a lacy carbon film. The vector/pDNA complexes were observed under JEM-100CX II TEM (Jeol, Japan) after negatively stained with 1.5 wt% phosphotungstic acid (pH 6.7).

2.3. Synthesis of PDMAEMA-capped ZnO QDs

PDMAEMA–ZnO QDs were synthesized in terms of the reported method [16]. First, a certain amount of zinc methacrylate ($Zn(MAA)_2$) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) were dissolved in 10 ml anhydrous ethanol under magnetic stirring. When the solution was heated to 80 °C, a small amount of 2, 2′-azobisisobutyronitrile (AIBN) was added to the reaction system. After refluxing for 2 min, the solution turned into milky white. Then 10 ml $LiOH{\cdot}H_2O$ ethanol solution was added and continuously refluxed for another 1 h. After cooling to room temperature, the solution was centrifuged under 5000 rpm for 15 min. The obtained white precipitate was redissolved in water and dialyzed (MWCO of 3500) against distilled and deionized water for 3 days. Finally the aqueous solution was filtrated and lyophilized to collect dry QDs. In this experiment, three PDMAEMA–ZnO QDs were prepared by varying $Zn(MAA)_2$ feed ratios as shown in Table 1. For comparison, PDMAEMA homopolymer was synthesized as well. The molecular weights of the copolymers on the shell layers of QDs were assessed by gel permeation chromatography (GPC Waters 510/M32) using tetrahydrofuran (THF) as a mobile phase at a flow rate of 1.0 ml/min at 35 °C after decomposing ZnO by a small amount of HCl. A series of monodisperse polystyrene standards (Polymer Laboratories Inc., MA) were used for calibration. $\overline{Mn}$ of PDMAEMA homopolymer was 5769; $\overline{M}_n$ values of PMAA-co-PDMAEMA copolymers on QD-1, QD-2 and QD-3 were 4761, 5104 and 4496, respectively.

2.4. Measurement of quantum yield (QY)

The quantum yield of the QDs was determined by a comparative method [17]. Two standard solutions, Fluorescein in 0.01 M NaOH and Rhodamine 6G in anhydrous ethanol were made as previously reported [16,18]. The test solutions were the QDs dissolved in water at different concentrations. All the absorbance values at the excitation wavelength of the solutions were measured by UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the solutions were recorded by FLS920 fluorometer at an excitation wavelength of 350 nm. The integrated fluorescence intensity is the area under the PL curve in the wavelength range from 400 to 800 nm. Then a graph is plotted using the integrated fluorescence intensity against the absorbance and a trend line was added for each curve with intercept at zero. The equation (1) was used to calculate the quantum yield [17].

$$\phi_X = \phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right) \quad (1)$$

Where the subscripts ST and *X* denote standard and test, respectively, ϕ is the fluorescence quantum yield, Grad is the gradient from the plot of integrated fluorescence intensity vs absorbance and η the refractive index of the solvent.

Table 1
Synthesis of different PDMAEMA-modified ZnO QDs.

Samples	DMAEMA (μl)	$Zn(MAA)_2$ (mg)	AIBN (mg)	$LiOH{\cdot}H_2O$ (mg)
PDMAEMA	840	0	8.2	21
QD-1	840	59	8.2	10.5
QD-2	840	118	8.2	21
QD-3	840	236	8.2	42

2.5. Preparation of vector/pDNA complexes

PDMAEMA–ZnO QD, PDMAEMA and DNA were separately dissolved in ultrapure water and the solutions were filtered with 0.22 μm sterile filters. Vector/pDNA complexes at varied ratios were then formulated by adding vector of desired concentrations to an equal volume of a defined pDNA solution, pipetting up and down to make the mixture homogeneously mixed. The mixtures were incubated at room temperature for 30 min to allow complex formation. In this study, the complexing ratio was expressed as the weight ratio of vector/pDNA.

2.6. Agarose gel electrophoresis

The vector/pDNA complexes at different weight ratios were prepared freshly as described above. 8 μl complex solution was mixed with a loading buffer, and loaded into 1% agarose gel containing ethidium bromide (0.5 μg/ml). The electrophoresis experiment was performed for 40 min in 1× TAE buffer at 100 V, and the image was captured through BioImaging Systems (UVP).

2.7. Cell culture and cytotoxicity assay

COS-7 cells (African green monkey kidney cells) were obtained from Peking Union Medical College (Beijing, China). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) with high glucose, containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2 humidified atmosphere.

The cytotoxicity of vectors was assessed through MTT assay. First, the cells were seeded in a 96-well plate at 2×10^4 cells/well and incubated overnight. Then the culture medium was removed and the vectors at increasing concentration from 0 to 800 μg/ml were added to each well. After 24 h the medium was replaced with 200 μl fresh medium containing 20 μl MTT (5 mg in PBS) and incubated for another 4 h. Finally all medium was removed and 150 μl/well DMSO was added, followed by shaking for 15 min. The absorbance of each well was measured at 570 nm on a ∑960 plate-reader (Metertech) with pure DMSO as a blank. Non-treated cell (in DMEM) was used as a control and the relative cell viability (mean% ± SD, $n = 3$) was expressed as $Abs_{sample}/Abs_{control} \times 100\%$.

2.8. In vitro transfection and luciferase assay

COS-7 cells were seeded at a density of 5×10^4 cells/well in 24-well plates and incubated for 24 h at 37 °C in 5% CO_2 humidified atmosphere. Prior to transfection the cell culture medium in each well was removed and replaced with 450 μl serum-free DMEM. Vector/pDNA complexes (50 μl, containing 2 μg DNA) at various weight ratios prepared as described above were then added to each well. After incubating at 37 °C in 5% CO_2 for 4 h, the medium containing complex solution was then replaced with 500 μl of fresh complete medium and the cells were incubated for an additional 48 h. Transfection tests were performed in triplicate. Following incubation, the culture medium was removed and the cells were washed with PBS twice. The cells in each well were treated for 15 min with 150 μl of reporter lysis buffer (RLB, Promega) followed by freeze–thaw cycles to ensure complete lysis. The lysate was centrifuged for 4 min at 13,000 rpm and the supernatant was collected for luminescence measurements. The luminescence of each sample was measured by 1420 Multilabel counter (Wallac, USA) using Bright-Glo™ luciferase assay system (Promega, USA) according to the manufacturer's protocol. The results were expressed as relative light units (RLU) per milligram of cell protein, and the protein concentration of each well was measured by a BCA protein assay (Pierce, Rockford, IL, USA).

2.9. Confocal microscopy

A laser scanning confocal microscope (Olympus FluoView FV1000) was used for live cell imaging. COS-7 cells were seeded onto the glass-bottomed culture dishes (35 mm, MetTek) at a density of 2×10^5 cells/dish and incubated at 37 °C in 5% CO_2 overnight. After that, the cells were transfected as described above (QD-2/pDNA complex containing 10 μg DNA with a weight ratio of 30:1). After a further 10 min and 3 h incubation, the cells were washed by PBS 3 times to remove the QD/pDNA complexes adsorbed on the outer surface of cell membrane. Finally 2 ml DMEM was added and the cells were imaged by the laser scanning confocal microscope under excitation wavelength of 350 nm.