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The effect of quantum dot size and poly(ethylenimine) coating on the efficiency of gene delivery into human mesenchymal stem cells

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ABSTRACT

Quantum dot (QDs) have been employed as bioimaging agents and delivery vehicles for gene therapeutics in several types of cells. In this study, we fabricated multiple QD bundled nanoparticles (NPs) to investigate the effect of QD size and poly(ethylenimine) (PEI) coating on the efficiency of gene delivery into human mesenchymal stem cells (hMSCs). Several types of QDs, which exhibit different ranges of particle size and fluorescence when employed, were coated with PEI to alter their negative charges and to enable them to be bundled into larger particles. Using specific wavelengths of QDs for bioimaging, gene-complexed QD bundled NPs were easily detected in the hMSCs using several different methods such as fluorescence-activated cell sorter, confocal laser scanning microscopy, and *in vivo* optical imaging. These PEI-coated, bundled QD NPs exhibited significantly higher gene transfection efficacy than single-type QDs. Particularly, the largest QD bundled NPs examined, QD655, had a much higher uptake capability and greater gene expression ability than the other QD NPs (QD525, QD565, and QD605). We believe that our findings help to enrich knowledge of design considerations that will aid in the engineering of QD NPs for stem cell application in the future.

1. Introduction

Rapid advances in nanotechnology have led to the development of nanostructured materials that have been used to improve both therapies and bioimaging of wound sites [1]. The development of therapies employing these materials represents a major opportunity for basic and applied bio-research. The nanostructural properties of these materials, which can be altered at the fabrication stage, have a strong influence on the microenvironment of the biological system in which they are employed, including the properties of the cells or tissues with which they interact. Specially, internalization of these particles via endocytosis by the cell membrane is highly influenced by particle size [2,3]. Although these particles enter the cell by endocytosis, they can be released from the cell by exocytosis. Therefore, it is necessary to modify the surfaces of nano-structures to enable a cascade of events to occur, which leads to the entry of the particles located and maintained in cytosols without exocytosis for bioimaging purpose.

Among the several types of nanostructures, some well-designed nanoparticles have primarily been tried for biomedical application due to their excellent physico-chemical properties such as specific optical and electronic properties [4,5]. This finding has led to the evaluation of inorganic nanostructured materials for use in drug delivery systems and bioimaging [6,7]. Semiconductor nanoparticles like quantum dots (QDs), which can serve as photoluminescent markers, can be applied for bioimaging [8–10]. Therefore, QDs have been used as potential markers for intracellular single-molecule imaging due to their superior detection abilities and their stability in maintaining specific colors in images [11,12]. However, although QDs are excellent molecular probes, the application of QDs to delivery vehicles for several therapeutic biomolecules (*e.g.,* protein, peptide, gene) is hampered by key limitations relating to surface modification issues [13].

To solve this problem, several types of trials have been performed to enable easy delivery of QDs into cells, including the use of QDs coupled with and conjugated to peptides [14], polymers [15], and nanogels [16]. They employ single QDs for encapsulation with the goal of obtaining a high fluorescence quantum yield for more sensitive and brighter detection in biological environment [17]. Recently, nanoprobe containing multiple QDs have also been developed for better physicochemical characteristics [18]. However,

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few studies have researched how the physicochemical properties (*e.g.*, size, shape and surface chemistry) of QDs can be engineered for successful gene delivery as well as for cell tracking in stem cells. This design bottleneck will impede the clinical translation of these nanotechnologies in bio-application [19]. Previously, we found that polyethylenimine (PEI) is an effective transfection carrier for gene delivery [20,21]. PEI has been used to coat and deliver poly(L-lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) into stem cells (SCs), enabling the endosomal escape of the particles and the release of the particles into the cytoplasm, followed by their reentry into the nuclei of SCs [22]. Inspired by our previous research, here we explore the use of PEI to organize QDs into larger bundled structure to apply as multifunctional imaging platform for translational research in stem cells.

The aim of this study was to evaluate the effect of size of QD and PEI coating on the efficiency of gene delivery into human mesenchymal stem cells (hMSCs) using multiple QD bundled NPs as multifunctional imaging platform for both gene delivery and cell tracking. In the usage of QDs in biomedical fields, with single form or aggregates forms, there are a few trials for stem cell researches [13]. When the QDs were applied for stem cells, they have been only used as a tracing probe when the cells were transplanted into animal model. Thus there is no research both macromolecular drug delivery and tracing into stem cells yet. In this study, several types of multiple QD bundled NPs, including QD525 (5 nm), QD565 (10 nm), QD605 (15 nm), and QD655 (20 nm), were fabricated via PEI coating using a simple coating method employing electrostatic attraction. The size and morphology of multiple QD bundled NPs were characterized by dynamic light scattering (DLS), field emission-scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM) at different ratio of PEI and QDs. The cellular internalization of multiple QD bundled NPs into hMSCs was evaluated by fluorescence activated cell sorter (FACS) and TEM. The transfection efficiency of multiple QD bundled NPs was investigated by Western blot, FACS, and confocal laser scanning microscopy (CLSM) analysis. Finally, to study the feasibility of multiple QD bundled NPs for gene delivery and cell tracking *in vivo* condition, multiple QD bundled NPs-transfected hMSCs were transplanted into Balb/c nude mice respectively and then detected using *in vivo* image station (IVIS).

2. Materials and methods

2.1. Materials

Linear polyethylenimine (PEI, 25 kDa) was purchased from Polyscience Inc. (Touhy Avenue, Niles, USA). Agarose, Tizma® base, glycine, potassium chloride, sodium chloride, Tween®20, TEMED (N,N,N′,N′-Tetramethylethylenediamine), 2-mercaptoethanol, ammonium persulfate, monoclonal anti-β-actin antibody, ethidium bromide (EtBr), 4′,6′-diamidine-2′-phenylindole dihydrochloride (DAPI), dimethyl sulfoxide (DMSO), and chloroform were purchased from the Sigma Chemical Company (St. Louis, MO). Both 30% (29:1) acrylamide/bisacrylamide stock solution and RIPA buffer were purchased from Bio-Rad Laboratories, Inc. (Royall St. Canton, USA). Qdot 525 ITK™ carboxyl quantum dots, Qdot 565 ITK™ carboxyl quantum dots, Qdot 605 ITK™ carboxyl quantum dots, Qdot 655 ITK™ carboxyl quantum dots, Qdot 655 ITK™ amino (PEG) quantum dots, alpha-minimum essential medium (α-MEM), antibiotic-antimycotic, and 0.25% trypsin were purchased from Invitrogen Life Technologies Corp. (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific Inc. (Wyman St., Waltham, USA). Green fluorescent protein (GFP) antibody and red fluorescent protein (RFP) antibody were purchased from Clontech Laboratories, Inc. (Madison, Wisconsin, USA).

2.2. Isolation and culture of hMSCs

hMSCs were purchased from Cambrex Ltd. (Cambrex, East Rutherford, NJ, USA). Adipose-derived mesenchymal stem cells (ADMSC) were kindly provided by Prof. Seung Who Kim (Asan Medical Center). Two types of MSCs were cultured in α-minimum essential medium (α-MEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% FBS and 100 U/ml penicillin G, 100 mg/ml streptomycin, and 0.25 mg/ml amphotericin B at 37 °C in a 5% CO_2 atmosphere. The medium was changed every 3 days and the cells were harvested with trypsine-EDTA (Invitrogen). The hMSCs were cultured 3–4 days before the transfection experiment. The cells were seeded in a six-well tissue culture plate at a density of 2×10^5 cells/well in 2 ml of α-MEM complete medium and incubated overnight at 37 °C in a humidified atmosphere under 5% CO_2. The multiple QD bundled NPs/pDNA complexes were used to transfect hMSCs for 6 h at 37 °C. The cells were then washed with 2 ml of phosphate buffered saline (pH 7.4) to remove any uncomplexed multiple QD bundled NPs/pDNA complexes.

2.3. Preparation of multiple QD bundled NPs

Preparation of multiple QD bundled NPs was performed as per the manufacturer's protocols. PEI (5 mg) was dissolved in HEPES-buffered saline for 1 h at 60 °C. Then, the PEI solutions were filtered through a 0.2 μm syringe filter (Advantec, Japan). QDs (0–2 nmol/ml) and PEI (5 μg/ml) were mixed with deionized water (200 μl) to produce the multiple QD bundled NPs solution. The multiple QD bundled NPs solution was then added to the plasmid DNA solution at various QDs concentration (0–2 nmol/ml) and vortexed gently.

2.4. Characterization of multiple QD bundled NPs

For affinity analysis of the multiple QD bundled NPs and pDNA complex, 1 μl of gel loading dye was added to each multiple QD bundled NPs/pDNA complex solution, and 30 μl of the resulting polyplex solution was loaded onto an agarose gel (1.2%, w/v) containing 1 μg/ml of ethidium bromide. The average diameters of the multiple QD bundled NPs were measured by DLS (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). In brief, the QDs were suspended in deionized water at a concentration of 2 nmol/ml. The mean hydrodynamic diameter was determined via cumulative analysis. The ζ-potential (surface charge) of the polymers and polyplexes was determined at 25 °C using DLS (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). Samples were prepared in phosphate-buffered saline (PBS) and diluted 1:8 with deionized water to ensure that the measurements were performed under conditions of low ionic strength where the surface charge of the particles could be measured accurately. The final concentration of the QDs was 2 nmol/ml. All data represent 15 measurements from one sample. Electron micrographs of multiple QD bundled NPs, including QD NPs (QD525, QD575, QD605, and QD655) coated with various concentrations of PEI (0–5 μg/ml), were produced by placing a drop of the sample onto copper mesh coated with an amorphous carbon film, followed by drying with a vacuum desiccator. The mean diameter and morphology of the various types of multiple QD bundled NPs/pDNA complexes were characterized by FE-SEM (S-4700; Hitachi, Japan). The surface morphologies of the multiple QD bundled NPs were imaged by TEM (H-7600, Hitachi Co. Ltd., Japan). The specimens were prepared by dropping multiple QD bundled NPs/pDNA solution onto a copper grid, followed by air drying at 37 °C for 1 h.

2.5. Stability of multiple QD bundled NPs

To confirm the stability of the multiple QD bundled NPs under various pH conditions, the particles were incubated at pH 4, 7, and 9 for 1–7 days with distilled water and cell culture media. The mean diameter and morphology of the multiple QD bundled NPs/pDNA complexes at pH 4, 7, and 9 were characterized via FE-SEM and DLS (Zetasizer Nano ZS, Malvern Instruments Ltd., UK).

2.6. Cellular uptake detection by FACS analysis

For analysis of cellular uptake, the hMSCs were transfected with multiple QD bundled NPs/pDNA for 1–6 h. The hMSCs were detached with trypsine-EDTA and resuspended in PBS using an FACS Calibur equipped with a 405 nm, 488 nm, and 642 nm laser (Guava easyCyte™ Flow Cytometer, Millipore Corp., USA). The data shown represent the mean fluorescent signals from 5000 cells.

2.7. Preparation of expression vectors

The NLS-EGFP expression plasmid (pEGFP) was generated by ligating the EGFP open reading frame derived from pEGFP-N3 into pcDNA3.1/hyg (Invitrogen, Carlsbad, CA, USA) followed by the insertion of SV40 NLS into the N-terminus of green fluorescence protein (EGFP). All plasmid constructs were verified by DNA sequencing. And NLS-DsRed1 expression plasmid (pRed) was generated by ligating the pDsRed1-C1 (Clontech, Mountain View, CA, USA) followed by the insertion of an SV40 nuclear localization signal (NLS, PKKKRKV) into the N-terminus of red fluorescence protein (pDsRed).

2.8. TEM images of hMSCs

The uptake of multiple QD bundled NPs into hMSCs was examined by TEM. First, the hMSCs were transfected with multiple QD bundled NPs/pDNA for 12 h. Then, the cells were fixed with 2.5% glutaraldehyde in PBS for 1–4 h at 4 °C. Secondary fixation was performed for 1 h at room temperature in 1% osmium tetroxide in distilled water. The cells were then incubated in propylene oxide:resin (1:1 mixture) for 1–2 h; the resin was mixed in plastic disposable beakers. The samples were embedded in a mold and polymerized in an oven at 60 °C for 24 h, and sectioned into 5 μm-thick slices using an ultra-microtome (Leica Microsystems Ltd., Germany). The sections were visualized using TEM (H-7600, Hitachi Co. Ltd., Japan).