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Intracellular delivery and activation of the genetically encoded photosensitizer Killer Red by quantum dots encapsulated in polymeric micelles

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ABSTRACT

We have prepared polymeric micelle-encapsulating quantum dots (QDots) for delivering the optically activatable protein Killer Red (KR) as a plasmid to cancer cells. QDots absorb light at a lower wavelength and emit light at a higher wavelength in the cell cytoplasm, activating the expressed KR. Once activated, KR triggers the generation of reactive oxygen species (ROS). We prepared cadmium selenide (CdSe)/zinc sulphide (ZnS) QDots and evaluated their optical properties. Subsequently, we performed morphology studies, elemental analysis, thermogravimetric analysis (TGA), and measurements of particle size and surface charge of prepared QDots encapsulated in PHEA-g-PEG-bPEI (PPP-QDot). Cellular uptake of PPP-QDot and PPP-QDot/KR nanoparticles was confirmed using confocal microscopy, and the cellular toxicity and transfection efficiency associated with uptake of PPP-QDot/KR nanoparticles were analyzed. KR expression in normal cells and cancer cells was confirmed using confocal microscopy and Western blotting. Cellular morphologies before and after intracellular activation of KR were observed using phase contrast, fluorescence, and confocal microscopy. Cell fate after exposure to blue light-emitting diode lighting was determined using apoptosis staining and a cell proliferation assay, confirming a suppression in proliferation and a reduction in metabolic activity. We determined that ROS generation contributed to cellular damage after treatment with PPP-QDot/KR nanoparticles and blue light exposure.

1. Introduction

Polymeric micelles have various advantages over other carriers for biomedical applications [1]. The core of a polymeric micelle has a hydrophobic nature that is compatible with hydrophobic substances, such as hydrophobic drugs, superparamagnetic iron oxide nanoparticles, and quantum dots (QDots). The surface corona of a polymeric micelle is hydrophilic as a result of conjugating with commonly utilized polymers such as polyethylene glycol (PEG) [2]. The hydrophilic surface of a polymeric micelle naturally gives a surface-smoothing effect, reducing its interaction with serum proteins, enhancing bio-compatibility, and lengthening the blood circulation time when administered *in vivo* [3,4].

When the surface of a polymeric micelle is designed with cationic polymers such as polyethylenimine (PEI) along with PEG, the polymeric micelle will acquire a positive charge from the protonated amine groups of PEI [5,6]. PEI complexes well with negatively charged plasmid DNA, which can be delivered efficiently inside cells and discharged in the cytoplasm as a result of the proton sponge effect.

The use of QDots for cell tracking is an established practice [7]. QDots can absorb and emit light even when they are inside cells [8,9]. Compared to dyes, which can quickly degrade, QDots are more stable and persistent and avoid the quenching effect [10]. The delivery of a therapeutic gene that can be specifically activated by an external light source has many advantages for normal cells, because they are less likely to be disturbed when the external light absorbent polymer with QDot does not reach them.

Killer Red (KR) is a genetically encoded photosensitizer that can be selectively activated by green light. Its activation results in the generation of reactive oxygen species (ROS) that observably damage the cell and trigger cell death [11–13]. KR is capable of

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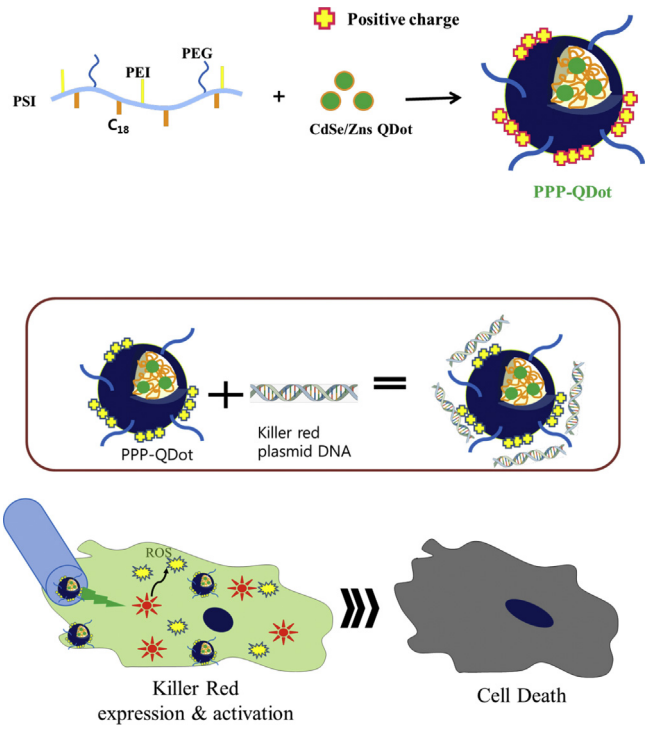

Scheme 1. Preparation of PPP-QDots and the mechanism of photoinduced cellular damage after delivery and expression of KR plasmid by PPP-QDots.

both singlet-oxygen and superoxide-ion generation upon irradiation with green light [14,15]. However, this process only affects cells that express KR proteins. If normal cells are prevented from uptaking the KR-encoding therapeutic plasmids, they will remain intact and healthy. Therefore, delivery of the therapeutic plasmid *via* polymeric micelles with multifunctional capacity can be an effective cancer treatment strategy [16].

Surface polymers can be conjugated with peptides, aptamers, or antibodies to enable specific targeting [17–20]. Imaging of polymeric micelles is performed *via* encapsulation of magnetic nanoparticles for magnetic resonance imaging (MRI) detection or QDots for optical detection [21,22]. Alternatively, hydrophobic drugs or contrast agents can be encapsulated in the core of the polymeric micelle [23]. The utility of the optically activatable gene is that it can be induced from outside the cell. Its multifunctionality should lead to more developments regarding this kind of cancer treatment.

The present study is novel because it employs a genetically encoded photosensitizer, KR, that is delivered with polymeric micelle-encapsulated QDots and that is activated intracellularly by QDots encapsulated in the same carrier. Previously, KR plasmid DNA without any carrier was delivered and activated directly with green light [15]. In this study, the KR plasmid was delivered with polymeric micelle-encapsulated QDots, and KR protein was activated locally with the same carrier. Specifically, PHEA-g-PEG-bPEI (PPP)-QDots were prepared to deliver the optically activatable KR plasmid to cancer cells. Upon plasmid delivery and protein expression, KR can be activated by blue light, which is absorbed by the CdSe/ZnS QDots encapsulated inside the polymeric micelle and emitted locally in the green wavelength within the cytoplasm of the cells. The activated KR protein triggers ROS generation, and as ROS increase intracellularly, they induce morphological changes that lead to cellular damage and apoptosis (Scheme 1).

The CdSe/ZnS-QDot was prepared and characterized and its optical properties were studied. We examined the morphology, performed elemental analysis and thermogravimetric (TGA) analysis, and measured the particle size and surface charge of the prepared PPP-QDots. Uptake of the PPP-QDot and PPP-QDot/plasmid nanoparticles was confirmed using confocal microscopy. The cellular toxicity and transfection efficiency following uptake of PPP-QDot/plasmid nanoparticles were analyzed. KR expression in normal cells and in cancer cells after PPP-QDot/KR nanoparticles treatment was confirmed using confocal microscopy and Western blotting. Cellular morphologies before and after blue light exposure were observed using phase contrast, fluorescence, and confocal microscopy. Cell fate after blue light exposure was tested using a fluorescent apoptosis marker and a proliferation assay. We confirmed that ROS generation was responsible for the cellular damage in MCF-7 breast adenocarcinoma cells treated with PPP-QDot/KR nanoparticles and irradiated with blue light.

2. Materials and methods

2.1. Materials

Stannous octate, trioctylphosphine oxide (TOPO), trioctylphosphine (TOP), cadmium oxide, cadmium chloride (technical grade), tellurium powder (−200 mesh), selenium powder, n-tetradecylphosphonic acid (TDPA), 1-octadecene (ODE), trioctylamine (TOA), dichloromethane (DCM), and hexadecylamine (HDA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethylcadmium ($CdMe_2$) and diethylzinc ($ZnEt_2$) were purchased from Fluka (Seelze, Germany).

2.2. Synthesis of CdSe/ZnS QDots

CdSe/ZnS nanocrystals were synthesized as follows. A mixture of CdO (0.026 g, 0.20 mmol) and lauric acid (0.260 g, 1.3 mmol) was heated to 180 °C to obtain a clear solution, then the mixture was cooled to room temperature. TOPO and hexadecylamine (3 g of each) were added to the flask, and the mixture was heated to 300 °C. The selenium precursor (0.316 g Se powder dissolved in 4 mL TOP) then was swiftly injected into a reaction flask. A mixture containing 100 μL $(TMS)_2S$ and 800 μL $Zn(Et)_2$ premixed in 2.0 mL TOP was injected into the solution at a rate of 0.1 mL/3 min at 160 °C. The mixture was reacted at 150 °C with stirring for 1 h. The solution then was cooled to room temperature, and the resulting CdSe/ZnS QDots were washed numerous times with chloroform and methanol.

2.3. Preparation and characterization of PPP-QDot polymeric micelles

We previously described the synthesis of poly(2-hydroxyethyl aspartamide) grafted PEG and bPEI (PPP) polymeric micelles in detail [24,25]. PPP was loaded with hydrophobic CdSe/ZnS QDots with a uniform morphology to prepare the PPP-QDot polymeric micelles. The PPP and QDots were dissolved in DMSO and dialyzed against water. The samples then were lyophilized and dissolved in water for further characterization. Transmission electron microscopy (TEM) was used to evaluate the morphology of the PPP-QDot micelles (JEM-2000 FXII, JEOL, Japan), elemental analysis of the micelles was conducted using an ELS-8000 (Otsuka Electronics, Japan) to measure the hydrodynamic size and zeta potential, and TGA analysis (Mettler-Toledo, SDT851, Columbus, OH, USA) was performed to determine the chemical composition.

2.4. Preparation of PPP-QDot/plasmid DNA nanoparticles

The plasmid DNA binding ability of the PPP-QDots was evaluated by electrophoresis through a 1.2% agarose gel containing ethidium bromide intercalating dye. The nitrogen-to-phosphate (N/P) molar