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Quantification of Quantum Dots in HUVECs by Confocal Laser Scanning Microscopy

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***Abstract*—Quantum dots (QDs) are emerged as a new class of fluorescent probes for many biological and biomedical applications. Comparing with conventional fluorescent probes, they have substantial advantages such as bright fluorescence, narrow emission, broad excitation band and high photostability. However, little is known about the toxicity of nanoscale particles to biological systems. In this study, the interaction between 3-Mercaptopropionic acid capped CdTe QDs and HUVECs was studied quantitatively in vitro. Fluorescent intensity of QDs in cells was measured by confocal fluorescence laser scanning microscopy. The results showed that the amount of QDs absorbed by cells is dependent on concentration and incubation time. Further, the viability of cells incubated with QDs was investigated using MTT assay. Dramatic dose-dependent decrease in cellular viability was observed.**

I. Introduction

WITH the great progress in nanotechnology, manufactured nanomaterials are being widely used in our daily life, such as nanoscale-air refreshing agents, sunscreens made with nanomaterials and nano-medicines, etc. Due to their unique size, nanoscale materials can filter through any gap larger than theirs. However, to date, little is known about the invasion of nanoparticles and the resulted biological toxicity. In previous studies, carbon particles of 35 nm in diameter can penetrate through brain blood barrier after inhalation[1, 2]. Gatti AM et al. observed micro- and nano-particles in colonic tissues. Their findings suggested that a possible link between the presence of such particles and the underlying pathology[3, 4]. Further, the uncoated fullerenes (C60), a hydrophobic nanomaterials, was reported to cause oxidative damage and depletion of glutathione in an aquatic species [5]. Thus, the safety issue of nanoscale materials is gaining more and more attentions. Since certain nanoscale particles are proved to be pathogenic to human beings, such as tiny dusts etc.[2, 6], it is essential to quantify the amount of manufactured nanomaterials that invaded into cells or human bodies[7]. 3D quantification of the uptake and distribution of nanoparticles in cells is essential to understand the cytotoxicity induced by nanoparticles.

Quantum dots (QDs), also called fluorescent semiconducter nanocrystals (diameter 2-10nm), are emerged as a new class of fluorescent probes for many biological and biomedical applications[8-11]. They have many substantial advantages over conventional organic fluorescent dyes or green fluorescent proteins (GFP) such as their bright fluorescence, narrow emission, broad excitation band, and high photostability[8, 9].

As one type of manufactured nanoparticles, the safety issue of QDs has been investigated. Although several groups have reported that QDs had no detectable toxicity to the labeled cells or the animals [11, 12], recent research on the potential toxicity of QDs revealed that cell viability decreased with increasing concentrations of QDs[13]. The cytotoxic effects of QDs was correlated with the particle surface[13, 14] and the release of Cd2+ of CdSe particles[15]. However, to date, there are no exiting studies on quantitative characterization of the interaction between cells and QDs.

In this study, 3-Mercaptopropionic acid (MPA) capped CdTe QDs and human umbilical vein endothelial cells (HUVECs) were used as a model to investigate the interaction between QDs and cells in vitro. Fluorescent QDs were quantified in cells by confocal laser scanning microscopy (CLSM). Further, the viability study of cellular response to QDs has also been performed using MTT assay.

II. Materials & Methodology

A. QDs Preparation

CdTe nanoclusters were prepared in aqueous solution using the reaction between Cd2+ and NaHTe solution in the presence of 3-Mercaptopropionic acid (MPA) as a stabilizer[16]. The typical molar ratio of Cd:Te:MPA was 4:1:9.6 in our experiments. For preparation of Cd precursor solutions, a solution of CdCl2 and MPA were mixed, and the pH of which was adjusted to 8 with 1 M NaOH. This solution was placed in a three-necked flask and was deaerated with N2 bubbling for 30 min. Under vigorous stirring, the prepared oxygen free NaHTe solution was injected. The resulting mixture solution was heated to 99-100℃ and refluxed for 12 hours to prepare the CdTe QDs whose emission peak position was at 590nm. The as-prepared nanocrystals were precipitated by adding 2-propanol to the solution. The precipitate was isolated by centrifugation and decantation. The wet precipitate was dried in vacuum.

UV-vis absorption and fluorescence spectra were measured with a Lambda 20 UV-visible spectrophotometer and a Varian Cary spectrometer (Perkin-Elmer, USA), respectively. All optical measurements were performed at

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room temperature under ambient conditions. Samples were prepared by diluting colloidal CdTe nanocrystal solutions with water.

B. Cell Culture

The human umbilical vein endothelial cells (HUVECs) were purchased from American Type Cell Culture (ATCC), and maintained in the medium200 proposed by ATCC (37°C, 5% CO2). For cellular uptake of QDs studies, cells were plated on circular glass coverslips (ϕ=16mm) placed in a 12-well culture plate (Corning, USA) at 1.0×104cells/ cm2. For cell viability studies, cell were plated on cell culture dishes (ϕ=30mm) at 1.0×104cells/ cm2.

C. QD Treatments

After incubation for 48h, the adherent cells were washed with PBS, then co-incubated with QDs in reduced-serum MEM (ATCC) to avoid interference of QDs with serum components for a final concentration of 0.005~0.01mg/ml of QDs, total volume of 500μl per well. After different incubation periods from 2h to 48h, cell monolayers were rinsed twice with PBS in order to remove any nonspecifically QDs adsorbed before the following experiment.

D. Confocal Imaging and Quantification of QDs Cellular Uptake

Confocal laser scanning microscope LSM510 Meta (Zeiss, Germany) was used, in this study, to obtain serial optical sections through whole cells that provide accurate information of fluorescence intensity. To image Qds distribution in the cells, argon laser (excitation 543nm) and emission filter 560 LP were used, and cells were observed using a 40×water immersion objective. Optical slicing was performed to collect 5~7 images throughout the cells (~1.5μm thick each slice) without fluorescence saturation. To quantify QDs uptake level of cells, the following method was employed. For each cell, the total fluorescent intensity of each slice $\sum_{j=1}^{k} I_i(j)$ was integrated. By averaging the total fluorescent intensity of all cells, the intensity $\overline{I_c}$ is correlated to the concentration of QDs inside cells.

$$\overline{I_c} = \frac{1}{n}\sum_{i=1}^{n}\sum_{j=1}^{k} I_i(j)$$

Where *n* denotes the selected cell number and *k* denotes optical slice number. Data were collected and analyzed by using Zeiss LSM v3.95 software.

E. Cell Viability Assay

The cell viability was studied using MTT assay. Each cell was plated into a 96-well culture plate (Corning, USA) at 1.0 × 104 cells/well (200μl/well). After 48h incubation, medium200 were removed and replaced by reduced-serum MEM (medium200 as control) containing QDs of different concentrations from 0.005-0.02 mg/ml. After 20h or 44h-treatment, 10μL stock MTT (5mg/ml) was added, and cells were then incubated for 4h at 37°C. Medium was removed, and cells were lysed with dimethylsulfoxide (Sigma). Absorbance was measured with a MODEL 680 microplate reader (Bio-Rad, USA) in a dual wavelength, with the measurement wavelength at 570 nm and the reference wavelength at 630 nm.

F. Statistical Analysis

Data were presented as mean ± S.E. The statistical significance was determined by Students' test ($P<0.05$).

III. Results & Conclusions

MPA-capped CdTe QDs were synthesized in water. Fig.1 shows their UV-vis absorption and photoluminescence spectra when dissolved in PBS (PH7.2), and the respective peak spectra were 543 and 590 nm.

Cells were incubated with QDs (0.005 or 0.01mg/ml) for different periods of time, cell uptake of MPA-CdTe QDs was predominantly located in the cytoplasm or around nucleus, which appeared as a highly fluorescent punctuated pattern and the cells remained normal morphology (Fig. 2), similar to that reported by Jaiswal JK[11]. But the mechanism of the phenomenon is unknown yet. The quantification result of the amount of QDs distributed inside cells was presented in Fig. 3. Each data point represented the mean fluorescent intensity value of nearly 200 cells at certain time. It indicated that the total fluorescent intensity increased with either the incubation time or the QDs concentration.

Subsequently, the cytotoxicity induced by different concentrations of QDs was investigated using MTT assay. After chronic QDs treatment of cells in either medium200 or serum-reduced MEM caused a dramatic dose-dependent decrease of cellular viability (Fig.4). In addition, Fig.4 shows that the viability of cells in the medium200 was much higher than that in the serum-reduced MEM when QDs concentration was 0.02 mg/ml. It suggested that QD-induced cytotoxicity might be reduced by the presence of serum proteins in the medium200, which was also observed by others[17]. The levels of QD-induced cytotoxicity are likely to be highly dependent on the specific particle, its surface, composition and size, and the type of cells[14, 15, 17-19].

In summary, this study presented a quantitative method to investigate the intracellular distribution of fluorescent nanoparticles, and especially their cellular uptake in different period. Further investigations on the metabolic pathway of QDs inside cells and the mechanisms of their potential cytotoxicity are in progress, which is important to broaden the range of QDs' application in biological systems.

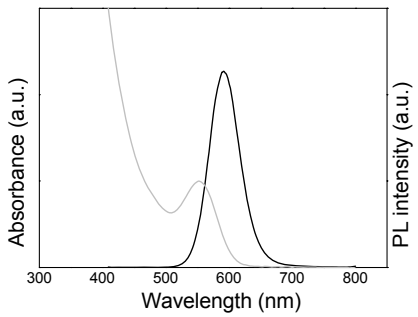

Fig.1 The UV-vis absorption and photoluminescence spectra of MPA-CdTe QDs. Gray and solid lines show the UV-vis absorption and photoluminescence spectra of MPA-CdTe QDs dissolved in PBS (PH7.2), respectively. The respective peak spectra were 543 and 590 nm.

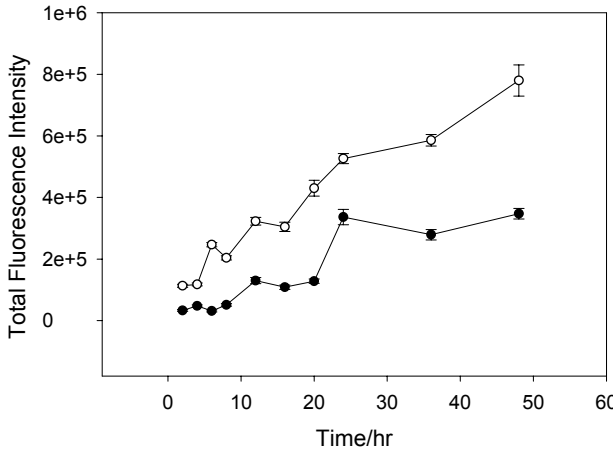

Fig.3 Quantification of QDs distributed in HUVECs by CLSM510. The amount of QDs inside cells exposed to 0.01mg/ml QDs (white) was larger than that of QDs inside cells exposed to 0.005 mg/ml QDs (black) at the same time. Results were presented in mean±SE.

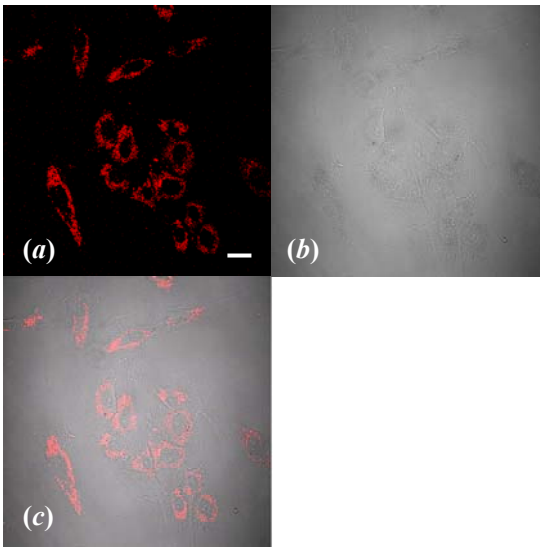

Fig.2 Fluorescence detection of HUVECs exposed to QDs (0.005 mg/ml) in reduced-serum MEM for 24hr: (*a*) under excitation of 543nm; (*b*) trans-illumination light; (*c*) superposition of images in (*a*) and (*b*); viewed with 40× water immersion objective. Scale bar: 20μm

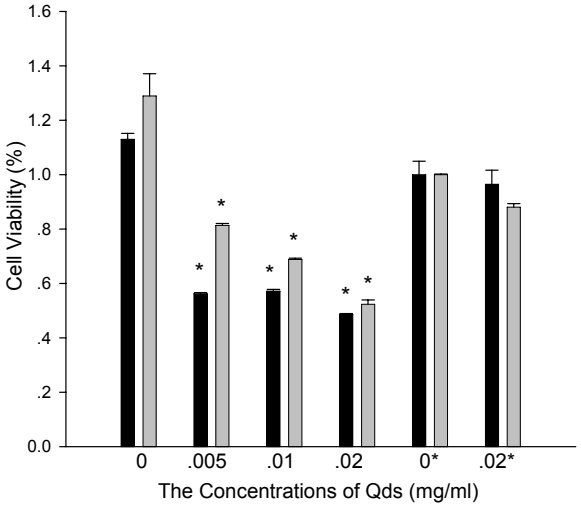

Fig.4 Viability of HUVECs exposed to QDs by MTT assay. Ticks with the asterisk (con*and .02*) in the horizontal axis denote that cells are cultured in medium200, while the others without asterisk denote that cells are cultured in serum-reduced MEM. Incubation time was 24hr (black) or 48hr (gray). Results were presented in mean±SE, P<0.05.

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