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*Original Article*

# Cytotoxicity of CdSe quantum dots and corresponding comparison with FITC in cell imaging efficiency

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**Abstract:** As a type of new emerging fluorescent nanoparticles, quantum dots applied in cellular imaging has elicited broad research interests. However, the wide application of quantum dots was limited by its potential bio-risk. In this study, a systematic investigation was carried out on the cytotoxicity of CdSe quantum dots, particularly focusing on the aspect of particles size, plasm concentration, and incubation period. Furthermore, the comparison between quantum dots and fluorescein isothiocyanate (FITC) on imaging efficiency was performed. MTT assay and flow cytometry proved the negligible cytotoxicity of CdSe quantum dots under the desired dyeing conditions. Given the excellent and stable optical properties of quantum dots such as high fluorescent quantum yield, broad absorption and narrow emission spectrum, the dyeing efficiency of quantum dots was superior to that of FITC. Therefore, quantum dots may be a better alternative with safe, effective and convenient labeling procedures for tissue imaging and immunohistochemistry.

**Keywords:** CdSe, quantum dots, FITC, cytotoxicity, fluorescent imaging

## Introduction

With the development of nano-technology, more and more nano-materials were prepared for biomedical applications, including drug delivery, oncotherapy, artificial replaceable tissues and so on [1-3]. Due to its good penetrating into cells and tissues, nanomaterials are prone to impact on intracellular physiological metabolism [4]. By virtue of this feature, nanomaterials may be a promising agent in clinical applications. Hence, the research on improving biocompatibility of biomedical nanomaterials is necessary before extensively applying in biomedical fields.

As one of novel fluorescent probes, quantum dots (QDs) composed of II-VI and III-V group elements were mainly used in cellular imaging by now. QDs was also proved of the potential of invasive tissue imaging [5-10]. Compared with the traditional organic dyes and fluorescent proteins, QDs performed better with the respect of a wilder absorption spectrum and a narrower emission spectrum. Meanwhile, QDs were of high quantum efficiency, resistance to quenching, and non-sensitivity to intracellular compositions (such as enzymes) [11]. The wider exciting spectrum makes the exciting light with a single wavelength suitable for more than one kind of QDs. In other words, a single excitation light source can satisfy the requirement of multichannel testing. In addition, the fluorescence intensity is 10~20 times higher than equal amount of FITC, and the durability is 100~1000 times longer when compared with FITC [12]. All these above properties make QDs extensively used in cellular imaging, and potentially useful for invasive imaging *in vivo*.

As a fluorescent probe with extensive application prospects, research on bio-safety of QDs are meaningful. In order to further explore the biocompatibility and utilize the fluorescent characteristics of CdSe QDs, MTT assays and flow cytometry were conducted to measure the effects of sizes, concentration, and incubation period on cellular cytotoxicity. Furthermore,

**Table 1.** Components of incubation samples in cytotoxicity test of CdSe QDs

| Final Concentration (nmol/mL) | 200 | 100 | 50 | 20 | 0 |
|---|---|---|---|---|---|
| Volume of Hela suspension (μL) | 180 | 180 | 180 | 180 | 180 |
| Volume of CdSe Solution (μL) | 20 | 10 | 5 | 2 | 0 |
| Volume of culture medium (μL) | 0 | 10 | 15 | 18 | 20 |

specific labeling effects on cell microtubule were compared between CdSe quantum dots and FITC via confocal calcium imaging system.

## Material and methods

*Agents and instruments*

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Hoechst33258 (B2883) were purchased from Sigma. DMEM powder and Fetal Bovine Serum were purchased from Gibco. Anti-α-tubulin (B-7, sc-5-286) and goat-anti-mouse IgG (KPL, 202-1806) were purchased from Shanghai season biotechnology co., LTD.

The 60-mm cell culture dishes, 24-well plates and 96-well plates were purchased from Corning. Slide and cover glass were purchased from Citotest Labware Manufacturing Co., Ltd. Disposable filters were purchased from Millipore. Instruments included cell incubator (Nuair, USA), centrifugal machine (Backman Optima L-10XP), microplate reader (Bio-Rad, USA), inverted microscope (Olympus, Japan), laser confocal fluorescence microscope (Olympus FV1000), multi-spectrum argon gas laser, and flow cytometer (BD FACS Calibur, USA).

*MTT assay*

MTT assay as the common method to measure cell viability was used in this study. Hela cells was utilized as the model cell. Passage between 2 and 5 was used to maintain consistency through this experiment. Cells were incubated at 37°C, 5% $CO_2$ in DMED medium supplement with 10% fetal bovine serum, 1% penicillin-streptomycin. Confluent cells were digested via trypsin-EDTA solution, and cells centrifugalized at 1000 rpm for 5 min. Then, the pellet was suspended by complete medium at concentration of $5\times10^4$ cells/mL. Concentrations of cells were set as **Table 1** in quadruplicates, and controls were of 180 μL cell suspension alone. After 12 h incubation, culture medium was removed, washed by cool PBS three times, and 100 μL fresh medium and 10 μL MTT (5 mg/mL) were added for another 4 h incubation. Blank cells were cultured in 90 μL culture medium for another 4 h. Absorptions were read by a microplate reader at 570 nm, and 655 nm were used as reference. Co-incubation with QDs with different size was performed following the same procedures. Three kinds of QDs with different exciting wavelength of 354 nm, 365 nm and 379 nm were selected. Incubation periods were set as 12 h, 24 h and 36 h.

*Flow cytometry*

For further study of the size effects, flow cytometry was used to evaluate the cytotoxicity as well. Hela cells in logarithmic phase were collected and prepared at concentration of $5\times10^4$ cells/mL. In a 24 well plate, QDs with three different sizes (CdSe-354, 365 and 379) were added, and final concentration was set as 20 nmol/L (2 mL for each well). In the positive control group, dexamethasone was added to promote cell apoptosis. All cells were cultured at 37°C in a humidified atmosphere with 5% $CO_2$ for 12 hours. The cells were collected and suspended in 50 μL PBS. Cells in positive control group were divided into four parts equally. One sample was blank without any dye; the second one was stained by 1:18 diluted Annexin V-APC; the third one was dyed with 1:50 diluted 7-AAD, and the last one was double-dyed. Experimental groups were double-dyed and then measured in flow cytometer.

*Cell fluorescent imaging*

Hela cells in logarithmic phase were used in fluorescent imaging of cell microtubule. One milliliter Hela cells at concentration of $5\times10^4$ cells/mL were added to each well of a 24-well plate, and then cultured at 37°C in a humidified atmosphere with 5% $CO_2$ for 4 hours for fully adherence. Cells were firstly rinsed by PBS and then fixed by mixture of 4% Triformol and 4% sucrose for 20 min. Secondly, cells were washed by PBS, and then cultured with 0.25% Triton for 15 min. Thirdly, triton were then washed by cool PBS, and 6% BSA were then added for blocking for another 45 min.

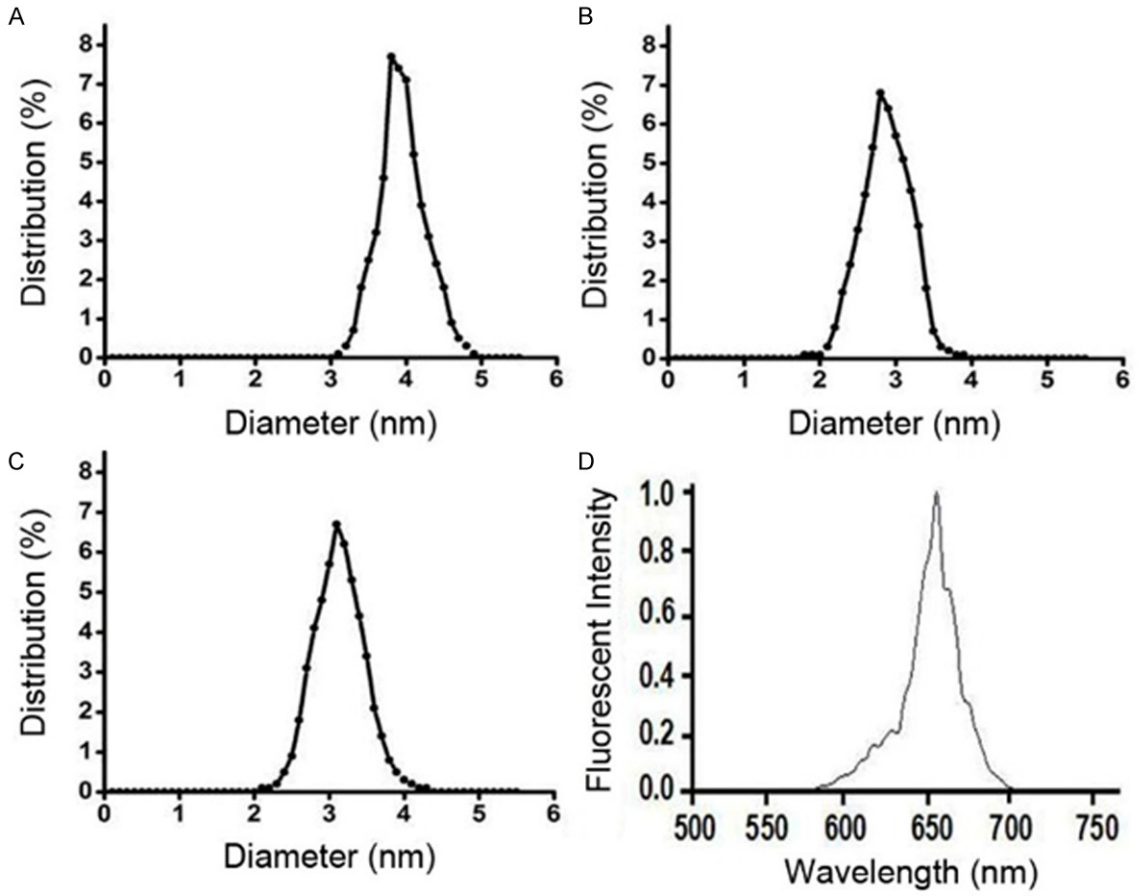


**Figure 1.** Characteristics of CdSe quantum dots. Size distributions of CdSe-354 (A), CdSe-365 (B), and CdSe-379 (C), and a typical absorption spectrum of CdSe-354 were provided (D).

For fluorescent probe labeling, cells were first cultured with 1:100 diluted anti-α-tubulin for 1 h. PBS-rinsed cells were then cultured with FITC or QDs labeled goat-anti-mouse IgG for another 1 hour. Cellular nucleus was finally dyed via 1:1000 diluted Hoechst33258 for 5 min and then washed by PBS. Samples were observed and photographed via confocal microscopy.

*Statistics*

Statistical analysis of the data was performed using the student's t-test using SPSS 19.0, and *p*-values less than 0.05 were considered as significant.

## Results

Distributions of dynamic light scattering-measured hydrodynamic diameters of CdSe-354, CdSe-365 and CdSe-379 were shown in **Figure 1A-C** with corresponding mean diameters of 3.78, 2.8 and 3.1 nm. A typical absorption spectrum of CdSe-354 was provided in **Figure 1D**.

*Cytotoxicity of quantum dots*

Based on the results showed in **Figure 2**, there was a proved concentration-dependent cytotoxicity of CdSeQDs. Cytotoxicity was proportional to QDs concentration in the tested range. There was a negligible cytotoxicity at concentration of 20 nM, which is twice of applied concentration of fluorescent cell imaging test. On the other side, no size-dependent cytotoxicity was observed. For time-dependence, cytotoxicity increased along with prolongation of incubation period, and tended to be stable after 12 h incubation. Therefore, cytotoxicity can be neglected in a typical fluorescent cell imaging procedure (10 nmol/L, 12 h), and the labeling

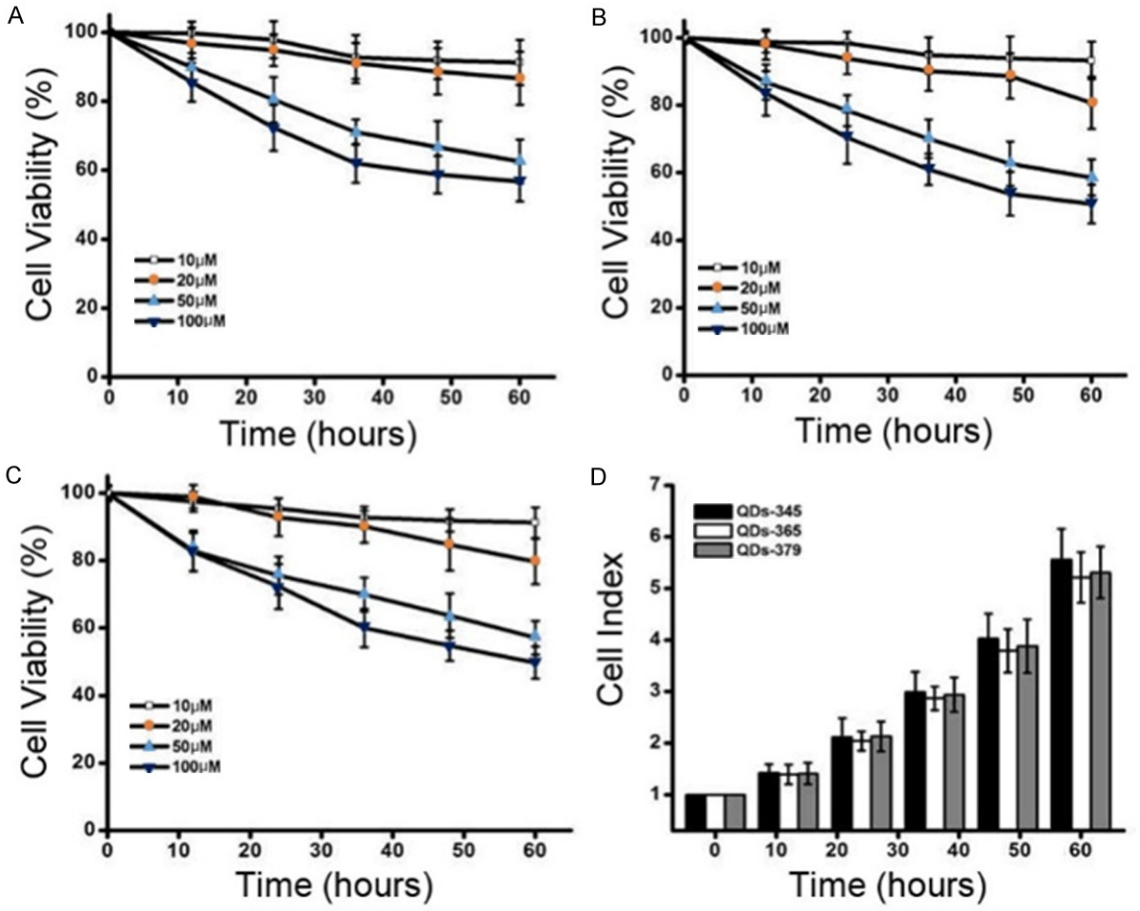


**Figure 2.** Cytotoxicity of QDs (MTT assays). Corresponding dependence on size, incubation period, and concentration for cells incubated with QDs-354 (A), QDs-365 (B) and QDs-379 (C), and size and incubation time dependence of cell proliferation at 20 nmol/L were provided (D).

effect to microtubule can be guaranteed satisfactorily.

Base on the result of flow cytometry, there was no significant cytotoxicity associated to the different sizes of QDs (**Figure 3**). After 12 h co-incubation with QDs-354 at the concentration of 20 nmol/L, there were 92.4% normal cells, 3.9% necrotic cells, and 0.9% apoptotic cells; For QDs-365, 90.2% normal cells, 5.6% necrotic cells, and 1.9% apoptotic cells were observed; In addition, there were 94% normal cells, 3.7% necrotic cells, and 0.7% apoptotic cells for those cultured with QDs-379.

It was proved that QDs posed great damage to macrophages through intracellular accumulation of QDs coupled with reactive oxygen species generation, particularly for QDs coated with PEG-$NH_2$ [13]. Different with macrophages, tumor cells with a better survivability were used in this research. Traditionally, 16 hours were needed for one generation of Hela cell division, furthermore, there were no obvious increase of cytotoxicity detected during 12 to 48 hours. Thus, no obviously negative effect on cell viability will be induced. Although emission spectrum of QDs vary with distinct sizes, there are no size-dependent toxicity. For practical applications, the simultaneous tests of multiple QDs components, which may correspond to various targets of one or more kinds of cells, or simultaneous physiological processes, may be realized by kinds of emission lights resulted from single excited light. Generally, QDs can be deemed as a kind of low-toxic, high-efficiency, and convenient cell fluorescent dyes.

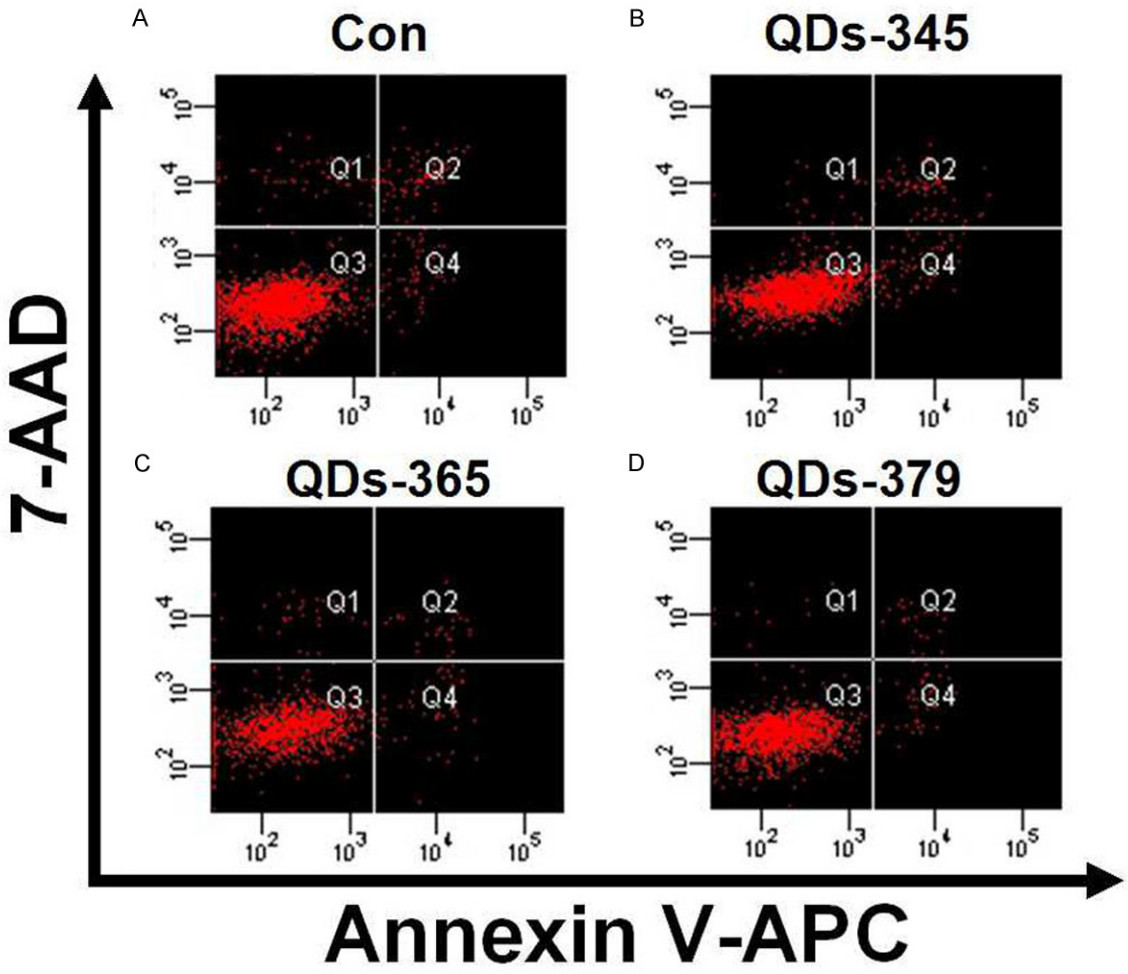


**Figure 3.** Flow cytometry for Hela cells incubated with QDs at 20 nmol/L for 12 h. A. Positive control; B. QDs-354; C. QDs-365; D. QDs-379. For the four quadrants, Q1 is apoptotic cells; Q2 is dying cells; Q3 is normal cells, and Q4 is necrotic cells.

*Imaging efficiency of QDs and FITC*

According to the comparison of cell microtubule imaging between FITC and QDs, a comparable display effects were exhibited in dying microtubule of Hela cell (**Figure 4**). Furthermore, the wider absorption spectrum makes QDs performed better than FITC to some extent. The narrow and symmetric emission spectrum of QDs can decrease the interference signal, so as to increase sensitivity of detection.

## Discussion

With respect to the advantages also reflected by above results of wide absorption spectrum, and narrow emission spectrum of quantum dots [14], peptide and antibody were successfully labeled with quantum dots [15, 16]. Besides, different with organic dyes, quantum dots performed stable and exhibited resistance to quenching, breaking the limitation of short detection period and benefiting dynamic live-cell imaging [17, 19]. Furthermore, QDs were more resistant to enzymes *in vivo* and with better biocompatibility than fluorescent dyes-labeled proteins. Additionally, due to the surface effect of nanostructures, QDs were more easily to attach to other molecules [20]. All these above items make QDs a better fluorescent dyes for cellular imaging and potential for *in vivo* imaging.

Compared the imaging efficiency with FITC, CdSe quantum dots may be a better alternative with safe, effective and convenient labeling procedures for cellular fluorescent imaging,

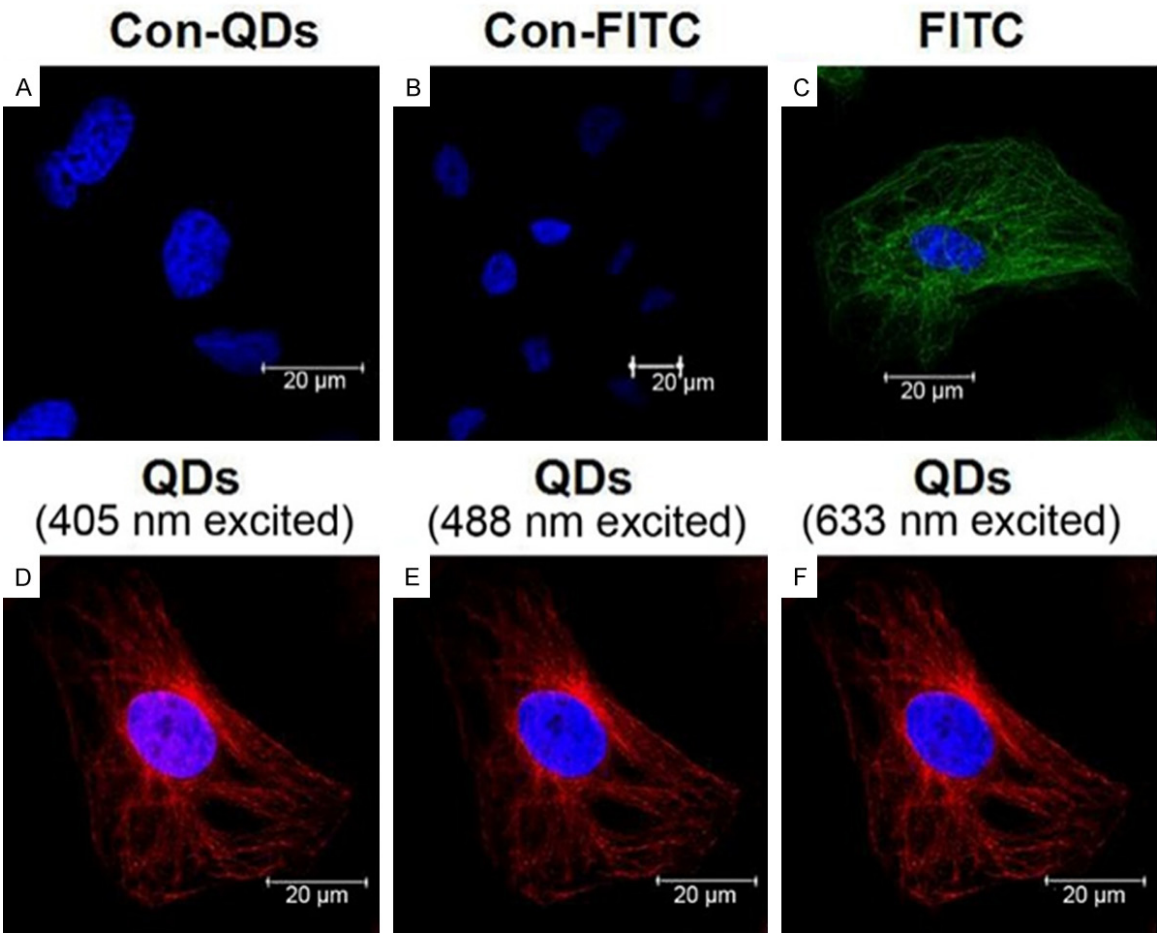


**Figure 4.** Fluorescent imaging of Hela cell microtubule dyed by QDs-365 or FITC labeled goat-anti-mouse IgG. Cell images were: A. QDs fluorescent cell imaging (no anti-α-tubulin added, negative control); B. FITC fluorescent cell imaging (no anti-α-tubulin added, negative control); C. FITC fluorescent cell imaging (488 nm exciting light); D. QDs fluorescent cell imaging (405 nm exciting light); E. QDs fluorescent cell imaging (488 nm exciting light); F. QDs fluorescent cell imaging (633 nm exciting light). Cell nucleus were dyed to blue by Hoechst33258.

where the cytotoxicity was negligible under the desired dying conditions.

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## Disclosure of conflict of interest

None.

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