Chapter

Toxicity of Quantum Dots

Gerardo González De la Cruz, Lourdes Rodríguez-Fragoso, Patricia Rodríguez-Fragoso and Anahi Rodríguez-López

Abstract

Quantum dots (QD) have been deeply studied due to their physicochemical and optical properties with important advantages of a wide range biomedical applications. Nevertheless, concern prevails about its toxic effects, mainly in those QD whose core contains cadmium. Therefore, there are reports about the toxicity caused by the release of ions of cadmium and the effects related to its tiny nanometric size. The aim of this chapter is to show the evaluations about the toxicity of QD, which include studies on viability, proliferation, uptake, and distribution *in vitro* and *in vivo* models. What are the worrying toxic effects of QD? There are reports about some mechanisms of toxicity caused by QD, such as immunological toxicity, cell death (apoptosis and necrosis), genotoxicity, among others. In addition, we discuss how coating QD with passivating agents that improve their biocompatibility. Likewise, this coating modifies their size and surface charge, which are fundamental aspects of the interaction with other biomolecules. We consider highlighting information about more precise techniques and methodologies that help us to understand how QD induce damage in several biological systems.

Keywords: quantum dots, cytotoxicity, cadmium, nanotoxicity, biocompatibility

1. Introduction

In recent decades, there have been countless publications on the use of nanomaterials, particularly in the biomedical area. The main use of semiconductor nanoparticles (NPs) lies in the development of formulations for the delivery of anticancer therapies, specifically targeting diseased tissues and organs. Moreover, quantum dots (QDs) provide remarkable specificity while avoiding damage to surrounding healthy cells and thus avoiding the dreaded side and adverse effects of current treatments. However, among the great applications and their attractive physicochemical and optical properties are a myriad of toxicological effects in biological systems [1]. QDs are inorganic semiconductors with a size range of 1–10 nm. Unlike other types of nanomaterials (NMs), QDs possess a unique and exceptional luminescent property. QDs have become the focus of a study by many researchers [2]. So far, QDs are the most promising option that have exhibited potential for applications in bioimaging (luminescence detection) [3, 4].

Quantum dots have properties, such as luminescent intensity, broad emission spectrum, tight size control, and selectivity, based on their composition. In addition, quantum dots have high resistance to photobleaching, physicochemical robustness, and better half-life than other conventional fluorochromes [5–9]. These

nanomaterials are constituted by central semiconductor core consisting of elements from groups II, VI, III-V, or IV-VI of the periodic table and mostly can be composed of heavy metals and toxic materials (e.g., Cd, Te and Hg, CdS, CdTe, CdSe, among others) [10, 11]. Because their main component is cadmium and because of their tiny size they imply a potential hazard, especially for medical applications. There are different types of cadmium-free quantum dots, such as InP/ZnS, CuInS2/ZnS, AgInS2/ZnS, silicon, and graphene. Although they are cadmium-free in their composition, they are still subject to rigorous toxicological studies [12].

In order to reduce the cytotoxicity of quantum dots, there are some strategies such as the use of some shells composed of ZnS, CdS, ZnSe, or even CdS/ZnS multishells. By covering the core not only improved luminescent effect but also reducing the toxicity by avoiding the release of heavy metal ions [13, 14]. Achieving functionalization of the QD core shell with a polymeric shell can give the desired biocompatibility and decrease its cytotoxic effects [15, 16]. Among some functionalized QDs, there are those coated with polymers such as dextrin or maltodextrin, which make the semiconductor able to target organs and can even be taken up by cellular organelles [17–19]. This advantage allows QD to be more specific and selective for applications for disease diagnosis and treatment purposes. However, the negative effects that QDs may have on cells are difficult to assess. QDs have higher fluorescence intensity, prolonged lifetime, specificity, and possess optical stability compared to conventional fluorochromes. In addition, the wavelength at which they emit is given by tight control of the core size. **Figure 1** shows the characteristic image of QDs emitting photoluminescence.

The characteristics of QDs include size, which is what determines the wavelength at which they emit, although in some cases it does not depend on their composition. Thus, QDs of smaller size (2 nm) emit in blue, QDs of 3–5 nm in green, 6–8 nm in orange, and sizes of approximately 8–10 nm in red [10]. The controversial mechanisms by which QDs are introduced into cells are of great interest among the scientific community and thus the molecular and physiological basis of cytotoxicity. These cytotoxic effects have been classified into *in vivo* and *in vitro*. Thus, cell culture-based tests have become the first choice for bioassessment of QD toxicity [20]. However, *in vitro* studies include assessments of cell membrane integrity, morphological changes, organelle dysfunction, and in some cases quantification of viable cells. Nevertheless,

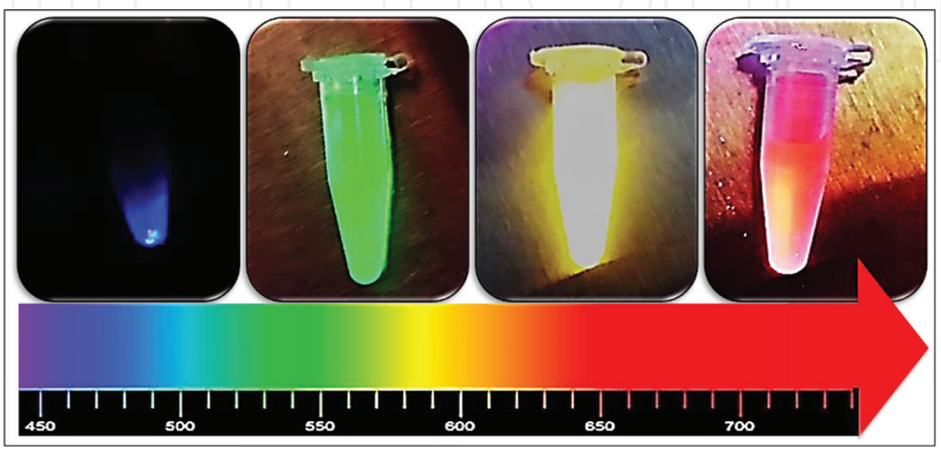

Figure 1.
Fluorescence image of cadmium QD. La emisión de fluorescencia es dependiente del tamaño de los QD. Por lo que, la fotoluminiscencia va del Azul Para aquellos QD más pequeños y hasta el rojo Para los de un núcleo mayor.

information on the behavior of quantum dots in a biological system is still scarce and does not emphasize the cell type-dependent toxicity induced by quantum dots. In this review, we have summarized the efforts in achieving a less toxic design, its advantages and disadvantages in the synthesis of single and bioconjugated quantum dots for application as nanovehicles.

2. Cytotoxic effects of quantum dots on diverse cell lines

The cell membrane is the first barrier that divides intracellular from extracellular mechanisms. The process by which QDs enter the cell is not well defined, although it includes anchoring of QDs to the cell membrane, transmembrane transport, distribution and localization within subcellular compartments, and intracellular accumulation. All these processes are linked to their future application, their potential toxic effects, and the adverse effects induced in a dose-time-dependent manner [21]. Tests such as i*n vitro* cytotoxicity are important because of the significant morphological changes caused by QDs at the cellular and subcellular levels. In recent years, a huge variety of *in vitro* studies suggest that QDs have toxic effects on cells at different levels [22, 23]. In addition, the passage of QDs across the cell membrane has been demonstrated, the effects are oxidative stress, direct damage to membrane, morphological alterations, and various types of cell death.

In vitro models are necessary for safety assessment in preclinical testing of nanomaterials for diagnostic purposes. Although some models for cytotoxicity are not sufficient due to lack of human cells available for culture or even lack of reproducibility in assays. Therefore, the predictability about the safety of a nanodrug is a difficult task for nanotoxicology researchers [24]. However, there are *in vitro* models considered as standard patterns for toxicological studies of nanomedicines such as the use of human renal Hek293 cells [25]. Over a decade, our research group has focused its interest on the study of dextrin-coated 3.5 nm sized cadmium sulfide QDs (CdS-dex) [26] and their potential biomedical application as is the case of doxorubicin-conjugated CdS-dex QDs (CdS-dex/dox) [27]. Therefore, we have established several *in vitro* tests using Hek293, HeLa (cervix adenocarcinoma), and HepG2 (hepatic cells) cells for preclinical studies on CdS-dextrin quantum dots and with maltodextrin. Therefore, our results demonstrate that CdS-dex QDs and CdS-dex/dox QDs induce exposure to dose-dependent cytotoxic effects. In addition to this, we consider that one of the main evaluations to be performed on QDs is the monitoring of their cellular uptake and distribution. We observed that Hek293, HeLa and HepG2 cells when being treated with concentrations of 0.01 and 1 μg/mL, CdS-dex QDs cross the cell membrane, induce morphological changes, and distribute uniformly at different cellular level. Due to their nanometer size, QDs caused cytotoxicity in the three different cell types by crossing the cell membrane. However, morphological changes varied significantly between Hek293, HepG2, and HeLa cells and the concentration of CdS-dex QDs (**Figure 2**). When QDs have contact with the extracellular membrane, they interact with components of the plasma membrane which allows them to somehow enter the cell by some mechanism such as endocytosis. Endocytosis engulfs the QDs by invagination of the membrane to form endocytic vesicles, which transport the QDs to subcellular compartments. Depending on the cell type, as well as some biomolecules involved in the process, endocytosis can occur in different types [28, 29]. Some authors refer to the uncertainty about the toxic effect that quantum dots may cause as they are transported through the bloodstream and leach into the kidneys.

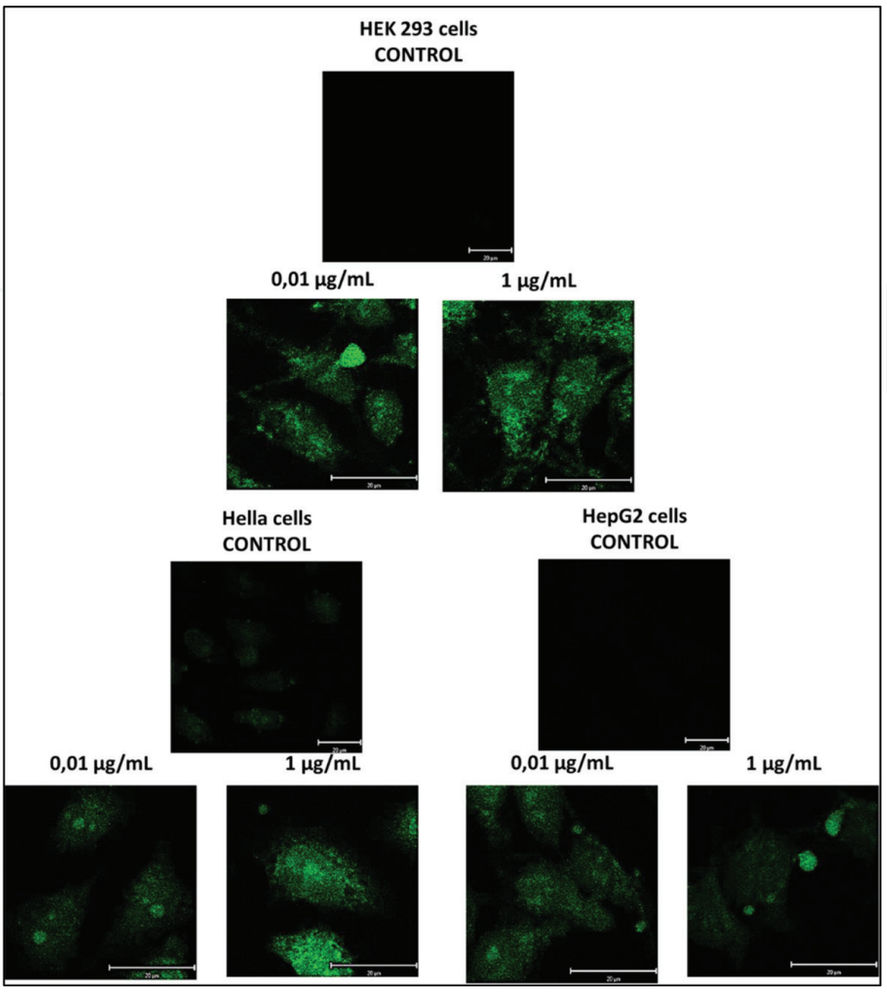

Figure 2.
Fluorescent microscopic visualization of CdS-dex QD in human cell lines. Cells were treated for 24 h with CdS-dex QD (0,01–1 µg/mL). Cells were seeded on slides by smearing and allowed to dry, then analyzed using confocal epifluorescence microscope. Green fluorescence shows the presence of QD surrounding the cytoplasm of Hek293, HeLa, and HepG2 cells. Scale bar 20 µm.

However, there is no information on the nephrotoxic effects of quantum dots both *in vitro* and *in vivo*. Nevertheless, some studies aim to understand the cytotoxic effect on renal cells caused by quantum dots. Therefore, quantum dots, such as titanium oxide (TiO_2), zinc oxide (ZnO), and cadmium sulfide (CdS), have been evaluated in tubular cells (HK-2) in which the cellular and molecular mechanism through oxidative stress induced by quantum dots was demonstrated. In which it was observed that the cytotoxicity of quantum dots was size and solubility dependent. Furthermore, quantum dots that were soluble such as CdS and ZnO were found to cause dose-dependent cell death and degradation/discharge of their ions, respectively [30].

In another investigation, carboxylated CdTe QDs were used and the induced cytotoxicity was evaluated in HeLa cells treated at concentrations from 0.1 to 1000 ng/mL during different exposure times. The effect of CdTe QDs on cell death type, genotoxic effect, and cellular uptake was also evaluated. In this study, they demonstrated that carboxylated QDs did not prove to be less cytotoxic compared to CdTe alone in a concentration-dependent manner. Furthermore, they concluded that CdTe-COOH QDs have genotoxic properties and antiproliferative effects in HeLa cells [31].

Although CdS-dex quantum dots produced different cytotoxic effects on human tumor cells, these effects are not necessarily benign. In fact, our study showed that these nanoparticles had the ability to enter even subcellular compartments. Thus, their biological behavior could trigger pathophysiological effects in a concentration-dependent intrinsic manner. Our CdS quantum dots are coated with a polymeric layer of dextrin. However, many nanomaterials are known to have an inorganic or polymer layer protecting the core to prevent degradation. Even so, heavy metal ions such as cadmium can be released through low stability [32–34]. Studies are needed to know if the cadmium core degrades and releases metal ions and what effects are related to this degradation.

Despite the remarkable effects caused by CdS-dex quantum dots, we clearly need to reinforce the studies and strategies that allow us to learn more about their toxicity. We are getting closer and closer to obtaining biocompatible semiconductor nanoparticles with useful capabilities in diagnosis, treatment, and monitoring of pathologies such as cancer.

Evidently, QDs have physicochemical properties and capabilities and characteristics similar to biological molecules that allow them to be used in biodiagnostics, bioimaging, and targeted drug delivery. For a drug to be effectively delivered using nanocarriers such as QDs, the core component of the QD, the drug or molecule with which it will bioconjugate, and the core shell must be considered. That is, this set of components must be carefully selected to have therapeutic efficiency and optimal safety for use in a biological system [35, 36]. Currently, QDs are considered a tool with promising uses and applications in nanomedicine. However, their cytotoxic effects remain among the main challenges regarding their biocompatibility. The QDs with the highest capacity to emit luminescence and with the highest efficiency in carrying molecules with active principle are those containing cadmium (Cd). However, one of the limitations for the use of Cd QDs in nanomedicine and clinical research is that it is suggested that the core disintegrates and is potentially toxic. That is, it has been considered that it is the core of the QD that largely determines the cytotoxic response and pathophysiological effects [37–39].

Some authors refer that the safety assessment of QDs alone or conjugated is of vital importance since it will allow predicting the effects when interacting with a biological system. They suggest that a nanomaterial is small enough to enter a cell and its cellular compartments, regardless of the route of administration [40–42]. For systemic drug delivery, the intravenous (IV) route is used, which is a major challenge in the development of nanotherapies [43]. The US Food and Drugs Administration (FDA) has approved NMs that have been studied in rigorous preclinical studies combining therapeutic and biological targets as drug delivery agents [44–46].

Our working group has been given the task of synthesizing colloidal CdS-dex/dox QD and evaluating on HeLa cell. We treated HeLa cells with CdS-dex and CdS-dex/dox to compare the selectividad of uptake alone as well as bioconjugated (1 μg/mL) in both cases and with doxorubicin at the same concentration. After 24 h of incubation and in order to investigate the cellular absortion of QD, cells were fixed on slides for visualization by confocal fluorescence microscopy. Through visualization of fluorescence and cellular uptake, we can observe that in cells treated with CdS-dex QDs without bioconjugation, there was a higher distribution in cytoplasm, nucleus, and nucleoli of the cell. However, this cellular uptake and distribution were not the same in the case of HeLa cells treated with doxorubicin and CdS-dex/dox. Nevertheless, in cells treated with doxorubicin and CdS-dex/dox, a significant increase in cell size was observed compared to cells treated with QDs alone. Although, QDs did not appear

homogeneous throughout the cytoplasm and with lower fluorescence intensity in the nucleus (**Figure 3**). They can also induce not only cytotoxic but also genotoxic effects in both normal and cancer cells [47–50].

Although, it has been shown that the effect after cellular uptake of various QDs depends on their size, shape, concentration, and cell type. The cytotoxic effect and mechanisms of nanotoxicity by the interaction of QDs with cells remain complex to assess and far from fully understood. However, this nanotoxicity has been shown to occur intracellularly or extracellularly [51]. QDs can even interact directly with biomolecules once inside the cell, due to their minute size. As a result of this interaction, an alteration in cellular equilibrium coexists, as well as irreversible morphological

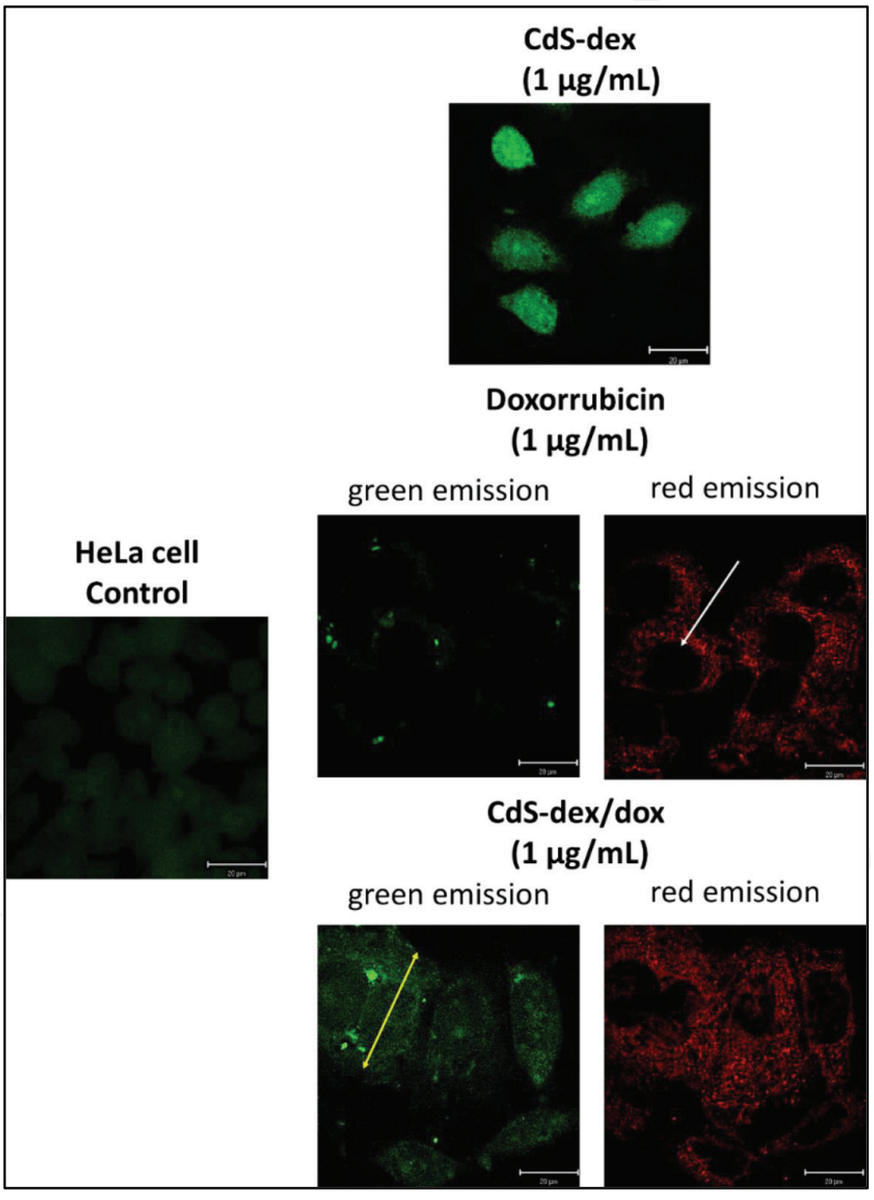

Figure 3.
Fluorescent microscopic visualization of doxorubicin, CdS-dex, and CdS-dex/dox QD in HeLa cell. Cells were treated for 24 h at 0,01–1 µg/mL concentration of doxorubicin, CdS-dex, and CdS-dex/dox QD. Cells were seeded on slides by smearing and allowed to dry, then analyzed using a confocal epifluorescence microscope. Green fluorescence shows the presence of CdS-dex QD. Red emission shows fluorescence in the presence of doxorubicin and CdS-dex/dox QD. The yellow arrow represents the increase in size and the white arrow indicates the absence of QD.

and functional damage [51]. Even if indirectly the outside of the interacts with QDs through membrane receptors that cause activation and inhibition of different signaling pathways, causing toxic reactions or cell death [52].

Therefore, the cytotoxicity of QDs is more complex than we can imagine, it can cause not only the interaction with heavy metals contained in QDs but the disintegration of the core and the release of Cd ions, which increases their toxic potential. Under this condition, researchers have expressed concern about the use of NM and the parameters to be evaluated for future medical applications. This question arises from the association of adverse effects derived from the ability of QDs to enter cells and lodge in various subcellular compartments. This implies that they could evade the defense mechanisms of the human body, cross biological barriers and even interact with components of blood circulation [53]. Moreover, the blood circulation is the primary passage of NMs to the distribution of target organs. Thus, vascular endothelial cells serve as the first barrier and are tasked with maintaining vascular integrity [54]. In a study with ZnO nanoparticles, it has been shown that they are capable of causing cytotoxicity in HUVEC cells due to the increase of intracellular reactive oxygen species (ROS) in a dose-dependent manner [55]. Our studies have shown that at concentrations of 0.01 μg/mL, CdS-dex QDs already cause cytotoxic effects in HUVEC cells. The QDs are distributed around the cytoplasm, producing an increase in cell size and completely changing the characteristic morphology of the endothelial cell (**Figure 4**). Although it does not penetrate into the nucleus and nucleoli, cellular uptake occurs in a dose-dependent manner. In addition, endosome formation is observed, suggesting that cell deformation and toxicity are caused by cellular stress following the passage of the QD into the cell. The cytotoxicity produced by QDs is the

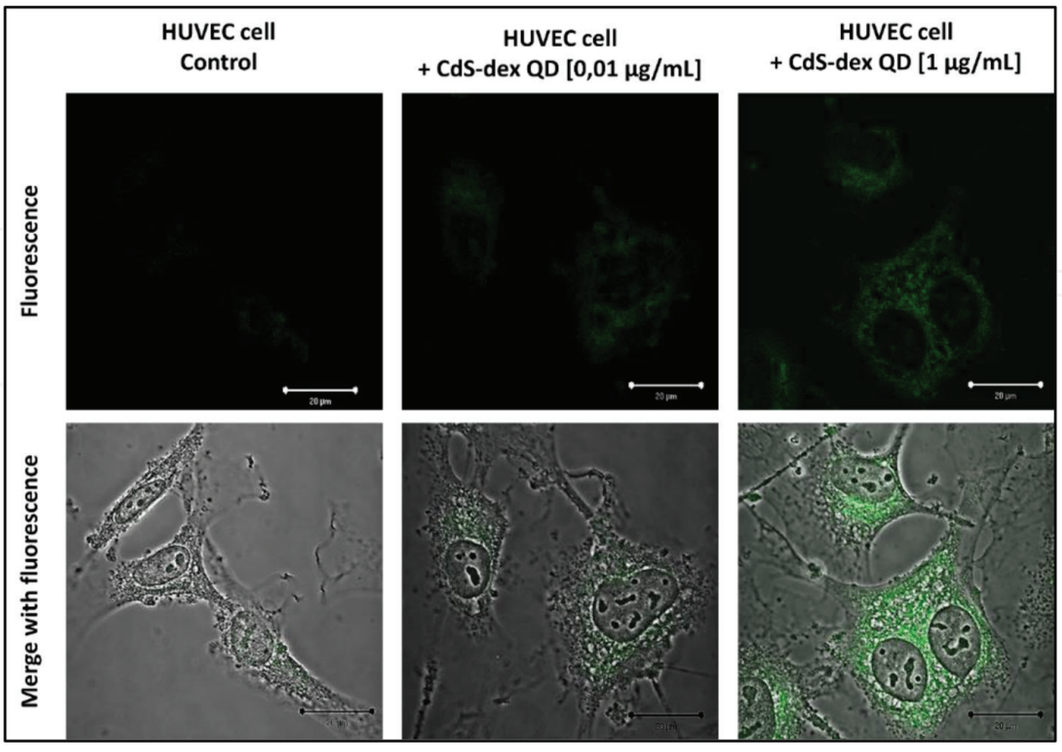

Figure 4.
Fluorescent microscopic visualization of HUVEC cells treated with CdS-dex QD at 0,01–1 μg/mL concentration and 24 h time exposure. Cells were seeded on slides by smearing and allowed to dry, then analyzed using a confocal epifluorescence microscope. Green fluorescence shows the presence of CdS-dex QD.

main parameter limiting their use in bioimaging research. The idea of applying QDs that produce morphological changes and ultimately cell death is a determining factor. Currently, joint efforts are being made for the development of innovative QDs capable of meeting the needs in healthcare areas. This progress in QD design and synthesis has resulted in improved safety *in vitro* studies. However, a myriad of factors that lead to cytotoxicity of QDs in normal, cancer, and endothelial cells remain in question. It has also been demonstrated that when QDs come into contact with organisms, they produce toxicity that is size-dependent, concentration threshold-dependent, and varies according to cytosensitivity [56]. However, factors such as concentration range are responsible for the intracellular distribution, which necessitates storage and bioaccumulation and thus increases cytotoxicity [57]. There is still a long way to go to achieve an accurate understanding and standardized parameters on safety for the use of quantum dots in the field of biomedicine.

In a whole decade, we have been dedicated to the design, synthesis, and nanotoxicological evaluation of quantum dots so we are very clear that, quantum dots can be improved in their design and composition. In addition, the nanoparticle size must be strictly controlled as it is one of the main factors influencing the toxicological effects of quantum dots [53]. The idea of having a complete profile of a type of nanomaterial is not unrealistic. However, it is necessary to demonstrate with studies on its preclinical evaluation. These evaluations include physicochemical characterization, *in vitro* evaluations with different types of human tumor and healthy cells, biodistribution, bioaccumulation, and pharmacokinetic studies. In addition, to perform exhaustive evaluations on its hemocompatibility as a starting point to rule out the toxic effect of a nanomaterial.

3. Conclusion

The development of newer drug delivery systems based on the use of quantum dots is one of the advantages for various disease treatments, such as cancer and gene therapy, as noted above. This modality allows for site-specific drug therapies and a higher safety profile. However, the pharmaceutical industry is far from knowing everything about the toxicological profile of all nanomaterials. However, nanotechnological challenges are evolving and it is necessary to focus our attention on the standardization of parameters for the evaluation of the cytotoxicity of nanomaterials such as quantum dots in order to broaden their safety range and thus ensure lower toxic effects. In the meantime, let us not forget that the key to the toxicity caused by quantum dots is given by the interaction of the elements that compose them and the biomolecules of the biological system. In the very near future, we can include scientific bases that tell us about physicochemical perspectives of quantum dots, better experimental conditions already standardized and reliable comparative analyses (*in vitro* and *in vivo*).

Appendices and nomenclature

NP	nanoparticles
QD	quantum dots
NM	nanomaterials
Cd	cadmium

Te	tellurium
Hg	mercury
CdS	cadmium sulfide
CdTe	cadmium telluride
CdSe	cadmium selenide
CdS-dex	sulfuro de cadmio core/capped dextrina
CdS-dex/dox	sulfuro de cadmio core/capped dextrina, with doxorubicin
HUVEC	umbilical cordon human cells
HepG2	hepatic cells
Hek293	kidney human cells
HeLa	adenocarcinoma of cervix
μg	micrograms
mL	milliliters

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