Biological Applications and Toxicity Minimization of Semiconductor Quantum Dots

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Review

Biological applications and toxicity minimization of semiconductor quantum dots

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Abstract

The extraordinary potential of semiconductor quantum dots (QDs) has resulted in their widespread application in various fields, from engineering technology and development of laboratory techniques to biomedical imaging and therapeutic strategies. However, the toxicity of QDs remains a concern and has limited their applications in human health. Better understanding the behavior of QDs as it relates to their composition will enable exploring their limitations and developing a strategy to control their toxicity for potential therapeutic applications. Here, approaches to minimize their toxicities according to the specific cell type, organ, or animal species are described. This review summarizes recent promising works at the levels of cells, organs, and whole organisms.

Potential of QDs in the biological fields

The multitude of researchers exploring the remarkable potential of **quantum dots** (QDs) (see Glossary, Box 1 and 2) in biomedical research, from tools in medical imaging to the development of sophisticated theranostic agents, indicate the great potential of QDs in this field. Among the variety of nanoprobes (upconversion nanoparticles, fluorescent proteins, and graphene-based nanomaterials), semiconductor QDs have distinguished themselves in the field of imaging due to their size and their optical properties (stable fluorescence, brightness) and cost-effectiveness relative to conventional fluorescent dyes. Their extremely small size (order of magnitude of biological molecules, 1 -10 nm) makes these QDs ideal candidates for identification and tracking of biomolecules and organelles inside the cell and especially in the nucleus, unlike upconversion nanoparticles that have a larger size (20-50 nm) and cannot reach the nucleus[1]. Moreover, unlike carbon dots, red fluorescence emission for QDs semiconductors, which is the preferred color in biological applications due to cells autofluorescence phenomena in the 400 to 600 nm range (yellow, green, blue), is possible with semiconductor QDs. As a result, despite the toxicity conferred by semiconductor QDs (although recently controversial for so-called "biocompatible" nanoparticles), better understanding the occurrence of their toxicity and strategies to minimize their toxicity would enable their better use. In targeted imaging, QDs targeting tumors or biomarkers play a crucial role in the detection, treatment, monitoring, and prognostic evaluation of certain diseases. However, for purely diagnostic applications, in which cell damage must be avoided, the potential toxicity caused by the release of metal ions remains a major limitation to the use of QDs, thereby hindering their translation from preclinical studies to clinical studies in biomedical imaging. A number of reviews have been published to address various questions, including the recent progress made on QD surface modification, describing their structure-activity relationships (Figure 1), mechanisms of uptake in cells, and biomedical applications. However, few reviews

have specifically presented a body of knowledge that enables a detailed overview on the behavior of QDs including or not means to minimize toxicity according to the specific cell type, tissue/organ, or animal species. This review aims to collate information on recent advances in the use of QDs in biological fields, with the objective of describing the various effects of QDs reported thus far, and to elucidate their behavior in living organisms, notably for future applications in humans.

Application of QDs at the cellular level

Stem cells (see Glossary) are able to multiply indefinitely *in vitro* in an undifferentiated state, and can differentiate in the presence of adequate factors. QDs have proved to be of great value in cell imaging, particularly in the monitoring of cell differentiation, and in functional studies. However, the biological effect may differ depending on the type of stem cell or differentiated cell (Table 1).

Stem cells

The hemocompatibility of QDs was assessed in hematopoietic stem cells by combining Tat Peptide with CdSe/ZnS-QDs, which made it possible to monitor transplantation successfully [2]. However, in differentiated hematopoietic cells (human blood neutrophil granulocytes, monocytes, lymphocytes, and platelets), harmful effects were observed that were specific to both their morphology (maximum accumulation of QDs with monocytes and minimum with lymphocytes) and function (despite the presence of the shell, neutrophils underwent cell death after phagocytosis of CdSe/ZnS-QDs, and CdTe-QD-induced platelet aggregation in the absence of plasma) [3,4].

Concerning epithelial stem cells, depending on QD composition, the effects may be either deleterious or beneficial. Several studies have provided explanations for the cytotoxic effects

of QDs, which occur notably via interaction of CdTe-QDs with aromatic rings and amine groups from trans-membrane proteins and the cytoskeleton[5], and the predomination of the overall cellular response, which is mediated by the minor non-endocytic uptake of CdSe/ZnS-QDs in BEAS-2B human bronchial epithelial cells [6]. Despite the use of multi-shell CdSe-CdS/ZnS-QDs in the Calu-3 human lung cell monolayer, mechanical stress and activation of Ca^{2+} influx were induced, affecting the lung barrier [7]. However, a different study showed that these QDs did not elicit noticeable changes in the long-term **TEER** (see Glossary) after a 70 day-exposure, suggesting their potential use for drug delivery at the surface of the lung epithelium for short periods [8]. Glass incorporation in CdSe-QDs in place of coating by greater polymer-enabled reduction of the cytotoxicity on HaCaT keratinocytes. The higher rigidity and density of glass relative to polymer enabled more efficient blocking of Cd and Se ions, making these QDs safer for biological applications [9].

Nerve stem cells (NSCs) appear to be more resistant than other stem cells. After uptake, mainly via microfilaments and microtubules, CdSe/ZnS-polymer-QDs and graphene-QDs had little effect on the growth and proliferation of primary NSCs within a week[10,11]. CdSe/ZnS-QDs did not affect either differentiation potential of NSCs or the protein expression of neuron- and astrocyte-derived primary nerve stem cells [11]. In the GT1-7 differentiated neuronal cell line, CdSe/ZnS-QDs were incorporated into only a limited fraction of the whole lysosomal compartment, inducing a slight but significant reduction in cell survival and proliferation [12].

Mesenchymal stem cells are important for synthesizing and repairing the musculoskeletal system (such as cartilage cells, bone cells, fat cells, synovial cells, or myoblasts). QDs have been mainly used for the characterization of these cell types, with a majority of the QDs having no negative impact on cell viability and differentiation [13,14]. The use of ZnS/CdSe-streptavidin-QDs conjugated with a Fab fragment of an anti-myc antibody enabled the tracking of GLUT4 movement in 3T3L1 adipocytes after stimulation by insulin or in an insulin-resistant

state [15]. The composition of CdSe/ZnS-polymer-QDs conjugated with a unique antibody against a heat shock chaperone (mortalin) allowed the successful tracking of mesenchymal stem cells labeled during tissue repair, following 26 weeks of allogeneic transplantation in a rabbit model of osteochondral defects [16]. The construction of a siRNA-coupled CdSe/ZnS-PEG-QDs has been successfully achieved, and has both demonstrated the involvement of *SOX9* in chondrogenesis and enabled the real-time monitoring of transfection [17]. CdTe-QDs have allowed the simultaneous characterization of two types of cadmium-induced cell death (apoptosis and autophagy) [18].

QD toxicity in male and female germ cells and during embryonic development has also been evaluated. At concentrations between 1 and 125 nM of CdSe-QDs, no toxicity was detected in germ cells (i.e., in terms of viability and motility of spermatozoa) and embryos. At 500 nM, toxic effects on both pre-and post-implantation embryo development and female germ cells (with decreased maturation and fertilization rates) were reported, which were reduced with the incorporation of a ZnS shell. The transfer across the placental barrier resulted in Cd accumulation in pups, which was reduced after coating with PEG or SiO2 [19].

Differentiated cells

Overall, Cd^{2+}-based QDs had deleterious effects in hepatic cells, including CdSe/ZnS-QDs-induced L02 hepatocyte pyroptosis, liver inflammation, and dysfunction[20], as well as CdTe-QD-induced mitochondrial damage in human hepatocellular carcinoma cells (HepG2), which were not solely attributable to cadmium released from QDs, as the observed effects were similar or greater to those of cadmium chloride[21]. However, graphene-QDs did not induce cytotoxicity in mouse primary hepatocytes [22].

An extensive investigation of the signaling mechanism involved in the induction of cytotoxicity of shell-less QDs has been performed in endothelial cells; notably, the pathways implicated in

CdTe-QDs-mediated mitochondrial-dependent apoptosis in human umbilical vein endothelial cells have been studied in detail [23]. Nevertheless, the use of shells or an amorphous form [24] seems sufficient to reduce their toxicity. Furthermore, additional applications may emerge following the discovery of stronger accumulation of CdSe/ZnS-QDs in slow-flowing, small caliber venous vessels than in fast-flowing, high caliber arterial vessels [25]and the large-scale internalization of CdSe/ZnS-QDs conjugated to monoclonal antibodies targeting the murine transferrin receptor (Ri7) by brain capillary endothelial cells [26].

The effects of unshelled QDs in nephrocytes have been discussed in detail. L-cysteine-capped CdTe-QDs decreased cell viability and modified the structure and activity of copper–zinc superoxide dismutase in mouse primary nephrocytes and hepatocytes. Notably, this effect was abrogated by the addition of ascorbic acid [27]. The study of pig renal cells revealed the involvement of the Wnt pathway in CdTe-QDs-induced damage [27]. The evaluation of the nephrotoxicity in HK-2 human epithelial tubular cells using various metallic QDs with differential solubility (TiO2, ZnO, and CdS) revealed that soluble CdS- and ZnO-QDs elicited cell death in a dose-dependent manner, and this effect was attributed to the release of metallic cations. However, insoluble TiO2-QDs had no cytotoxic effect [28]. Targeting bladder urothelial cancer cells was performed successfully using CdSe/ZnS-QDs conjugated with the prostate stem cell antigen [29].

Recently, promising applications in both diagnostics and therapy of microbial infections have been reported. QD-specific antibodies designed for use as biosensors via the association of rabbit anti-p38 antibody and CdSe/ZnS-QDs, were shown to successfully infiltrate porous silicon whose pores contained immobilized Egp38 antigen. These QDs were used to detect *Echinococcus granulosus* for early diagnosis of hydatid disease [30]. Photoexcited QDs have been used to kill a wide range of multidrug-resistant bacterial clinical isolates, including methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Escherichia coli*, extended-

spectrum β-lactamase-producing *Klebsiella pneumoniae*, and *Salmonella typhimurium*, while leaving mammalian cells intact. Their proposed application in clinical phototherapy is suggested for the treatment of infections [31].

Application of QDs in organs

Ex vivo investigation would enable elucidation of QD-induced effects on a collection of specific cells in their environment. The different applications of QDs were grouped by organ system in this review (Table 2).

Integumentary system

Several studies have investigated QD penetration through the skin. In one of these studies, CdSe/ZnS- mPEG-5000-QDs were injected subcutaneously into CDF1 mice or deposited onto human skin. The results showed that QD diffusion in the dermis is limited by the basement membrane and dense connective tissue fibers; this resulted in negligible QD penetration into the epidermis, hair follicles, sebaceous and sweat glands, nerves, and blood vessels. The total penetration of QDs could only be achieved via a 10-minute massage of damaged or stripped stratum corneum. An increase in pH (8.3) of QD formulations enabled some degree of QD penetration of intact skin [32–34]. Furthermore, simultaneous use of cell therapy and graphene QDs accelerated the repair of skin lesions [35].

Brain system

Owing to their nanoscale size, which enables them to cross the blood–brain barrier, QDs represent a revolutionary theranostic tool for the brain system. CdSe/ZnS-streptavidin-QDs injected by stereotaxis into the mouse brain were able to specifically label the microglia in primary cortical cultures via macrophage scavenger receptors and mannose receptors present

177 on their surface, and did not induce toxicity (i.e. induction of inflammation) in the short term
178 [36]. Recently, graphene-QDs were shown to successfully reduce symptoms in mice primed to
179 develop Parkinson's disease by inhibiting fibrillization of α-syn and interacting directly with
180 mature fibrils, thus triggering their disaggregation. These QDs were also to inhibit the
181 aggregation of Aβ peptides which leads to Alzheimer's disease. These promising results
182 constitute a considerable advance in the treatment of complex pathologies for which no
183 effective therapies are available[37,38].
184
185 *Digestive system*
186 The digestive system is essential for the assimilation of nutrients, immune and endocrine
187 defenses, and the metabolism of endogenous or exogenous molecules (e.g., drugs). A study
188 using CdTe/CdS-QDs administrated by intravenous injection showed that QDs were retained
189 in the sinusoids and selectively taken up by sinusoidal cells (Kupffer cells and liver sinusoidal
190 endothelial cells) within 3 h, but not by hepatocytes[39]. However, the absorption of QDs at
191 the intestinal level has been poorly studied. Previous studies focusing on nanoparticle
192 absorption in general describe the requirement for well-defined characteristics to cross the
193 mucus barrier and the intestinal epithelium lining the intestinal lumen. In nematodes
194 (representative of intestine models), CdTe-QDs influenced lipid metabolism via alteration of
195 the molecular basis of both synthesis and degradation of fatty acid, but not degradation of
196 phospholipids, and increased in fat storage in intestine [40]. The spleens of rats were also
197 exposed by CdSe/CdS-PEG-QDs coupled or not by folic acid with different doses(0–300 nM),
198 and both were revealed to be non-toxic. Moreover, the addition of folic acid significantly
199 improved the uptake of QDs in a dose-dependent manner, highlighting the importance of
200 surface coating properties [41]. VEGFR2-CdSe/ZnS-QDs were used to successfully distinguish
201 between diseased and non-diseased tissue in the colons of carcinogen-treated mice with high

202 sensitivity and specificity. However, the complete removal of the contrast agent by flushing is
203 difficult to achieve [42,43].
204
205 *Pulmonary system*
206 The respiratory system is the preferred site for exchange of gas and airborne particles between
207 the body and the external environment. The evaluation of the efficacy or toxicology of QDs in
208 the pulmonary system is therefore essential. A short-term exposure to 240 μg CdO-QDs/m^3 for
209 seven days (3 h/day) caused pulmonary injury and tissue remodeling in mice, and altered
210 immune function [44]. Intratracheal instillation of CdSe/ZnS-QDs or CdSe/ZnS-PEG-QDs
211 persistently induced acute neutrophil infiltration, followed by interstitial lymphocyte
212 infiltration, transiently reduced pulmonary function, and a granulomatous reaction on day 17.
213 Although the granulomatous reaction disappeared for CdSe/ZnS-PEG-QDs at day 90,
214 PEGylation was not sufficient to reduce the risk of side effects associated with QDs [45]. Upon
215 further exploration, genetic predisposition factors and atopy (in this case dust mite allergy) were
216 found to induce impaired pulmonary mechanics by exposure to QDs [46,47].
217
218 *Urinary system*
219 The urinary system is essential for the excretion of endogenous and exogenous substances that
220 are either catabolized or intact. Studies of the kidneys of mice have shown that CdTe-QDs
221 deplete glutathione levels, which reduce the ability of the liver and kidneys to eliminate
222 hydroxyl and superoxide anion radicals, thus inducing oxidative damage to tissues. However,
223 a partial recovery was observed after 28 days [48,49]. Free CdSe/ZnS-QDs and anti-CD47-
224 CdSe/ZnS-QDs were intravesically instilled in mice. Examination of the *in vivo* biodistribution
225 of anti-CD47-CdSe/ZnS-QDs revealed the absence of significant accumulation of QDs outside

of the bladder, and no acute toxicity up to 7 days post-administration. Mice showed extravesical biodistribution of QDs under some conditions; however, this was rare [50].

Cardiovascular system

The cardiovascular system transports blood from the heart to the peripheral regions of the body and vice versa, and carries a wide variety of substances. The ideal geometry and functionalization capabilities of QDs make them an excellent tool for visualizing the cardiovascular system. Accordingly, CdSe/ZnS-QDs have proved their utility in the study of sarcomere dynamics during excitation-contraction coupling in healthy and diseased cardiac muscle and in the whole heart [51]. By combining *in vivo* cryotechniques and the injection of glutathione-coated QDs into kidneys, QD distribution in the vascular system was achieved, primarily in glomerular blood capillaries for a few seconds and extending to peritubular blood capillaries by five seconds [52]. With regard to therapy, the amorphous form of SeQDs has proved to be effective in prevention of endothelial dysfunction and atherosclerosis [24].

Musculoskeletal system

The musculoskeletal system gives form, stability, and movement to the human body. The application of QDs on this system thus far has been confined to imaging. The use of CdSe/ZnS-streptavidin-QDs bound to biotin-phalloidin, which labels skeletal actin filaments, has enabled rapid, quantitative, and inexpensive imaging of sarcomeric movements, notably step-size measurement for low-duty cycle muscle myosin [51,53]. A recent study reported that QDs were mainly found in the synovial membrane, but significantly less on cartilage, one week after being intra-articularly injected into the joints of healthy or osteoarthritis horses [14]. Among the selected articles, no studies were conducted to evaluate the spread of QDs in the bone system, and QD accumulation in bone remains to be defined.

Applications of QDs in animals

Invertebrates

In order to evaluate the integrity of water ecosystems, two aquatic species, polyp *Hydra vulgaris* and coral *Stylophora pistillata*, representative of freshwater and sea habitats, respectively, were exposed to CdSe/ZnS-polymer-QDs and CdTe-QDs at concentrations exceeding the Cd^{2+} content in seawater. Despite the presence of a shell and polymer, CdSe/ZnS-polymer-QDs induced dramatic changes, including morphological and global transcriptional changes, albeit less than those induce by CdTe-QDs [54–57]. In order to track the visualization of cyclin E within cells of developing *Xenopus laevis* embryos in real time, CdSe/ZnS-QDs have been coupled to cell-cycle regulatory proteins (recombinant (His6)-cyclin E protein), and their accumulation in the nucleus at midblastula transition (6 h post-fertilization) has been confirmed [58].

Drosophila, which has high genetic homology with the human genome and has innate immunity signaling pathways that are similar to those of mammals, is an excellent model for genotoxicology and immunotoxicology. QDs were administered via the ingestion of QD-supplemented food (CdSe/ZnS- or InP/ZnS-QDs, 100 and 500 pM) and stress response (hsp70, hsp83), genotoxic stress (p53), and apoptotic cell death (Dredd) genes were observed following the administration of CdSe/ZnS-QDs. However, no significant response was observed following the administration of InP/ZnS-QDs [59].

Small vertebrates

Although alternatives to animal studies should be favored, the advantages provided by studies in small animals are not negligible (*i.e.,* transposition of whole biological systems). Various QDs (CdSe/ZnS-QDs, PbS/CdS/ZnS-QDs, CdTe/ZnS-QDs, CdTe-QDs, and QD innovative

276 structures) and modes of administration (intravenous, intraperitoneal, intratracheal, plantar, and
277 oral) were evaluated in mice and rats (Table 3). CdTe-QDs affected steroid hormone and lysine
278 biosyntheses and taurine metabolism [60]. CdSe/ZnS-QDs injected intravenously in mice at a
279 concentration up to 10 mg/kg body weight (BW) did not induce significant toxic effects during
280 four weeks of administration; however, a dose of 20 mg/kg BW caused death in two-thirds of
281 mice shortly after the start of experiment [61]. Biodistribution kinetics of intravenously injected
282 PbS/CdS/ZnS-QDs at 0.04 mg/mL revealed the presence of QDs in the lungs after short time
283 periods (first 0-30 s) followed by accumulation in the liver (60 – 120 s) with complete
284 disappearance of fluorescence at the injection site. After 25 days, elimination by the urinary
285 system lead to the total elimination of the QDs [62,63]. Similarly, biodistribution studies of a
286 single intravenous dose
287 (0.2 μmol/kg) of CdTe/ZnS-QDs defined an elimination half-life of 12 - 14 h [64]. After
288 intraperitoneal injection of CdSe/CdS-QDs every 3 days for a period of 15 days, an increase in
289 the levels of LDH, NADPH, and IL-6 pro-inflammatory cytokines was specifically detected in
290 the plasma, liver, and spleen [65]. Globally, QD accumulation in the liver, kidney, and spleen
291 was observed in the four previously cited studies, regardless of route of administration. The
292 injection dose, particle size, and surface charge have been identified as factors affecting mean
293 residence time, apparent volume of distribution, and clearance, particularly in the case of Ag2S
294 QDs [66]. The assessment of pulmonary toxicity following intratracheal injection of CdSe/ZnS-
295 QDs revealed lung injury and inflammation in a dose-dependent manner, which peaked at days
296 7 and 14 post-exposure, with a holding time of QD fluorescence of 1 week. The built-up of
297 Cd^{2+} occurred only in lung-associated lymph nodes and kidneys for 28 days [67]. An overdose
298 (50 mg/kg) of phospholipid micelle-encapsulated CdSe-CdS/ZnS-PEG-QDs over 112 days (16
299 weeks) did not induce toxicity, despite the accumulation of QDs in the liver and spleen in
300 Kunming mice [68]. The evaluation of orally administered 0.2 mg/kg and 0.4 mg/kg BW/day

of CdTe-QDs/quercetin complex for 3 weeks highlighted the utility of QDs as nano-carriers by reducing inflammation significantly and promoting cartilage regeneration [69]. Intraperitoneal injection (10 mg/kg) of graphene QDs effectively ameliorated autoimmune encephalomyelitis [70]. The evaluation of CdSe/ZnS-QDs (10, 20, and 40 mg/kg BW injection) on the male reproductive system at different stages of development was performed in 32 male mice (adult group) and 24 pregnant mice (embryo group) on day 8 of gestation, and no significant effect on fetal testis development and on adult groups at up 20 mg/kg BW was observed. However, at 40 mg/kg BW, a decrease in lamina propria, destruction in interstitial tissue, deformation of seminiferous tubules, and a reduction in number of spermatogonia, spermatocytes, and spermatids was observed [71–73]. Embryotoxicity following the injection CdTe-QDs on the 13th day of gestation in pregnant rats was assessed in 121 fetuses and via histological analysis of placentas. This study revealed dose-dependent survival rates of fetuses (Table 3) due to placental damage rather than QD penetration and accumulation in the fetuses [74], and an impairment on the first two generations of placenta growth [75].

Large vertebrates

Biomedical research in large animal models is rare, especially in non-human primates, which are unfortunately irreplaceable as models of certain diseases owing to their close similarity to humans.

In the cellular therapy field, assessment of cell migration and adhesion is of paramount importance. CdSe/ZnS-polymer-QDs were used to successfully label and assess the adhesion of goat adipose tissue-derived stem cells (g-ADSCs) after 30, 60, and 90 days of cell infusion in the goat mammary glands. The labeling did not affect cellularity and morphology, and was maintained long-term, even after freezing of liquid NO_2. The health status of goats following QD administration has not been specified. [76].

Both phospholipid micelle-encapsulated CdSe/CdS/ZnS-QDs (ph-QDs) [77] and F-127-encapsulated-silicon-QDs [78] were administrated to monkeys (25 and 200 mg/kg BW, respectively) via short intravenous transfusion (30 min). For both QDs, no signs of toxicity were clearly detected in terms of general conditions (body mass, physical, mental, and nutritional behavior), biological parameters (biochemical, no signs of infection or allergic or toxic reactions, liver, and renal). The histological analyses have been unchanged despite QD accumulation in kidney, liver, and spleen, indicating that the breakdown and QDs clearance is quite slow, unlike in mice in which there were signs of delayed and time-dependent increased liver damage (inflammation, proliferation of Kupffer cells, multifocal cholestasis, and spotty necrosis of hepatic cells). The fate of the two types of QDs could not be determined [77,78]. The injection of ph-QDs in pregnant monkeys at a gestational age of 100 days resulted in a miscarriage rate of 60% after having crossed the placenta from the mother to fetus and induced acute hepatocellular injury in the mother after a week of administration [79].

Concluding Remarks and Future Perspectives

The rapid expansion of the popularity of QDs has resulted in the publication of a large number of studies on the subject, with high variability in the structure and composition of the QDs studied, thus making synthesis and comparisons difficult (see Outstanding Questions). Nevertheless, 50% of the studies are based on CdSe/ZnS-polymer-QDs produced by the same supplier, which presents both an advantage (comparability of articles) and a limitation (fewer studies on the structure of other semiconductor QDs).

At the cellular level, overall toxic effects appear at a certain threshold concentration that differs according to the sensitivity of each cell type [109]. Stem cells appear to be more resistant under certain conditions than differentiated cells, and the presence of QDs does not seem to affect stem cell differentiation. Depending on the intended application (*in vitro* imaging, diagnostic,

or theranostic), it is essential to define the optimal concentration range to obtain desired effects and toxicity thresholds for each study, and not conclude, without due investigation, that QDs show global cytotoxicity. Interestingly, the results with innovative QD structures incorporating new materials are encouraging for safer use in medical imaging.

With regard to organs, the lungs, kidneys, and the liver are the most affected by toxicity at high concentrations due to QD accumulation. The involvement of these organs is globally observed for all nanoparticles, probably inherent to their size, rather than specifically for QDs [80]. As observed for a number of anticancer and antiretroviral drugs, accumulation in organs might be associated with potential toxicities, but preliminary precautions can be taken (improvement of hydration for nephrotoxic risk, addition of adjuvant protective organ) and does not necessarily constitute an obstacle to their use. Additional studies are needed to adjust QD concentrations according to their use. Surprisingly, the brain seems to be more resistant to the toxic effects of QDs; the same observations have been made at the cellular level, and revolutionary applications in neurodegenerative diseases are being explored.

Based on the observed negative effects on the ecosystem, QDs should be used with the same caution as chemotherapy treatments. The studies carried out on small and large animals are encouraging, taking into account the benefit/risk balance; however, animal studies on concrete cases of pathologies have yet to be conducted.

Regarding strategies for minimizing toxicity, in the case of stem cells, the addition of a shell reduces toxicity, especially by means of polymerization. Biocompatibility has been achieved by incorporating QDs in glass. For differentiated cells, the addition of a shell associated with polymerization is in some cases not sufficient; the use of QDs in amorphous form or the addition of adjuvant alleviates the effects. Other winning strategies such as functionalization or infiltration into silicone have minimized toxicity. Except for the pulmonary system, where the toxicity can be exacerbated due to factors such as genetic predisposition, the addition of shells

associated with polymerization is sufficient to limit the toxicity in the organs. In invertebrates, all cadmium-based QDs, even when combined with a shell, polymerization and functionalization, show toxicity. Only a change to another chemical element in another group reduces the toxicity. For small animals, the preferred means of toxicity minimization is the addition of a multishell. For large vertebrates, all minimization strategies have been successfully applied at once, and these QDs have been proven to be non-toxic, except in the case of pregnancy. Despite their toxicity, semiconductor QDs have been noted for their ability to respond to a wide range of applications. The toxicity of QDs can be exploited favorably to destroy cells that have lost their capacity to die (i.e., tumors) via a controlled toxicology strategy. A strategy of diffusion limitation can also be considered by playing on a galenic formulation without modifying the structure of the QD.

Before their potential is fully exploited in biological application, in-depth studies of pharmacokinetics, taking into account all routes of administration, are necessary and must be supplemented by regimens with repeated administrations. The fate of QDs in the body must be further clarified; particularly, the mechanism of elimination must be elucidated.

To reach clinical and industrial translation by establishing a pharmaceutical product for human clinical trials, close cooperation between different disciplines (chemist, physicists, biologists, pharmacists, pharmacologists, clinicians) and legal instances is paramount in order to take into account biological and environmental considerations and reglementary requirements (Figure 2). For this purpose, it is necessary to agree on a standardized and large-scale QDs preparation method with good and reproducible performance and to define a set of standard quality control for the characterization of QDs. However, between batches, there may always be a lack of reproducibility that should be overcome by additional controls and may be validated by functional tests. Another challenge is to ensure regulations to qualify the raw material in pharmaceutical grade; it is necessary to ensure the absence of heavy metals. However, heavy

401 metals are constituents of semiconductor QDs. Without demonstrating the effectiveness of the
402 toxicity minimization strategies or an argument in favor of controlled toxicology weighing the
403 benefits/risks for certain cases, accompanied by progress in legal regulation, the development
404 of a pharmaceutical preparation that is employable in the clinical setting cannot be envisaged.
405
406 Competing Financial Interests: The authors declare having no conflicts of interest related to this
407 paper.
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409
410

Box 1. Structure-activity relationship of QDs

A QD is a nanocrystal made of semiconductor materials consisting of a few million atoms (but only a small number of free electrons, ≤ 100) that is small enough to exhibit quantum mechanical properties [81]. QDs can be composed of metallic atoms (e.g. Ni, Co, Pt, Au) or mostly of semiconductor materials, such as elements of periodic group II – VI (CdTe, CdSe, CdS, ZnSe, ZnS, PbS, PbSe, SnTe) [82] or from III – V group such as In and Ga [83]. In addition, QDs can surround by shell often to reduce ion release and therefore, its toxicity [84]. QDs can also be functionalized by adding a terminal function such as - NH_2 or - COOH allowing their hydrophilization [85], polymers to reduce their toxicity or improve their absorption [86], peptides [87] or antibodies [88] to specifically target a biological element (Figure 1).

Box 2. Characterization of quantum dots

Initially, their optical properties are evaluated by UV-visible [89] and photoluminescence spectroscopy [90,91], which is a fast and nondestructive technique allowing for the excitation, absorption, and emission spectra used for calculation of quantum yield to be determined [92]. The band gap studies were determined by optical diffuse reflectance spectra measurement [93,94]. The size and morphology of QDs can be estimated by scanning or transmission electron microscopy [95], dynamic light scattering (DLS) [96,97], or mathematic approaches from absorption edges using Henglein empirical curve [98]. To define the functionalization, Fourier transformed infrared (FTIR) spectroscopic measurements can be used [99]. The analysis of structure and elemental composition can be performed by X-ray diffraction [83,100] and energy dispersive X-ray analysis [101,102], respectively. Non-destructive techniques such as Raman spectroscopy explore the confined electronic structure of QDs [103–105]. QDs dosage suspension can be achieved by high pressure liquid chromatography via size exclusion chromatography with a fluorimetric detector [106]. The metal ion content in final QDs was also

436 assayed by inductively coupled plasma atomic emission spectrometry [107], and QD behavior
437 was studied by electrochemical methods [108].
438
439

Glossary

Long-term transepithelial electrical resistance TEER: commonly used *in vitro* for the assessment of the integrity of the epithelial cell layers.

Quantum dots: The term quantum comes from these mechanical properties at the atomic and subatomic scale with zero-dimensional as a fixed point.

Stem cells: consist of three forms: (i) adult stem cells (ASC) also called stem cells specific to the tissues contained in the body (e.g., hematopoietic, epithelial, neuronal, mesenchymal), (ii) embryonic stem cells or (iii) embryonic germ cells.

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722 Figure legends

723

724 Figure 1: Schematic illustration of the structure-activity relationship of QDs. Since the
725 inorganic core is biologically inactive, a hydrophilization step is necessary for its use in
726 biological applications. In order to minimize core-induced toxicity, surface modification
727 strategies may be envisaged (i.e., a capping, a polymerization and a functionalization).

728

729 Figure 2: Impact of QDs toxicity including biological and environmental considerations and
730 reglementary requirements for QD use in human.

731

732

733 Table 1: Biological effects depending on the structure of QDs and the type of stem cells or differentiated cells.

Core material	Shell material	Functionalization/Encapsulation	Type of cell	Outcome	Reference
Stem cells					
CdTe-QDs	-	-	Epithelial	Toxicity	[6]
	-	-	Synoviocytes	Monitoring, toxicity	[18]
CdSe-QDs	-	-	Embryonic	Concentration dependent toxicity	[19]
	ZnS	-	Epithelial	Toxicity	[6]
		-	Embryonic	↙ toxicity	[19]
		-	Neuronal	Slight toxicity	[11]
		Anti-myc antibody	Adipocytes	Monitoring	[15]
		Anti-mortalin antibody	Chondrocytes	Monitoring	[16]
		Tat Peptide	Hematopoietic	Monitoring	[2]
	CdS/ZnS	-	Epithelial	± toxicity	[7,8]
	ZnS - polymer	-	Embryonic	↙ toxicity	[19]
		-	Neuronal	No toxicity under threshold detection	[10,11]
		SiSOX9	Chondrocytes	Monitoring	[17]
	-incorporated glass	-	Epithelial	No toxicity under threshold detection	[9]
Graphene-QDs	-	-	Neuronal	No toxicity under threshold detection	[10]
Differentiated cells					
CdTe-QDs	-	-	Endothelial	Toxicity	[21]
	-	-	Platelets	Toxicity	[4]
	-	-	Renal	Toxicity	[28]
	-	L-cysteine	Renal	Toxicity	[27]
CdSe-QDs	ZnS	-	Monocytes, neutrophils, granulocytes	Toxicity	[3]
		-	Lymphocytes	No toxicity under threshold detection	[3]
		-	Hepatocytes	Toxicity	[20]
		-	Urothelial	No toxicity under threshold detection	[29]
		Photoexcited	Multidrug-resistant bacteria	Bactericidal	[31]
		Anti-Ri7 antibody	Endothelial	Built-up	[25]
		Anti-p38 antibody infiltrated in porous silicon pores immobilized Egp38 antigen	Parasites	Detection	[30]
	ZnS - PEG	-	Endothelial	Built-up	[24]
Graphene-QDs	-	-	Hepatocytes	No toxicity under threshold detection	[22]

734 Table 2: Biological effects depending on the structure of QDs and the organs.

Core material	Shell material	Functionalization	Type of organ	Outcome	Reference
CdTe-QDs	-	-	kidney	Toxicity	[48,49]
	-	-	Intestine	Toxicity	[40]
	CdS	-	Liver	Monitoring	[39]
CdO-QDs	-	-	Lung	Toxicity	[44]
CdSe-QDs	ZnS	-	Brain	No toxicity under threshold detection, visualization	[36]
		-	Heart	Monitoring	[51]
		-	Bladder	No toxicity under threshold detection, no diffusion	[46]
		-	Lung	Toxicity	[45]
		-	Joint	No toxicity under threshold detection	[14]
		GSH	Vascular	Monitoring	[52]
		VEGFR2	Tumor	Detection	[42,43]
		Biotinylated phalloidin	Muscle	Monitoring	[51,53]
		Anti-CD47 antibody	Bladder	No toxicity under threshold detection, no diffusion	[50]
	ZnS - polymer	-	Skin	No penetration	[32-34]
		-	Lung	Toxicity	[41]
		-	Spleen	No toxicity under threshold detection	[41]
		Folic acid	Spleen	Built-up	[41]
Graphene-QDs	-	-	Skin	Therapy	[35]
	-	-	Brain	Therapy	[37-38]

735

736

737 Table 3: Biological effects depending on the structure of QDs and the animal species.

Core material	Shell material	Functionalization /Encapsulation	Animal species	Outcome	Reference
CdTe-QDs	-	-	Mice	Toxicity	[60]
	-	-	Pregnant rat	Dose-dependent embryotoxicity	[74-75]
	-	Quercetin complex	Rat	Therapy	[69]
	ZnS	-	Mice	Half-life = 12 – 14h	[64]
CdSe-QDs	ZnS or CdS	-	Drosophila	Toxicity	[59]
		-	Mice	Dose-dependent toxicity	[61]
		-	Rat	Toxicity	[65]
		-	Male reproductive system in mice	Dose-dependent toxicity	[71-73]
		(His6)-Cyclin E protein	Aquatic species	Monitoring	[58]
	ZnS - polymer	-	Aquatic species	Toxicity	[54-57]
		-	Goat	Monitoring	[76]
		Phospholipid micelle-encapsulated	Mice	No toxicity under threshold detection	[68]
	PbS/ZnS	-	Mice	No toxicity under threshold detection	[62;63]
		Phospholipid micelle-encapsulated	Primate	No toxicity under threshold detection	[77]
			Pregnant primate	Toxicity	[79]
InP-QDs	ZnS	-	Drosophila	No toxicity under threshold detection	[59]
Graphene-QDs	-	-	Rat	Therapy	[70]
Silicon-QDs	- polymer	-	Primate	No toxicity under threshold detection	[78]

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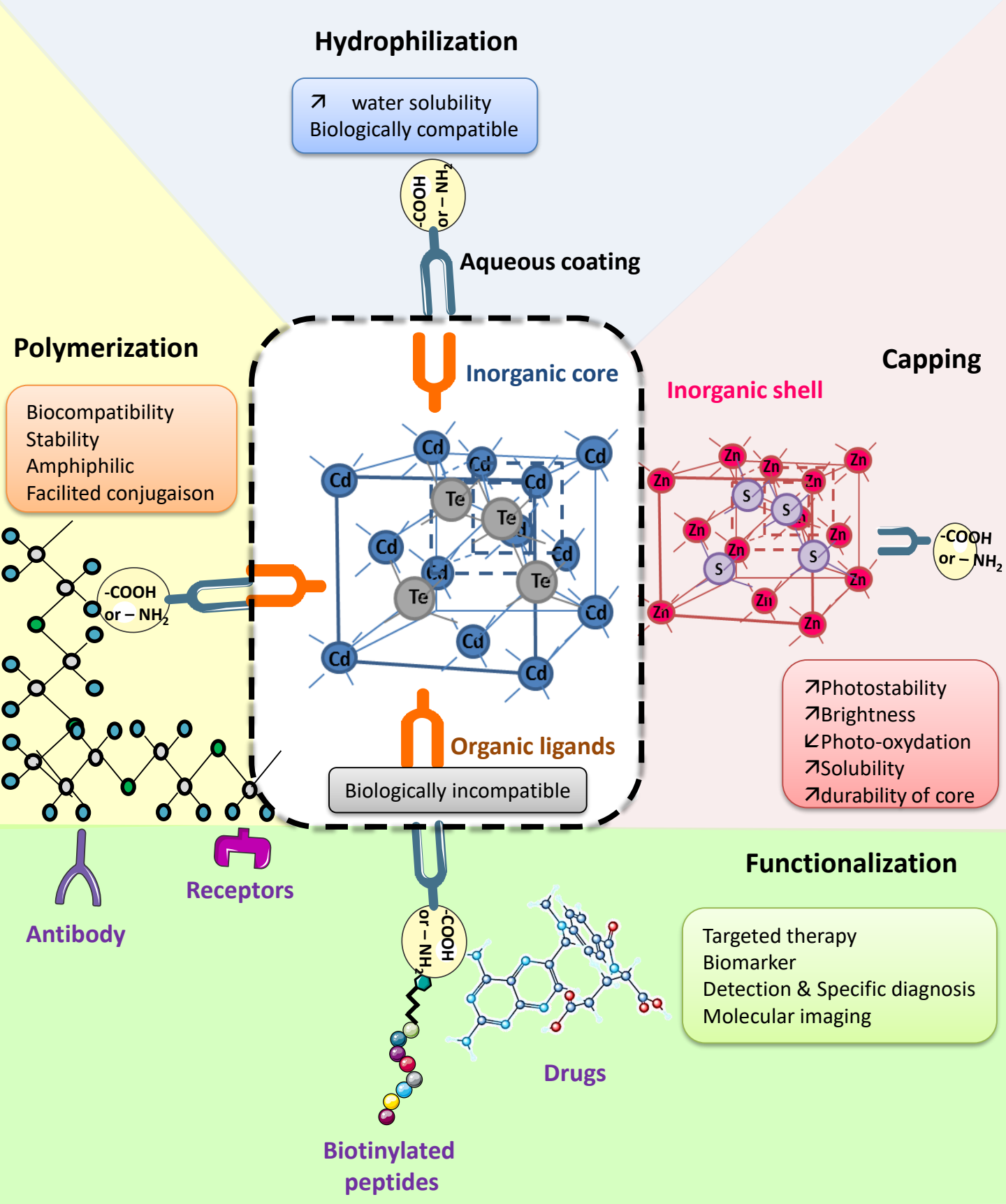
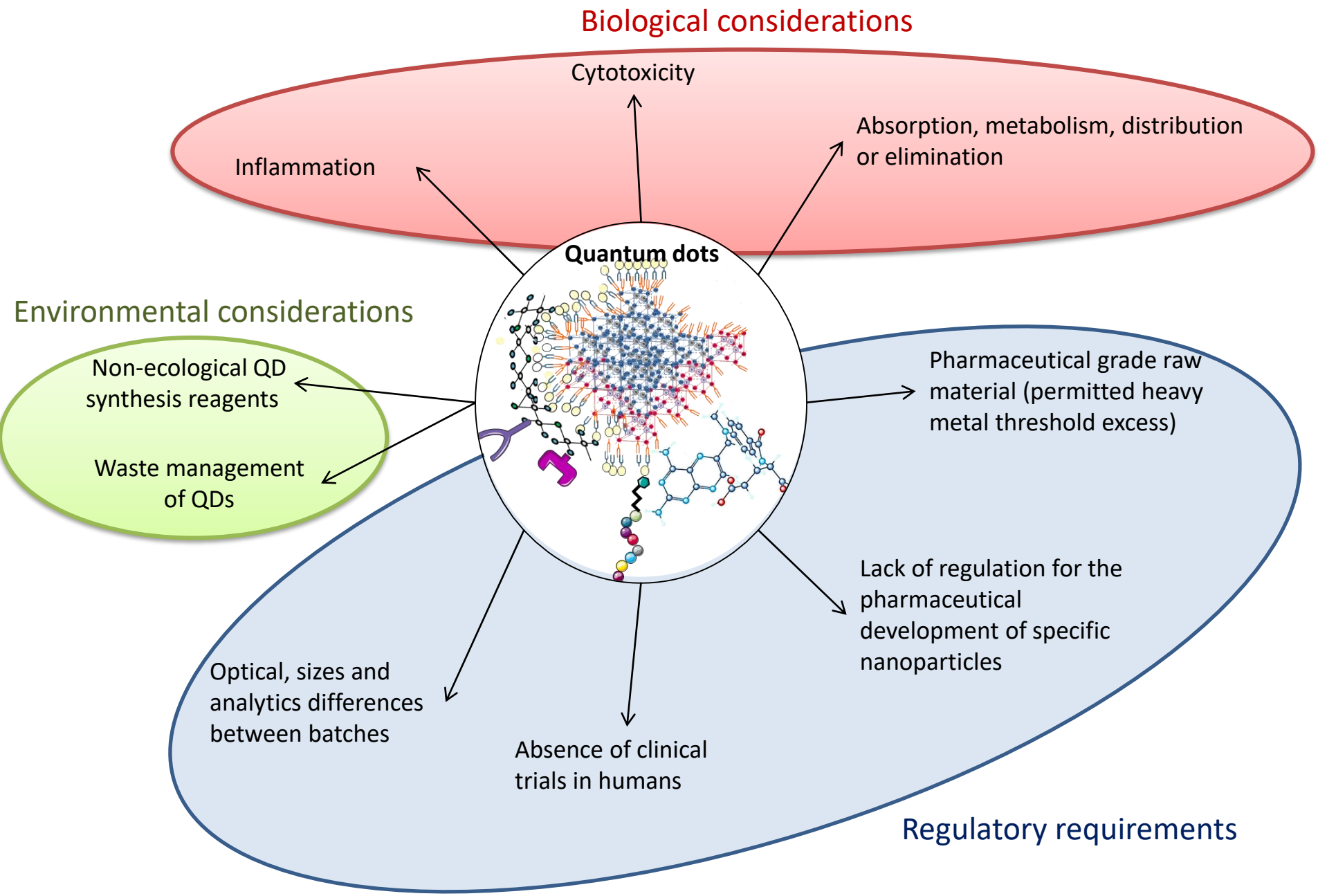

Hydrophilization
↗ water solubility
Biologically compatible
-COOH or – NH2
Aqueous coating
Polymerization
Biocompatibility
Stability
Amphiphilic
Facilited conjugaison
-COOH or – NH2
Inorganic core
Cd
Te
Organic ligands
Biologically incompatible
Capping
Inorganic shell
Zn
S
-COOH or – NH2
↗Photostability
↗Brightness
↙Photo-oxydation
↗Solubility
↗durability of core
Antibody
Receptors
Functionalization
-COOH or – NH2
Drugs
Targeted therapy
Biomarker
Detection & Specific diagnosis
Molecular imaging
Biotinylated peptides


Biological considerations
Cytotoxicity
Inflammation
Absorption, metabolism, distribution or elimination
Quantum dots
Environmental considerations
Non-ecological QD synthesis reagents
Waste management of QDs
Pharmaceutical grade raw material (permitted heavy metal threshold excess)
Lack of regulation for the pharmaceutical development of specific nanoparticles
Optical, sizes and analytics differences between batches
Absence of clinical trials in humans
Regulatory requirements