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# *In vivo* toxicity assessment of non-cadmium quantum dots in BALB/c mice

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**Abstract**

Along with widespread usage of QDs in electronic and biomedical industries, the likelihood of QDs exposure to the environment and humans is deemed to occur when the QD products are degraded or handled as waste for processing. To date, there are very few toxicological reports available in the literature for non-cadmium QDs in animal models. In this work, we studied the long term *in vivo* toxicity of InP/ZnS QDs in BALB/c mice. The biodistribution, body weight, hematology, blood biochemistry, and organ histology were determined at a very high dosage (25 mg/kg) of InP/ZnS QDs over 84 days period. Our results manifested that the QDs formulation did not result in observable toxicity *in vivo* within the evaluation period, thereby suggesting that the InP/ZnS QDs can be utilized as optical probes or nanocarrier for selected *in vivo* biological applications when an optimized dosage is employed.

***From the Clinical Editor:*** This study investigated the toxicity of quantum dots in BALB/c mice, and concluded that no organotoxicity was detectable despite of using high concentration of InP/ZnS quantum dots with prolonged exposure of 3 months.


*Key words:* Biodistribution; Quantum dots; Toxicity; Indium phosphide

Quantum dots (QDs) are semiconductor nanocrystals which consist of semiconductor materials such as CdTe, CdSe, CdS, and PbS.[1,2] In comparison to traditional organic fluorophores, QDs provide many attractive optical properties, such as size-tunable fluorescence, high quantum yield of fluorescence, broad excitation spectra and narrow emission spectra.[2] In the last decade, hundreds of kilograms of QDs have been produced each year and they are widely used in biological labeling and diagnostics, solar harvesting, optical sensing, imaging, and lighting applications.[3-7] Thus, there is great concern about the exposure of QDs to the environmental and humans and whether such exposure will cause harmful impact to the nature and human health. To shed light on the impact of QDs to biological systems, the QDs community has started to evaluate the toxicity of QD formulations using various kinds of *in vitro* and *in vivo* models such as cell lines, mice, rat, pig, and non-human primates.[8,9]

Collectively speaking, majority of the QD cytotoxicity studies is focused on cadmium-based nanocrystals[10-12] and very little information can be obtained for other types of QDs such as PbSe, PbS, InAs and InP. In practice, the cytotoxicity assessment parameters of QDs are focused on the basis of their chemical and physical parameters, such as their inherent chemical composition, shape, size, and surface charge.[10,13] So far, most of the reported QD toxicity is still relying on cell culture models such as human embryonic kidney HEK293 cells,[14]

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human keratinocytes HaCaT cells[15] and other types of cells.[16,17] A fraction of these *in vitro* studies has demonstrated that QDs are showing harmful effects on the cells when the QDs are degrading in the biological environment and such observations have initiated a serious debate about whether or not to pursue the translation of QDs into clinical research and applications. Sung et al showed that long-term exposure to CdTe QDs caused functional impairments in live MCF-7 cells and this induced cell death via mechanisms involving both the released of $Cd^{2+}$ and ROS generation from the QDs accompanied by lysosomal enlargement and intracellular redistribution.[18] Similarly, Li et al also found that unmodified CdS QDs induced cytotoxicity by mechanisms through intracellular ROS production, GSH depletion, and cadmium ions ($Cd^{2+}$) released from the particles.[19] Tang et al showed that unmodified CdSe QDs can promote the elevation of cytoplasmic calcium levels in primary rat hippocampal neuros, involving both extracellular $Ca^{2+}$ influx and internal $Ca^{2+}$ release.[20] This may cause interruption to the neuron system. Angela et al showed that CdTe QDs caused cytotoxicity by upregulating the Fas receptor and lipid peroxidation in the cells that lead to impaired neuroblastoma cell functions.[21] All these studies pointed out that careful surface passivation of QDs play an important role in lowering the possibility of the breakdown of the QDs. In comparison to the reported QDs cytotoxicity, the *in vivo* toxicity studies showed an opposite trend when the QD formulations were injected to the small animals. For example, Tanya et al performed a systematic animal toxicity study of CdSe/ZnS core/shell QDs in Sprague–Dawley rats and they showed that the QD formulations did not cause appreciable toxicity even after 80 days of QDs treatment to the animals.[22] Su et al studied the *in vivo* distribution, pharmacokinetics, and toxicity of aqueous synthesized CdTe QDs in female BALB/c mice and demonstrated no overt histological and biochemical changes in mice after long exposure time.[23] Jorina et al conducted an acute *in vivo* toxicity study with commercially available CdSe/ZnS QDs with different surface modifications and found that QDs at high doses caused pulmonary vascular thrombosis and this was possibly due to the activation of coagulation cascade.[24]

In our previous work, we performed the toxicity assessment of phospholipid micelle encapsulated CdSe/CdS/ZnS in Kunming mice and in nonhuman primates.[25] The results show that the phospholipid micelle-encapsulated QDs formulation did not result in noticeable toxicity for both animal models. Serum analysis has shown no changes in the treated mice or monkeys when compared to the treated ones with PBS buffer solution. Furthermore, histological analysis of tissues from major organs demonstrated that there are no acute toxic effects of the QDs in mice and monkeys. Nonetheless, fluorescence imaging and chemical analysis confirmed that majority of the QDs remained in the liver and spleen after 3 to 4 months of treatment.

InP QDs have appeared to be a less hazardous nanocrystal when compared to cadmium-based nanoparticles since they have greater degree of covalent bonding, comparing to those made up of group II-VI elements. However, their cytotoxicity has not been well investigated and examined; detailed studies are needed if the QDs community would want to pursue them for clinical use. Chibli et al reported that they have found a considerable amount of superoxide and a small amount of hydroxyl radical formed under visible illumination of InP/ZnS QDs with a single ZnS shell. The authors demonstrated that a double thickness shell coating on the InP QD will reduce the reactive oxygen species concentration by at least two folds. Survival assays in various cell lines suggested that a significant reduction of toxicity was observed when the thicker shell of InP QDs was used in the testing. This study indicates that InP QDs can be served as a useful alternative to replace cadmium-based QDs for *in vivo* applications. Nevertheless, it is crucial for the QDs community to evaluate the InP QD formulation in detail before they can be safely applied in clinical setting. To our knowledge, very limited data related to *in vivo* toxicity of InP QDs are available in the literature. Thus, there is an urgent need to understand the long term *in vivo* toxicity of InP QDs.

In this work, we systematically study the long term *in vivo* toxicity of InP/ZnS QDs in BALB/c mice. The biodistribution, body weight, hematology, blood biochemistry, and organ histology were determined at a relatively high concentration (25 mg/kg) of InP/ZnS QDs over a period of 84 days (12 weeks). We found that InP/ZnS QDs were accumulated in spleen and liver over 84 days post-injection and accumulation of indium element from injected QDs still remained at major organs even after 84 days of injection. No weight changes were observed for the mice treated with InP/ZnS QDs formulation. Hematological and biochemical analysis has shown no changes in treated mice when compared to the ones treated with phosphate buffer saline (PBS) solution. Histological analysis of tissues from major organs indicated that there are no acute toxic effects of the QDs on the mice *in vivo*. These results suggest that InP/ZnS QDs can serve as optical probes for specific clinical applications.

## Methods

### *Preparation and characterization of InP/ZnS QDs*

InP/ZnS quantum dots (trace tristrimethylsilylphosphine and oleylamine as ligands on the surface) were purchased from NN-Labs, LLC (AR Fayetteville). The as received QDs were dispersed in toluene. To enable aqueous dispersion PEGylated phospholipid encapsulation was performed. Briefly, the InP/ZnS QDs were washed with ethanol and re-dispersed in chloroform. The InP/ZnS QDs were then mixed with mPEG-DSPE (Laysan Bio MW 5000) at a weight ratio of 1:5 in chloroform. The solvent was then evaporated with a rotary evaporator under vacuum where the sample flask was immersed in water bath at room temperature. The resulted lipidic film was dispersed with PBS buffer assisted by ultrasound sonication. Possible aggregations in the solution were removed by a 0.2 μm syringe filter. The final product was stored at 4 °C and covered with aluminum foil to avoid light exposure. For sample characterization, a Shimadzu model UV-2450 spectrophotometer and a Fluorolog-3 Spectrofluorometer (NJ Edison) were used to get the extinction and photoluminescence spectra, respectively. The QD sample was loaded into a quartz cuvette for measurements using PBS buffer as reference. The hydrodynamic size distribution and zeta potential of the QD formulation were analyzed using the particle size analyzer (Brookhaven Instruments 90Plus). Transmission Electron Microscopy (TEM) images were obtained using a JEOL model JEM 2010 microscope with an acceleration voltage of 200 kV. The specimens were

prepared by drop-casting the sample dispersions onto carbon coated 300 mesh copper grids; then the excess solvent was absorbed by a filter paper underneath. All measurements were carried out at room temperature.

*Animal injection and weight measurements*

BALB/c mice were obtained from the Medical Laboratory Animal Center of Guangdong Province and handled with protocols approved by the Laboratory Animals Center of Shenzhen University. All the animal experiments and maintenance were approved by the Laboratory Animal Ethics Committee of Shenzhen University. BALB/c mice were obtained at 8 weeks of age and were housed 5 per cage in a 12 h/12 h light/dark cycle. All animals were fed with water and standard laboratory chow. The mice were injected with 200 μL of buffer saline or buffered QD dispersion. At different periods after injection (0 to 84 days), the mice were weighed and evaluated for behavioral changes.

*Frozen section and histology*

After the sacrifice, the major organs (heart, liver, spleen, lung, kidney, brain) of the mice are harvested. Frozen sections from these organs were made using a freezing microtome (Leica cm3050). The distribution of InP/ZnS QDs was observed under a fluorescent microscope (Olympus BX51). For histopathological analysis, the mouse organs are embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E staining). The H&E staining slices are examined under a light microscopy (Olympus BX51) by a clinical pathologist.

*Inductively coupled plasma mass spectrometry (ICP-MS) analysis*

Blood and tissue samples were collected from animals and kept at −80 °C. Before ICP-MS analysis, the samples were digested with 6 ml 65% $HNO_3$ and 2 ml 30% $H_2O_2$ at 200 °C for 30 min by a microwave acid digestion apparatus (Milestone ETHOS ONE). After digestion, the solution was diluted 10 times with deionized water in order to reduce the concentration of $HNO_3$. Then the standard elemental concentrations of indium were using an ICP-MS system (Agilent 7500C1)

*Blood routine examination and serum biochemistry*

Blood samples are harvested from mice injected with InP/ZnS QDs for 1 day, 3 days, 5 days, 7 days, 14 days, 28 days, 56 days and 84 days and mice injected with saline buffer. Blood is collected from the orbital sinus by removing the eyeball from the socket quickly. Blood routine examination is performed using the whole blood by a routine blood test instrument (Mindray RJ-0C107223), and indicators for kidney and liver function are measured using serum by a blood biochemistry analyzer (Mindray BS-220).

## Results

Figure 1, *A* shows the extinction and photoluminescence spectra of the aqueous dispersion of InP/ZnS QDs. The QDs dispersion showed an emission peak at 627 nm. The inset of Figure 1, *A* shows the TEM image of the QDs. The QDs have an average size of around 5-6 nm and are moderately monodispersed. Dynamic light scattering was used to evaluate the hydrodynamic diameter of the InP/ZnS QDs. The average hydrodynamic diameter of QDs was about 58.0 ± 1.83 nm (Figure 1, *B*) and the zeta potential of the formulation was determined to be around −8.38 ± 0.07 mV. No aggregation was observed when they were injected into the mice. In this study, 80 BALB/c mice were used (40 mice for the treated group and 40 for the control group). For the treated/control group, the mice received either 25 mg $kg^{-1}$ of QD/PBS or buffer solution alone via tail vein injection, respectively. The body weight was continuously monitored to investigate the long-term toxic effects of InP/ZnS QDs in mice. As shown in Figure 1, *C*, no significant differences were observed in the average body weight between the treated group and control group for 84 days after intravenous injection of InP/ZnS QDs. This suggests that the InP/ZnS QDs were well tolerable in mice. In addition, no changes in eating, drinking and physical features (e.g. hair color and glossiness) were observed in the mice treated with QDs. These results suggest that the InP/ZnS QDs formulation is highly biocompatible for our test subjects.

In order to investigate the distribution of QD formulations in BALB/c mice, their major organs (heart, liver, spleen, lung, kidney, brain) were removed for fluorescence imaging at different time points after mice were intravenously injected with 25 mg/kg of QDs. Our results showed that very strong fluorescence emitted by QDs was observed in the liver and spleen even after 84 days of injection (Figure 2), but only little or almost no fluorescence occurred in the heart, lung, kidney and brain (data not shown). These results manifest that these majority of QDs were taken up by the spleen and the liver and remain intact in these two organs with sustainable fluorescence emitting ability. To further quantify the accumulation and biodistribution of InP QDs, the concentrations of indium in blood and tissues from QDs were measured by ICP-MS analysis. Figure 3, *B* shows the indium concentration in blood circulation at 0.5 h, 1 h, 2 h, 4 h, 6 h and 24 h after injection. At 0.5 h, the indium concentration was 1.809 ± 0.359 μg/ml, but at 6 h, the indium concentration decreased to 0.0726 ± 0.0177 μg/ml. The blood clearance profile revealed that the QDs can be cleared from the blood quickly. Figure 3, *C* shows the indium concentration in major organs. Indium element cannot be detectable in tissues in untreated animals. Indium element form QDs had predominantly accumulated in the spleen and liver, where the indium concentrations on Day 3 after injection were 12.506 ± 2.492 μg/g and 3.521 ± 0.852 μg/g, respectively. In addition, indium element also can be detected in kidney, lung, heart and brain, where the indium concentrations on Day 3 were 0.427 ± 0.131 μg/g, 0.175 ± 0.038 μg/g, 0.048 ± 0.017 μg/g and 0.006 ± 0.002 μg/g, respectively. Yet as time passed, the indium concentration in spleen, liver and kidney decreased gradually. But even on Day 84, the indium component still obviously accumulated in the spleen, liver and kidney, where the indium concentrations were 2.915 ± 0.728 μg/g, 0.482 ± 0.051 μg/g and 0.046 ± 0.011 μg/g, respectively. These results suggest that the injected indium remains in the animals' major organs after 84 days.

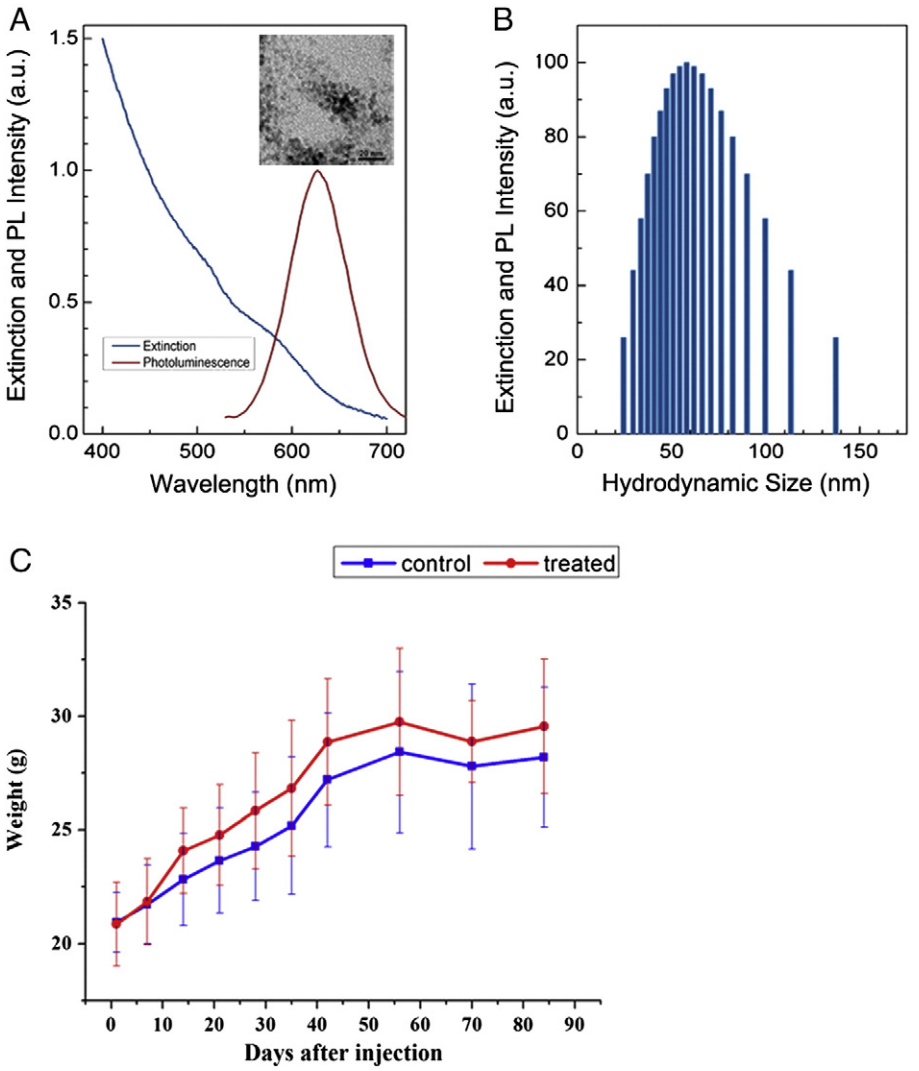


Figure 1. Characterization of PEGylated phospholipid encapsulated InP/ZnS QDs. **(A)** Extinction and photoluminescence (PL) spectra; inset is a TEM image of InP/ZnS QDs, with an average size of 5-6 nm. **(B)** Hydrodynamic size distribution. **(C)** The weights of mice were monitored after the injection of QD/buffer solution.

To further evaluate the toxicity of QDs *in vivo*, we performed the blood routine examination for the animals treated with either QDs or PBS buffer solution. When QDs are intravenously administered into the body, the first physiological system they interact with is the blood fluids and their respective components (e.g. blood cells and hemoglobin). QDs are particulate materials within the size range of large proteins and viruses, and consequently they may arouse an inflammatory response and influence the activity of the immune system, which will alter the related hematological factors such as white blood cell count.[26,27] In addition, the degradation of QDs surface coatings or the exposure of the core material may occur and could lead to the changes in hematology indicators such as red blood cells or hemoglobin.[28] Therefore, it is crucial for us to determine the standard hematology markers such as quantity change or shape distribution of the blood cells and components for evaluating the impact of InP/ZnS QDs in the body. In our work, the major biomarkers including red blood cell count (RBC); white blood cell count (WBC); Platelet (Plt); hematocrit (Hct); Hemoglobin (Hb); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); mean platelet volume (MPV); mean Corpuscular Volume (MCV); platelet distribution width (PDW); coefficient of variation of RBC volume distributing width (RDW-CV); and standard deviation of RBC volume distributing width (RDW-SD) were monitored over the 84 day period. The results showed that all measured factors were within normal ranges (Figure 4) and did not indicate any adverse trend associated with QDs treatment. This suggests that InP/ZnS QDs formulation does not promote any abnormalities of the blood cells and components in the blood fluids.

Once the QDs are cleared from the blood stream, they will migrate to the liver and kidneys. In most cases, exogenous substances are metabolized and cleared mainly through liver and kidney. We hypothesize that the overloading of foreign substances in liver and kidney may possibly cause function damage to these organs. We thus try to investigate and understand the impact of QDs accumulation in liver and kidney. Some questions that we would like to answer through this

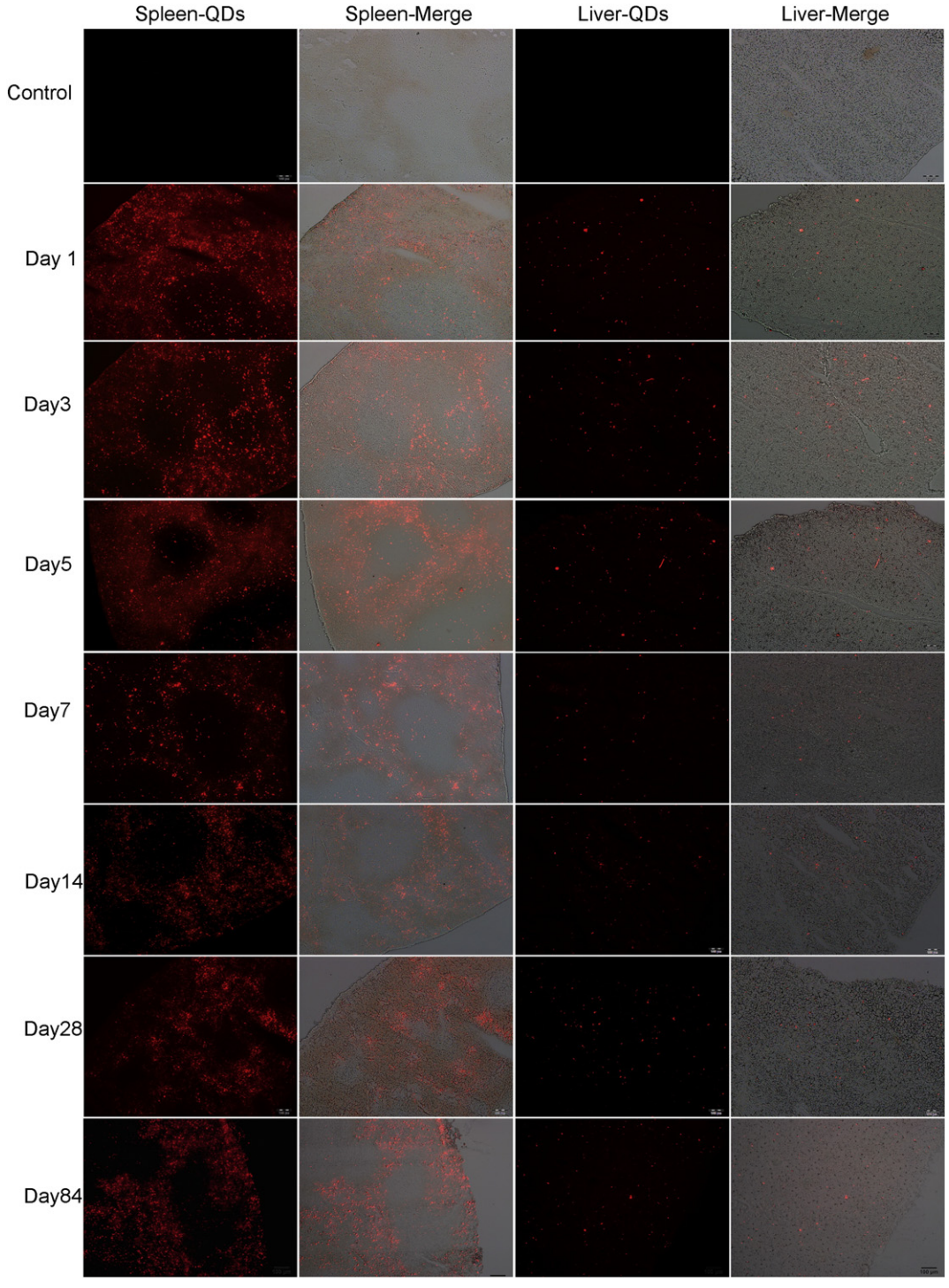


Figure 2. Fluorescence images of spleen and liver of the treated mice at different time points.

experiment are: whether the QDs will degrade in liver and kidney? If the QDs do degrade, what kind of impact will it cause to the systems? Lastly, we would also like to know whether the QDs are able to be excreted from the body. By measuring a variety of factors in the serum, it is possible to assess liver and kidney function, hepatocellular injury, and cholestasis. In this work, the representative serum biomarkers including the ratio of albumin and globulin (A/G), albumin (ALB); alkaline phosphatase (ALP); alanine transaminase (ALT); aspartate transaminase (AST); creatinine (CREA); globulin (GLB); glucose (GLU); lactate dehydrogenase (LDH); triglyceride (TG); total protein (TP) and uric acid (UA) were determined for mice treated with InP/ZnS QDs dispersion over the 84 day period. The results are shown in Figure 5 and demonstrate that no significant toxicity symptom is observed for liver and kidney. More importantly, this experiment confirmed that majority of the QDs are remained

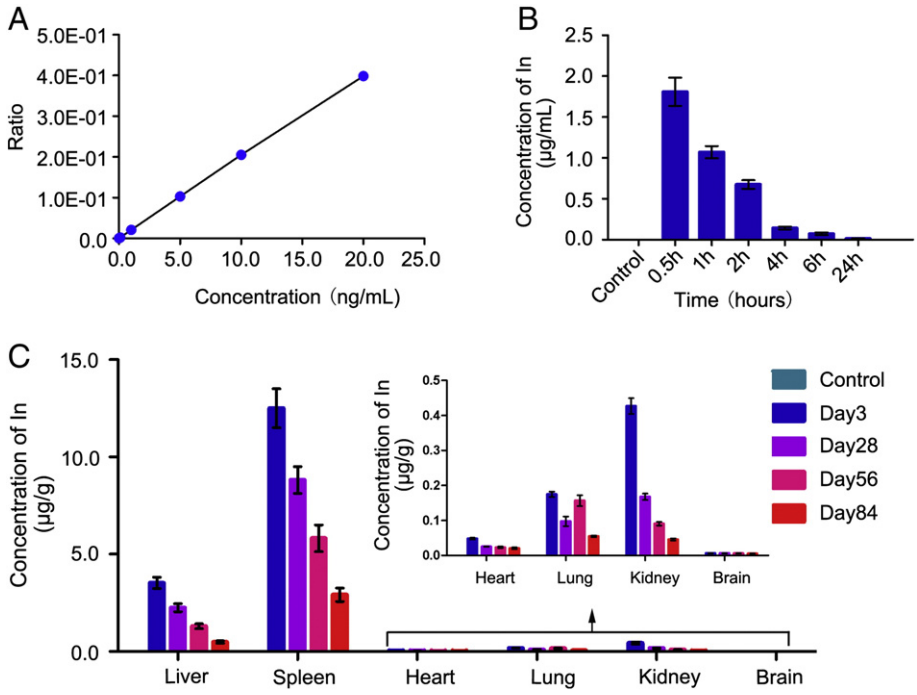


Figure 3. ICP-MS analysis of the blood samples and the major organs of treated mice. **(A)** Standard curve of indium determined by ICP-MS analysis. **(B)** ICP-MS analysis of blood samples at 0.5 h, 1 h, 2 h, 4 h, 6 h, and 24 h post-injection of quantum dots. **(C)** *In vivo* biodistribution of indium in Liver, Spleen, Heart, Kidney, Lung and Brain at indicated time points. Values are the means ± SD, n = 3.

intact in the mice body for more than 84 days but they are not able to excrete from the mice body.

In addition to hematological and biochemical markers investigation, hematoxylin and eosin (H&E) staining of the heart, liver, spleen, lung, kidney, and brain tissue sections was performed to observe the histological changes at different days post-injection of the InP/ZnS QDs. As shown in Figure 6, there were no apparent histopathological abnormalities related to treatment of these animals with the InP/ZnS QDs. Although accumulation of QDs in the frozen sections of spleen and liver existed even 84 days after injection, no pathological injury in the structure of the spleen and liver was observed. These results indicate that the InP/ZnS QDs formulation has low tissue toxicity.

## Discussion

Despite the promising application of QDs in industrial and medicinal fields, concerns have been raised about their possible adverse health effects and toxicity. The toxicity assessment of nanomaterials can be conducted by both *in vitro* model and *in vivo* system. To date, the majority of toxicity investigations related to QDs are performed using *in vitro* cell models.[29] Despite these *in vitro* studies, there are few toxicological reports of QDs using animal model,[22-25] which is a more preferable system to use for the toxicological evaluation of a nanomaterial since the biological aspects of the animal model are very similar to human biology.[30] In general, the *in vitro* models cannot simulate the complexity of an *in vivo* system or provide meaningful information about the response of a physiological system to a foreign substance. For example, Sayes demonstrated a lack of correlation when comparing the data between *in vitro* and *in vivo* toxicity assessments for fullerenes.[31] Similarly, for carbon nanotubes, Manna et al reported *in vitro* toxicity in Human Keratinocytes,[32] whereas Schipper et al found no significant toxicity in mice.[33] Toxicity *in vivo* depends on many factors such as dosage, methods of administration, metabolism, excretion, and immune response of the animal models. The toxicological profiles of QDs might also be influenced by inherent chemical constitution, size, shape, charge distribution, aggregation, and surface modification. Here, we demonstrated a systematic framework to study the long term *in vivo* toxicity of InP/ZnS QDs at concentrations as high as 25 mg/kg with the average size of 58.0 nm and the zeta potential of −8.38 mV. This experimental model could be applied to other engineered nanoparticles and a continuation of this study would result in a complete toxicological assessment of QDs.

The biodistribution of QDs and their corresponding released concentrations of heavy metals are important key parameters for evaluating their toxicity *in vivo*. Several studies have reported the biodistribution of different types of cadmium-based QD formulations in mice and rat models. Fitzpatrick et al reported that the fluorescence of CdSe/ZnS QDs was able to be detected in spleen, liver and lymph nodes of BALB/c and nude mice even after two years of injection and these experiments were carried out by using both whole-body and microscopy fluorescence techniques.[34] Recently, our group reported the QD toxicity in non-human primate, and our results revealed that most of the initial dose of cadmium remained in the liver, spleen and kidneys after 90 days.[25] Consistent with this study, we found that the

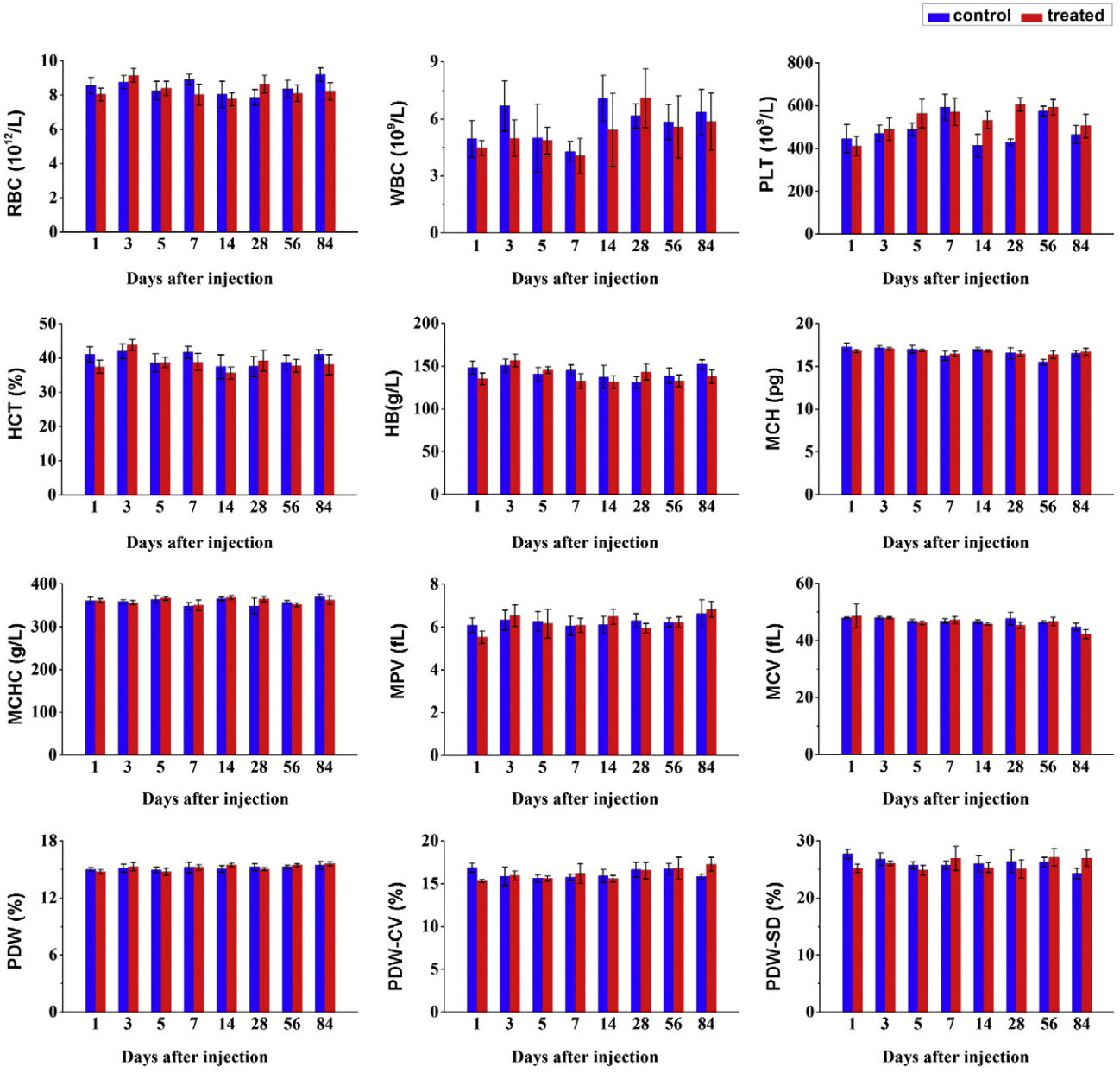


Figure 4. Hematology markers were monitored over the 84-day period of treatment. Abbreviations: red blood cell count, RBC; white blood cell count, WBC; Platelet, Plt; hematocrit, Hct; Hemoglobin, Hb; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; mean platelet volume, MPV; mean Corpuscular Volume, MCV; platelet distribution width, PDW; coefficient of variation of RBC volume distributing width, RDW-CV; standard deviation of RBC volume distributing width, RDW-SD.

fluorescence of InP/ZnS QDs injected into BALB/c can be detected in the liver and spleen for up to 12 weeks post injection. Contrary to our findings, Choi et al showed that no accumulation of QDs was observed in the liver and kidney of Sprague–Dawley (SD) male rats and CD-1 male mice post-injection with ~5.5 nm of QDs.[35] Choi et al suggested that the hydrodynamic size of the particles plays an important role in the excretion process in the body. In their case, they explained that the majority of the QDs were removed by renal excretion since the particles size is within the kidney filtration size limit. Su et al also reported that the biodistribution trend of QDs is size-dependent and they have discovered that aqueous synthesized QDs with larger sizes were found to be accumulated in the spleen instead of kidney.[23] The surface coating of the QDs is another key factor in influencing the *in vivo* biodistribution and toxicity of QDs.[36] For instance, if high positively charged QDs are used for *in vivo* testing, they may cause disruption in the blood serum components since they can interact with the slightly negative charged proteins, amino acid, peptide, etc. in the blood. Also, it is worth mentioning that, sometimes, not being able to observe fluorescence of QDs from the organs (tissue sections or whole body imaging) of treated mice and rats does not mean that the components of QDs have

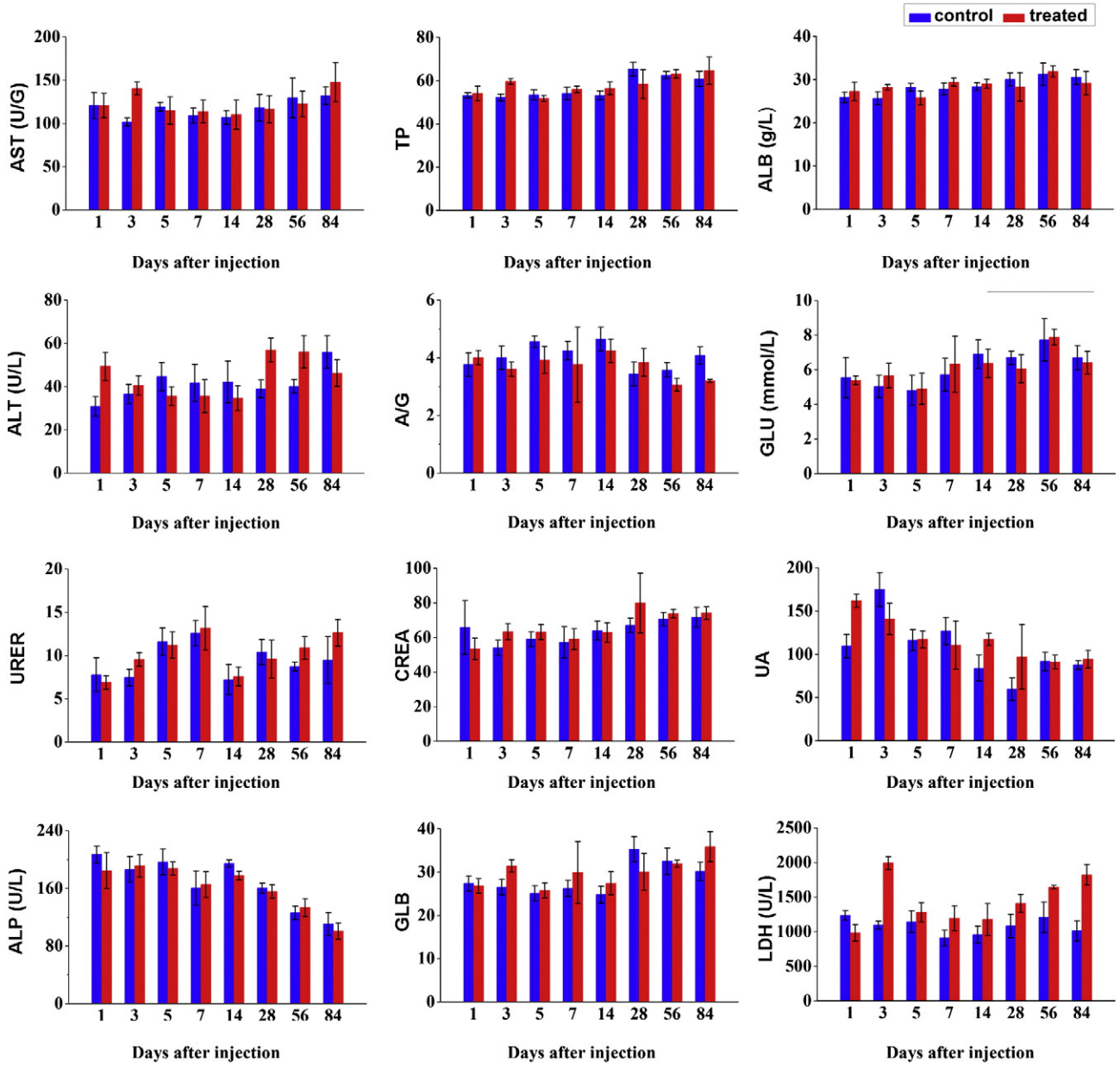


Figure 5. The serum biochemical indicators were measured over the 84-day period of treatment. Abbreviations: the ratio of albumin and globulin, A/G; albumin, ALB; alkaline phosphatase, ALP; alanine transaminase, ALT; aspartate transaminase, AST; creatinine, CREA; globulin, GLB; glucose, GLU; lactate dehydrogenase, LDH; triglyceride, TG; total protein, TP; uric acid, UA.

been removed from the body. In some cases, very likely, their fluorescence is quenched because they have been broken. To address this issue, further investigations such as ICP-MS analysis are needed to monitor the concentration of heavy metal that remained in the organs. In this study, our results show that indium element accumulated in major organs besides spleen and kidney.

A series of mechanisms is available in the body to encounter invasion from many foreign substances. These include delivering of foreign substances to body tissues for distribution or degradation process; biotransformation of the substances mediated by enzymes located primarily, but not exclusively, in liver microsomes; and excretion via the kidneys, biliary tract, lungs, secretory glands and other organs.[37] Many foreign substances are capable of evoking some degree of liver and kidney injury because liver plays the central role in biotransformation and kidney is the major organ for excretion of foreign compounds in the body. Several factors such as size, chemical composition, surface coating, solubility, shape, and aggregation are responsible for the biological impacts and excretion of nanoparticles via kidney.[26] These parameters interfere with the clearance of nanoparticles from the body by influencing foreign nanoparticles cellular uptake, protein binding and distribution in the cells.[38] In this study, we found that the major fraction of InP QDs was accumulated in the liver and spleen for over 12 weeks

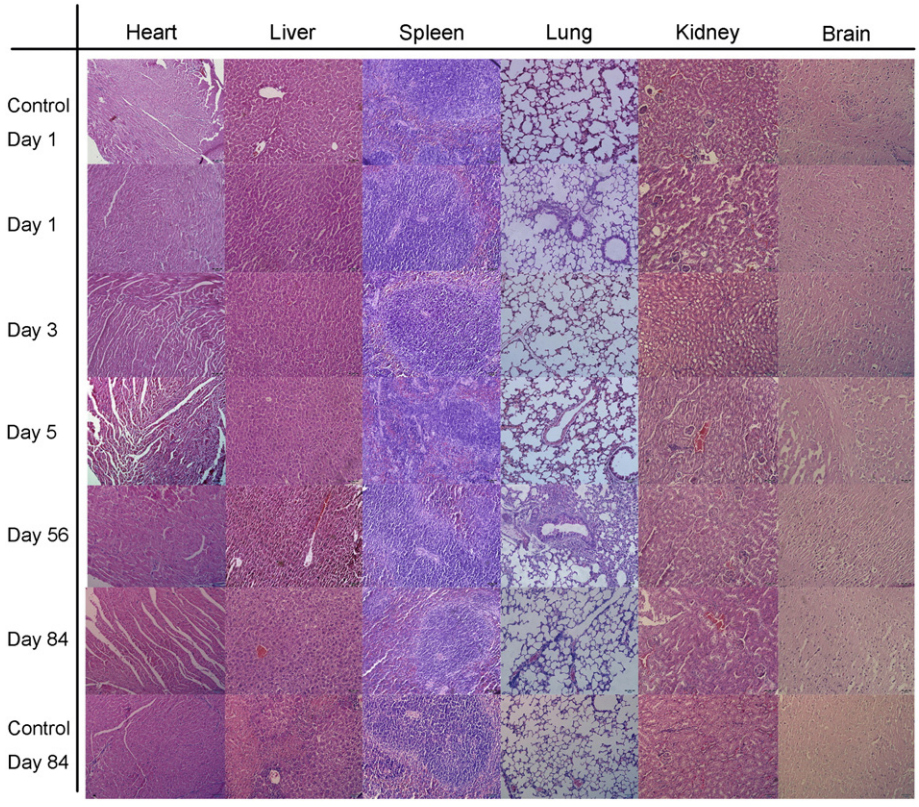


Figure 6. Representative histological images from the major organs of the mice at different time points post intravenous injection of the InP/ZnS QDs, compared with the untreated control at day 1 and day 84.

without being cleared by the kidney. These particles stayed intact in these organs and without causing any injuries or inflammation to liver, kidney and spleen tissues as demonstrated by our histological analysis. Similarly, Lin et al and Yang et al reported the continued dosage increase of the QD705 nanocrystals in the liver, kidney, and spleen between 1 and 28 days without any appreciable excretion or metabolism from the body 28 days post-injection.[39,40] This indicates that majority of our prepared QDs are well protected by their surface coating and only a small fraction of QDs degradation is observed within the examination period as demonstrated in the ICP-MS study. In general, if breakdown of QDs takes place, this will generate surface trap states on the particle thereby affecting the electron-hole recombination process and leading to poor fluorescence intensity. However, such scenario is not happening in liver and spleen as we are still being able to observe high fluorescence intensity of QDs even after 12 weeks in the body. Another reason that the InP nanocrystals are not prone to degrade in the biological environment might be due to their chemical bonding in crystal form. Commonly, it is known that III-V semiconductor nanocrystals have better structural robustness when compared to II-VI semiconductors. This results from the greater degree of covalent bonding in the III-V semiconductors such as InP, compared to those made up of group II-VI elements such as CdSe and CdTe. Such structural robustness of InP nanocrystals will improve their optical stability and minimize their possibility of breaking down and releasing heavy metals to the biological systems.

According to the studies above, we suggest that the hydrodynamic diameter and surface coating of InP QDs are crucial parameters to be considered for *in vivo* applications. For example, if one wants to employ them for targeted imaging and excrete from the body after performing their tasks, one can engineer ultra-small InP QDs with hydrodynamic diameter less than 5.5 nm for such usage. One the other hand, if excretion of particles is not the primary considered factor in the *in vivo* application, for instance, traceable drug delivery therapy of deadly illnesses, one can prepare large micelle nanoparticles that contain QDs, drugs, and targeting moieties for achieving the objectives. Overall, we view InP QDs as an excellent candidate of optical contrast agents for future nanoimaging and targeted drug delivery applications.

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