

Chapter 2

Cytotoxicity of Photoactive Nanoparticles

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Abstract This chapter describes the cytotoxicity of photoactive materials (specifically, quantum dots, noble metal nanoparticles (including gold and silver), and fluorescent silica nanoparticles). A thorough representation of in vitro and in vivo toxicity studies is presented. Since the toxicity on photoactive nanomaterials described in this chapter has developed rapidly and has attracted a great amount of interest, it is expected that many novel developments and applications of photoactive nanomaterials will ensue in the near future.

Abbreviations

Ag NPs:	Silver nanoparticles;
Au NPs:	Gold nanoparticles;
BSA:	Bovine serum albumin;
CdSe:	Cadmium selenide;
CdTe:	Cadmium telluride;
EC ₅₀ :	Effective concentration;
GSH:	Glutathione;
LC ₅₀ :	Lethal concentration;
MMP:	Mitochondrial membrane potential;
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;
MPA:	Mercaptopropionic acid;
MUA:	Mercapto-undecanoic acid;
NAC:	N-Acetylcysteine;
NPs:	Nanoparticles;
PEG:	Polyethylene glycol;
QDs:	Quantum dots;
ROS:	Reactive oxygen species;

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Silica NPs: Silica nanoparticles;
SOPC: Phosphatidylcholine;
SOPS: Phosphatidylserine;
ZnS: Zinc sulfide.

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2.1 Introduction

Photoactive nanomaterials are one of the most important nanomaterials for functioning as highly sensitive labeling reagents in various bioapplications (Cao et al. 2001; Dubertret et al. 2002; Zhao et al. 2003a, 2004; Liang et al. 2005). Current developments have demonstrated that photoactive nanomaterials provide significant strong and photostable optical signals reporting the presence of trace amounts of analytes. In comparison with traditional optical labeling techniques, which usually use fluorescent molecules to signal target analytes, the photoactive nanoparticle enhances detectable signals 10–10000 times (Mirkin et al. 1996). So far, these nanomaterials have been used in the detection of trace amounts of DNA, mRNA, and proteins (Cao et al. 2002, 2003; Lagerholm et al. 2004). Most importantly, the photoactive nanomaterials have demonstrated great potential as highly efficient labeling reagents for in vitro and in vivo study of various cells (Michalet et al. 2005; Santra et al. 2005; Yi et al. 2005).

In the application of photoactive nanomaterials with living cells, a major concern, is whether these nanomaterials would cause toxic effects to living systems. Recently, some scientists have started working on the cytotoxicity of photoactive nanomaterials. Although current cytotoxicity studies are at the initial stage, results are significant for the further development and application of photoactive nanomaterials in the biological and biomedical fields.

In this chapter, some recent cytotoxicity studies on several typical photoactive nanomaterials will be reviewed. These nanomaterials include quantum dots (QDs), noble metal nanoparticles (gold nanoparticles (Au NPs) and silver nanoparticles

(Ag NPs)), and fluorescent silica nanoparticles (Silica NPs). Various factors that affect the cytotoxic properties of nanomaterials will be discussed briefly, such as the release of metal ions and particle size. Meanwhile, the general strategies of reducing/eliminating the cytotoxicity of these nanomaterials will be summarized in this chapter as well.

2.2 Quantum Dots

Quantum dots (QDs) or semiconductor nanocrystals are considered to be one of the best substitutes for conventional organic fluorescent materials. Currently, the most widely used QDs are made from cadmium and selenium. The diameter of the QDs is around several nanometers. Because of their composition and size, QDs are endowed with certain physically tailorable chemical properties, such as a tunable fluorescence emission wavelength and a strong resistance against photo-bleaching. Therefore, QDs have been recruited as fluorescent probes in biological and biochemical studies.

Although cadmium selenide quantum dots (CdSe QDs) seem to be the next generation of fluorescent materials for medical uses, their toxicity might be a major concern. Since cadmium is an acutely toxic heavy metal even at very low concentrations, the potential toxicity properties of Cd QDs should be evaluated prior to their real applications in the field of biomedicine and biology. So far, a few literature articles have reported the cytotoxic effects and DNA damage of Cd QDs (CdSe QDs and cadmium telluride (CdTe) QDs). The toxic properties of other heavy metal QDs, for example, PbS QDs, have not been reported yet. Thus, this chapter will only focus on the cytotoxicity of cadmium QDs and the major factors that cause such toxic effects.

2.2.1 Cytotoxic Effects of Cd QDs

2.2.1.1 Release of Cd²⁺ Ions

The toxicity of Cd QDs was first studied by Derfus et al. (2004). Primary hepatocytes were incubated with CdSe QDs for 24 hrs following a measurement of cell mitochondrial activity through an MTT viability assay. Severe cytotoxic effects of CdSe QDs were observed even at a low concentration of 62.5 $\mu\text{g}\cdot\text{mL}^{-1}$ of QDs under certain conditions, such as perturbing the CdSe QDs with UV-light, oxygen or hydrogen peroxide. The release of free Cd²⁺ ions from CdSe QDs after surface oxidation was a key reason for their cytotoxicity. The experiments clearly demonstrated that the extent of cytotoxic effects was correlated with the concentration of the released Cd²⁺.

To reduce the cytotoxic effect from the surface oxidation, proper postcoating of CdSe QDs was an effective solution. In the effort of achieving this goal, zinc sulfide (ZnS) and bovine serum albumin (BSA) surface coated CdSe QDs were developed. The ZnS- and BSA-coated CdSe QDs showed a significant decrease of their

cytotoxicity, but it was not completely eliminated. Kirchner et al. (2005) further proved this hypothesis by testing the detachment of NRK fibroblasts from the cell culture substrate upon incubation of the cells with QDs. They found the stability of the postcoating shell of the CdSe QDs was crucial to the QDs cytotoxicity. A stable and well-covered shell reduced the release of Cd^{2+} dramatically. For example, the ZnS shell of CdSe/ZnS QDs increased the critical concentration (no toxic effects were observed at this concentration of QDs) by almost a factor of 10. The effect of a silica shell on the QDs was also investigated. The results demonstrated that a stable crosslinked silica shell successfully reduced the cytotoxic effects of QDs. Furthermore, as larger polymer compounds were employed for the second postcoating silica-coated QDs, the toxic effect of QDs became undetectable. At concentrations of 30 μM of surface atoms of Cd, both polyethylene glycol (PEG) silica-coated CdSe and CdSe/ZnS QDs showed no toxic effects.

Lovrić et al. (2005) found a similar cytotoxic effect of CdTe QDs as that of CdSe QDs. With the hypothesis that free Cd^{2+} ions was a major cause for their cytotoxicity, two antioxidants, N-Acetylcysteine (NAC) and Trolox, were employed to pretreat P12 cells for 2 hrs. Based on previous studies, NAC and Trolox protected cells against Cd^{2+} induced cell death. The QD-induced reduction in cell metabolic activity completely disappeared in the NAC pretreated cells. However, Trolox showed no improvement regarding the QD-induced cytotoxicity. This result suggested that the release of free Cd^{2+} ions was one major factor, but not the only cause for cytotoxicity. Lovrić et al. (2005) listed three possible mechanisms that NAC reduced cytotoxicity: (a) Stabilized the QDs in the media by being absorbed onto the QDs' surface; (b) Enhanced glutathione (GSH) expression of cells to prevent QD-induced cytotoxicity; and (c) Activated key antiapoptotic signal transduction pathways that lead to transcription of genes involved in cell survival. The stabilization function of the postcoating method has also been demonstrated in BSA-coated CdTe QDs.

2.2.1.2 Chemical Properties of Surface Molecules

In addition to the release of free Cd^{2+} ions from Cd QDs, some surface-covering molecules have also contributed to the cytotoxic effects of QDs. Hoshino et al. (2004) investigated the cytotoxic effects of CdSe/ZnS QDs with different surface-covering groups using the comet assay, flow cytometry, and the MTT viability assay. WTK1 cells and Vero cells were employed as target cells to incubate with CdSe/ZnS QDs that were coated with mercapto-undecanoic acid (MUA) (QD-COOH), cysteamine (QD-NH₂), or thioglycerol (QD-OH) groups. After 2 hrs of incubation of the cells with the QDs, the MUA-coated QDs (QD-COOH) showed severe cytotoxicity at doses greater than 100 $\mu\text{g}\cdot\text{mL}^{-1}$ of QDs, while the thioglycerol-coated QDs showed slightly cytotoxic effects to the target cells. These three groups of QDs, which have the same core composition but different surface chemical molecules, demonstrated obvious different cytotoxic effects. Based on this result, Hoshino et al. concluded that the chemical properties of surface-covering molecules on the QDs affected the toxic effects of QDs significantly.

2.2.1.3 Size Effect

The different sizes of QDs have showed different cytotoxic effects. Shiohara et al. (2004) compared the cytotoxic effects of three different sized QDs: QD520 (green fluorescence QD), QD570 (yellow fluorescence QD), and QD640 (red fluorescence QD). QD520, which has the smallest size among the three QDs, showed the highest extent of cytotoxic effects. The explanation for the size effect was that the mobility of the QDs inside the cells depends on its size. The small size gave QDs a better mobility than the larger ones. Thus, the small QDs had more of a chance to contact cells and further caused a higher extent of cytotoxicity.

The size effect was also observed during a cytotoxicity study of CdTe QDs. Lovrić et al. (2005) found the green fluorescence CdTe QDs (2.2 ± 0.1 nm in diameter) exhibited higher cytotoxic effects than the red fluorescence QDs (5.2 ± 0.1 nm in diameter). The green CdTe QDs formed stable colloids in solution for more than one month, while the red ones aggregated easily. The actual size of the red fluorescence QDs might be larger than 5.2 nm. Using a fluorescence microscope, the interaction of the CdTe QDs with cells was monitored in situ. The green fluorescence QDs penetrated in the nucleus membranes of N9 cells and remained inside the nucleus after 1 hr of incubation. At the same conditions, the red fluorescence QDs stayed in the cytosol, but could not enter the nucleus. Interestingly, when the green fluorescence QDs were post-coated with macrobiomolecules such as BSA, these larger sized QDs were not able to enter the nucleus. The results suggested that the cytotoxicity of QDs was reduced by increasing particles size.

2.2.1.4 Other Effects

The stability of coating ligands on the QDs affected their cytotoxicity. Kirchner et al. (2005) investigated different compounds coated on CdSe QDs, in which binding forces between QDs and coating ligands were different. The results showed that less stable polymer-coated CdSe QDs and CdSe/ZnS QDs had larger cytotoxic effects than the corresponding mercaptopropionic acid (MPA)-coated QDs. The previous experiments confirmed that MPA was immobilized in a stable manner onto CdSe QDs. Thus, to reduce the cytotoxicity of QDs, the improvement of coating ligand stability on QDs is an effective approach.

In addition, the extent of QD cytotoxicity was related to the type of cell. Shiohara et al. (2004) tested the cytotoxic effects of MUA-QDs (CdSe QDs) to three cell lines – Vero cells (African green monkey's kidney cells), Hela cells, and primary human hepatocytes. The QDs caused much less damage to Vero cells than the other two cell lines at the same experimental conditions.

2.2.2 DNA Damage by QDs

It has been reported that some QDs cause DNA damage. Water soluble CdSe/ZnS QDs damage super-coiled double-stranded DNA through DNA nicking through

incubation of the QDs with the DNA. The nicking effect resulted in the breaking of deoxyribose units and the uncoiling of the double strands of DNA. Green and Howman (2005) discovered this phenomenon using a plasmid nicking assay. Fifty-six percent of DNA was damaged after 1 hr of incubation with MPA-coated CdSe/ZnS QDs under UV light. Meanwhile, in the same condition, the control samples without QDs showed only 5% of DNA damage. Furthermore, when the cell samples were treated with QDs in the dark (no UV radiation), 29% of DNA strands were damaged after 1 hr of incubation. Green et al. concluded that the obvious DNA damage was caused by free radicals released during oxidation of QDs. The ESR spectrum confirmed this hypothesis when comparing the QDs before and after radiation. An increase of the free radical signals was determined after radiation of QDs indicating more free radicals were formed by QDs. Thus, the oxidation reaction contributed to DNA damage when using QDs.

In summary, some QDs have shown significant toxic effects to cells and DNA strands. These effects might be a significant obstacle for the further applications of QDs in the field of biomedicine. To reduce the toxic effects of QDs, proper protections like postcoating QDs should be employed.

2.3 Noble Metal Nanoparticles

Nanostructures made from noble metals, Au or Ag, have been employed as photoactive labels in a variety of biosensing systems. The noble metal NPs have strong, size-dependent optical properties and UV-visible extinction bands. Thus, different sized metal NPs give different colors. From this point of view, metal NPs have similar properties with QDs with the major exception that their nominal size is much bigger than QDs. However, compared to QDs, gold and silver nanoparticles have several advantages, particularly their ease of synthesis and ability to bind to various target molecules. So far, gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) have been developed and used for a wide variety of ultrasensitive chemical and biological analyses.

To explore the safety issue of gold and silver nanoparticles, the cytotoxic effects of silver and gold nanoparticles were studied recently. The effects of dosage, surface molecule properties, and sizes were investigated. The cytotoxic effects of these noble metal nanoparticles were much less than QDs, but detectable in certain situations. Some resolutions for the elimination of Au/Ag NPs toxic effects will be summarized in this section.

2.3.1 Silver Nanoparticles

2.3.1.1 Oxidative Stress

Reactive oxygen species (ROS) damage DNA and RNA strands in cells through participation in the cell apoptosis processes. Due to the redox reactive property of noble

metals, Ag NPs increased the concentration of ROS, and thus caused cell death. This phenomenon was observed by Hussain et al. (2005). In the concentration range of 5–50 $\mu\text{g}\cdot\text{mL}^{-1}$ of silver nanoparticles (Ag NPs), a significant cytotoxic effect of Ag NPs (15 and 100 nm) was determined using the MTT assay after 24 hrs of incubation of Ag NPs with BRL 3A rat liver cells. This result was further confirmed by the membrane leakage of lactate dehydrogenase (LDH) assay for the concentration range of 10–50 $\mu\text{g}\cdot\text{mL}^{-1}$ Ag NPs. To address the cytotoxicity rationale, the ROS levels in cells were investigated at different time periods. The maximum ROS level was determined at 6 hrs of treatment of the cells with the Ag NPs. The ROS amounts were increased by 10 fold in the cells after incubating them with 25 $\mu\text{g}\cdot\text{mL}^{-1}$ silver nanoparticles. After 24 hrs of treating cells with Ag NPs, the mitochondrial membrane potential (MMP) of these BRL 3A cells significantly decreased (80%), and GSH was also reduced (70%). Therefore, the oxidative stress was an important cause for silver NP induced cytotoxicity. Braydich-Stolle et al. (2005) reported similar silver NP induced cytotoxicity in mammalian germline stem cells (mouse C18-4 cells). The Ag NPs reduced mitochondrial function and increased the membrane leakage. However, some mechanisms are still unknown, such as how these Ag NPs deplete GSH levels and increase ROS concentration.

2.3.1.2 Size Effect

Ag NPs have demonstrated an opposite size effect with QDs. As the size of Ag NPs decreased, the toxic effect as decreased as well. Hussain et al. (2005) found that the 100 nm Ag NPs exhibited higher cytotoxicity than that of 15 nm Ag nanoparticles. In the LDH assay, the EC_{50} (effective concentration) value of 100 nm silver NPs was $24\pm 9.25 \mu\text{g}\cdot\text{mL}^{-1}$. The data was much lower than that of the EC_{50} of 15 nm NPs ($50\pm 10.25 \mu\text{g}\cdot\text{mL}^{-1}$). However, the differences of EC_{50} values between 15 and 100 nm Ag NPs in the MTT assay were much smaller (100 nm Ag NPs: $19\pm 5.2 \mu\text{g}\cdot\text{mL}^{-1}$, 15 nm Ag NPs: $24\pm 7.25 \mu\text{g}\cdot\text{mL}^{-1}$). So far, the mechanism of the size effect is not clear.

2.3.1.3 Other Effects

The cytotoxicity of Ag NPs was slightly different depending on cell lines. Braydich-Stolle et al. (2005) compared the cytotoxic effects between C18-4 cells and BRL 3A cells. The LDH EC_{50} of Ag NPs in C18-4 cells was $0.25 \mu\text{g}\cdot\text{mL}^{-1}$. In contrast, the LDH EC_{50} of Ag NPs in BRL 3A cells was $50 \mu\text{g}\cdot\text{mL}^{-1}$; therefore, C18-4 cells were more sensitive than BRL 3A cells to 15 nm Ag NPs. Nevertheless, the treatment conditions were not identical (C18-4 cells: 48 hrs treatment, BRL 3A cells: 24 hrs treatment).

Until now, the effect of surface molecules on Ag NPs cytotoxic properties has not been reported. However, a study of surface molecules on Au NPs has been carried out as reviewed below. The results provided fundamental information that might be relevant to Ag NPs as well.

2.3.2 Gold Nanoparticles

2.3.2.1 Effect of Surface Modification

Pure gold nanoparticles (Au NPs) have little toxic effects due to the nature of inert elements. However, surface modification of NPs is necessary to obtain certain properties for nanoparticle applications. The surface molecules give nanoparticles' additional properties that might result in cytotoxicity of the nanoparticles. Goodman et al. (2004) compared the toxicity of cationic and anionic functionalized Au NPs (diameter of core nanoparticles was 2 nm) to Cos-1 cells by determining cell LC_{50} values (Lethal Concentration). Results showed that the cationic molecule covered Au NPs exhibited higher cytotoxic effects than that of the anionic modified molecules. After incubation of the cells with cationic molecules coated Au NPs for 1 hr, the LC_{50} value of Cos-1 cells reached $1.0 \pm 0.5 \mu\text{M}$. The LC_{50} value of the cells incubated with anionic coated Au NPs was greater than $7.37 \mu\text{M}$ after 24 hrs of incubation. To further confirm the result, red blood cells and *Escherichia coli* (*E. coli*) bacterial cells were tested using the same method. The results were the same as that of Cos-1 cells. The difference in cytotoxicity between the two coated Au NPs resulted from the different extent of cell membrane adhesion or cell lysis caused by the NPs. Due to the negative charges in all cells, the cationic molecule coated NPs had strong electrostatic attractions with the cells. As a result of this attraction, the NPs were drawn into the cell membranes. The hypothesis was proved by a vesicle-disruption assay. Two vesicles, SOPC (phosphatidylcholine) and SOPS (phosphatidylserine), were used in the assay. SOPS was an overall negatively charged vesicle and SOPC was a neutral vesicle. The results showed that the cationic NPs lysed negative charged SOPS more efficiently than the anionic NPs. Meanwhile, the neutrally charged SOPC showed a reversed trend for anionic and cationic nanoparticles. The assay confirmed that different surface charges of Au NPs resulted in different cell lysing efficiencies.

2.3.2.2 Effects of Cell Types and Stability of the Surface Molecules

Cell types and the stability of coating ligands on the NPs also affected the cytotoxicity of Au NPs. Goodman et al. (2004) determined the cytotoxicity of Au NPs towards two types of cells — Cos-1 cells and *E. coli* bacterial cells at the same conditions. The LC_{50} value of Cos-1 cell was $1.0 \pm 0.5 \mu\text{M}$ after 1 hr of incubation with cationic Au NPs, while the *E. coli* bacterial cells showed a 2- to 3-fold increase in LC_{50} value. The possible explanation for this difference was that cell wall surrounding the *E. coli* bacterial cells protects the cell against the penetration of NPs. Thus, a higher concentration of Au NPs was needed to fully rupture the bacterial cells.

The stability of coating ligands on NPs affected its cytotoxicity. As described in the section of Ag NPs, stable surface ligands reduced NPs toxic effects. Kirchner et al. (2005) coated Au NPs with an inert polymer. The cytotoxic effects of the Au NPs with such stable surface ligands were much lower than that of Au NPs.

2.4 Fluorescent Silica Nanoparticles

Fluorescent nanoparticles provide highly luminescent signals due to the relatively high quantum yield of dye molecules doped inside the nanoparticles. Various organic dye-doped polymer microparticles have been developed (Ito et al. 2001; Kwon et al. 2002; Zhou et al. 2002). However, so far, little toxicity studies on this type of nanoparticle have been reported. Silica-based fluorescent nanoparticles have been rapidly developed in recent years. Tan's group in the University of Florida has made use of the fluorescent silica nanoparticles for a wide variety of applications, including the biological field (Zhao et al. 2003b; Wang et al. 2005; Chang et al. 2005). The fluorescent silica nanoparticle consists of thousands of dye molecules in a silica matrix. Due to such a large number of dye molecules, the dye-doped silica nanoparticles provide highly luminescent signals when used as optical probes. Recent applications of the fluorescent nanoparticles have demonstrated a great potential for the NPs towards becoming a revolutionary labeling materials for bioanalysis. Therefore, an investigation of the cytotoxic properties of the fluorescent silica nanoparticles is critical to direct further applications of the fluorescent NPs in the field of biomedicine.

When comparing the compositions and structures of QDs/noble metal nanoparticles to that of the fluorescent silica nanoparticles, fluorescent silica NPs contain no redox active metal atoms, which have proved to be a major cause of the cytotoxic effects of QDs (cadmium) and Ag NPs (silver). Based on the current initial research, it seemed like the fluorescent silica NPs exhibited a much lower toxicity than QDs and noble metal NPs as summarized below.

2.4.1 Low Cytotoxicity of Silica Nanoparticles

Li et al. (2002) investigated the cytotoxicity of fluorescent silica NPs to COS-7 cells using MTT assays. After being treated with the silica NPs (50 nm in diameter), COS-7 cells showed no apparent cytotoxic effects as the nanoparticle concentration was lower than $1560 \mu\text{g}\cdot\text{mL}^{-1}$. As the concentration of silica NPs was over $1560 \mu\text{g}\cdot\text{mL}^{-1}$, the number of living cells decreased drastically. Luo et al. (2004) also observed the low cytotoxic property of silica NPs through an MTT assay. They studied both pure silica nanoparticles and superfect contained silica NPs. The results showed that pure silica nanoparticles had no effect on cell proliferation; but the superfect contained silica NPs (concentration of NPs: $2 \times 10^8 \text{ NPs mL}^{-1}$, superfect: $7.5 \mu\text{g}$) inhibited cell proliferation about 30% after a 2 hr incubation of the cells with the NPs.

Recently our group has studied the cytotoxicity of carboxyl group coated fluorescent silica NPs to Mouse A549 cells using various bioassays. The cells were incubated with the NPs over a period of 72 hrs. The results exhibited little cytotoxicity of silica NPs to the living cells. Compared to the negative control samples (without fluorescent silica NPs), the nanoparticle treated cells retained similar percentages of survival cells (60%). These preliminary results have demonstrated a

great potential of using the fluorescent silica nanoparticles in the biomedical field with low cytotoxicity.

2.4.2 Protection of DNA from Cleavage

In addition to the lower cytotoxic effect, fluorescent silica nanoparticles have demonstrated a unique property – protection of DNA strands from cleavage. He et al. (2003) reported that amino-modified silica NPs (diameter: 45 ± 4 nm) efficiently protected plasmid DNA from enzymatic cleavage. They first investigated the cleavage effect of DNA was cleavage enzyme – Dnase I on two groups of DNA strands. One of the DNA strands incubated with the nanoparticles prior to the reaction with Dnase I. The results showed that the enzyme could not cleave this group of DNA strands. However, the other group of DNA strands without incubation with NPs were cleaved to many small strands. It seemed that the nanoparticles protected the DNA from cleavage. To confirm the results, they further tested the Dnase I cleavage effect on plasmid DNA. The nanoparticle conjugated plasmid DNA was still able to release green fluorescence protein (GFP) indicating the presence of intact plasmid DNA. The mechanism of nanoparticle protection of DNA strands from cleavage is not clear.

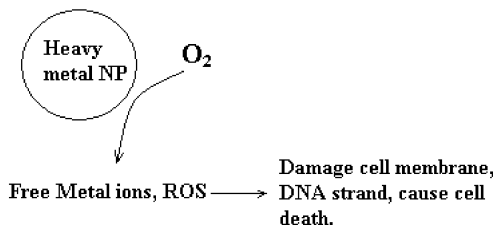
2.5 Summary

Photoactive nanomaterials have exhibited different extents of cytotoxic effects. A summary of the cytotoxicity properties of some typical photoactive nanomaterials is listed in Table 2.1. In general, the toxic metal-contained nanomaterials, QDs,

Table 2.1 Summary of factors affecting cytotoxicity of photoactive NPs

Nanomaterials	Composition	Size	Surface molecule	Cell line
Cd QDs	Heavy metal, toxic	Increases cytotoxicity as size decreases	Reduces/increases cytotoxicity	Cytotoxicity is related to cell lines
Noble metal NPs				
Ag NPs	Release of ROS, toxic	Decreases cytotoxicity as size decreases	N/A	Cytotoxicity is related to cell lines
Au NPs	Not toxic	N/A	Increase cytotoxicity	Cytotoxicity is related to cell lines
Fluorescent silica NPs	Not toxic	No effect	N/A	No effect

Fig. 2.1 Suggested mechanism of redox active property induced cytotoxicity of heavy metal nanoparticles



exhibited the highest cytotoxic effects among current popular photoactive nanomaterials. For example, the cadmium-contained QDs (CdSe QDs, CdTe QDs) caused acute cytotoxic effects at a low concentration over a short time period ($100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ QDs in 24 hr incubation). Redox active metal nanoparticles have also showed significant cytotoxic properties (Fig. 2.1). Silver NPs was an example of such redox metal materials. The fluorescent silica nanoparticles showed the lowest cytotoxic effects among current typical photoactive nanomaterials. At low concentrations, silica nanoparticles showed no apparent cytotoxic effects.

The cytotoxic effects can be reduced by changing nanomaterial compositions and sizes. The sizes of photoactive NPs were in the range of 1–100 nm. The smaller the size the NPs, the lower the cytotoxic effect. The penetration ability of the nanomaterials into cells increased as the size decreased. The larger nanomaterials stayed in the cytosol, while the smaller ones entered the nucleus of cells. Currently three kinds of photoactive nanomaterials, QDs, noble metal NPs, and fluorescent silica NPs, were all able to enter cell membranes but only smaller ones entered the nucleus when observed under fluorescent microscopes. This size effect was observed in each type of nanomaterial. However, silver nanoparticles were reported with a reverse trend.

The coating technique was one effective strategy to reduce or prevent the cytotoxic effects of NPs. Among all the coating materials used, the biocompatible materials, such as silica and BSA, have demonstrated a great potential to reduce the cytotoxicity of nanomaterials. In addition, cytotoxicity of NPs was related to the type of cell. The same type of nanomaterials might exhibit diverse toxic effects to different cell lines.

Different mechanisms of cytotoxic effects of photoactive nanomaterials was reported. The release of toxic cadmium ions was a major hypothesis for the cytotoxic effects of QDs. The presence of redox active elements in metal nanoparticles could cause toxic effects to living cells. Kirchner suggested (Kirchner et al. 2005) that the surface concentration of metal atoms should be used in the study of QDs cytotoxicity instead of using QDs concentration. This surface concentration correlated the ability of QDs to release cadmium ions better than the QD concentration. The smaller QDs had higher surface-to-volume ratios. Comparison of the surface concentrations of differently sized QDs of the same amounts showed, the smaller QDs had a higher amount of surface molecules. Thus, the smaller QDs caused higher cytotoxic effects.

Since the toxicity studies on photoactive nanomaterials described in this chapter has developed rapidly and has attracted great interest in such a short period of time, we expect many novel investigations in this field that will direct developments and applications of photoactive nanomaterials in bioanalysis within the next several years.

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