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# Biological Applications of ZnO Nanoparticles

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**Abstract:** Over the past few years, ZnO nanoparticles have attracted great attention due to their biocompatibility and low cost. A number of investigations demonstrated their potential applications in biotechnology and biomedicine. This review presents the current biological applications of ZnO nanoparticles, including biological imaging, drug releasing and biosensing, as well as their advantages and limitations in these areas. In addition, the toxicity of ZnO nanoparticles is discussed in comparison with other conventional nanoparticles.

**Keywords:** Bioimaging, biosensing, drug carrier, nanocrystals, toxicity, ZnO.

## 1. INTRODUCTION

Semiconductor nanocrystals have unique physical and chemical properties that show significant advantages in biological and biomedical applications, especially in bioimaging, drug delivery and biosensing fields. These properties are based on the size similarity with biomolecules such as proteins and polynucleic acids, large surface to volume ratios, fluorescent and magnetic behaviors, and quantum size effects [1-6]. However, most semiconductor nanocrystals have not been applied practically in biological and medical areas because of their potential toxicity and poor biocompability. In the past decades, plenty of investigations have been done to explore biocompatible substitute materials. ZnO nanoparticles, as low cost and low toxic materials, have shown promising performances in biomedical experiments.

Here, we present a brief review of current research activities that concentrate on the biomedical applications of ZnO nanocrystals, including bioimaging, drug delivery and biosensing applications. We also discuss the toxicity of ZnO nanocrystals and give a future outlook.

## 2. ZNO NANOPARTICLES FOR BIOLOGICAL IMAGING

Various imaging technologies have become increasingly important to understand the information of biological and clinical phenomena in cells or on molecular level. Current bioimaging technologies include fluorescence imaging, magnetic resonance imaging, computed tomography, ultrasound, and positron emission tomography [7]. Among these imaging technologies, fluorescence imaging technologies have been widely used in preclinical researches for its low expense, high sensitivity, no radiation and facile measurement. Since cells are almost transparent to visible light and individual macromolecules are too small to be observed with optical microscopy, it is very important to take advantage of fluorescence probes to visualize biomolecules and compartments within cells. In comparison with conventional fluorescence probes of organic dyes, photoluminescent nanocrystals exhibit great advantages due to their properties of high quantum yield, broad absorption, narrow and symmetric emission band, large effective Stokes shifts, high resistance to photobleaching and chemical degradation, size-tunable luminescence and so forth [1, 3, 6, 8-12]. However, the traditional CdSe and CdTe nanocrystals have great toxicity to the biological systems. Although various protections have been developed, the leakage of Cd ions through the shell defect is still observed, and the destructive reactive oxygen species (ROS) are easily produced by these nanoparticles, especially under light irradiation [13-17].

As one kind of versatile materials, ZnO nanocrystals have attracted great attention of scientists, not only because of their exceptional semi-conducting, optical and piezoelectric properties, but also because of their biological safety and low cost. Zinc is a very important trace element in human body and plays an important role in many biological systems. The average adult body contains 3.0–4.5 $\times 10^{-2}$mmol of zinc and adult men and women need 9.5mg and 7.0mg of $Zn^{2+}$ per day, respectively [18]. ZnO is listed as safe matter by the US Food and Drug Administration (21CFR182.8991). Therefore, ZnO holds a tremendous potential for biological and biomedical applications.

However, only a few literatures have reported the successful biolabeling applications of ZnO nanocrystals so far. The main reason is that the conventional ZnO nanoparticles are unstable in water. The physical or chemical properties of ZnO nanoparticles are often determined by the synthetic methods and modification materials. For example, ZnO nanocrystals prepared by sol-gel methods usually exhibit strong visible fluorescence. Such products have a potential for biological applications [22]. Several sol-gel methods have been developed to synthesize ZnO nanocrystals through hydrolysis of zinc salts in alcohol solvents, i.e. ethanol [23], triethylene glycol (TEG) [24-27], diethylene glycol (DEG)

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**Table 1. Current researches on biological imaging applications of ZnO nanomaterials.**

| Surface modification materials | Models | Mode | Imaging modality | Ref. |
|---|---|---|---|---|
| Vinyltriethoxysilane, TEOS, APTES | Hela cells | *In vitro* | Confocal scanning laser microscopy | [21] |
| No surface treatment or modification | Blood cells of Zebrafish | *In vitro* | Nonlinear optical imaging | [52] |
| | The seeds of *A. thaliana*. | *In vivo* | | |
| Using phospholipid micelles as the stabilizer and treated with target folic acid (FA) | KB cells | *In vitro* | Nonresonant nonlinear optical imaging | [53] |
| PEGMEMA | BALB/ca nude mice | *In vivo* | Laser confocal microscope | [20] |
| APTES, diglycolic anhydride | MDA-MB-231 cells | *In vitro* | Fluorescence microcopy | [54] |
| 3-mercaptopropionic acid, Maleimide-polyethylene, glycol-succinimidylcarboxy methyl ester, PEG-RGD | U87MG and MCF-7 cells | *In vitro* | Fluorescence microscopy and positron emission tomography | [18] |
| | Female Balb/c mice | *In vivo* | | |
| No modification | K562 cells | *In vitro* | fluorescence microscopy equipped with laser beams | [55] |
| $TiO_2$ or TEOS | The mung bean (*Vigna radiate*) seeds | *In vivo* | Confocal scanning laser microscopy | [56] |
| APTES, Grafted by carbon nanoparticles | *S. aureus* | *In vivo* | Fluorescence microscopy | [57] |
| Poly(2-(dimethylamino)ethyl methacrylate | COS-7 cells | *In vitro* | Confocal scanning laser microscopy | [58] |
| AEAPS | Hela cells | *In vitro* | Confocal scanning laser micros-copy;Magnetic resonance imaging | [59] |
| PEGMEMA | QGY 7763 | *In vitro* | Confocal scanning laser microscopy | [19] |
| TEOS and 3-[2-(aminoethyl)aminopropyl] trimethoxysilane | NIH/3T3 | *In vitro* | Confocal scanning disk microscopy | [44] |
| Poly(amidoamine) dendrimers | *Escherichia coli* MG1655 | *In vivo* | Laser scanning head coupled with an inverted microscopy | [46] |
| APTES, FA, Doxorubicin | Hela cells | *In vitro* | Confocal scanning laser microscopy | [60] |
| Stearate and TREG | NIH/3T3 | *In vitro* | Confocal scanning disk microscopy | [24] |

[28], tetraethylene glycol [28], 2-propanol [29] or polyethylene glycol (PEG) [31, 32]. But these products are bare and the luminescent centers on the ZnO nanocrystals surface are easily destroyed by water molecules. Furthermore, they tend to aggregate or undergo Ostwald ripening owing to their high surface energy [32]. Surface modification is widely employed to solve these problems. The hydroxyl groups on the surface of ZnO nanocrystals make them functionalized readily by surface coating materials, such as oleic acid (OA) [25, 26, 33], polystyrene (PS) [34, 35], poly(methylmethacrylate) (PMMA) [34, 36], (3-(2,3-epoxy-propoxy)propyl)trimethoxysilane [37], polyvinylpyrrolidone (PVP) [38, 39] *etc*. These modified ZnO nanocrystals are stable but usually show blue emission and they are only dispersed in organic solvents, which was unfit for biological experiments. Water-dispersible ZnO nanocrystals have been developed, *via* using modification materials of poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) [19], poly(ethylene glycol methyl ether) (PEGME) [40], hyperbranched polymers [41], silane coupling agents [21, 26, 32, 37, 42-47], poly(amidoamine) (PAMAM) dendrons [46] *etc*. There are still unresolved problems. For example, the quantum yield of the ZnO nanocrystals often decreases sharply after modification. Although the luminescence mechanism of ZnO nanocrystals has not been understood clearly, a widely accepted one is that the visible luminescence is from the surface defects of ZnO nanocrystals [22, 32, 48-50]. In the processes of modification, surface defects may be passivated by ligands and thus leading to some decrease of luminescence intensity of ZnO nanocrystals [32, 48, 50, 51]. Furthermore, the luminescence stability of ZnO nanocrystals is not solved perfectly, especially when dispersed in the buffer solutions or cell mediums [21]. Another question is how to graft functional groups on ZnO nanoparticle surface for further bioconjugation. ZnO nanomaterials with $–NH_2$ groups, -COOH groups or –SH groups are optimal for further bioconjugation, but the bioconjugation conditions are always limited by the stability of ZnO nanocrystals. For these reasons, few articles reported the bioimaging of ZnO nanocrystals especially *in vivo* applications, as shown in (Table **1**).

Our group obtained water-stable ZnO@PEGMEMA quantum dots(QDs) and successfully applied them as a fluorescence probe *in vitro* (Fig. **1**) and *in vivo* (Fig. **2**). To our knowledge, this is the first time using ZnO QDs as fluorescence probes *in vitro*. Thereafter, we further improved the

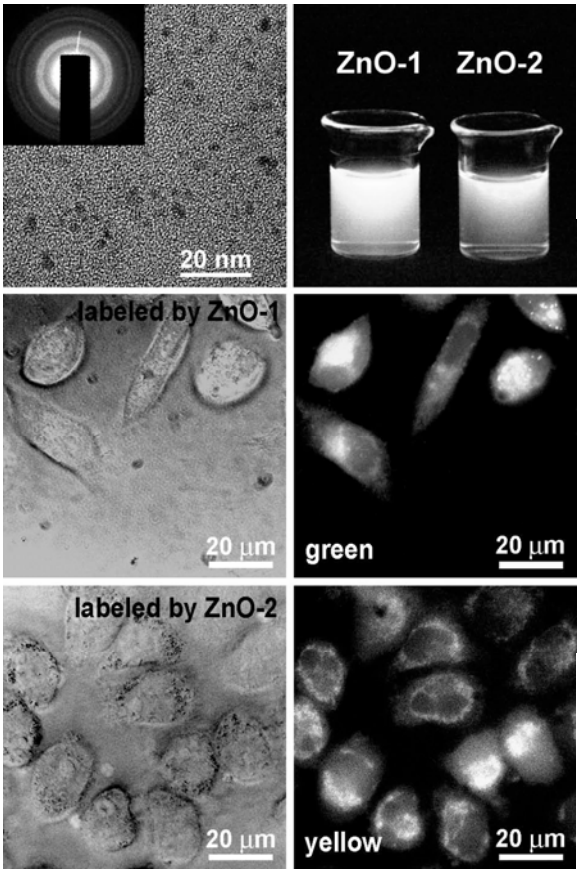


**Fig. (1).** The upper part are the HRTEM image of ZnO-1 with the inset of ED pattern (left) and the aqueous solutions of ZnO-1 and ZnO-2 under a UV light (right); The middle part and the lower part are the DIC pictures (left) and the fluorescent images (right) of the cancer cells labeled by ZnO-1 and ZnO-2 respectively. Reprinted with permission from Ref. [19]. Copyright 2008 American Chemical Society.

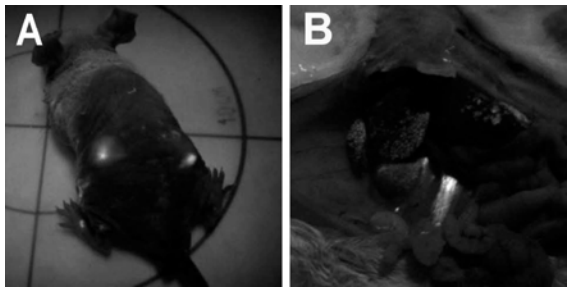


**Fig. (2).** (**A**) A mouse under UV light after intradermal injection of ZnO@polymer nanoparticles. (**B**) An intravenously injected mouse was sacrificed and imaged under UV light. Note that ZnO fluorescencelocates mainly in the aorta, liver and kidney. Reprinted with permission from Ref. [20], Copyright 2011 John Wiley & Sons, Ltd.

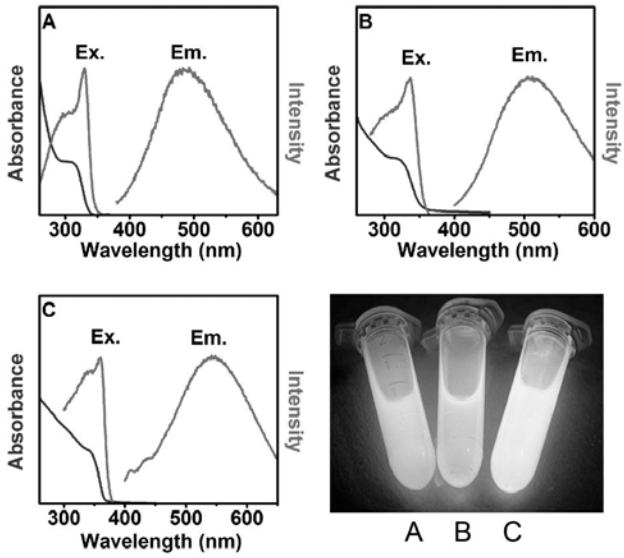


**Fig. (3).** Photoluminescent spectra and absorption spectra of (**A**) ZnO-A@silica (blue-emitting), (**B**) ZnO-B@silica (green-emitting) and (**C**) ZnO-C@silica (yellow-emitting) in water, with a photograph of these samples under a UV lamp. Reprinted with permission from Ref. [21]. Copyright 2012 Royal Society of Chemistry.

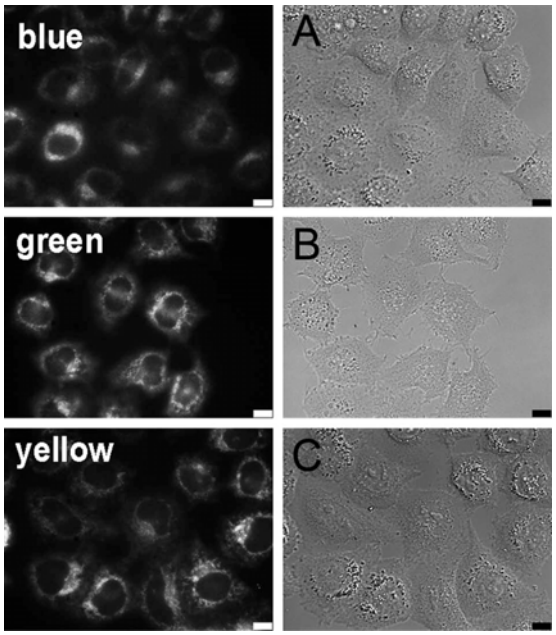


**Fig. (4).** Confocal luminescence images of HeLa cells incubated with (**A**) ZnO-A@silica (blue-emitting), (**B**) ZnO-B@silica (green-emitting) and (**C**) ZnO-C@silica (yellow-emitting) under UV light of 365 nm. The left pictures arefluorescent images of HeLa cells while the right pictures are the corresponding DIC images. Reprinted with permission from Ref. [21]. Copyright 2012.-9.Royal Society of Chemistry.

stability of ZnO QDs by a three-step silanization method and got remarkable products, which can be stable in water, PBS and cell medium, ensuring the feasibility of their further bioconjugation and applications (Figs. **3** and **4**).

Most bioimaging applications in the above reports are based on single photon UV excitation, which is not effective for deep tissue imaging *in vivo* due to the reduced penetration depth, absorption and scattering of optical signals. Short wavelength excitation often leads to autofluorescence of cells and tissues, making imaging and tracking more difficult. Besides, the blinking phenomenon of these nanoparticles makes it difficult to implement [61-63].

Although it is limited to use ZnO nanocrystals as bioimaging probes under a linear excitation condition, their non-

linear properties make up these limitations. Nonlinear optical processes for live cells and tissues imaging include two (or multi) photon excited fluorescence, second and third harmonic generation, vibration coherent anti-stokes Raman scattering and so on [53, 62]. The nonlinear nature of interactions can provide a high 3-D spatial resolution, improve signal-to-noise ratio, increase the imaging depth by using near infrared excitation, and reduce thermal interaction and stress in biological systems.

The most common nonlinear optical process in bioimaging is two photon excited fluorescence, which is a resonant process. Two-photon excitation or multiphoton fluorescence imaging using infrared excitation allows ZnO nanoparticles to overcome the barrier of high-energy excitation wavelengths, and harmonic generation processes help eliminate blinking [52, 62, 63]. However, an efficient two photon excited fluorescence is correlated with two photon resonance and limited to specific wavelengths. Fortunately, researchers have developed a new bioimaging modality as supplementary by using second harmonic generation (SHG). SHG is a property of certain crystals and molecules that exhibit birefringence and noncentrosymmery under lattice inversion, and it occurs under strict phase matching conditions in conventional optical materials [52]. More importantly, SHG is a nonresonance nonlinear process and can offer new advantages over two photon bioimaging in terms of no requirement of conventional phase matching, no cell damage because of their nonradiative decay pathway, no autofluorescence by the proper choice of the excitation wavelength depending on the constituents of the appropriate biological media, and no need for confocal microscopy [64, 65].

Biocompatible ZnO nanocrystals have not only multiphoton absorption ability, but also SHG for their noncentrosymmetric structure [66]. The SHG created in high-quality ZnO nanoparticles can be efficient for bioimaging *in vitro* and for tracking and imaging targets to a certain depth *in vivo*. Kachynski *et al.* [53] first reported utilization of ZnO nanocrystals as targeted nonlinear optical probes for bioimaging (Fig. **5**). They demonstrated the application of photostable water-dispersible ZnO-FA nanoparticles encapsulated in phospholipid micelles for high contrast nonresonant nonlinear optical bioimaging in human KB cells, which are known to overexpress receptors for folic acid. The SFG and SHG imaging signals are rather robust from live KB cells treated with targeted ZnO nanoparticles when compared with those of the nontargeted ZnO nanocrystals. Urban *et al.* [52] demonstrated the imaging of the SHG of highly optical nonlinear susceptible ZnO nanoparticles *in vitro* in the blood cells of zebrafish. More important fact was that they successfully got the SHG imaging *in vivo* of a germinating roots and shoots of Arabidopsis plants, which is not possible to observe using conventional fluorescence microscopy, for the seed coat prevents the penetration of UV-visible light. As the intensity of the ZnO SHG has a positive correlation with crystal quality, it is important to prepare high quality ZnO nanocrystals. The preparation of uniform isotropic water-dispersed of ZnO nanocrystals with high crystal quality will be an important research field in the future.

Multimodal nanoprobes can provide more accurate and detailed information than monomodal ones. For example,

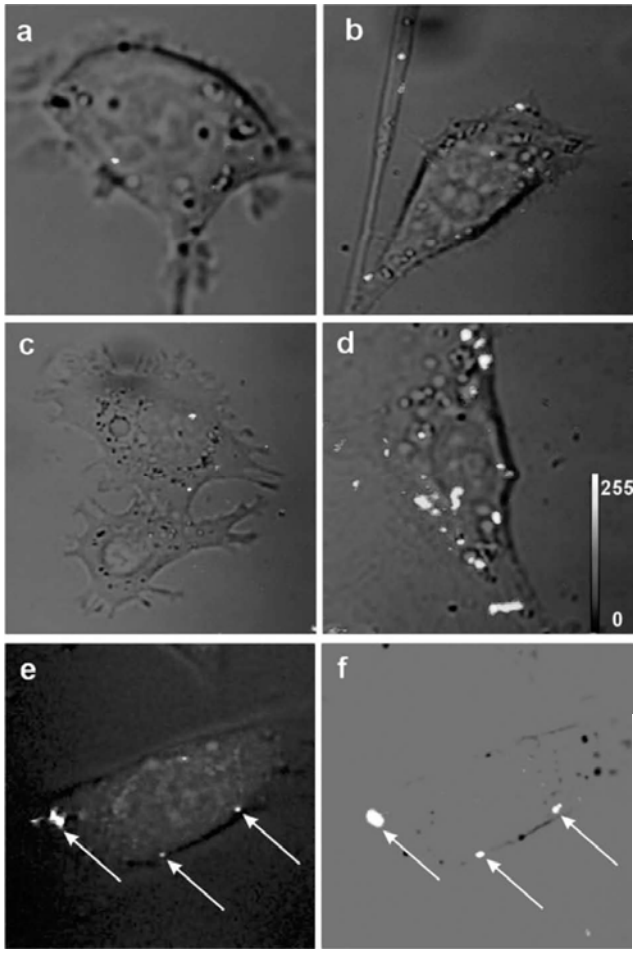


**Fig. (5).** (**a**-**d**) SFG images of KB cells treated by ZnO nanoparticles not targeted (**a** and **c**) and targeted with FA (**b** and **d**) after 1 h (**a** and **b**) and 3 h (**c** and **d**) of incubation. The intensity-coded SFG images (see scale inset in panel **d**) were super imposed on the transmission 1064 nm green background images. (**e**) FWM image without transmission background and (**f**) corresponding SFG image of KB cells. Reprinted with permission from Ref. [53], Copyright 2010 American Chemical Society.

articles about MRI and fluorescence imaging dual mode nanoprobes are increasing in the past years [7, 59, 68-73]. Noninvasive MRI is one of most powerful techniques for both clinic and basic researches. It can penetrate deep into tissue, and provide a wealth of spatial and temporal resolution. Besides, it does no harm to the patient. The drawback is its low sensitivity. In contrast, fluorescence imaging has much higher sensitivity and a potential for real-time imaging but limited depth perception. Therefore, it is a promising approach to integrate magnetic resonance and optical imaging functionalities into one nanocrystal to overcome both of their limitations. Though large amount of researches focus on doped-ZnO nanocrystals, very few of them concern their multimodal imaging applications. Wu *et al*. [74] reported the synthesis and surface modification of Co-doped ZnO nanocrystals and proved their applications as dual color imaging agents on human osteosarcoma (Mg-63). Liu *et al*. [59] fabricated Gd-doped ZnO QDs with silica coating and found that Gd doping caused yellow emission significantly enhanced. They proved the successful labeling of Hela cells in

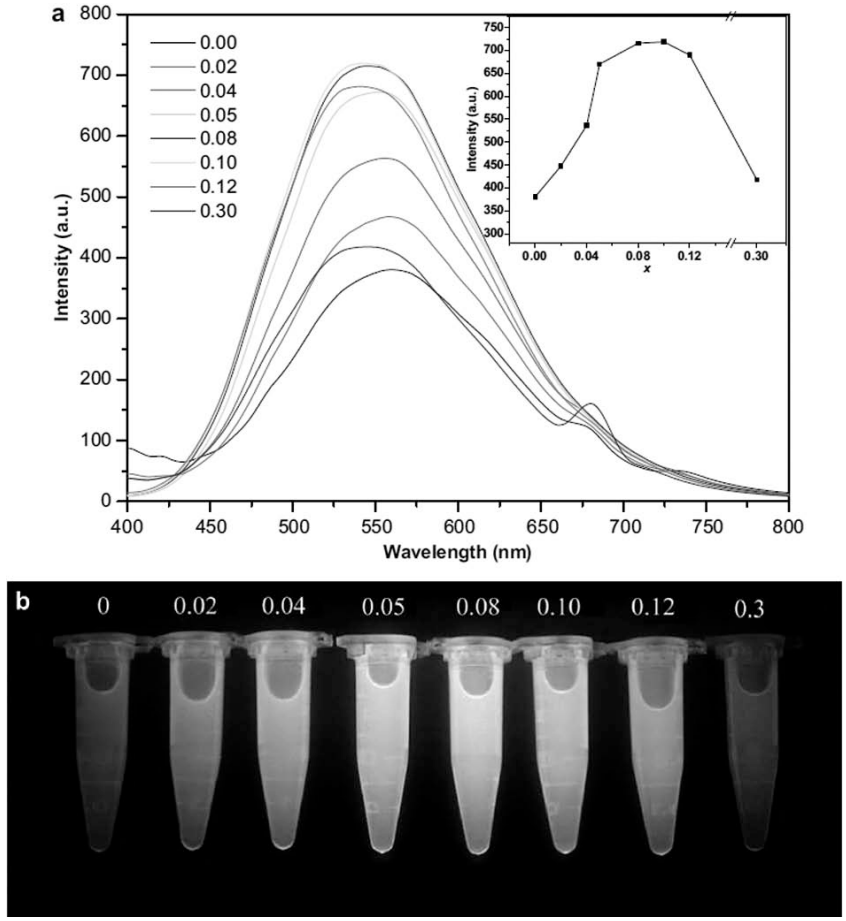


**Fig. (6).** (**a**) Fluorescence emission spectra of Gd-doped ZnO QDs with different molar ratios of Gd/Zn at an excitation wavelength of 340 nm. (**b**) Fluorescence of Gd-doped ZnO QDs with different molar ratios of Gd/Zn under UV light at 365 nm. Reprinted with permission from Ref. [59], Copyright 2011 Elsevier.

short time using these QDs as fluorescence and magnetic resonance imaging dual mode nanoprobes, which exert strong positive contrast effect with a large longitudinal relaxivity ($r_1$) of water proton of 16$mm^{-1}s^{-1}$ in MRI studies (Figs. **6** and **7**).

## 3. ZNO NANOPARTICLES FOR DRUG DELIVERY

Anticancer drugs in the traditional chemotherapy often show low efficacy and toxic adverse effects because they have no selectivity between cancer cells and healthy cells. Nanocarriers, which can recognize cancer cells or tumor issues, have been employed extensively to deliver anticancer drugs to overcome such drawbacks [75]. Deliberate modification of nanocarriers with ligands can make the drug-nanocarries system bind to receptors over-expressed on tumor cells specifically. The interaction between drug and nanocarries include physical adsorption, electrostatic interaction, π-π stacking and so on [11, 75, 76]. All of the above interaction forces are weak non-convalent forces and the large surface to volume ratio does a favor to these interactions. When the drug-nanocarriers reach the target sites, the host and guests interactions are broken to release the drug, and thus the drug concentrations at the target sits are improved and the relevant toxicity and adverse effects towards normal cells and tissues are suppressed. Biocompatible ZnO nanocrystals degrade readily in moderately acidic environment, making them suitable for pH-responsive systems. The extracellular mildly acidic environment in solid tumor tissues and intracellular compartments such as endosomes and lysosomes provide ideal conditions for drug-ZnO nanocarriers.

Doxorubicin (DOX), commonly used in cancer chemotherapy, is widely investigated in the pH-responsive DOX-ZnO nanocarriers systems. These systems could be advantageous, since the relatively low pH in tumors will specifically stimulate the DOX release in the target site (Figs. **8** and **9**). Yuan *et al*. [77] fabricated a kind of blue-emitting ZnO-QD–chitosan–folate carrier with long-term stability and water dispersed for tumor-targeted drug (DOX) delivery and found the drug-loading efficiency was 75%. Muhammad *et al*. [60] fabricated pH-responsive ZnO-FA (QDs) with water-stable and highly luminescent as biolabelings and targeted carriers for DOX. The surface folic acid ligands lead the drug-nanocarries system to binding to tumor cells. This ZnO QDs remained stable at physiological pH, but in acidic intracellular environments of cancer cells, DOX was instantly released through complex dissociation and dissolution of ZnO QDs, and consequently, killing the cancer cells. Pulsatile release by ultrasound irradiation can be also used in DOX-ZnO nanocarriers systems for controlled and targeted drug delivery, as Barick and coworkers [76] found. Furthermore, ZnO nanocrystals in the DOX-ZnO nanocarriers systems not only

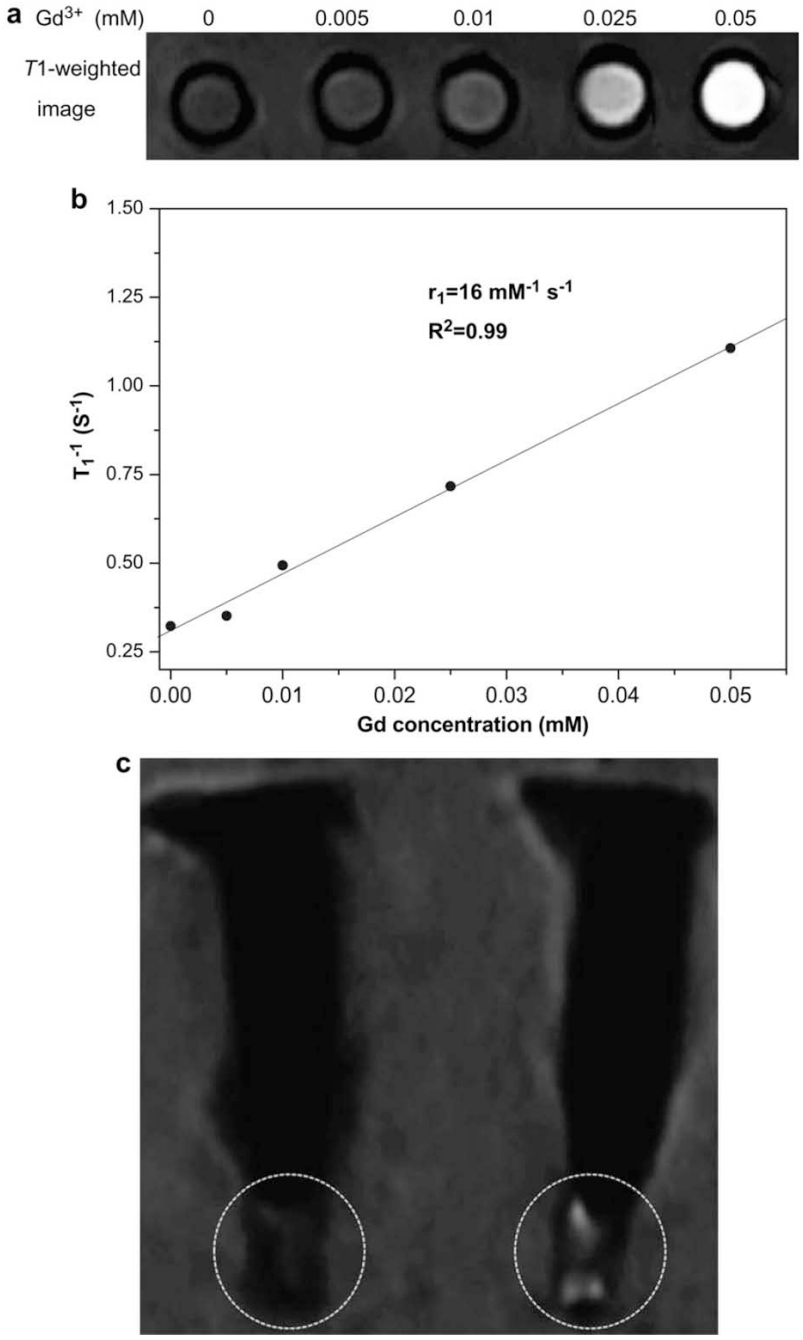


**Fig. (7).** (**a**) $T_1$-weighted magnetic resonance image for various $Gd^{3+}$ concentrations of Gd-doped ZnO QDs (molar ratios of Gd/Zn: 0.08) in water from a 1.5 T clinical MRI system. (**b**) The linear relationship between $T_1$ relaxation rates ($1/T_1$) and $Gd^{3+}$ ion concentrations for Gd-doped ZnO QDs (molar ratios of Gd/Zn: 0.08). (**c**) $T_1$-weighted image of blank HeLa cells pellet (left) and HeLa cells incubated with Gd-doped ZnO QDs at 0.01 M $Gd^{3+}$ ions for 2 h. Reprinted with permission from Ref. [67], Copyright 2011 Elsevier.

behave as nanocarriers but also exhibited significant antitumor activities [78]. Our recent investigation demonstrated that the cytotoxicity of ZnO@Polymer-DOX increased significantly when compared with DOX and ZnO@Polymer [79]. The combination of DOX with ZnO QDs help DOX uptaken by cancer cells to reach higher concentration inside cells, and the decomposition of ZnO release toxic $Zn^{2+}$ ions and reactive oxygen species (ROS) to enhance the cytotoxicity, as shown in (Fig. **9**). Therefore, it is possible to apply drug-ZnO nanocarriers systems for chemotherapy.

Zhang *et al*. [80] combined ZnO nanorods with anticancer drug daunorubicin (DNR) and applied them in photodynamic therapy (PDT) and demonstrated that this combination system improved the anti-tumor activity remarkably with UV illumination. The notable photodynamic activity of ZnO nanorods could considerably increase human hepatocarcinoma cells (SMMC -7721 cells) injury mediated by ROS. Hackenberg and coworkers [81] proved UVA-1-activated ZnO-NPs in combination with paclitaxel and cisplatin induce tumor-selective cell death in human squamous cell carcinoma

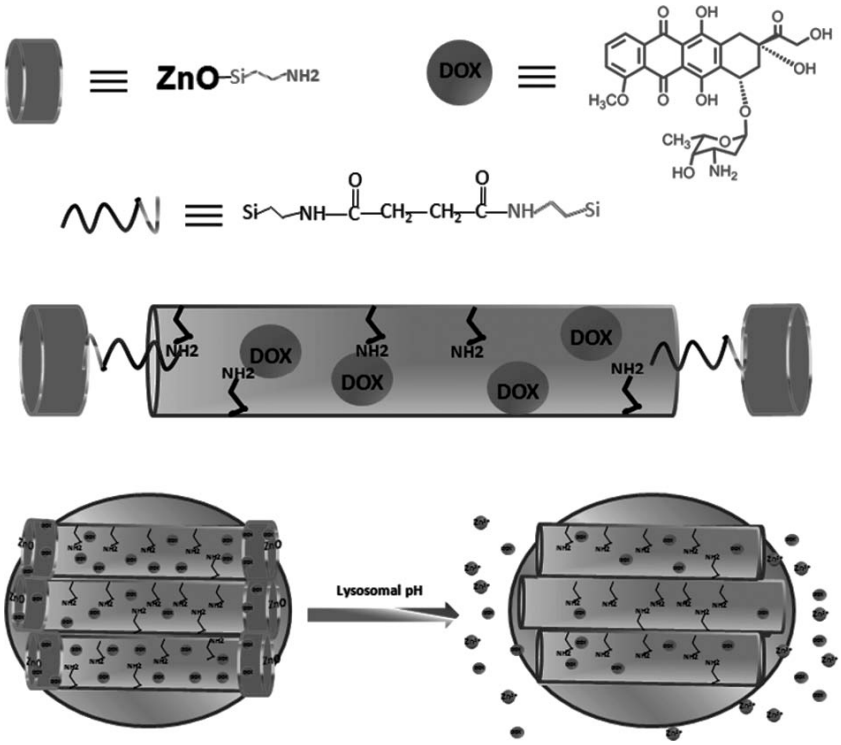


**Fig. (8).** Schematic illustration of the synthesis of ZnO@MSNs-DOX and working protocol for pH-triggered release of the anticancer drug (DOX) from ZnO@MSNs-DOX to the cytosol *via* selective dissolution of ZnO QDs in the acidic intracellular compartments of cancer cells. Reprinted with permission from Ref. [78], Copyright 2011 American Chemical Society.

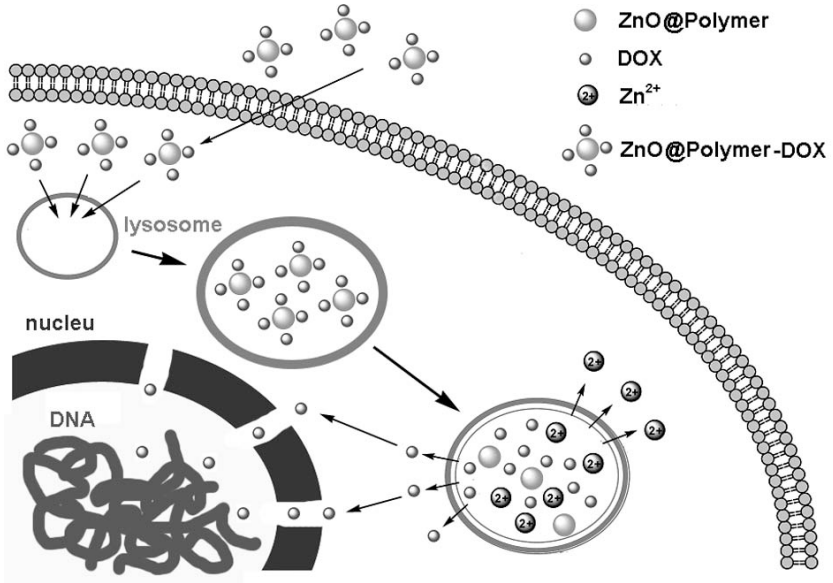


**Fig. (9).** Schematic illustration of the cytotoxicity mechanism of ZnO@Polymer-DOX.Reprinted with permission from Ref. [79], Copyright 2013 John Wiley & Sons, Ltd.

(HNSCC) *in vitro*, and indicated that photocatalytic therapy of HNSCC with ZnO-NPs could enhance the cytotoxic action of chemotherapeutic agents synergistically, especially under UV excitation condition. Muhammad *et al*. [82] demonstrated the potential of ZnO NPs in photodynamic therapeutic applications. They conjugated nanoporous zinc oxide (ZnO NPs) with Photofrin for efficient intracellular drug delivery in photodynamic therapy. These ZnO NPs complex could emit 625 nm red light in the presence of Photofrin with 240 nm UV light excited intracellularly, and activated a chemical reaction that produced reactive oxygen species (ROS), leading to the death of A-549 lung carcinoma cells within a few minutes. Moreover, the ZnO NPs conjugated with Photofrin under UV light exposure displayed valuable cytotoxic effects as compared to Photofrin alone.

Though lots of investigations reported the size of ZnO nanocrystal contributes little to their toxicity, some found

that under UV irradiation, the effect of size fact is significant. Guo and coworkers [83] explored the cytotoxic effect of anticancer drug daunorubicin on leukemia cancer cells in the absence or presence of different sized ZnO nanoparticles, with or without UV irradiation. They found that the combination of ZnO nanoparticles and daunorubicin under UV irradiation have synergistic cytotoxic effect on leukemia cancer cells. The cytotoxicity of ZnO nanoparticles to leukemia K562 and K562/A02 cancer cells was dose-dependent. With the aid of ZnO nanoparticles, cellular uptake of daunorubicin apparently enhanced. UV irradiation could enhance the proliferation suppression ability of ZnO nanoparticles on cancer cells and the cytotoxicity suppression of daunorubicin on both leukemia cell lines exposed to the ZnO nanoparticles solutions. Li and coworkers [84] explored the cytotoxicity and photodynamic effect of different-sized ZnO nanoparticles to target cells and demonstrated that ZnO nanoparticles exerted dose-dependent and time-dependent cytotoxicity for cancer cells like hepatocellular carcinoma SMMC-7721 cells *in vitro*. The size-depended effect was not clear in the scope from 20 to 100 nm without UV irradiation. UV irradiation could enhance the suppression ability of ZnO nanoparticles on cancer cells proliferation, and these effects were in the size-dependent manner, while the smaller the nanoparticle size, the higher the cytotoxicity of cancer cell proliferation caused by ZnO nanoparticle. Furthermore, when ZnO nanoparticles combined with daunorubicin, the related cytotoxicity of anticancer agents on cancer cells was evidently enhanced. Palanikumar and coworkers [85] used ZnO nanoparticles as a carrier for amoxicillin drug delivery system. The amoxicillin-loaded zinc oxide nanoparticles have good antibacterial activities against infectious Gram-positive and Gram-negative bacteria. The antimicrobial property increases with increasing in the drug loading, which depends on the size of nanoparticles, concentrations of drug, and stirring time.

However, the size effect mechanism of the drug-ZnO nanocarriers system under UV irradiation is not clear. We hypothesize that the smaller ZnO nanocrystals have larger surface to volume ratio, so more defects on the surface induce more ROS, and $Zn^{2+}$ ions are easier to release, and thus leading to more serious toxicity. To utillize this drug-ZnO nanocarriers system, we can prepare different ZnO nanocrystals with required characteristics. As Xiao and coworkers [86] found that zinc oxide-zinc sulfide quantum dots (ZnO-ZnS QDs) could increase the affinities for protein selectively, the specificity of the drug-ZnO nanocarriers system will have significant applications in the future. Furthermore, using ZnO nanocrystals as drug-nanocarriers will realize real-time monitoring for drug delivery.

## 4. ZNO NANOPARTICLES FOR BIOSENSING

The sensing of biological agents, diseases, and toxic materials is an important goal for biomedical diagnosis, forensic analysis, and environmental monitoring. The practical applications of biosensing technologies, including colorimetric sensing, fluorescence sensing, and electrochemical sensing require their high quality of sensitivity, selectivity and stability. Take enzyme-linked immunosorbent assay (ELISA) for example, this assay often uses molecular fluorophores as labels, which have low photoluminescence stability. And photobleaching may reduce the accuracy of the sensing information. Besides, the low abundance of protein and the limited number of procedures for protein amplification result in low sensitivity. In contrary, the unique physicochemical properties of semiconductor nanocrystals make them promising candidates for sensing application. The high photoluminecence stability ensures them fluorescence sensing application and nanostructure have unique advantages in immobilization enzymes and retaining their bioactivity as a result of the high surface area for higher enzyme loading, thus improving the sensitivity.

When combined with eletrochemical sensor application, semiconductor nanostructures provide the direct electron transfer between the enzyme's active sites and the electrode, therefore, offer more accuracy information by integrating fluorescent sensor and electrochemical sensor. Semiconductor ZnO nanocrystals present as one of the most promising materials for biosensing application, not only because of their good photochemical and electrochemical properties, but also for their biocompatibility and low cost. ZnO has been utilized for immobilization of proteins, enzymes and antigens for accelerated electron transfer between desired immobilized biomolecules and electrode. As ZnO has a high isoelectric point (IEP) of about 9.5, it is suitable for adsorption of a low IEP protein or enzyme such as glucose oxidase [87], tyrosinase [88], transferring [54], rabbit-immunoglubin antibodies (r-IgGs) and bovine serum albumin(BSA) [89] in proper solutions. Chakraborti *et al.* [90] showed that ZnO NPs are capable of disrupting protein-protein association. ZnO NPs bind to the largest cleft on the protein surface, thereby helping it to retain the secondary structures to a greater degree and exhibit enzymatic activity even under denaturing conditions.

Some clinical diagnoses require enzyme sensors offering high sensitivity and relatively narrow dynamic detection range, but some of them need wide linear response range for the detection. Since the ZnO nanowire glucose sensor has a great feature of the correlations of $K_M$ and linear response sensitivity (LRS) with the enzyme loadings over a wide range, it could be easily tailored to meet the requirements. For example, investigation reported that BSA/r-IgGs/nano-ZnO/indium-tin-oxide (ITO) immunoelectrode exhibits linearity as 0.006-0.01nM/$dm^3$ with detection limit of 0.006nM/$dm^3$ for ochratoxin-A (OTA) [89]. The single-crystal ZnO nantube (ZNT)/ITO-based biosensor exhibits wide linear calibration ranges from 10 μM to 4.2 mM, and a low limit of detection (LOD) at 10 μM for sensing of glucose [91]. The linear range of ZnO/tyrosinase biosensor for phenol determination was from $1.5\times10^{7}$ to $6.5\times10^{5}$mol $L^{-1}$ with a detection limit of $5.0\times10^{8}$mol $L^{-1}$ [88]. Single ZnO nanofiber based glucose biosensor showed a linear range from 0.25 to 19 mM with a low limit of detection (LOD) of 1 μM [92].

The ZnO nanostructures based biosensors exhibited good performances in terms of response rate, sensitivity, operational stability, and fabrication simplicity. As the robust mechanical adhesion and electrical contact between the nanostructured ZnO and the electrodes realize the direct electron transfer between the electrode surface and the redox protein,

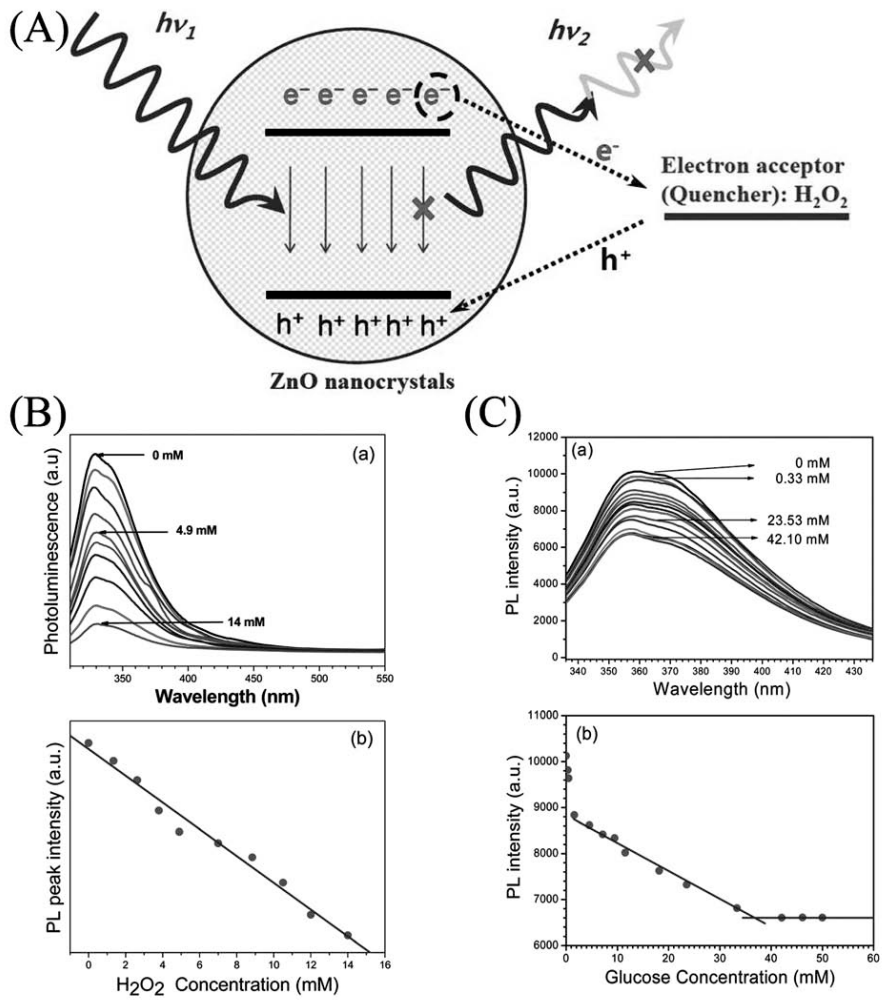


**Fig. (10).** (**A**) Schematic diagram presenting a collisional quenching mechanism causing decrease in PL intensity of ZnO nanocrystals. (**B**) Characteristic PL response of GOx-immobilized ZnO nanocrystals to glucose concentration (**a**) and PL peak intensity variation with glucose concentration (**b**). (**C**) Variation in photoluminescence (PL) spectra with hydrogen peroxide concentration (**a**) and linear decrease in PL intensity with hydrogen peroxide concentration (**b**). Reprinted with permission from Ref. [93], Copyright 2011 Royal Society of Chemistry.

ZnO nanocrystals can provide a potential powerful platform for biosensing application. However, most of current investigations focused on electrochemical biosensing application, but scarely on colormetric sensing and fluorescence sensing applications (Fig. **10**). One of the main reasons is that the problems of fluorescence stability are still unsolved. In the future, multi-modal application of ZnO nanocrystals will be a main investigation direction. When integrating the applications of bioimaging, biosensing and drug delivery, more accurate information by real-time monitor will be collected to understand the mechanism of diseases.

## 5. TOXICITY OF ZNO NANOPARTICLES

Because of the enormous application promising of nanocrystals, great progress has been made with intensive investigations focusing on synthesis and modification. However, researches on their toxicity don't keep up the same pace. Although large bulk ZnO materials are safe, nano-level ZnO materials may exhibit different toxicity due to their small size and large surface to volume ratio. Before putting them into practical applications in biology and biomedicine, it is very important to understand how the nanocrystals affect organisms, human beings and environment. Large amount of investigations demonstrated that ZnO nanocrystals are more toxic than $Al_2O_3$, $SiO_2$ and $TiO_2$ nanoparticles, though they are relatively biocompatible when compared with Cd-based QDs (Table **2**). The toxicity of ZnO nanocrystals could ascribe to the release of $Zn^{2+}$ ions and excess ROS generation (Fig. **11**) [47, 83, 94-125]. But, most of these investigations focus on bare ZnO nanocrystals and there are some contradictions referring to which one play a major role in determining the toxicity. For example, some studies demonstrated that the size of ZnO nanocrystals play an important role in determining their toxicity, however, the others found that there are no different effects between ZnO nanocrystals with different sizes [83, 84, 94, 103, 108, 119, 126]. Another problem is there are not standard methods to evaluate the toxicity of nanocrystals. Considering the various resistant behaviors of different organisms, and the discrepant organism responses under different environment, such as culture medium or growth intensity [97, 120], we cannot make a comparison between those results of reports properly.

**Table 2. Current researches on cytotoxicity of NPs.**

| NPs | Models | Size of NPs | Treatment | Viability | Ref. |
|---|---|---|---|---|---|
| MPA-CdTe | MCF-7 cells | No data | 10 μg/mL and 1 hour | ~10% | [127] |
| Cys-CdTe | | | | ~20% | |
| NAC-CdTe | | | | ~40% | |
| Cye-CdSe/ZnS | | | | ~90% | |
| Cys-CdTe | SMMC-7721 cells | ~3.5 nm | 35.9 nM and 24 hours | 50% | [128] |
| MPA-CdSe/ZnSe | BALA/3T3 cells | 4.63 nm | 0.746 nM and 24 hours | ~100% | [129] |
| GA-CdSe/ZnSe | | 65.9 nm | 0.746 nM and 24 hours | ~1% | |
| QSA-CdSe/ZnS | HaCaT cells | 7-13 nm | 400 nm and 24 hours | ~100% | [130] |
| QSH-CdSe/ZnS | | | | | |
| QEI-CdSe/ZnS | | 8.19 nm | 5.14 nmol/L and 24 hours | 50% | |
| QEI-CdSe/ZnS | | 10.07 nm | 3.06 nmol/L and 24 hours | | |
| QEI-CdSe/ZnS | | 12.78 nm | 23.36 nmol/L and 24 hours | | |
| $CuInS_2$/ZnS | | 11.14 nm | 433.89 nmol/L and 24 hours | | |
| MSA-CdTe | HUVECs cells | 4 nm | 10 μg/mL and 24 hours | 50% | [131] |
| F-68-CdSe | HepG2 cells | 159 nm | 400 ppm and 72 hours, | more than 80% | [132] |
| SDS- CdSe | | 178 nm | 100 ppm and 72 hours | ~0% | |
| CTAB-CdSe | | 266 nm | 50 ppm and 12 hours | no more than 50% | |
| MPA-CdSe | zebrafish | 3.5 nm | 1.98 mg/L and 120 hours | 50% | [133] |
| TGA-CdTe | zebrafish | 3.5 nm | 185.9 nM and 120 hours | 50% | [134] |
| MPA-CdTe | *Escherichia coli* | No data | 7.4-8.8 × $10^{-8}$ mol/L | 50% | [135] |
| MPA-CdSe/ZnSe | *Daphnia magna* | 65.9 nm | 70.4 μg/L and 48 hours without UV-B irradiation | 50% | [136] |
| | | | 17.3 μg/L and 48 hours with UV-B irradiation | | |
| GA- CdSe/ZnSe | | | 95.9 μg/L and 48 hours without UV-B irradiation | | |
| | | | 58.5 μg/L and 48 hours with UV-B irradiation | | |
| TGA-CdTe | *Hydra valgaris* | 3.2 nm | 1.4 mg/L and 24 hours | 50% | [137] |
| ZnO | HepG2 cells | 30 nm | 20 μg/mL and 24 hours | 43% | [138] |
| PEGMEMA-ZnO | QGY 7763 cells | 3-4 nm | 0.2 mg/mL and 24 hours | more than 90% | [19] |
| $ZnO/SiO_2$ | NIH/3T3 cells | 50 nm | 30 μg/mL and 24 hours | more than 85% | [44] |
| TREG-ZnO | NIH/3T3 cells | 2-9 nm | 20 μg/mL and 24 hours | more than 90% | [24] |
| ZnO | WIL2-NS cells | 30 nm | 15.9 mg/L and 24 hours | 50% | [102] |
| OA-ZnO | | | 28.2 mg/L and 24 hours | | |
| PMAA-ZnO | | | 41.4 mg/L and 24 hours | | |
| Medium-ZnO | | | 41.8 mg/L and 24 hours | | |

**Table (2) contd…**

| NPs | Models | Size of NPs | Treatment | Viability | Ref. |
|---|---|---|---|---|---|
| ZnO | HELF cells | 20-40 nm | 20 mg/L and 72 hours | lower than 10% | [98] |
| ZnO | BEAS-2B cells | 10-40 nm | 40 µg/mL and 24 hours | ~20% for both high and low density cells. | [97] |
| | L-929 cells | | 20 µg/mL and 24 hours | ~0% for low density cells. | |
| | | | | ~20% for high density | |
| | CRL-292 cells | | 30 µg/mL and 24 hours | ~0% for low density cells. | |
| | | | | ~70% for high density cells. | |
| | C2C12 cells | | 30 µg/mL and 24 hours | ~0% for both high and low density cells. | |
| ZnO | LoVo cells | 50-70 nm | 5 µg/mL and 48 hours | less than 50% | [106] |
| ZnO | Mouse macrophage Ana-1 cells | 100 nm | 43.95 µg/mL and 24 hours | 50% | [139] |
| | | 30 nm | 40.41 µg/mL and 24 hours | | |
| | | 10-30 nm | 30.95 µg/mL and 24 hours | | |
| ZnO | Lymphocyte | 8 nm | 5 nM and 24 hours | 50% | [140] |
| | NK cells | | 1 nM and 24 hours | | |
| | monocytes | | 0.3 nM and 24 hours | | |
| ZnO | HFL1 cells | 20 nm | 0.5 mg/mL and 48 hours | less than 50% | [125] |
| $TiO_2$ | | 21 nm | | ~60% | |
| $SiO_2$ | | 20 nm | | ~60% | |
| $Al_2O_3$ | | 13 nm | | ~90% | |
| ZnO | *Euglena gracilis* | 10.4 nm | $10^{-3}$ M and 10 days | 10% | [123] |
| TOPO-ZnO | | 15.3 nm | | 10% | |
| Brij-76-ZnO | | 12.7 nm | | 10% | |
| ZnO | *Anabaena flos-aquae* | 10.4 nm | | 75% | |
| TOPO-ZnO | | 15.3 nm | | 25% | |
| Brij-76-ZnO | | 12.7 nm | | 75% | |
| ZnO | *Pseudokirchneriella subcapitata* | 50-70 nm | 0.04 mg Zn/L and 72 hours | 50% | [141] |
| $TiO_2$ | | 25-70 nm | 5.83 mg Ti/L and 72 hours | | |
| CuO | | 30 nm | 0.71 mg Cu/L and 72hours | | |

**Table (2) contd…**

| NPs | Models | Size of NPs | Treatment | Viability | Ref. |
|---|---|---|---|---|---|
| ZnO | *Vibrio fischeri* | 50-70 nm | 1.9 mg/L and 30 min | 50% | [119] |
| CuO | | 30 nm | 79 mg/L and 30 min | | |
| $TiO_2$ | | 25-70 nm | 20000 mg/L and 30 min | | |
| ZnO | *Daphnia magna* | 50-70 nm | 3.2 mg/L and 24 hours | | |
| CuO | | 30 nm | 3.2 mg/L and 24 hours | | |
| $TiO_2$ | | 25-70 nm | 20000 mg/L and 24 hours | | |
| ZnO | *Thamnocephaluspla tyurus* | 50-70 nm | 0.18 mg/L and 24 hours | | |
| CuO | | 30 nm | 2.1 mg/L and 24 hours | | |
| $TiO_2$ | | 25-70 nm | 20000 mg/L and 24 hours | | |
| ZnO | *Caenorhabditis elegans* | 20 nm | 2.3 mg/L and 24 hours | 50% | [142] |
| $Al_2O_3$ | | 60 nm | 82 mg/L and 24 hours | | |
| $TiO_2$ | | 50 nm | 80 mg/L and 24 hours | | |
| ZnO | *Saccharomyces cerevisiae* | 50-70 nm | 131 mg/L and 24 hours | 50% | [118] |
| CuO | | 30 nm | 13.4 mg/L and 24 hours | | |
| $TiO_2$ | | 25-70 nm | 20000 mg/L and 24 hours | ~100% | |

Current cytotoxicity assays include 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for colorimetric detection of mitochondrial activity, lactate dehydrogenase (LDH) assay for colorimetric detection of LDH release, annexic V/propidium iodide for fluorimetric detection of apoptosis marker and necrosis marker, neutral red for colorimetric detection of intact lysosomes, 2',7'-dichlorodihydrofluorescen diacetate (DCFH-DA) for fluorimetric detection of ROS production and so on [21, 96, 98, 99, 106, 143, 144]. But, most reports didn't mention how to avoid the deviation caused by the interaction between ZnO nanocrystals and dyes molecules. Nevertheless, some scientists recently developed researches on the molecular mechanism of the ZnO nanocrystals toxicity and demonstrated ZnO nanocrystals may induce apoptosis by p53 pathway [145, 146] or inflammatory responses by NF-$\kappa$B signal way [147]. ZnO nanocrystals may cause cell death as well as carcinogenic effect through damaging DNA molecular [96, 99, 102, 105, 111]. Some researchers even found that nanocrystals can damage DNA without contacting the cells directly [148]. This phenomenon is worthy of our note in order to avoid long-term adverse effect, especially when applied *in vivo*. However, current researches are insufficient because most toxicity studies are based on bare ZnO nanocrytals *in vitro*, while there may be little correlation between the toxicity *in vitro* and that *in vivo*. Furthermore, the surface modification materials may change the toxicity of ZnO nanocrystals significantly [123, 149].

It is an urgent task to solve these problems for most ZnO nanocrystals applied in biology and biomedicine are modified. The investigations on the toxicity *in vivo* are very difficult due to the complex environment in biological systems. Zhang *et al*. [150] developed a method to predict oxidative stress and acute pulmonary inflammation using metal oxide nanoparticle band gap but not tradition toxicity test assay. But to understand the distribution and clearance of nanocrystals *in vivo* will be the basic work in determining their toxicity and their future investigation directions. Combining synthesis and modification work with toxicity studies will be a promising approach to promote the applications of nanocrystals.

Just as every coin has two sides, toxic nanocrystals can be utilized as anticancer and antibacterial agents. Resistance to drugs is a serious problem existing in clinic therapy. As discussed above, the toxicity of ZnO nanocrystals mainly due to the release of metal ion and ROS, especially under UV exposure. Therefore, less resistance to drug may occur for cancer cells, when comparing drugs loaded on ZnO nanoparticles and those traditional anti-cancer agents. Besides, ZnO nanoparticles can be modified with specific groups, and thus, target delivery will be realized to minimize the side effects.

## 6. CONCLUSIONS

ZnO nanocrystals have been tested in a wide range of biological and biomedical applications, especially in bioimaging, drug delivery and biosensinig fields. Nevertheless, ZnO nanocrystals have far from exhausted their biological and biomedical potentials. Multimodel applications will be a major direction in the future. To realize their practice applications, one important problem is how to obtain water-dispersible ZnO nanocrytals with high quality, including high stability, efficient luminescent intensity and good biocompatibility. Another meaningful issue is how to avoid

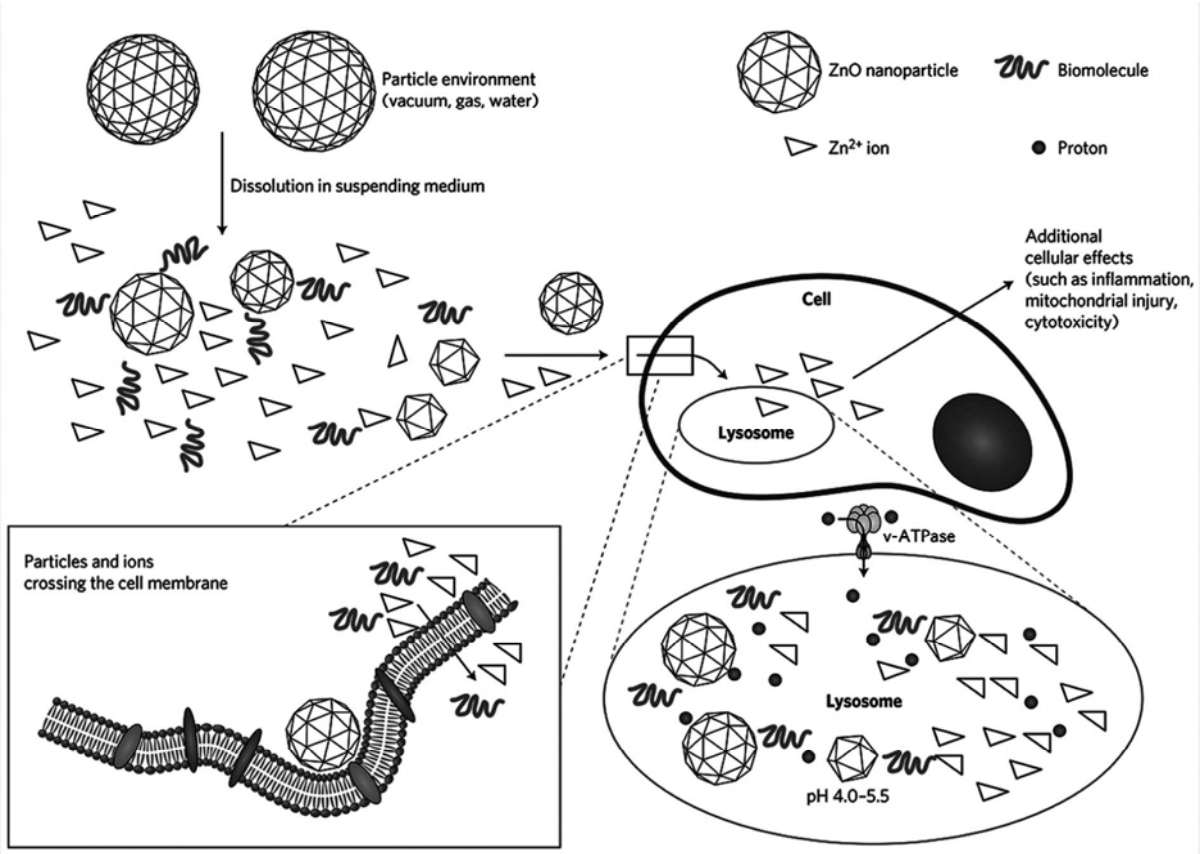


**Fig. (11).** Influence of ZnO on lysosomal function. ZnO dissolution through interactions at sequential nano-bio interfaces in the extracellular environment and the acidifying lysosome generates cellular toxicity through the release of toxic $Zn^{2+}$ ions. Release of $Zn^{2+}$ in the lysosome and the intracellular environment can induce a series of harmful cellular outcomes, such as lysosomal damage, mitochondrial perturbation, ROS production, excitation of pro-inflammatory cytokine and chemokine production. Reprinted with permission from Ref. [144], Copyright 2009 Nature Publishing Group.

compatibility. Another meaningful issue is how to avoid or utilize the toxicity of ZnO nanocrytals in a practical biomedical test. All these interesting issues are attracting scientists to put forward researches on ZnO nanoparticles.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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## PATIENT'S CONSENT

Declared None

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