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The nanotoxicity investigation of optical nanoparticles to cultured cells in vitro

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ABSTRACT

Optical nanoparticles (NPs) have the potential to provide new tools for diagnosis and treatment of human diseases, however, their nanotoxicity and biological characteristics are still unclear. Here, we prepared a series of typical NPs (including gold nanospheres, gold nanorods, silver nanopheres, silver triangular nanoplates and quantum dots) with different material and surface chemical modification for nanotoxicity test. Cell proliferation was investigated by SRB assay where the NPs were co-cultured with cancer cells. It was found that NPs' toxicity was highly correlated to different factors—material selection, physical size/surface area, shape, and surface chemical property, etc. This work has the potential to provide a uniform and systematic information when they are applied as probes in biological and medical fields.

1. Introduction

With rapidly growing interests in nano-research, the toxicity of nanoparticles (NPs) is becoming an increasingly important issue in nanotechnology [11]. However, little is known about the health risks and toxicity of these nanomaterials [11]. In addition, due to the increased production and use of NPs in various fields, the side effect of NPs is an emerging and growing concern both academically and socially [11]. Recommendations for the use of NPs in various fields have emerged as a result of these initial toxicity studies [11]. Several previous studies have investigated the toxicity of NPs based on various factors, such as shape, size, surface chemistry, chemical composition, surface activity and solubility [3,13,16,14]. For example, series of investigations have been finished on the nanotoxicology of carbon nanomaterials, ferric oxide NPs and TiO_2 NPs [9,34,29].

The biomedical applications of NPs, especially the NPs with optical properties, have been extensively studied in the past decade, especially in the term of drug and biolabeling, biosensors, cancer treatment and diagnostic tools

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[18,11,35]. Due to the special physical–chemical properties, the applications of NPs with optical properties in biological fields are widely developed, e.g., metal plasmonic NPs gold nanospheres (GNSs), gold nanorods (GNRs), silver nanospheres (SNSs), silver triangular nanoplates (STNPs), etc., and semiconductor quantum dots (QDs) (CdSe, CdTe, etc.). The nanotoxicology of these NPs is particularly significant due to the consideration of biological safety. Prasad et al. [21] have evaluated the cytotoxic and genotoxic assessment of glycolipid-reduced/-capped gold and silver spherical NPs. They found that gold NPs are more cytocompatible than similar silver NPs, which demonstrated that silver NPs could cause more DNA damage compared to gold NPs. Chan et al. [8] have found that the gold nanorods after the surface coating with poly (diallyldimethylammonium chloride)-poly(4-styrenesulfonic acid) system have negligible impact on cell function, while a very low number of genes experience any significant change in expression via the effect of surface chemistry on gold nanorods uptake, toxicity, and gene expression in mammalian cells. Clift et al. [4] have investigated that different surface-coated QDs will cause oxidative stress and affect macrophage cell signaling in vitro. Ying et al. [24] have proved that the cellular interaction induced toxicity of the QDs is dose-dependent. At the same time, the cationic and hydrophobic QDs have stronger interactions with cells than the anionic QDs. More important, the cationic QDs are the most toxic, but their toxicity could be reduced by introducing poly(ethylene glycol) (PEG) on the QDs surface. Su et al. investigated the cytotoxicity of aqueous-synthesized CdTe QDs and the relative contributions from released cadmium ions and demonstrated that after the surface passivation with CdS and ZnSe shell, the cytotoxicity of the CdTe QDs will be greatly suppressed [22,23].

To date, the nanotoxicity studies are mainly focused on single variable quantity for the control experiments and at the same time all the results are investigated separately [3,16,14]. Furthermore, the used NPs are often surface-modified with substitution of the original surface group from the classical synthesis batch, where the experiments are less applied and followed [21,8,24]. However, a more rigorous evaluation of nanotoxicology in the same platform is needed in order to confidently regulate the safe use of all forms of NPs with optical utilities. The nanotoxicology investigation of all the popularly optical NPs in the same platform or system has not been published till now.

In this work, we prepared the biological-compatible aqueous gold nanospheres, gold nanorods, silver nanospheres, silver triangular nanoplates, CdTe/CdS core/shell QDs and then investigated the nanotoxicity of these NPs in both of human hepatocarcinoma cell line HepG2 and human cervical carcinoma Hela cells. Those two cell line have stronger endocytosis comparing with other cell lines and were commonly used to evaluate cytotocixity of antitumor drugs with Sulforhodamine B (SRB) assay [12,27,20]. Nanoparticles' toxicity is highly correlated to different factors, including material selection, physical size/surface area, appearance shape, and surface chemical property. From our results, the nanotoxicity of all the nanopartilces is dosage-dependent, but the nanotoxicity of the same-material metal nanopartilces with different shape is different (e.g., SNSs vs. STNPs, GNSs vs. GNRs); the nanotoxicity of the same-shape nanopartilces with different material is different (e.g., SNSs vs. GNSs vs. QDs); the nanotoxicity of the same-material and shape metal nanopartilces with different surface group is different (e.g., citrate capped GNSs vs. CTAB capped GNSs); the nanotoxicity of the same-material and shape nanopartilces with different size is different (e.g., 3 nm QDs vs. 5 nm QDs, 15 nm GNSs vs. 30 nm GNSs), and the nanotoxicity of the same nanopartilces to different cell line is different (e.g., Hela cells vs. HepG2 cells). This work has systematically investigated the nanotoxicity of these optical NPs and has the potential to provide a uniform and systematic information or reference when these NPs are applied as probes in biological and medical fields.

2. Materials and methods

2.1. Chemicals and reagents

Fetal bovine serum (FBS), DMEM high glucose medium, penicillin and streptomycin were obtained from Beyotime Institute of Biotechnology (Haimen, China). Sulforhodamine B (SRB), trichloroacetic acid (TCA), chloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$, ≥99.9%) and sodium borohydride ($NaBH_4$, ≥98%), were purchased from Sigma–Aldrich (St. Louis, MO). Hexadecyltrimethylammonium bromide (CTAB, ~99%) and L-ascorbic acid (≥99.5%) were purchased from Sigma. Silver nitrate ($AgNO_3$, ≥99%) was purchased from Fluka. Deionized water was purified through a Milli-Q water purification system and the resistivity was 18.2 MΩ cm.

2.2. Synthesis and characterization of gold nanospheres

GNSs were prepared according to the standard sodium citrate reduction method. Typically, 45 mL deionized water and 5 mL $HAuCl_4$ (2.5 mM) aqueous solutions were mixed in a three-neck flask and heated to 100 °C. Subsequently, 1% sodium citrate solution was quickly added into the flask. The color of the solution rapidly changed from tint yellow to black and red or deep purple. After 1 h, the GNSs were cooled to room temperature waiting for the next nanotoxicity measurement. The size could be tuned by changing the volume of the additional sodium citrate. As shown in the field-emission scanning electron microscope (FESEM) of Fig. 1A and B, the size of as-prepared GNSs was about 15 nm and 30 nm, respectively. The plasmon absorption peaks were localized at 520 nm and 525 nm (shown in Fig. 2A).

2.3. Synthesis and characterization of gold nanorods

The synthesis of gold nanorods was a traditionally seed-mediated growth procedure [15,17,32], in which Au salt was reduced initially with a strong reducing agent, in water at room temperature. Simply, the Au seed particles were prepared by reduction of $HAuCl_4$ (0.25 mM) in CTAB solution with the reduced reagent-ice-cold sodium borohydride. Subsequent reduction of more metal salt

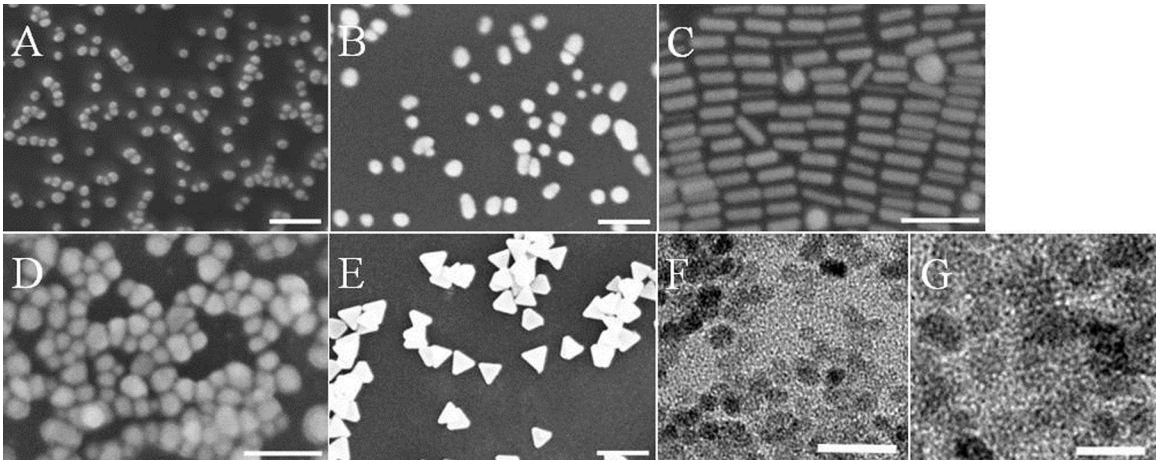

Fig. 1. FESEM results of 15 (A) and 30 nm (B) GNSs, 15 nm × 44 nm GNRs (C), 30 nm SNSs (D) 60 nm STNPs (E) and TEM results of 2.5 nm GQDs (F) and 5 nm RQDs (G). The scale bar in (A)–(D) is 100 nm. The scale bar in (E) is 150 nm. The scale bar in (F) and (G) is 10 nm.

with a weak reducing agent, in the presence of structure-directing additives, leads to the controlled formation of gold nanorods. Briefly, a 25 mL growth solution was prepared by reduction of 0.5 mM $HAuCl_4$ in a solution containing CTAB, 0.05 mM silver nitrate and 176 μL of ascorbic acid. The color of the solution rapidly changed from golden to colorless after the introduction of ascorbic acid. 36 μL of the seed solution was then added into the growth solution and the color of the solution slowly changed from colorless to deep purple. Ultimately, the bilayer CTAB-coated gold nanorods were obtained after several hours' stirring. As shown in the FESEM of Fig. 1C, the size of the prepared nanorods was mainly 15 nm in width and 44 nm in length with plasmon absorption peaks at 517 (transverse plasmon band) and 714 nm (longitudinal plasmon band) (shown in Fig. 2B).

2.4. Synthesis and characterization of silver nanospheres

SNSs were prepared according to the standard sodium citrate reduction method. Typically, 25 mL deionized water and 0.25 mL $AgNO_3$ (10 mM) aqueous solutions were mixed in a conical flask at room temperature. Subsequently, 1% sodium citrate solution was quickly added into the flask. The color of the solution rapidly changed from colorless to pale yellow. After 1 h, the SNSs were prepared and waited for the next nanotoxicity measurement. As shown in the FESEM of Fig. 1D, the size of as-prepared SNSs was about

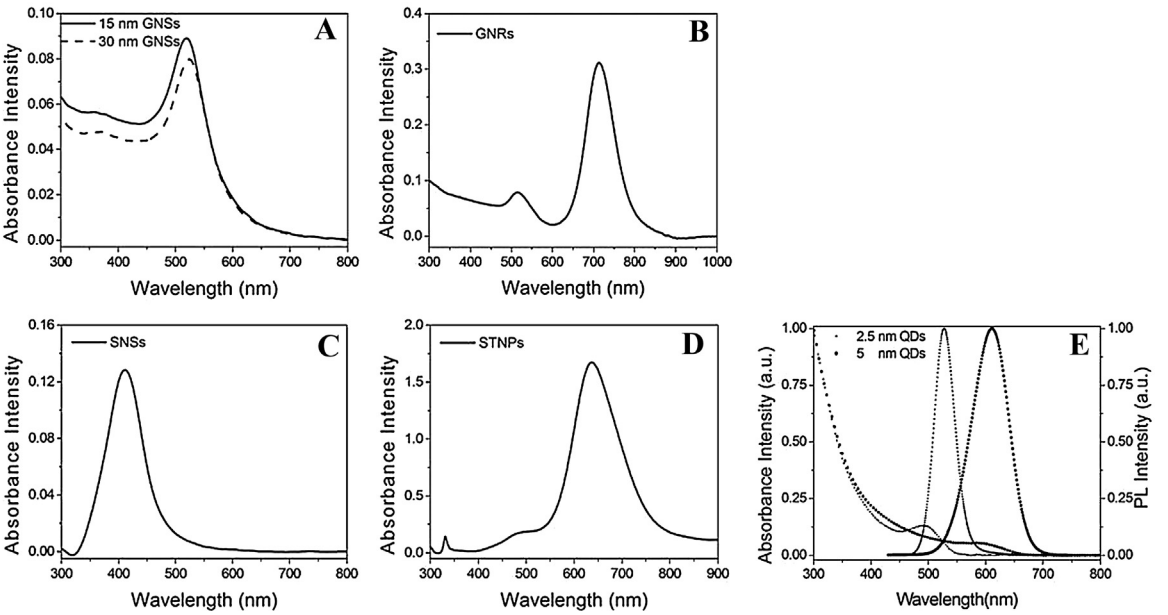

Fig. 2. The plasmon absorption spectra of 15 and 30 nm GNSs (A), 15 nm × 44 nm GNRs (B), 30 nm SNSs (C), 60 nm STNPs (D) and the absorption and emission spectra of 2.5 nm GQDs and 5 nm RQDs (E).

30 nm. The plasmon absorption peaks were localized at 411 nm (shown in Fig. 2C).

2.5. Synthesis and characterization of silver triangular nanoplates

A typical experiment was carried out by the photoconversion method [33,25]. First, silver seeds were prepared by dropwise addition of $NaBH_4$ solution (8 mM, 1 mL) to an aqueous solution of $AgNO_3$ (0.1 mM, 99 mL) in the presence of trisodium citrate (1 mM) under vigorous stirring. Next, the yellow silver seeds (100 mL, in glass conical flask with a cover) were exposed under a sodium lamp (NAV-T 70 model from Osram China Lighting Co., Ltd.). The irradiation power density of the sodium lamp was determined to be 80.6 mW/cm^2 as measured using a Gentec-EO Solo 2 energy and power meter at the position of silver solution. The color of silver colloids changed from yellow to green and blue during the irradiation process. After 2 h, the STNPs were prepared and waited for the next nanotoxicity measurement. As shown in the FESEM of Fig. 1E, the size of as-prepared STNPs was about 60 nm. The in-plate dipole plasmon resonance absorption peaks are localized at 636 nm (shown in Fig. 2D).

2.6. Synthesis and characterization of CdTe/CdS core/shell QDs

The 3-mercaptopropionic acid stabilized CdTe/CdS core/shell QDs were prepared according to our previous work [31]. The green and red CdTe/CdS core/shell QDs were emitted at 527 and 611 nm (Fig. 2E) with sizes of about 2.5 nm and 5 nm (shown in Fig. 1F and G). The absorption spectra showed that there is a gradually enhanced absorption below the band gap, which property is different from that of the metal NPs due to the native absorption energy level of the semiconductor QDs.

2.7. Cell culture and SRB assay

Human hepatocellular carcinoma HepG2 cells and Human cervical cancer Hela cells were maintained in DMEM high glucose medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin in an atmosphere of 95% air and 5% CO_2 at 37 °C. The SRB assay is routinely used for cytotoxicity determination, based on the measurement of live cell protein content. It was performed in 96-well plates in octuplicate after 48 h treatment. In brief, cells were seeded at a density of 5×10^3 cells/well overnight, and treated with the indicated concentrations of NPs for 48 h. Afterwards, 100 μL of 20% TCA was added to the culture medium in each well and refrigerated at 4 °C for 3 h, then the supernatant was discarded and the plate was washed for 5 times with water and air dried. 100 μL of SRB solution 0.4% (w/v) in 1% acetic acid was added to each well and incubated for 30 min at room temperature. Unbounded SRB was flicked off the plates and air dried the plates. Bound SRB was solubilized with 150 μL of 10 mM Tris–HCl to each well and the plate was shaked for 5 min. The optical density (OD) at 570 nm wavelength was measured and the ratio of cell viability to control group was calculated from the SRB data. The concern that various nanaoparticles, such as GNSs, GNRs and QDs in 570 nm would interfere with the SRB determination was resolved by rinsing off the excess NPs which resided outside the cell or adhered to the dead cells in SRB assay procedure [28]. The control group also went through the same process for SRB assay.

3. Results

3.1. Effect of gold nanoparticles on cell viability of HepG2 and Hela cells

As shown in the FESEM of Fig. 1A and B, the size of as-prepared GNSs was about 15 nm and 30 nm, respectively. The plasmon absorption peaks were localized at 520 nm and 525 nm (shown in Fig. 2A), the red-shift of the absorption peaks reflected the size effect of gold NPs clearly. As shown in the FESEM of Fig. 1C, the size of the prepared nanorods was mainly 15 nm in width and 44 nm in length with plasmon absorption peaks at 517 (transverse plasmon band) and 714 nm (longitudinal plasmon band) (shown in Fig. 2B). As shown in Fig. 3A, the cell viability of 15 nm and 30 nm citrate sodium capped gold nanospheres in HepG2 cells was assessed after 48 h treatment with a series of concentrations of the NPs from 0 to 200 pM. It was shown that 15 nm GNSs did not influence cell growth even in the 200 pM concentration, however, 30 nm GNSs showed the reduction of HepG2 cells proliferation ratio by 15.7% in 50 pM, 21.9% in 100 pM and 28.9% in 200 pM compared with control group. The 15 nm and 30 nm GNSs on cell growth in Hela cells showed almost the same effect as HepG2 cells (data not shown). The results indicated that the toxicity of GNSs was increased by the size-dependent manner (Fig. 3A). Moreover, the influences of CTAB-capped gold nanorods on cell viability were measured in HepG2 and Hela cells with the same cultural conditions. It showed that a significant damage was exhibited both in cell proliferation and cell morphology. As shown in Fig. 3B, 0.625 pM of GNRs induced 15.8% and 22.1% reduction of cell survival rate in HepG2 and Hela cells respectively. The median inhibition concentration (IC50) of GNRs in HepG2 cells is only 1.75 pM, which is significant less than that of 30 nm GNSs (more than 200 pM). Shrinked cell membrane and cell debris were observed in HepG2 cells treated with 12.5 pM CTAB capped GNSs and GNRs for 48 h (Fig. 3D), which cellular morphology was greatly different from the control sample or the citrate capped GNSs. To elucidate the cause of significant difference in cytotoxicity between GNSs and GNRs, the impact of 45 nm CTAB capped GNSs was detected in HepG2 cells. The result was shown in Fig. 3C, 45 nm GNSs with the same surface group inhibited cell proliferation with the same trend to GNRs, which showed the obviously higher nanotoxicity than citrate capped GNSs, indicated that it was the surface ligand, not the shape played a key role on cell survival in this case. When the concentration was higher than 0.125 pM, the CTAB capped GNSs (45 nm) showed higher nanotoxicity compared to the GNRs (15 nm × 44 nm), which was due to the larger surface area of the GNSs contacted with the HepG2 cells.

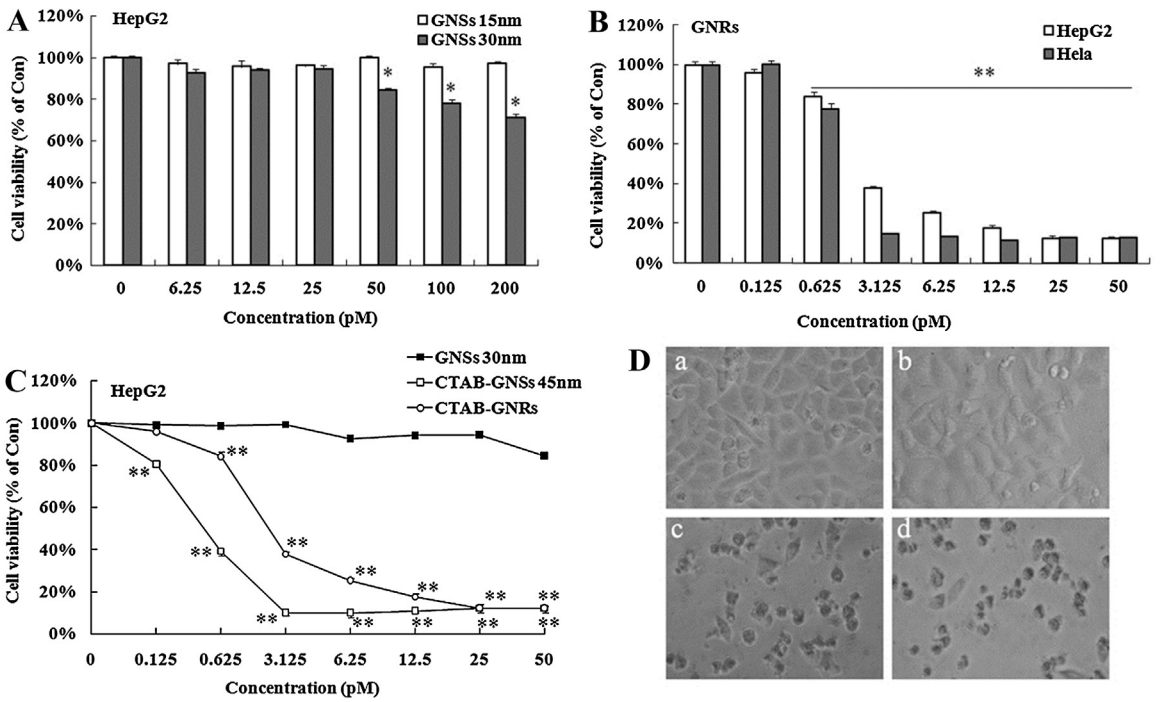

Fig. 3. Effect of gold NPs on cell viability and cell morphology in HepG2 and Hela cells. (A) 15 and 30 nm GNSs on cell viability in HepG2 cells. (B) GNRs on cell viability in HepG2 and Hela cells. (C) Comparison of 30 nm GNSs, CTAB-GNSs (45 nm) and CTAB-GNRs (15 nm × 44 nm) on cell viability in HepG2 cells. (D) HepG2 cell morphology in control (a), 30 nm GNSs (b), 45 nm CTAB-GNSs (c) and 15 nm × 44 nm CTAB-GNRs (d) after 48 h-treatment with the same concentration of 12.5 pM. The cell viability of control cells were set arbitrarily to 100%. The data plotted are mean ± standard error (SE), $n = 8$. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

3.2. Effect of silver nanoparticles on cell viability of Hela cells and HepG2 cells

As shown in the FESEM of Fig. 1D, the size of as-prepared SNSs was about 30 nm. The plasmon absorption peaks were localized at 411 nm (shown in Fig. 2C). As shown in the FESEM of Fig. 1E, the size of as-prepared STNPs was about 60 nm. The in-plate dipole plasmon resonance absorption peaks are localized at 636 nm (shown in Fig. 2D). As shown in Fig. 4A, silver nanospheres (30 nm) and silver triangular nanoplates (60 nm) did not influence HepG2 cell growth when the concentration was less than 200 μg/ml. About 14% decrease in cell viability was shown when the concentration reaches 400 μg/ml. The cell growth curve was almost overlapping between SNSs and STNPs, indicating that HepG2 cell has the same response with the different shape of silver nanopaticles. Interestingly, for Hela cell lines, the cell growth curve of STNPs and SNSs began to bifurcate at the point of the concentration of 50 μg/ml, and STNPs showed increasing inhibitory percent of 11.8%, 15.6% and 31.9% more than SNSs in 100, 200 and 400 μg/ml, suggesting that Hela cells were more susceptible to STNPs comparing with the SNSs. In general, Hela cells was more sensitive to silver NPs than HepG2 cells in cell viability as well as cell morphology changed (shown in Fig. 4B), which was similar with the nanotoxicity of GNRs. The finding indicated the toxicity of silver NPs was of cell and shape specific.

3.3. Effect of CdTe/CdS core/shell QDs on cell proliferation of HepG2 cells Characterization

The green and red CdTe/CdS core/shell QDs were emitted at 527 and 611 nm (Fig. 2E) with sizes of about 2.5 nm and 5 nm (shown in Fig. 1F and G), the red-shift of the absorption and emission peaks reflected the quantum size effect of QDs clearly. The effect of CdTe/CdS core/shell QDs with different size (2.5 nm green QDs and 5 nm red QDs) on cell proliferation was detected in the concentration from 0.02 to 2 μM. The result showed that the cell growth was gradually inhibited with the increase of QDs concentration. 5 nm QDs was more toxic than 2.5 nm QDs at the same concentration level, indicating the size was the important factor for QDs in cytotoxicity. The results were shown in Fig. 5.

4. Discussion

Cellular endocytosis, cellular delivery, and subcellular targeting inducing nanotoxicity of a nanoparticle can be influenced in several ways [24]. First, a nanoparticle has a high surface to-volume ratio, and thus any small change in the particle size, shape and/or surface functional group may lead to significant alteration in cellular interaction. Consequently, the cellular uptake, cytotoxicity, and subcellular localization of NPs are highly sensitive to the particle size, shape, surface charge, hydrophobicity, and nature of the

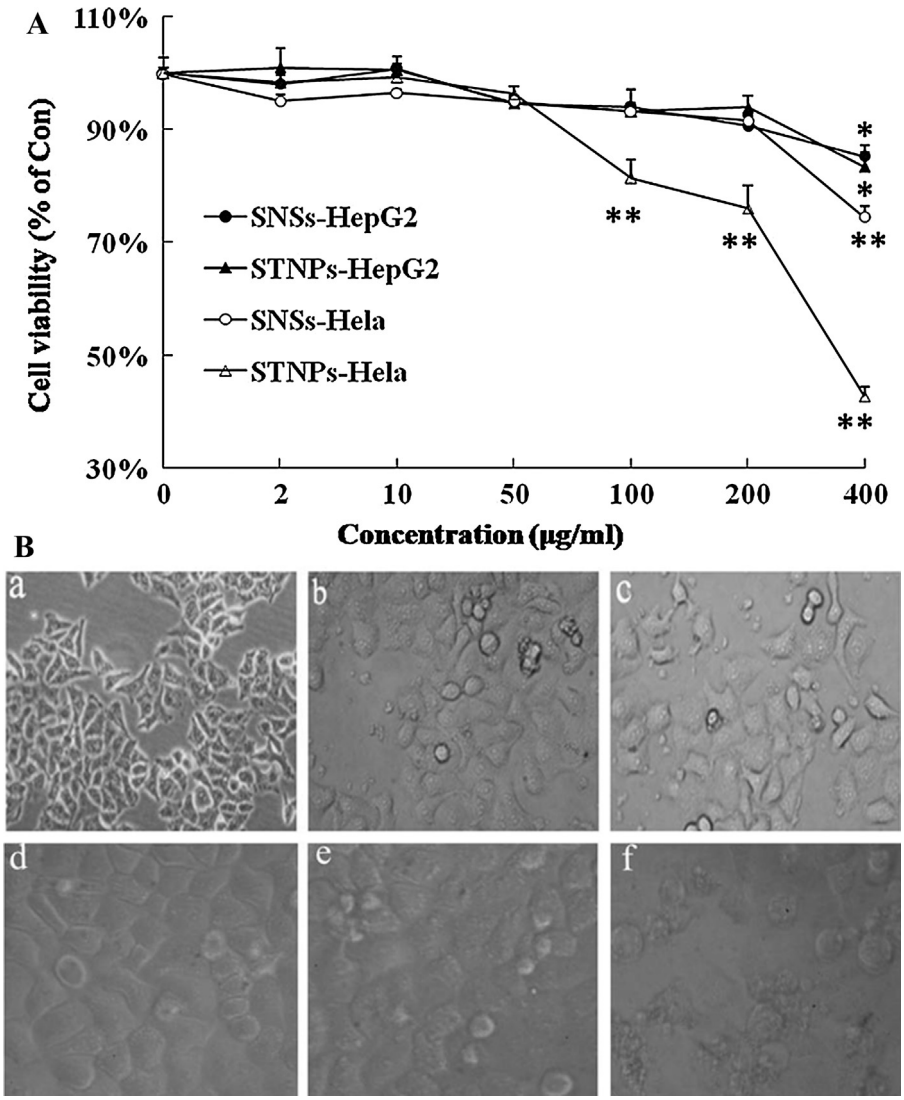

Fig. 4. Effect of silver NPs on cell viability and cell morphology in HepG2 and Hela cells. (A) SNSs and STNPs on cell viability in HepG2 and Hela cells. (B) HepG2 cell morphology in control (a), 200 μg/ml SNSs (b), 200 μg/ml STNPs (c) and Hela cells morphology in control (d), 200 μg/ml SNSs (e), 200 μg/ml STNPs (f) after 48 h-treatment. The cell viability of control cells were set arbitrarily to 100%. The data plotted are mean ± standard error (SE), $n = 8$. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

surface ligands. Second, a larger particle size and a higher surface charge would often induce high nonspecific cellular uptake of NPs, which further complicated the uptake activity of affinity ligands by reducing the specificity of NPs. Third, NPs often end up at lysosomes and prevent subcellular targeting. As a result, based on the same material, shape, and surface group, the larger size GNSs (or QDs) were more toxic than smaller size GNSs (or QDs) at same dosage administration concentration. It is reported that the surface charge plays a significant role in the process of cellular uptake [1,8]. This means that a larger particle size and a higher surface charge would often induce high nonspecific cellular uptake of NPs [24]. As a result, the larger size and a higher surface charge NPs will cause a relative obvious interaction with the cell line and lead to a serious cell toxicity. Considering the same material, different shape would cause differently toxic results, e.g., STNPs were more toxic than SNSs. Comparing with the influence of shape of the NPs, surface ligand functioned more crucial effect on the nanotoxicity of cells, e.g., CTAB capped GNSs and GNRs were obviously more toxic than citrate sodium capped GNSs because CTAB, as well-known cationic surfactant, could increase cell membrane permeability and conjugate with nuclear acid [19]. On the other hand, the CTAB can damage mitochondria, and then induce apoptosis [19].

A growing body of evidences indicated that the mechanism of NPs on cytotoxicity is mostly related with the induction of apoptosis in living system. However, the initiators and signaling pathways of apoptosis are different, which is depending on the special natures of NPs. Gao et al. [6] reported gold NPs caused hydrogen oxygen accumulation by cytosolic glutathione (GSH) depletion and subsequently activated mitochondrial apoptosis pathway.

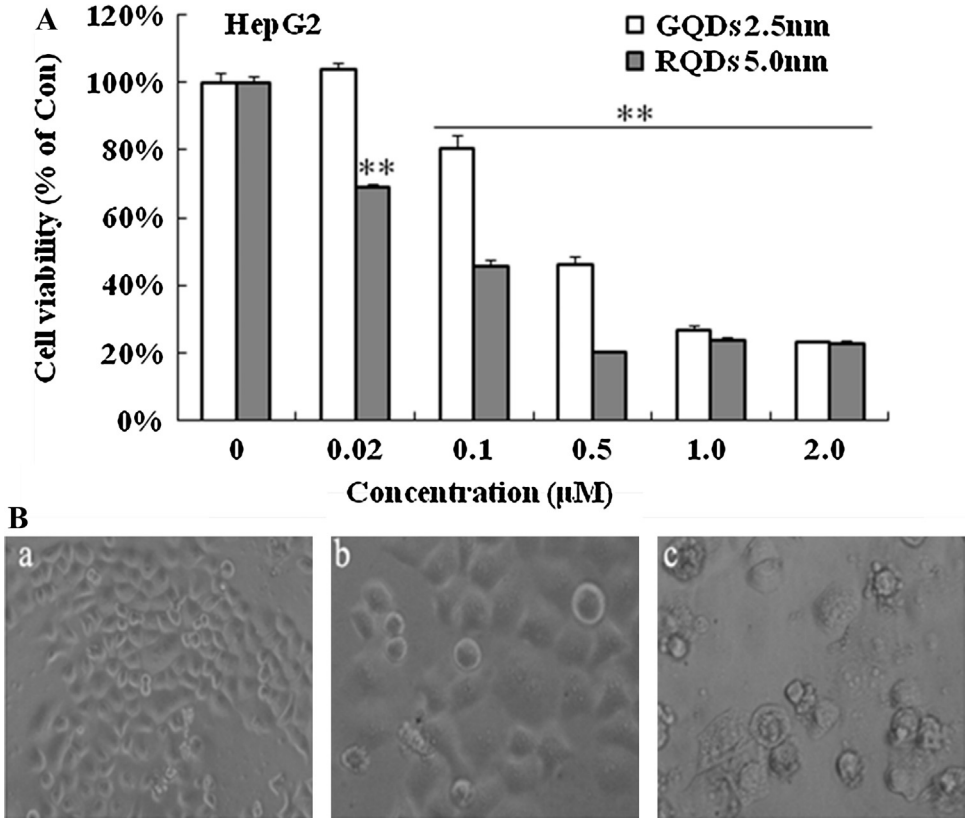

Fig. 5. Effect of 2.5 nm and 5 nm CdTe/CdS core/shell QDs on cell viability and cell morphology in HepG2 cells. (A) 2.5 nm and 5 nm CdTe/CdS core/shell QDs on cell viability in HepG2 cells. (B) HepG2 cells morphology in control (a), GQDs (b) and RQDs (c) for 48 h-treatment with the same concentration of 0.1 μM. The cell proliferation of control cells were set arbitrarily to 100%. The data plotted are mean ± standard error (SE), $n = 8$. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

Kang et al. [10] stated that gold NPs localizing in cell nuclei would induce DNA damage and cytokinesis arrest. Moreover, the toxicity of silver NPs and QDs were depended on the dissolved ion concentration (such as silver ion, cadmium ion, etc.) and surface coating [26,2]. Furthermore, the production of intracellular reactive oxygen species (ROS), increased expression of cleaved caspase proteins and p53 activation were commonly involved in the mechanisms of apoptosis induced by NPs [5,7,30]. In the present study, Hela cell is more sensitive to STNPs than SNSs and HepG2 cell is more susceptible to GNRs than GNSs, indicating that there is cell selective response to the shape of NPs.

The traditional gold NPs have been widely applied in the biological and medical fields for a long time since they are considered as owning better biocompatibility, on the contrary, the CdSe and CdTe QDs are always controversial on toxicological issues because of the heavy metal property of cadimium although Su et al. have demonstrated that after the surface passivation with CdS and ZnSe shell, the cytotoxicity of the CdTe QDs will be greatly suppressed [22,23]. Surprisingly, we found that when the nanotoxicity of GNSs (30 nm) and QDs (5 nm) is on the same level (~70% cell viability), the dosage administration concentrations of GNSs and QDs are 0.2 nM and 20 nM, respectively. This indicates that the nanotoxicity of GNSs is 100 times higher than QDs. Since it is reported that the toxicity of the silver NPs are more serious than the gold NPs since silver NPs cause more DNA damage compared to gold NPs [21]. Furthermore, we have proved that the CTAB capped GNRs are more toxic than the GNSs. We can draw a conclusion that size and surface properties but not the elemental composition of the NPs themselves are more significant on the cellular nanotoxicity, which may provide a novel judgment on the nanotoxicity of aqueous cadmium-containing QDs.

In conclusion, we have prepared the biological-compatible aqueous gold nanospheres, gold nanorods, silver nanospheres, silver triangular nanoplates, CdTe/CdS core/shell QDs according to the traditional method, and then we investigated the nanotoxicity of these NPs in human hepatocarcinoma cell line HepG2 and human cervical carcinoma Hela cells. NPs' toxicity is highly correlated to many characteristics, including material selection, physical size/surface area, appearance shape, and surface chemical property. We observed that the nanotoxicity of all the NPs is dosage-dependent, but the nanotoxicity of the same-material metal NPs with different shape is different (e.g., SNSs vs. STNPs, GNSs vs. GNRs), the nanotoxicity of the same-shape NPs with different material is different (e.g., SNSs vs. GNSs vs. QDs), the nanotoxicity of the same-material and shape metal NPs with different surface group is different (e.g., citrate capped GNSs vs. CTAB capped GNSs), the nanotoxicity of the same-material and shape

NPs with different size is different (e.g., 3 nm QDs vs. 5 nm QDs, 15 nm GNSs vs. 30 nm GNSs), and the nanotoxicity of the same NPs to different cell line is different (e.g., Hela cells vs. HepG2 cells). Based on the findings, it seems that not only the dose is a key parameter that affects toxicity of NPs, but also other properties such as particle size, roughness, shape and charge are the important factors impacting cytotoxicity. This work has systematically investigated the nanotoxicity of NPs owning powerful optical properties, and has the potential to provide a uniform and systematic information estimation or reference when these NPs are applied as probes in biological and medical fields.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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