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Research Article

Cisplatin Loaded Hyaluronic Acid Modified TiO_2 Nanoparticles for Neoadjuvant Chemotherapy of Ovarian Cancer

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Novel tumor-targeting titanium dioxide (TiO_2) nanoparticles modified with hyaluronic acid (HA) were developed to explore the feasibility of exploiting the pH-responsive drug release property of TiO_2 and the tumor-targeting ability of HA to construct a tumor-targeting cisplatin (CDDP) delivery system (HA-TiO_2) for potential neoadjuvant chemotherapy of ovarian cancer. The experimental results indicated that CDDP release from the HA-TiO_2 nanoparticles was significantly accelerated by decreasing pH from 7.4 to 5.0, which is of particular benefit to cancer therapy. CDDP-loaded HA-TiO_2 nanoparticles increased the accumulation of CDDP in A2780 ovarian cancer cells via HA-mediated endocytosis and exhibited superior anticancer activity *in vitro*. *In vivo* real-time imaging assay revealed that HA-TiO_2 nanoparticles possessed preferable tumor-targeting ability which might potentially minimize the toxic side effects of CDDP in clinical application.

1. Introduction

Ovarian cancer is a commonly fatal disease which is responsible for 4% of deaths from cancer in women. Primary debulking surgery before initiation of chemotherapy has been the standard of care for patients with advanced ovarian cancer [1].

One of the most critical issues in neoadjuvant chemotherapy of ovarian cancer is the effectiveness of drug delivery. Conventional chemotherapeutic agents were distributed nonspecifically in the body when given intravenously. They affect both cancerous and normal cells, thereby limiting the dose achievable within the tumor and also resulting in suboptimal treatment due to excess toxicity [2]. The development of nanotechnology has shed light on tumor-targeted delivery of anticancer drugs. The nanosized particles can passively accumulate at the tumor site via the so-called enhanced permeability and retention effect [3]. Moreover, advanced tumor-targeting effect can be further achieved by attaching or grafting certain tumor-targeting ligand to the particles [4, 5]. Hyaluronic acid (HA), also called hyaluronan, is a naturally occurring polysaccharide present in the extracellular matrix and synovial fluids. In particular, since HA can specifically bind to various cancer cells, many studies have focused on the pharmaceutical applications of HA as a targeting ligand for anticancer therapeutics [6, 7]. In particular, ovarian cancer has been proved to be a cancer whose evolution process is closely related to HA [8, 9].

To date, a variety of nanocarriers have been successfully developed with various formulations and on the basis of many different materials. This includes polymer based polymeric micelles, lipid based liposomes, and some solid nanoparticles comprised of inorganic skeleton [10–12]. Titanium dioxide nanoparticles (TiO_2 NPs) have been proven to be a suitable candidate for biomedical applications due to their many merits such as the fact that TiO_2 NPs possess good optical and electronic properties and the catalyst is stable, nontoxic, cheap, and biologically and chemically inert [13, 14]. At the same time, the high surface area of TiO_2 NPs can guarantee preferable loading of drug [15]. As a result, TiO_2 NPs have been identified as a promising drug carrier in anticancer therapy [16, 17].

Among various clinical applied chemotherapy drugs, cisplatin (cis-diamminedichloroplatinum (II), CDDP) has been demonstrated to be a promising one which shows preferable anticancer effect on ovarian cancer and has already been adopted in neoadjuvant chemotherapy of ovarian cancer for many years [18, 19]. In this study, with the aim of effectively delivering CDDP to the ovarian cancer site, we employed TiO_2 NPs as a solid nano-sized vector. The TiO_2 NPs were functionalized with HA to formulate HA-TiO_2 nanoparticles with the ability to specifically bind with ovarian cancer cells. At last, the HA-TiO_2 nanoparticles were loaded with CDDP to construct a tumor-targeted delivery system with the hope to achieve preferable neoadjuvant chemotherapy of ovarian cancer.

2. Materials and Methods

2.1. Materials. Titanium (IV) isopropoxide (TIP) (95%) was obtained from Alfa Aesar (Lancs, UK). Pluronic block copolymer F127, hyaluronic acid (molecular weight = 10 kDa), cisplatin (CDDP), coumarin-6 (C6), DiR iodide (DiR), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals used were from Nanjing Chemical reagent Co., Ltd., and of analytical grades.

2.2. Cell Culture. A2780 human ovarian cancer cells, obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), were maintained in RPMI 1640 medium (Sigma, St. Louis, MO) containing 10% (v/v) fetal bovine serum (Hyclone, USA), 100 U/mL penicillin G, and 100 μg/mL streptomycin with a humidified atmosphere of 5% (v/v) CO_2 and a temperature of 37°C.

2.3. Animal Model. Female BALB/c nude mice were purchased from Shanghai Laboratory Animal Center (SLAC, China) and maintained at standard conditions of temperature, humidity, and a 12 : 12 h light-dark cycle daily with free access to food and water. All animal experiments were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The A2780 tumor-bearing mice models were established by inoculating a suspension of 1×10^6 A2780 cells in 100 μL physiological saline into the subcutaneous dorsa of BALB/c nude mice. Tumor-bearing mice were included for study when the tumor volume reached 80–100 mm^3 (calculated by the formula: $V = a^2 \times b/2$, where a and b are the shortest and longest diameter, resp.).

2.4. Preparation of HA-TiO_2 Nanoparticles

2.4.1. Preparation of TiO_2 Nanoparticles. The TiO_2 nanoparticles were synthesized via a sol-gel route as previously reported [20]. F127 was selected as a structure-directing agent and TIP as a TiO_2 source. Briefly, 16 g F127 was dissolved in proper amount of ethanol and then mixed with 2.4 mL water (Milli-Q). Another 7.4 mL of TIP was added to the solution at ambient temperature with vigorous stirring for 1 h. After that, the solution was kept at room temperature without stirring for 12 h. Finally, 2 g precursor sample was dispersed in certain amount of ethanol and stirred for 2 h at 70°C. After stirring, samples were collected by centrifugation and extracted for three times in this way, followed by vacuum desiccation at 40°C overnight. Moreover, the resulting product was dried at 200°C for one day to remove ethanol and F127 completely.

2.4.2. Preparation of HA-TiO_2 Nanoparticles. 80 mg of TiO_2 nanoparticles was suspended in HA phosphate buffer (PBS, pH 7.4, 10 mg/mL), and then the solution was stirred for 5 h at room temperature. These HA-TiO_2 nanoparticles were then collected by centrifugation (5000 g for 10 min) and were dried by vacuum desiccation.

2.5. Drug Loading. For a typical drug loading experiment, CDDP (2 mg) in 1 mL of methanol was added to 5 mL water/methanol (1 : 1, v/v) suspension of HA-TiO_2 nanoparticles (10 mg/mL). The above reaction mixture was continuously agitated with a stirrer at 50 g for 1 h to construct drug delivery system of CDDP-loaded HA-TiO_2 nanoparticles. CDDP-loaded HA-TiO_2 nanoparticles were isolated from the free-standing drug molecules through centrifugation at 5000 g for 30 minutes and the supernatant was determined by high-performance liquid chromatography (HPLC, Agilent 1200 series) to determine drug loading. The HPLC was loaded with ODS 120A column (4.6 × 250 mm) at a constant temperature of 45°C. The mobile phase consisted of 6 mM 1-octanesulfonate, 6 mM tetra-n-butylammonium hydrogen sulfate, and 20 mM KH_2PO_4, which was adjusted to pH 5.0 with 1 M NaOH. The applied flow rate was 0.7 mL/min. The spectrophotometric detection was set at 301 nm as described before [21]. Encapsulation efficiency and loading efficiency were calculated by the following equations:

$$\begin{aligned}&\text{Encapsulation efficiency (\%)}\\&= \left(\frac{\text{Amount of drug in CDDP-loaded HA-TiO}_2\text{ nanoparticles}}{\text{initial amount of drug}}\right)\\&\times 100,\\&\text{Loading efficiency (\%)}\\&= \left(\frac{\text{Amount of drug in CDDP-loaded HA-TiO}_2\text{ nanoparticles}}{\text{CDDP-loaded HA-TiO}_2\text{ nanoparticles}}\right)\\&\times 100.\end{aligned} \tag{1}$$

2.6. Morphology, Particle Size, and Zeta Potential Analysis. The morphologies were observed by transmission electron microscopy (TEM) under a JEM-2100 Electron Microscope (JEOL, Tokyo, Japan). The size distributions, mean particle size, and polydispersity index (PDI) were assessed by dynamic light scattering (DLS) (Brookhaven Instruments Corp., USA) by measuring the autocorrelation function at 90°. The zeta potential of the nanoparticles was determined using a Zeta plus zeta potential analyzer (Brookhaven Instruments Corp., USA) at 25°C.

2.7. In Vitro Drug Release. Release behavior of CDDP from CDDP-loaded HA-TiO_2 nanoparticles was investigated at pH 5.0 (approximate pH in endosomes or lysosomes), pH 6.0 (pH of the environment around the tumor), and pH 7.4 (pH of physiological blood). CDDP-loaded HA-TiO_2 nanoparticles (20 mg) were dispersed in PBS (pH 7.4, 5 mL) and transferred into a dialysis bag (Spectra/Por, USA, WMCO 3500 Da). The dialysis bag was then immersed in 95 mL of PBS at different pH values and kept in a horizontal laboratory shaker maintained at 50 g and 37°C. At predetermined time points, sample (2 mL) of the medium was removed, the same volume of fresh medium was added. The amount of released CDDP in the medium was then determined by HPLC.

2.8. Cellular Uptake of HA-TiO_2 Nanoparticles. To evaluate the cellular uptake of TiO_2 nanoparticles and HA-TiO_2 nanoparticles in A2780 human ovarian carcinoma cell line, fluorescent probe coumarin-6 (C6) was encapsulated in the nanoparticles in the same way as for the CDDP-loaded nanoparticles (the C6 loading of the two formulations was 0.17% which was determined by fluorescence spectrophotometer (RF-5301 PC; Shimadzu, Japan)). A2780 cells were seeded in confocal dishes (Φ = 15 mm) at a density of 1×10^5 cells/dish and cultured overnight. The dishes were then replaced with 1 mL of the serum-free medium containing C6-loaded TiO_2 nanoparticles and C6-loaded HA-TiO_2 nanoparticles (C6 content: 500 ng/mL). To investigate whether nanoparticles were taken up through HA-mediated endocytosis, cells were incubated with 10 mg/mL of free HA polymer for 1 hour prior to nanoparticles addition. After 2, 4, and 6 hours of incubation, the culture media were removed, and the cells were rinsed with PBS thrice. Subsequently, the cells were fixed with 4% formaldehyde for 20 minutes and observed under a Leica confocal laser scanning microscope (CLSM) (TCS SP5; Leica, Germany). The fluorescence intensity was quantitatively determined by flow cytometer (FCM) (BD FACSCalibur, USA).

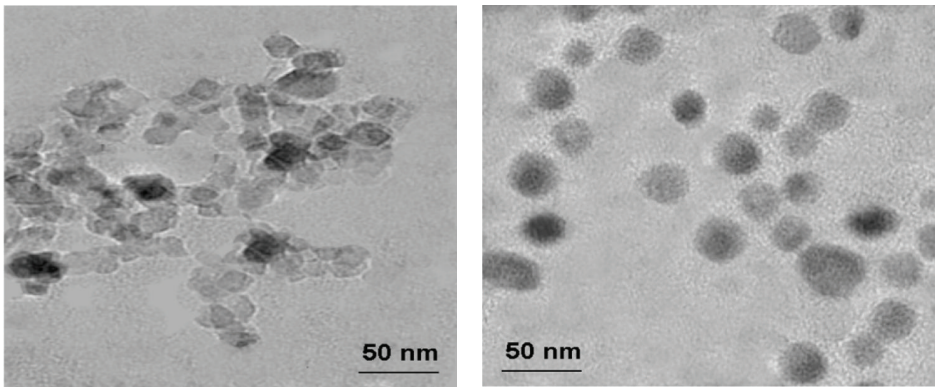
2.9. Assay of Anticancer Activity. Cytotoxicity of free nanoparticles and CDDP-loaded nanoparticles was studied. The A2780 cells were harvested by trypsinization and resuspended at a concentration of 1×10^4 cells/mL in fresh culture medium. The cells were then seeded into 96-well flat-bottomed tissue-culture plates and incubated for 48 h in the presence of different samples. The free TiO_2 nanoparticles and free HA-TiO_2 nanoparticles were diluted with culture medium to obtain concentrations in the range of 1–50 μg/mL; CDDP sample solutions were diluted with culture medium to obtain CDDP concentrations in the range of 0.01–10 μg/mL. The cytotoxic effects of free TiO_2 nanoparticles, free HA-TiO_2 nanoparticles, CDDP, CDDP-loaded TiO_2 nanoparticles, and CDDP-loaded HA-TiO_2 nanoparticles were evaluated by adding a mixture of 100 μL of each sample and 100 μL of culture medium to each well. After 48 h of incubation, cell viability was evaluated by MTT assay.

2.10. In Vivo Distribution of HA-TiO_2 Nanoparticles. Near-infrared fluorescent (NIR) probe DiR iodide (DiR) was encapsulated in the nanoparticles in the same way as for the C6-loaded nanoparticles. The A2780 tumor-bearing mice were injected in the tail vein with DiR-loaded HA-TiO_2 nanoparticles (as the experimental group) and DiR-loaded TiO_2 nanoparticles (as the control group), respectively (at a concentration of 1.0 mg/mL, 200 μL). The *in vivo* tumor-targeting efficacy and biodistribution of different nanoparticles at predetermined time intervals were evaluated using *In Vivo* Imaging System (FXPRO, Kodak, USA) equipped with DiR filter sets (excitation/emission, 720/790 nm). After live imaging, the mice were sacrificed, and the tumor tissues as well as major organs (heart, liver, spleen, lung, and kidney) were excised for *ex vivo* imaging using the same imaging system.

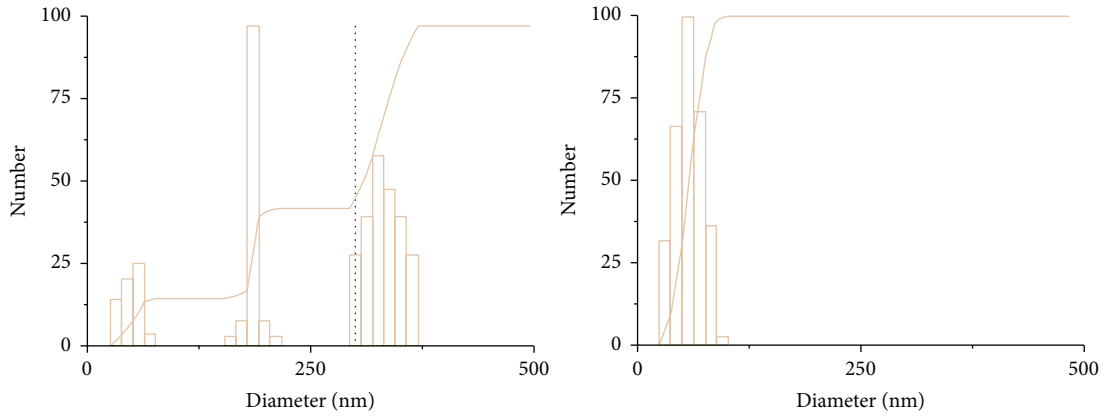
3. Results and Discussion

3.1. Characterization of Nanoparticles. TEM images of TiO_2 nanoparticles and HA-TiO_2 nanoparticles are displayed in Figure 1(a). Although observed TiO_2 nanoparticles had a spherical shape with a diameter of about 25 nm, most of the TiO_2 nanoparticles appeared to be agglomerated and formed large particle with a diameter over 300 nm, which might be due to their large surface area and high interface energy. Compared with TiO_2 nanoparticles, HA-TiO_2 nanoparticles showed no agglomeration with a diameter around 30 nm. The particle size measurement also confirmed the results. As depicted in Figure 1(b), the size of TiO_2 nanoparticles appeared to have multiple size sections with a PDI over 0.4 and a mean diameter of 277 nm. In contrast, the HA-TiO_2 nanoparticles were more homogeneous with a diameter of 50 nm and a PDI of 0.123. The zeta potential results (Figure 1(c)) showed that HA-TiO_2 nanoparticles exerted higher surface charge than TiO_2 nanoparticles (−30 mV versus −12 mV). It has been reported that applying polyanion on the surface of nanoparticles can change their surface to be negatively charged [22]. As in our experiment, the HA modification increased the surface charge of TiO_2 nanoparticles, which might, in turn, alleviate the agglomeration and result in well dispersity of HA-TiO_2 nanoparticles. On the other hand, it is well known that small particle size, especially under 100 nm, is expected to prevent uptake by the reticuloendothelial system and facilitates extravasation at leaky sites of capillaries, leading to passive accumulation in certain tissues like tumor [23]. Our results indicated that the HA-TiO_2 nanoparticles had dimensions suitable for escaping rapid renal excretion, as well as avoiding components of the reticular endothelial system. Thus, potentially passive targeting of drugs to tumors was facilitated via enhanced permeation and retention effect, and drug accumulation in tumor cells after endocytosis might be increased.

3.2. Drug Loading. CDDP is a cell cycle nonspecific antineoplastic drug and used to treat various types of cancers, including sarcomas, some carcinomas (e.g., small cell lung cancer and ovarian cancer), and lymphomas and germ cell tumors. High dosages are required for an effective therapy. Reports have demonstrated that TiO_2 nanotubes in aqueous solution showed high affinity to CDDP, which could suck



(a)



(b)

	Mean particle size (nm)	Zeta potential (mV)
TiO_2 nanoparticles	277.6 ± 7.2	−12.1 ± 1.4
HA-TiO_2 nanoparticles	50.6 ± 5.4	−30.5 ± 3.9

(c)

Figure 1: TEM images (a) of TiO_2 nanoparticles (left) and HA-TiO_2 nanoparticles (right). Particle size distribution (b) of TiO_2 nanoparticles (left) and HA-TiO_2 nanoparticles (right). Mean particle size and zeta potential measurements (c) of TiO_2 nanoparticles and HA-TiO_2 nanoparticles. Scale bar: 50 nm.

CDDP and retains it in the TiO_2 skeleton [24]. Herein, we employed TiO_2 nanoparticles as a carrier to load CDDP. Encapsulation efficiency and loading efficiency of CDDP-loaded HA-TiO_2 nanoparticles were assessed and calculated as 68.76% ± 5.67% and 25.36% ± 5.31%, respectively (data not shown). Results show that CDDP-loaded HA-TiO_2 nanoparticles might efficiently act as anticancer drug delivery system (DDS).

3.3. pH-Responsive Drug Release. Drug entrapment can be possible through complexation with transition metal ions. Since transition metals show variable valence with respect to pH of its surrounding environment, it is possible that the drug release can be regulated to site specificity through adjustment of pH in case of the transition metal encapsulating the drug [25]. Therefore, we were with the opinion that TiO_2 nanoparticles loaded with CDDP could be able to be directed for cancerous cells as its pH is acidic in nature. Moreover, the release might be further enhanced in endosomes and lysosomes so that the effective toxicity will be enhanced.

As shown in Figure 2, release profile of CDDP depended on both the medium pH and release time. Drug release at pH 7.4 was slow and sustained, with release ratio within 22% in 48 hours. However, at lower pH, CDDP release rate became much faster, with approximately 68% (pH 6.0) and 90% (pH 5.0) of the drug released within 48 hours. Protonation of the drug (dissociation of CDDP-loaded TiO_2 nanoparticles) occurred at lower pH, which released absorbed CDDP drug molecules into the medium. It was hypothesized that, at normal physiological conditions (pH 7.4), most CDDP will remain in the carrier for a considerable time period, suggesting the potential for prolonged CDDP retention time

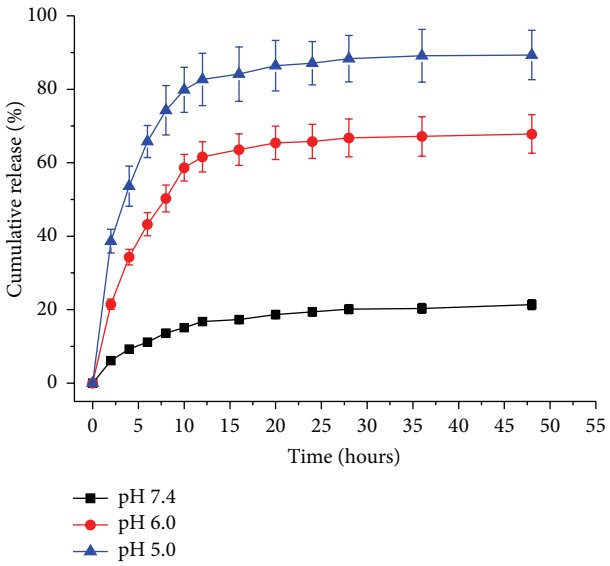


FIGURE 2: *In vitro* CDDP release behavior of CDDP-loaded HA-TiO_2 nanoparticles at pH 7.4, 6.0, and 5.0.

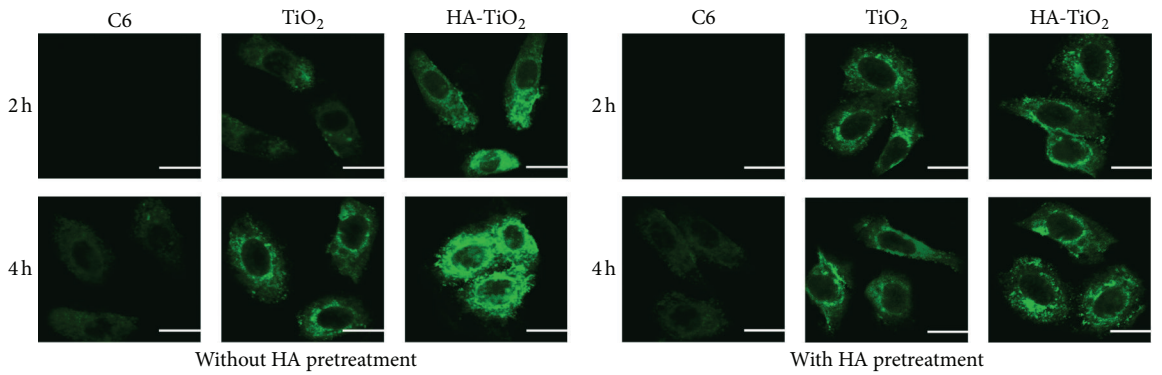
in blood circulation, and thereby could greatly reduce the side effects of CDDP to normal tissues. Moreover, once CDDP-loaded TiO_2 nanoparticles are taken up by tumor cells via endocytotic process, an increased release might occur at lower local pH, that is, surrounding the tumor site or inside the endosome and lysosome of tumor cells, which might lead to significant improvement of therapeutic effect.

3.4. Cellular Uptake of HA-TiO_2 Nanoparticles. It has been demonstrated that the HA segment exposed to the surface of the nanoparticles can target some of the receptors which were excessively expressed in ovarian cancer cells [26]. In order to address the concept of HA mediated-targeting, C6 as a model fluorescent molecule was entrapped in the nanoparticles and the endocytosis process was monitored by fluorescence microscopy. The fluorescence intensity of C6 was further quantified by FCM at different time points. A2780 ovarian cancer cells were employed for cellular uptake evaluation [27].

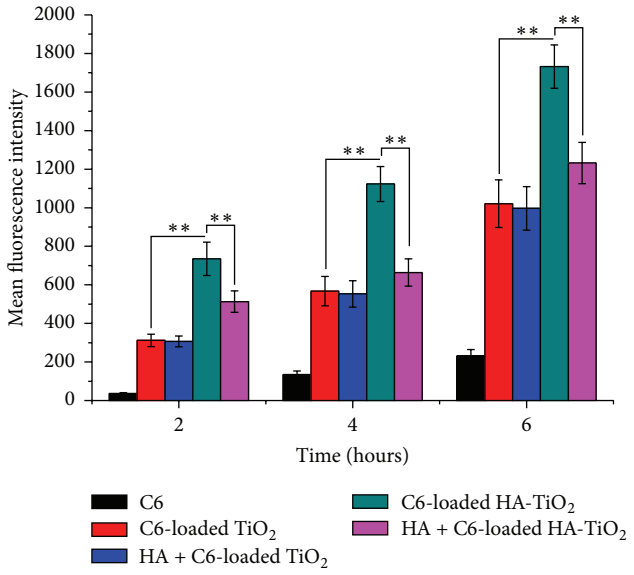
As shown in Figure 3(a), CLSM revealed higher green C6 fluorescence signals in the cells of HA-TiO_2 group than that of TiO_2 group, indicating that HA-TiO_2 nanoparticles have more effectively entered the cells. It was worth mentioning that under the same conditions, the C6-loaded nanoparticles displayed remarkably higher intensity compared with free C6, suggesting that DDS can enhance the uptake efficiency of free drugs. The intracellular uptake was also quantitatively analyzed by FCM, as displayed in Figure 3(b). The fluorescence intensities of C6-loaded TiO_2 nanoparticles and C6-loaded HA-TiO_2 nanoparticles were approximately 4.21-fold and 8.31-fold higher, respectively, than that of free C6 after incubation for 4 hours. However, after HA pretreatment, the fluorescence intensity of C6-loaded HA-TiO_2 group suffered a great decline while the fluorescence intensity of C6-loaded TiO_2 group remained almost the same level. These results clearly suggest that C6-loaded HA-TiO_2 nanoparticles were internalized into cells via HA-mediated endocytosis. In addition, the fluorescence signal gradually became stronger with extended incubation time, indicating that the intracellular uptake of the nanoparticles was in accordance with a time-dependent manner.

3.5. Assay of Anticancer Activity. To further explore the anticancer efficiency of the novel pH-responsive DDS based on TiO_2 nanoparticles for CDDP, A2780 cells were cultured with free CDDP at different concentrations (0.01, 0.1, 1, and 10 μg/mL) and CDDP-loaded TiO_2 nanoparticles and CDDP-loaded HA-TiO_2 nanoparticles with equivalent CDDP concentration for 48 hours. Cytotoxicity results were estimated by MTT assay (Figure 4). At the same time, cytotoxicity test of the nanoparticles without CDDP is also conducted as the first-level evaluation (nanoparticle concentrations range from 1 to 50 μg/mL, Figure 4(a)). When treated by TiO_2 nanoparticles and HA-TiO_2 nanoparticles, more than 90% of the cells survived at the highest dose, which is consistent with a previous report [28]. The low cytotoxicity of TiO_2 nanoparticles and HA-TiO_2 nanoparticles thus ensures a wide potential range of applications in the field of biomedical science and cancer therapy. Compared with CDDP alone, CDDP-loaded TiO_2 nanoparticles showed certain cytotoxicity on A2780 cells. Moreover, CDDP-loaded HA-TiO_2 nanoparticles exhibited a more increased cytotoxicity, which was even superior to CDDP alone. Results also demonstrated that, with increasing concentrations of CDDP, lethality increased, suggesting a dose-dependent effect *in vitro*. Increased cytotoxicity of CDDP-loaded HA-TiO_2 nanoparticles might be due to the improved CDDP cellular uptake mediated by HA (as illustrated above) and to the burst release of CDDP from HA-TiO_2. At normal physiological conditions (pH 7.4), it is expected from the release experiment that most CDDP in HA-TiO_2 nanoparticles will remain in the carrier for a considerable time period. In the endocytosis process, pH values drop from 7.4 to 6.5 or even as low as 4.5. At this low pH, protonation of the drug occurs. Protonation will then trigger the release of absorbed drug molecules which facilitates the drug release process. Consequently, a burst release of CDDP from CDDP-loaded HA-TiO_2 nanoparticles occurs once the carrier is taken up by the tumor cells via endocytic process. Therefore, a sufficiently high concentration of CDDP can be generated within a reasonably short period of time, thereby greatly promoting the cell-killing effects efficiently.

3.6. In Vivo Imaging. HA modification was expected to increase the accumulation of HA-TiO_2 nanoparticles at the tumor site. Here, the fluorescence intensity of DiR was monitored using an NIR fluorescence imaging system for 6 h to evaluate the targeting ability of different nanoparticles in A2780 tumor-bearing nude mice. Figure 5(a) showed the *in vivo* images at the tumor site after intravenous injection of complexes at different time points. Images recorded at 1, 3, and 6 h showed significant differences in targeting efficacy



(a)



(b)

FIGURE 3: *In vitro* cellular internalization evaluation of HA-TiO_2 nanoparticles. (a) Cellular uptake of free C6, C6-loaded TiO_2 nanoparticles, and C6-loaded HA-TiO_2 nanoparticles with and without pretreatment with free HA polymer, in A2780 cells as a function of the incubation time (2 and 4 hours at 37°C). (b) Quantitative flow cytometric analysis of the intracellular uptake of free C6, C6-loaded TiO_2 nanoparticles, and C6-loaded HA-TiO_2 nanoparticles with and without HA pretreatment in A2780 cells for 2, 4, and 6 hours of incubation. Data were expressed as mean ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$. Scale bar: 20 μm.

between TiO_2 nanoparticles and HA-TiO_2 nanoparticles. In detail, the fluorescence intensity of HA-TiO_2 nanoparticles at the tumor site was all markedly stronger than that of TiO_2 nanoparticles at each time point. The fluorescence intensity of M HA-TiO_2 nanoparticles at 1 h, especially, was almost the same as that of TiO_2 nanoparticles at 6 h, indicating the preferable accumulation of HA-TiO_2 nanoparticles at the tumor site. The powerful tumor targetability of HA-TiO_2 nanoparticles might be ascribed to a combination of EPR effect and HA-mediated endocytosis mechanism. The *ex vivo* imaging of tumor and major organs demonstrated that TiO_2 nanoparticles were mainly distributed in the liver and kidney, which, due to their large particles size and low surface charge, might in part be attributed to their poor tumor targetability.

4. Conclusion

In summary, a new type of tumor-targeting DDS (HA-TiO_2) was developed through modification of TiO_2 with HA to explore the potential feasibility of modified TiO_2 in targeted CDDP delivery. The experimental results indicated that CDDP release from the HA-TiO_2 nanoparticles was significantly accelerated by decreasing pH from 7.4 to 5.0, which is of particular benefit to cancer therapy. CDDP-loaded HA-TiO_2 nanoparticles increased the accumulation of CDDP in A2780 ovarian cancer cells via HA-mediated endocytosis and exhibited superior anticancer activity *in vitro*. *In vivo* real-time imaging assay revealed that HA-TiO_2 nanoparticles possessed preferable tumor-targeting ability which might

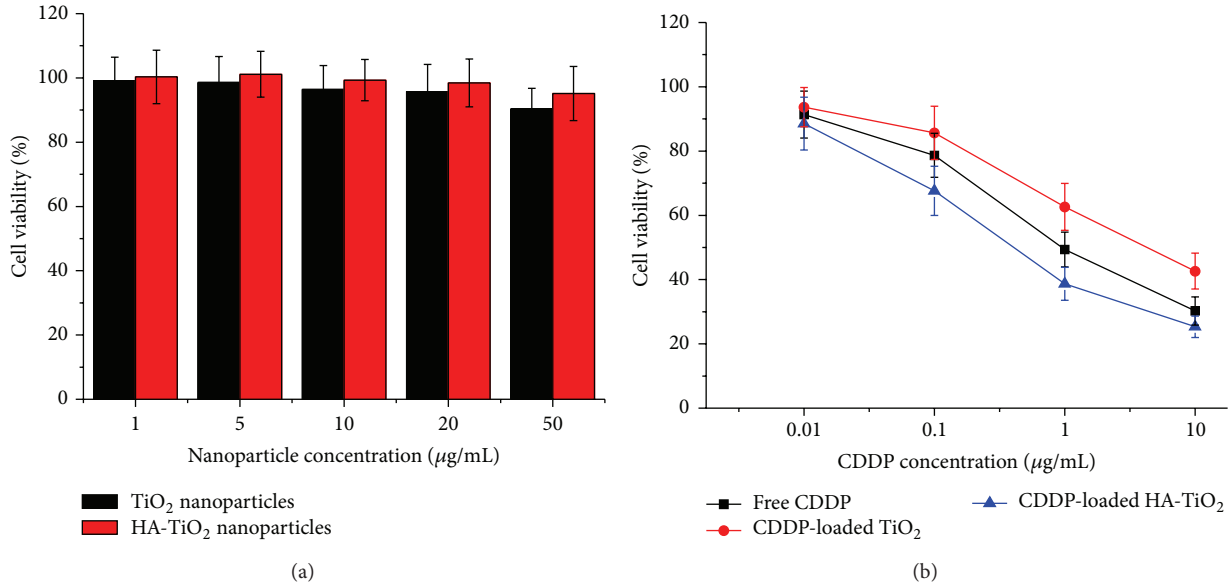


FIGURE 4: (a) Cell viabilities of A2780 cells incubated with free TiO_2 nanoparticles and HA-TiO_2 nanoparticles at various concentrations (1–50 μg/mL) for 48 h. (b) Cell viabilities of A2780 cells incubated with free CDDP, CDDP-loaded TiO_2 nanoparticles, and CDDP-loaded HA-TiO_2 nanoparticles at various concentrations (0.01–10 μg/mL) for 48 h. Data were expressed as mean ± SD ($n = 5$). $^{*}P < 0.05$ and $^{**}P < 0.01$.

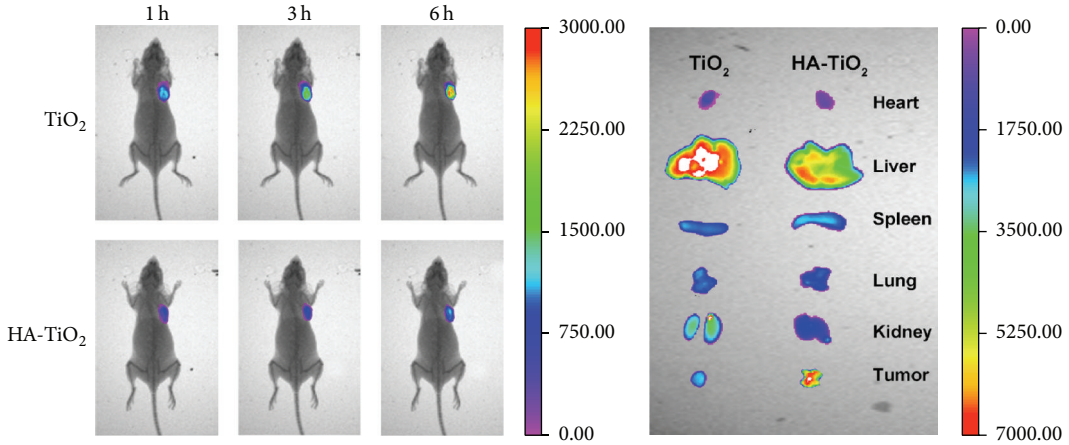


FIGURE 5: (a) *In vivo* time-dependent tumor-targeting images after intravenous injection of DiR-loaded TiO_2 nanoparticles and DiR-loaded HA-TiO_2 nanoparticles in A2780 tumor-bearing mice and (b) representative *ex vivo* NIR fluorescence images of dissected tumors and major organs at 6 h after injection.

potentially minimize the toxic side effects of CDDP in clinical application.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgment

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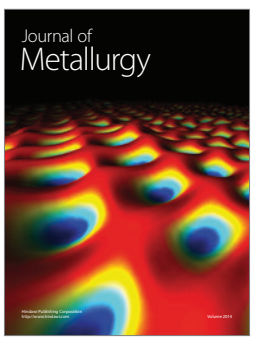

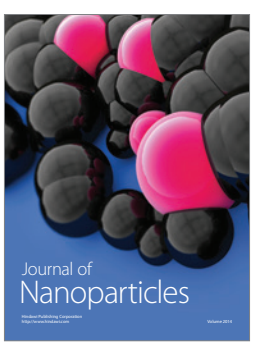

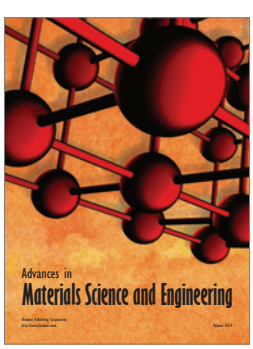

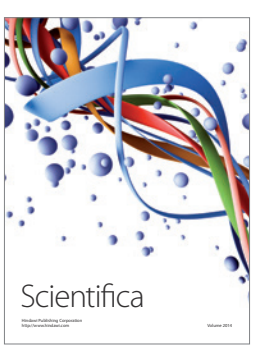

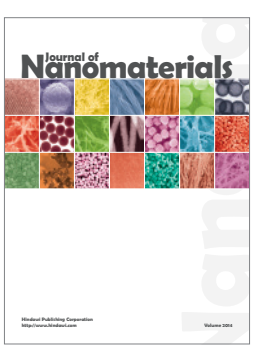

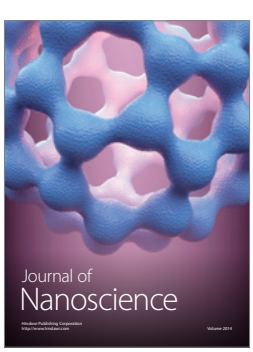

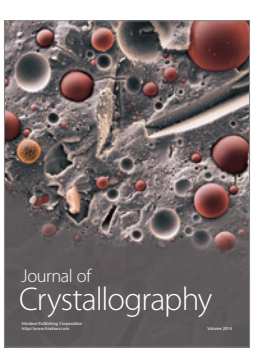

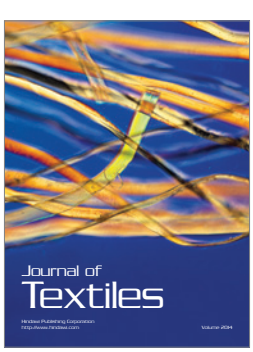

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