A novel thermal and pH responsive drug delivery system based on ZnO@PNIPAM hybrid nanoparticles

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Abstract

A smart ZnO@PNIPAM hybrid was prepared by grafting thermal responsive poly(N-isopropylacrylamide) (PNIPAM) on zinc oxide (ZnO) nanoparticles via surface-initiated atom transfer radical polymerization (ATRP). The thermal gravimetric analysis (TGA) shows that the grafting amount of PNIPAM was about 38%, and the SEM images show that the PNIPAM chains can prevent the aggregation of ZnO nanoparticles. The responsive properties of ZnO@PNIPAM were measured by photoluminescence spectra, and the results demonstrate that the PNIPAM chains grafted on ZnO surfaces can realize reversible thermal responsive and photoluminescence properties. An anticancer drug, doxorubicin (Dox), was used as a model drug and loaded into the hybrid nanoparticles, and an in vitro drug release test implied that ZnO@PNIPAM could work as a thermal responsive drug delivery system. Furthermore, pH sensitive drug releases were carried out in acetate buffer at pH 5.0 and pH 6.0 and in water at pH 7.0, and the results showed evident pH dependency, showing its pH responsive properties.

Keywords:
ZnO, ATRP, Thermal responsive, Drug delivery, Surface modification

1. Introduction

Chemotherapy is one of the common methods to treat cancer, however most traditional chemotherapeutic agents have concentration dependent toxicity in non-cancerous tissues according to the therapeutic index [1]. Recent advances in nanotechnology provide a promising way to impact many longstanding challenges in medicine, such as selective nanoparticle (NP) drug delivery [2]. This nanotechnology is supposed to improve the performance of the existing treatments through their altered pharmacokinetics biodistribution profiles [3]. An ideal design of nanocarriers is the one that can carry drugs effectively to the targeted cells and release their payload over an extended period to achieve a clinical response [4]. In order to realize controlled release, “smart” drug delivery systems based on pH [5], temperature [6] and redox sensitive materials [7] have been investigated widely.

Among many reported drug carriers, ZnO NPs are an outstanding candidate due to their nontoxic and biodegradable properties. On the other hand, ZnO is an indispensable trace element for adults, and ZnO can slowly dissolve in acidic condition (e.g., in tumor cells/microenvironment), and work as a pH sensitive carrier [8]. For instance, Xiong’s group reported the preparation of pH-responsive ZnO@polymer core-shell nanocarriers via the copolymerization of methyl methacrylate modified ZnO and acrylamide, and the results demonstrated that ZnO composites are biocompatible and biodegradable, ensuring its safety for healthy tissues [9]. In addition, Xiong and his coworkers also prepared water soluble ZnO NPs, which showed tunable photoluminescent properties and thus can be used in cell imaging [10]. However, the poor water-solubility and easy aggregation phenomena of ZnO have hindered its application as an important nanomaterial. This defect can be reduced by coating ZnO NPs with hydrophilic polymer chains, which may enhance the colloidal stability and handling for chemoligation (targeting moieties) and increase blood circulation lifetimes [2]. For example, Zhang and co-workers prepared stable aqueous ZnO NPs with co-polymerization of methyl methacrylate (MMA) and vinyltriethoxysilane (VTES) on ZnO surface, and then 3-aminopropyltriethoxysilane was linked to the vinyltriethoxysilane. This hydrophobic core of MMA and VTES could protect ZnO tightly from water to avoid ZnO aggregation, and also protect the luminescence centers on ZnO quantum dots’ surface [11]. Woo et al. coated ZnO with polyethylene glycol biscalboxymethyl (PEG(EOH)2) to get water soluble ZnO NPs [12]. Among the several hydrophilic polymers, poly(N-isopropylacrylamide) (PNIPAM) is the thermal responsive material. In addition, PNIPAM has shown excellent biocompatible properties and can tune the cell attachment or detachment by the change of temperatures [13], and thus it has been widely used in biomedical fields [14,15].
In this work, a thermal responsive ZnO@PNIPAM hybrid was synthesized by surface-initiated atom transfer radical polymerization (ATRP) of NIPAM. The general approach is shown in Scheme 1. Firstly, ZnO NPs were treated with 3-aminopropyltriethoxysilane, and then reacted with 2-bromoisobutyryl bromide. The ATRP reaction was conducted on the surface of ZnO with 2-bromoisobutyrate-functionalized ZnO NPs as the initiator. Due to the thermal responsive property of PNIPAM and the acid biodegradability property of ZnO, the prepared ZnO@PNIPAM may work as a double responsive drug delivery system.

2. Experimental

2.1. Materials

Zinc acetate dehydrate (Zn(CH₃COO)₂·2H₂O, ≥ 98%, AR), sodium hydroxide (NaOH, AR) and ethanol absolute (C₂H₄O₂, ≥ 99.7%, AR) were purchased from Tianjin Damao Chemical Reagent Factory. 2-Bromoisobutyryl bromide (≥ 98%) was purchased from ACROS. These chemicals were used as received without further purification. Copper (I) bromide (CuBr) was purified by stirring with glacial acetic acid, followed by washing with absolute ethanol and acetone, and then dried under vacuum. Triethylamine (TEA, AR) was dried by distillation from calcium hydride. Tetrahydrofuran (THF, AR) was dried with sodium wire and redistilled before use. N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA) and N-isopropylacrylamide (NIPAM, 97%) were obtained from Sigma-Aldrich and used without further purification. Doxorubicin hydrochloride (Dox) was purchased from Beijing Huafeng United Technology Co., Ltd.

2.2. Preparation of amino-functionalized ZnO (ZnO–NH₂) NPs

ZnO–NH₂ NPs were synthesized according to Shi’s work [16]. Zn(CH₃COO)₂·2H₂O (4.3902 g, 20.00 mmol) was dissolved in 200 mL of absolute ethanol, and then the solution was refluxed at 80 °C for 2 h under continuous stirring. The as-obtained solution was cooled to 0 °C in an ice water bath, and then APTES (0.80 mL, 3.4 mmol) was added and intensely stirred for 20 min to ensure adequate dispersion. The Zn⁺⁺ precursor and NaOH (0.8 g, 20.00 mmol) ethanol solution were mixed at 0 °C under vigorous stirring for 30 min to obtain a turbid solution. The sediments were washed with ethanol for several times and then collected by centrifugation. The ZnO–NH₂ NPs were finally obtained by lyophilization of the collected sediments.

2.3. Synthesis of 2-bromoisobutyrate functionalized ZnO (ZnO-Br) NPs

ZnO–NH₂ NPs (500 mg) and dry TEA (0.7 mL, 5.0 mmol) were dispersed in 100 mL anhydrous THF. After cooling to 0 °C, 2-bromoisobutyryl bromide (0.5 mL, 4.0 mmol) was added dropwise. The solution was stirred at 0 °C for 0.5 h and then at room temperature for 12 h. The raw product was collected by centrifugation and washed for several times, and then the final product was obtained by lyophilization.

2.4. Preparation of ZnO@PNIPAM NPs

ZnO-Br NPs (400 mg), PMDETA (60.0 μL, 0.30 mmol) and NIPAM (1.0 g, 8.84 mmol) were dispersed in 10 mL deionized water. The mixture was dispersed by ultrasonication. The oxygen was removed by bubbling with nitrogen (N₂) for 30 min, and then CuBr (43 mg, 0.30 mmol) was added under N₂ atmosphere. Then, the mixture was kept in a 30 °C oil bath under stirring with N₂ bubbling throughout the polymerization process for 24 h. Finally, the white product was collected by centrifugation and washed with deionized water to remove the remained monomer and homopolymer. The final product (ZnO@PNIPAM) was obtained by lyophilization.

2.5. Drug loading and release

ZnO@PNIPAM (12 mg) was dispersed in doxorubicin hydrochloride solution at a concentration of 0.552 mg/mL while shaking overnight at room temperature, and then the NPs were collected by centrifugation. The amount of doxorubicin loaded on the NPs was analyzed using UV–Vis spectroscopy. The characteristic absorbance of Dox (480 nm)
was recorded and compared with a standard curve of drug concentrations varying from 0 to 45 mg/mL.

For the in vitro drug release studies, a prescribed amount of drug-loaded NPs dispersed in deionized water, and then suspended in dialysis bags was placed into 5 mL phosphate-buffered saline (PBS), and incubated at room temperature and 37 °C (for temperature-triggered release), separately. The pH-response behavior was examined by release experiments at acetate buffer with different pH values. The amount of released drug was measured by UV–Vis spectroscopy. The drug concentration was determined according to the standard curves for the drug solution at different temperatures.

2.6. Characterization

Scanning electron microscopy (SEM) was used to observe the NPs’ morphology using a QuanTA-200F environmental scanning electron microscope (FEI). Fourier transform infrared (FTIR) analysis was carried out using KBr discs in the region of 4000–500 cm\(^{-1}\) by a Fourier transform infrared (FT-IR) spectrophotometer (Shimadzu IR prestige-21, Japan). The photoluminescence (PL) spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. Thermal gravimetric analysis (TGA) was performed on a PerkinElmer TGA 7 analyzer from 25 °C to 600 °C with heating rate of 20 °C/min under nitrogen. Ultraviolet–visible (UV–Vis) absorption spectra were performed on a Lambda 750 spectrophotometer (PerkinElmer, USA). The particle sizes of NPs were determined by a Britain Malvern PSA (NANO2590) submicron particle size analyzer with angle detection at 90°.

3. Results and discussion

The surface grafted PNIPAM can be studied by FTIR spectroscopy. As shown in Fig. 1, compared with the IR spectrum of ZnO (Fig. 1a), new peaks appeared in the IR spectra of functionalized ZnO samples. The absorption band at 1648 cm\(^{-1}\) was ascribed to the C\(_\text{=O}\) stretch of 2-bromoisobutyrate residues on the surface of ZnO-Br. The peaks at 1366 and 1388 cm\(^{-1}\) were ascribed to the vibrations of C–H groups on the two methyl groups in 2-bromoisobutyrate residues. When PNIPAM was grafted on the surface of ZnO, significantly enhanced absorption peaks appeared at 1647 cm\(^{-1}\) (C\(_\text{=O}\) stretching), 1543 cm\(^{-1}\) (N–H bending), and 1366 and 1388 cm\(^{-1}\) (C–H bending), demonstrating that the PNIPAM was successfully connected on the surface of ZnO.

The grafting amount of PNIPAM on the surface of ZnO could be measured by thermal gravimetric analysis (TGA). As shown in Fig. 2, the bare ZnO NPs show a weight loss of about 8.22% as heating from 15 °C to 600 °C, due to the removal of absorbed water and decomposition of the hydroxide group. The weight loss of ZnO-Br and ZnO@PNIPAM was 12.9% and 51.2%, respectively, which were derived from the evaporation of adsorbed water and decomposition of organic groups. When compared with the weight loss of ZnO-Br and ZnO@PNIPAM, the grafting amount of PNIPAM was calculated to be 38.3%.

![Fig. 3. TEM images of ZnO (a) and ZnO@PNIPAM (b) (the shell thickness was denoted in b, shown between the dotted lines).](image)

![Fig. 4. SEM images of ZnO (a) and ZnO@PNIPAM (b).](image)
As shown in Fig. 3, the diameter of ZnO nanoparticles was about 4–6 nm, while the diameter of ZnO@PNIPAM was about 10–12 nm. Furthermore, it can be seen that the thickness of PNIPAM was about 5–6 nm (Fig. 3b). The grafted PNIPAM chains may prevent the aggregation of ZnO NPs and realize its homogeneous dispersion. As shown in Fig. 4a, the ZnO NPs aggregate heavily, while ZnO@PNIPAM can disperse very well (shown in Fig. 4b). Further, the PNIPAM chains on the surface of ZnO may also affect its photoluminescent properties. In addition, due to the thermal responsive properties of PNIPAM, ZnO@PNIPAM may also have thermal responsive properties. Fig. 5a and b presents the room temperature photoluminescence (PL) spectra of ZnO and PL of ZnO@PNIPAM, respectively. It is noticed that PNIPAM grafted on the ZnO NPs increased the defect emission characteristics. The result indicates that the surface modification of ZnO NPs by PNIPAM can induce more scattering centers per cross-sectional unit area, and hence increase the luminescence from ZnO@PNIPAM due to multiple path excitations. When ZnO@PNIPAM NPs were heated to 40 °C, the intensity of its PL spectra decreases a lot (Fig. 5c), which is mainly induced by the shrinking of PMIPAM chains on the surface of ZnO, and thus it is demonstrated that the ZnO@PNIPAM NPs possess thermal responsive properties.

![Normalized Intensity vs Wavelength](image1)

**Fig. 5.** PL spectra of bare ZnO (a) nanoparticles, ZnO@PNIPAM at 25 °C (b) and ZnO@PNIPAM at 42 °C (c).

![Normalized Intensity vs Temperature](image2)

**Fig. 6.** PL spectra of ZnO@PNIPAM at different temperatures from 28 °C to 50 °C (a) and the corresponding PL intensity at 533 nm from 28 °C to 50 °C (b).

![Intensity vs Cycles](image3)

**Fig. 7.** Reversible changes of PL emission of ZnO@PNIPAM at 22 °C and 37 °C.
To further study the thermal responsive properties of ZnO@PNIPAM, the PL spectra under irradiation at 325 nm at different temperatures were investigated (Fig. 6a), and the relations between the corresponding peak intensity at 533 nm and the temperature were shown in Fig. 6b. As shown in Fig. 6b, when heated above 32 °C, the PL intensity reduced dramatically. This demonstrates that the LCST was 32 °C. Interestingly, from Fig. 7, it can be seen that the defect emission intensity at 533 nm can be recovered completely by cooling the sample solution, and it was a reversible change between heating and cooling processes. After several continuous cycles, the photoluminescence did not show any evident change.

The particle size of ZnO@PNIPAM can change with the variance of temperature. As shown in Fig. 8a, the average hydrodynamic diameter ($D_h$) of ZnO@PNIPAM was about 401 nm at 25 °C. When the solution was heated to 42 °C, the $D_h$ decreased to 252 nm (Fig. 8b). This phenomenon is derived from the changes of PNIPAM conformation that when the temperature is at 25 °C, the PNIPAM was hydrophilic. While it is heated to 42 °C, the PNIPAM would transfer to hydrophobic state and collapsed on the ZnO surface, and thus the particle size would decrease heavily. As a matter of fact, the result corresponds with the changes in PL intensity showed in Fig. 5.

The potential application of ZnO@PNIPAM was investigated by in vitro drug release experiments. Dox is commonly used to treat tumors as an antineoplastic agent. The time-dependent release of doxorubicin hydrochloride with different temperatures was evaluated by dialysis method by choosing ZnO@PNIPAM as a sample in phosphate buffered saline (PBS, pH 7.4). The release performance was observed for 70 h, and the release profiles were shown in Fig. 9. The actual loading level of doxorubicin hydrochloride in the NPs is calculated to be 16.48% in weight. Fig. 9a showed the in vitro release profiles of doxorubicin hydrochloride at 15 °C. The enhancement of releasing drugs at 37 °C can be observed in Fig. 9b. The phenomenon corresponded strictly to the facial state of the grafted PNIPAM, and the result indicates that the release rate increased above the transition temperature of the PNIPAM.

The pH-responsive release behavior was studied by UV/Vis absorption spectroscopy. Fig. 10 shows the release profile at different pH values. When the release experiment was carried out at pH = 7, the release was very slow, with only 20% Dox being released. When the pH was tuned to 6, some ZnO core began to decompose, and an evident increase of release speed was observed. When the medium was tuned to pH = 5, the decomposition speed of ZnO was enhanced, and thus the release speed increased a lot, about 50% Dox released from the drug carrier. Then, 12 h later, about 80% Dox was released. From these results, we can conclude that ZnO@PNIPAM can work as a pH responsive drug delivery system. Since there is a mildly acidic environment in the tumor tissues as well as within the endo/lysosomal compartments of tumor cells [17], if ZnO@PNIPAM NPs were used to treat tumor cells, it

![Fig. 8. Hydrodynamic diameters ($D_h$) of ZnO@PNIPAM in the aqueous medium at 25 °C (a) and 42 °C (b).](image)

![Fig. 9. Time-dependent release of Dox from ZnO@PNIPAM at different temperatures.](image)

![Fig. 10. Time-dependent release of Dox from ZnO@PNIPAM in different pH values.](image)
would realize a faster release inside tumor cells, which would be beneficial to killing tumor cells.

4. Conclusions

In this work, a thermal responsive ZnO@PNIPAM hybrid was prepared via the surface initiated ATRP method. The ZnO@PNIPAM hybrid showed thermal responsive photoluminescence properties, and the PNIPAM chains can prevent the aggregation of ZnO NPs and realize its homogeneous dispersion in aqueous solution. The ZnO@PNIPAM hybrid also showed pH responsive properties, which is realized through the solubility of ZnO in acidic environments.

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References