Polymer Chemistry

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

pH-responsive polymeric Janus container for controlled drug delivery

Ziguang Zhao[a,b], Feiyan Zhu[b,c], Xiaozhong Qu[b,c], Qiuhua Wu[a], Qian Wang[b], Guolin Zhang[a]*, and Fuxin Liang[b]*

[a] *Liaoning Provincial Key Laboratory for Green Synthesis and Preparative Chemistry of Advanced Materials (Liaoning University), College of Chemistry, Liaoning University, Shenyang 110036, China. Fax: 8624-62202380; Tel: 8624-62207848; E-mail: glzhang@lnu.edu.cn (G. Z.).*
[b] *State Key Laboratory of Polymer Physics and Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. Fax: 8610-62559373; Tel: 8610-82619206; E-mail: liangfuxin@iccas.ac.cn (F. L.).*
[c] *University of Chinese Academy of Sciences, Beijing 100049, China.*

In this study, we develop a novel approach to fabricate an pH-responsive polymeric Janus hollow spheres (JHSs) with bi-layers structure by two-steps polymerization with silica particles as template. The inner layer is the biodegradable poly(ε-caprolactone) (PCL) brush and the outer layer is cross-linked pH-responsive poly(diethylaminoethylmethacrylate) (PDEAEMA). So these as-synthesized JHSs can selectively loaded oil-soluble materials into the cavities and controllably release them by changing pH. Doxorubicin (DOX) is selected as model drug which is loaded into the cavity of the JHSs and showed faster release at acidic pH than at physiological pH. It was revealed that the intracellular uptake of JHSs at the pH in tumorous tissues was significantly larger than that under the same conditions at pH of the normal physiological environment.

KEYWORDS: Janus hollow spheres; pH-responsive; Container; Controlled drug release

Introduction

Janus objects with two different compositions compartmentalized distinctly onto the same one have gained increasing interests in both academic and industrial fields due to their diversity of promising performances.[1-6] There are many pursuing applications such as building blocks of complex superstructures, particle surfactants, optical nano-probes, stimuli-response, electrical display, and self-propulsion nano-motor.

Recently, amphiphilic Janus hollow spheres (JHSs) have become attractive due to their asymmetric shell with two compositions localized on the interior and exterior surfaces of the shell, respectively.[7,8] They can serve as specific containers thus desired compounds can be selectively captured thereby from their surroundings due to different affinity. The performance is promising in controlled loading and release and confined catalytic reaction.[9,10] Similar to JHSs structure, organic amphiphilic micells have been extensively synthesized based on self-assembly of

amphiphilic small molecules or block copolymers.[11,12] Although they demonstrate a series of attractive properties in drug delivery systems, such as good biocompatibility and high stability in vitro and in vivo, and have been described as promising materials in applications such as biosensors, tissue engineering, or selective drug delivery, the shells are rather weak. JHSs can serve as micelle mimetic containers but are more robust. They are promising compounds for controlled loading/release and confined catalysis. Catalytic nanoparticles could be pre-loaded inside the cavity of JHSs to fabricate a micro-reactor, which can provides an easier approach for selective enrichment of reagents in a confined environment for further reaction.[10] Janus hollow spheres of polymer-inorganic bi-layered composites have been synthesized by a sequential grafting onto the vinyl-group-terminated interior surface of the JHSs.[9] The currently reported JHSs are mainly composed of inorganics and not easily deformable. Polymers are a rational alternative. If shell could be replaced by polymers, JHSs with diverse composition and thus performance will be obtained. Polymers will endow JHSs flexibility and the composition, thickness and microstructure of the shell can be easily adjusted. Especially, if polymers are responsive, the as-synthesized JHSs should be responsive. Meanwhile, poly(2-(diethylamino) ethyl methacrylate) (PDEAEMA) is a cationic polyelectrolyte with a pK_a of 7.2. It is soluble in acidic solution by protonation of the pendant amine groups but becomes hydrophobic at neutral or basic pH.[13-15] This feature makes it a promising pH-sensitive material for antitumor drug delivery and various copolymers such as star[16] and brush[17] of PDEAEMA have been developed as anticancer vehicles for improved cancer therapy.

Herein, we develop a facile approach to fabricate pH-responsive polymeric JHSs by surface-initiated ring-opening polymerization (ROP) techniques and atom transfer radical polymerization (ATRP) with silica nanoparticle (SNPs) as sacrificial template. The inner layer is the biodegradable poly(ε-caprolactone) (PCL) brush and the outer layer is composed of crosslinked pH-responsive PDEAEMA. Base on ROP and ATRP methods, silica nanoparticles are used as sacrificial templates to graft PCL and PDEAEMA respectively forming bi-layers polymeric shell. After etching the silica core with NH_4F/HF buffer solution, polymeric JHSs are obtained. Using this approach, we can well control the thickness, size and composition of the JHSs by adjusting the molecular weight of the polymers, the size of the template particles and the kinds of the as-used monomers. The hydrophobic PCL brush forms the biodegradable inner layer and the partial crosslinked PDEAEMA forms pH-responsive outer layer of the JHSs, respectively. For the hydrophobic PCL interior surface of the JHSs, it can selectively load hydrophobic materials into the cavities. Meanwhile, the transfer of materials through the PDEAEMA outer layer could be controlled by adjusting pH for its pH response. Thus these as-formed JHSs are

favorable to be utilized as drug carriers. For instance, DOX is employed as a model drug to test the drug loading and releasing properties. These JHSs can act as containers to load lipophilic DOX into the cavities for the hydrophobic PCL interior surface, and the release process can be controlled by tuning pH for the pH-responsive PDEAEMA outer layer. Meanwhile, the surface wettability of the JHSs is corresponding to environment pH. So the intracellular uptake of the JHSs also can be controlled by adjusting pH.

Experimental section

Materials

Tetraethylorthosilicate (TEOS), 3-aminopropyldimethylethoxysilane, glycidol, ε-caprolactone (ε-CL), stannous octoate ($Sn(Oct)_2$), 2-bromoisobutyryl bromide, triethyl-amine, N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA), copper bromide (CuBr), diethylaminoethyl methacrylate (DEAEMA), and ethyleneglycol dimethacrylate (EGDMA), were purchased from ACROS. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) was purchased from Sigma. Fluorescein isothiocyanate (FITC) was purchased from Beijing Fanbo Science & Technology Co., Ltd. (China). Fetal bovine serum (FBS, Gibco) was purchased from Co., Ltd. (China). Ammonia solution ($NH_3 \cdot H_2O$, 28 wt.-%), enthanol, xylene, toluene, dichloromethane, N,N-dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Doxorubicin hydrochloride (DOX·HCl) was purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd. (Hubei China) and used as received. CuBr was purified by stirring in acetic acid and washed with ethanol for three times. The ε-CL was dried over calcium hydride and distilled under reduced pressure before use. DEAEMA were distilled under reduced pressure. DMF and THF were refluxed with CaH_2 and then distilled prior to use. All other reagents were used as received without further purification.

Preparation of amino functionalized SNP (SNP-NH_2)

Uniform and monodisperse silica nanoparticles (SNPs) with 500 nm were prepared according to the classical stöber method.[18] Briefly, 15 g of TEOS was added into a mixture of ethanol (110 mL), deionized water (19.5 mL), and 28 wt.-% ammonia solutions (50 mL). The reaction mixture was stirred for 12 h. The particles were obtained (500 nm). Then a suspension (16 mL) of 10 wt.-% SNP aqueous was added to a round-bottom flask with 3-aminopropyldimethylethoxysilane (1.0 g, 6.0 mmol) and ammonium hydroxide solution (1 mL, 16.0 mmol). The reaction mixture was stirred for 24 h. Then the products were thoroughly washed via centrifugation/redispersion cycles with ethanol for 5 times. The products were dried at 40 °C under a vacuum for 24 h to give the desired SNP-NH_2.

Preparation of hydroxide functionalized SNP (SNP-OH)

0.5 g of SNP-NH_2 was put into 25 mL of methanol and then 0.2 mL of glycidol was added into the mixture.[19] The reaction was performed at 60 °C for 12 h under a nitrogen atmosphere. The particles were obtained by centrifugation at 8000 rpm for 3 mins followed by washing with THF 5 times and then dried at 40 °C under a vacuum for 24 h.

Preparation of PCL brushes grafted SNP (SNP-*g*-PCL)

The SNP-*g*-PCL was prepared through ring-opening polymerization (ROP) on the silica surface.[20] The polymerization procedure was shown as follows. 0.05 g of SNP-OH was dispersed in 10 mL of dried xylene. 0.5 mL (4.64 mmol) of ε-CL and 18 mg (0.044 mmol) of $Sn(Oct)_2$ were added. The mixture was degassed after three circles of freeze-pump-thaw and then immersed into an oil bath string at 120 °C for 48 h under a nitrogen atmosphere. After the mixture was exposed to air to terminate the polymerization and cooled to room temperature. The products were purified by centrifugation and ultrasonic dispersion three times in xylene and THF, respectively, and finally dried at 40 °C under a vacuum for 24 h.

Preparation of tertiary-bromine terminated PCL polymer brushes grafted SNPs (SNP-*g*-PCL-Br)

0.05 g of the PCL-*g*-SNP was dispersed in 20 mL of 2% (v/v) dry THF solution of triethylamine followed by addition of 0.5 mL of 2-bromoisobutyryl bromide. The mixture was kept at 0 °C for 0.5 h and at room temperature for another 24 h. The modified particles were isolated by centrifugation at 8000 rpm for 3 mins and washed with dichloromethane and toluene several times to remove excess 2-bromoisobutyryl bromide, and then dried under a vacuum oven.

Preparation of SNP-*g*-PCL-*b*-cPDEAEMA

0.05 g of SNP-*g*-PCL-Br was dispersed in 6 mL of DMF under unltrasonication in a glass tube under stirring. After addition of 0.8 g (4.0 mmol) of DEAEMA, 0.11 g (0.56 mmol) of EGDMA, and 0.018 g (0.01 mmol) of PMEDTA, the mixture was degassed by three cycles of freeze-pump-thaw. In a frozen state, 0.014 g (0.01 mmol) of CuBr was added under the atmosphere of nitrogen. The tube was subjected to another freeze-pump-thaw and then sealed under vacuum. The reaction was performed at 70 °C for 24 h and then terminated by exposed to air. The particles were purified after three cycles of centrifugation and redispersion in THF and methanol, respectively and dried for 12 h at 40 °C in a vacuum to oven.

Preparation of Janus hollow spheres

The inorganic silica core was etched by 8 M NH_4F / 2 M HF buffer (pH=5). 0.05 g of SNP-*g*-PCL-*b*-cPDEAEMA was dispensed and stirred in 5 mL of NH_4F/HF buffer at room

temperature for 12 h to remove the silica core. The excess NH_4F, HF, and SiF_4 were removed by dialysis in deionized water for 1 day. Finally, the JHSs were obtained by freeze-drying process.

DOX-loaded process into Janus hollow spheres

DOX-loaded JHSs were formed using the permeation method. 5.0 mg of DOX·HCl was stirred with a 1-fold excess of TEA in 5 mL of DMF overnight to obtain DOX base. 5.0 mg of JHSs was dispensed in another 5 mL of DMF and then mixed with the DOX solution, followed by stirring for 24 h at room temperature. The DOX-loaded JHSs were obtained by centrifugation at 12000 rpm for 10 min and washed 5 times with 10 mL of phosphate-buffered saline (PBS, pH 7.4) to remove the physically absorbed DOX. The amount of loaded drug for JHSs was determined by a fluorescence spectrophotometer at 480 nm. The concentration of DOX was calculated according to a standard curve of pure DOX dissolved in PBS. The drug loading capacity was calculated using formulas 1.

$$\text{Drug loading}(\%) = \frac{\text{weight of DOX loaded drug}}{\text{weight of dried capsules}} \times 100\% \quad (1)$$

In vitro release of DOX from JHSs

The in vitro DOX release profiles from JHSs were determined in buffers with pH values of 5.5, 6.5 and 7.4. In a typical experiment, 5.0 mg of DOX-loaded JHSs was suspended in 5.0 mL of PBS (pH 7.4 or 6.5) or acetate buffer (pH 5.5). The resulted solutions were moved to dialysis bag (molecular weight cut-off 3500-4000) and placed in the beakers containing 15 mL of PBS or acetate buffer and stirred at room temperature. At predetermined time intervals, a 5 mL of sample (V_e) was taken out and an equal volume of fresh buffer was added to maintain the total volume. The amounts of released DOX in different buffers were monitored by fluorescence spectrophotometer at 480 nm. The cumulative percent drug release (Er) was calculated based on Eq. (2).

$$E_r(\%) = \frac{V_e \sum_1^{n-1} C_i + V_0 C_n}{m_{DOX}} \times 100 \quad (2)$$

Where m_{DOX} represents the amount of DOX in the JHSs, V_0 is the volume of the release medium (V_0 = 20 mL), and C_n represents the concentration of DOX in the nth sample. The in vitro release experiments were carried out in triplicate at each pH to get the final release curves.

Cytotoxicity measurements of JHSs

Cytotoxicity test of JHSs was carried out by the cell counting kit-8 (CCK-8) assay. U87MG cells were cultured with 96-well plates at a density of 10^4 cells per well for 24 h. The samples were

prepared in a series of desired concentrations at pH 7.4 and pH 6.5, respectively. Every experimental well was added to the samples for 24 h. The culture medium was replaced with CCK-8 reagent solution, and cells were further incubated for 2 h. The absorbance of each well was measured at 450 nm using a microplate reader. Cell survival was expressed as the absorbance relative to that of the untreated controls.

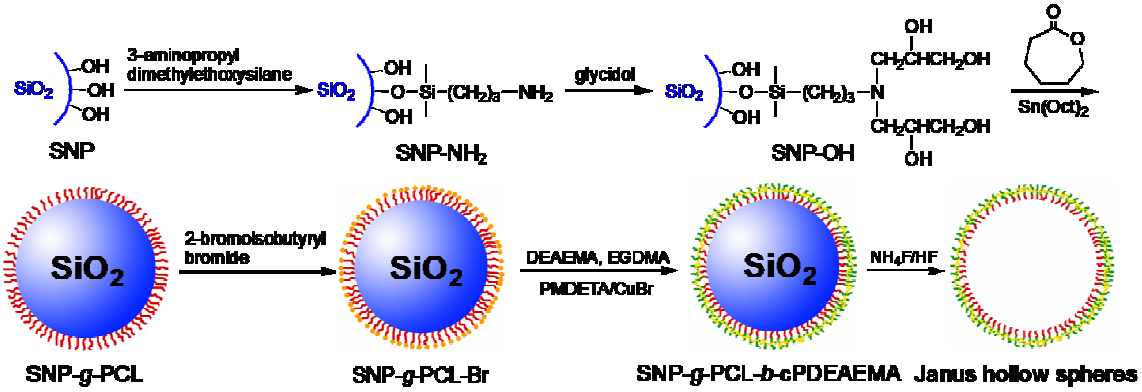
Intracellular uptake of JHSs

L929 cells were cultured with RPMI-1640 medium supplemented with 10% FBS and 1% penicillin and streptomycin, followed by incubation under 5% CO_2 and 95% humidified atmosphere at 37 °C. The cells were further cultured for 48 h. The cells were harvested by trypsinization using a 0.05% (w/v) trypsin solution (1×10^5 cell/mL), to a 35-mm plate for 24 h. The FITC-labeled JHSs were dispensed in RPMI-1640 medium (0.1 mg/mL) at pH 7.4 and pH 6.5, respectively, and the samples were added into 35-mm cell culture plate. After culturing for 8 h and 24 h, L929 cells was fixed in 4% paraformaldehyde for 20 min, and the nucleus was stained with DAPI for 10 min. Samples were thoroughly washed three times with PBS and imaged with the confocal laser scanning microscopy (CLSM) with a 100×oil objective, 405 nm (DAPI) and 488 nm (FITC) excitation wavelength.

Characterization

The samples for transmission electron microscopy (TEM) (JEOL 100 CX operating at 100 kV) observation were prepared by spreading very dilute dispersions in ethanol onto carbon-coated copper grids and dried at room temperature. Scanning electron microscopy (SEM) measurement was performed on a Hitachi S-4800 apparatus operated at an accelerating voltage of 15 kV. The samples were ambient dried and vacuum sputtered with Pt. Energy-dispersive X-ray (EDX) analysis was preformed on a Hitachi S-4800 SEM equipped with an EDX analyzer operated at an accelerating voltage of 15 kV. Fourier transform infrared (FT-IR) spectroscopy was performed after scanning samples for 32 times using a Bruker EQUINOX 55 spectrometer (resolution: 4 cm−1) with the sample/KBr pressed pellets. Thermogravimetric analysis (TGA) was performed using the Perkin-Elmer Pyris 1 TGA under air at a heating rate of 10 °C/min. X-ray photoelectron spectroscopy (XPS) was performed on the Thermo Scientific ESCALAB 250Xi using 200 W monochromated Al Kα radiation. The 500 μm X-ray spot was used for XPS analysis. The base pressure in the analysis chamber was about 3×10^{-10} mbar. Typically the hydrocarbon C1s line at 284.8 eV from adventitious carbon is used for energy referencing. Particle sizes were analyzed using a dynamic light scattering (DLS) instrument (Malvern Nano Zetasizer ZS 90) with a scattering angle of 176.1°. The JHSs or particles were dispersed in water (1.0 or 5.0 mg/mL) and was determined in triplicate, and the errors have been estimated to be ≤5%. The measurements

were taken after the samples had been dispersed in distilled water (1.0 or 5.0 mg/mL). The zeta potentials were determined by electrophoretic measurement carried out with the same instrument (Malvern Nano Zetasizer ZS 90). The measurements were performed three times for each sample at 25 °C. The cytotoxicity of samples were measured using a microplate reader (Thermo MULTISKAN MK3). Fluorescence spectra were obtained using a Hitachi F-4500 fluorescence spectrophotometer and the excitation spectra of DOX solution were scanned from 550 to 650 nm at room temperature, with an emission wavelength of 480 nm. The confocal laser scanning microscopy (CLSM) was carried out on Olympus FV1000-IX81 confocal laser biological microscope system.



Scheme 1 Synthesis of the polymeric JHSs.

Results and discussion

In this work, we fabricate intelligent polymeric JHSs by surface-initiated ROP and followed by ATRP with SNPs as templates. This synthesis procedure is summarized in Scheme 1. Generally, the surface modification based on surface-initiated LRP techniques can be divided into two categories—"grafting to" and "grafting from" approaches—and both of them have advantages and limitations. In this process, we employ "grafting from" rather than "grafting to" technique since "grafting from" technique can control the functionality, density, and thickness of the polymer on the surface of template[21] and results in higher grafting density[22, 23] and then more stable JHSs after removal of the SNPs template.

Preparation of hydroxide functionalized SNPs

In this study, the diameter of as-used templates SNPs is about 500 nm (Figure 3a). As shown in Scheme 1, a two-step approach are employed to prepare hydroxide functionalized SNPs. The SNPs are modified with 3-aminopropyldimethylethoxysilane followed with glycidol, and then form hydroxyl groups functionalized SNPs. The FT-IR spectrum proves the existence of SNPs-OH (Figure 1b). The characteristic peaks of C-H groups at 2800-3000 cm^{-1}. The quantity of

N increases from 0.00 of SNPs to 1.09 wt.-% of SNPs-NH_2 for the existence of amine groups according to the EDX analysis (Table 1). From the elemental analysis by XPS measurement (Table SI1†), the content of O increases from 23.86 at.-% to 29.08 at.-% after modification with glycidol for the increase of hydroxyl groups. All of these evidences strongly confirmed that the SNPs-OH is successfully prepared.

Table 1 Results of EDX analysis of hybrid SNPs

Sample	Weight (%)				
	Si	O	C	N	Br
SNPs	49.91	50.09	0.00	0.00	0.00
SNPs-NH_2	48.68	46.20	4.03	1.09	0.00
SNPs-OH	46.30	46.72	6.09	0.89	0.00
SNPs-*g*-PCL	44.01	45.23	10.43	0.33	0.00
SNPs-*g*-PCL-Br	42.82	44.35	11.75	0.23	0.85
SNPs-*g*-PCL-*b*-cPDEAEMA	40.28	43.52	15.28	0.64	0.28

Preparation of SNPs-*g*-PCL

After that, SNPs-*g*-PCL is synthesized via surface-initiated ROP of ε-CL initiated by hydroxyl groups of the as-formed SNPs-OH. There is a strong peak at 1730 cm^{-1} related to carbonyl groups of PCL in the FT-IR spectrum of SNP-*g*-PCL (Figure 1c). The content of C increases from 6.09 wt.-% to 10.43 wt.-% in the EDX analysis (Table 1) due to the existence of the PCL composition. PCL content is 8.10 wt.-% from the TGA analysis (Figure 2b). The thickness of PCL layer is about 5 nm (Figure 3c). It is confirmed that the PCL polymer chain is successfully grafted onto the surface of the hydroxide side of the SNPs-OH.

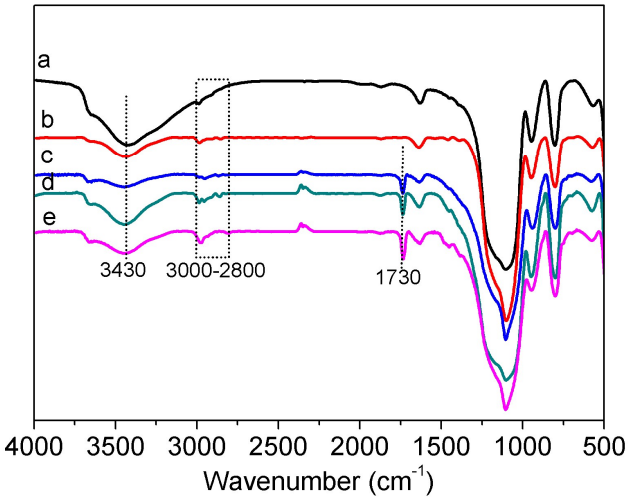


Fig. 1 IR spectra of different samples: (a) SNPs-NH_2, (b) SNPs-OH, (c) SNPs-*g*-PCL, (d) SNPs-*g*-PCL-Br and (e) SNPs-*g*-PCL-*b*-cPDEAEMA.

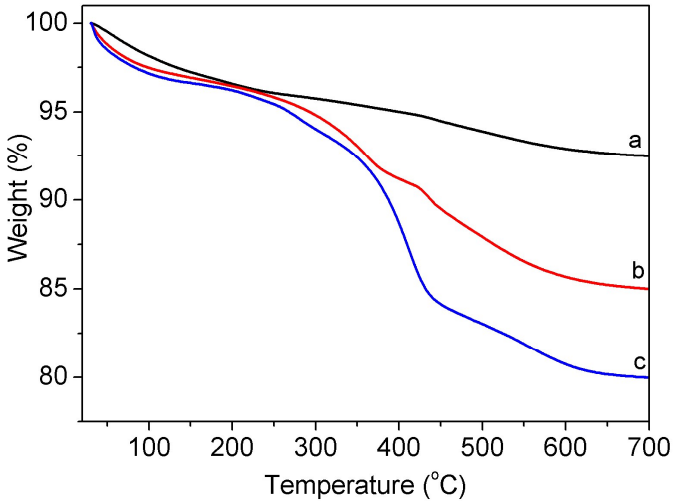


Fig. 2 TGA curves of different samples: (a) SNPs, (b) SNPs-*g*-PCL and (c) SNPs-*g*-PCL-*b*-cPDEAEMA.

Preparation of SNPs-*g*-PCL-*b*-cPDEAEMA

The SNP-*g*-PCL-Br is prepared via a reaction between hydroxyls in the end of PCL and C(O)-Br groups and then used to initiate the ATRP of DEAEMA and EGDMA to achieve a crosslinked pH-responsive PDEAEMA shell. Br element, which is not present in the PCL-*g*-SNP, is detected in SNP-*g*-PCL-Br in the EDX analyses (Table 1). Meanwhile, the characteristic peak of 68 ev (Br 3d) in the XPS (Figure SI1† c, d) indicates the existence of Br element. All there evidences prove that the SNP-*g*-PCL-Br is successful prepared.

Subsequently, the crosslinked PDEAEMA layer is grafted onto the Br- side of the SNP-*g*-PCL-Br via ATRP of DEAEMA and EGDMA monomers. The DEAEMA is a pH-responsive functional monomer and the EGDMA is a cross-linker which is employed to enhance the stability of JHSs. The molar ratio of DEAEMA to EGDMA, which influences the structure and properties of the modified SNPs and then the JHSs, is crucial in the system. It is reasonable that lower molar ratio result in higher cross-link densities and then more stable but less or even without pH-responsive JHSs. So the final molar ratio of 7:1 is chose in this reaction. After the ATRP, there is an obvious increase on the uniform thickness of the polymer shell (about 10 nm) as deduced from the TEM images (Figure 3d). The peaks of 1730 cm^{-1} and 2800~3000 cm^{-1} of SNPs-*g*-PCL-*b*-cPDEAEMA is stronger than that of SNP-*g*-PCL-Br and the peak of 1454

cm^{-1} is the character of $-CH_3$ in PDEAEMA (Figure 1d). From the TGA, cross-linked PDEAEMA and PCL contents are 7.1 wt.-% and 8.1wt.-%, respectively.

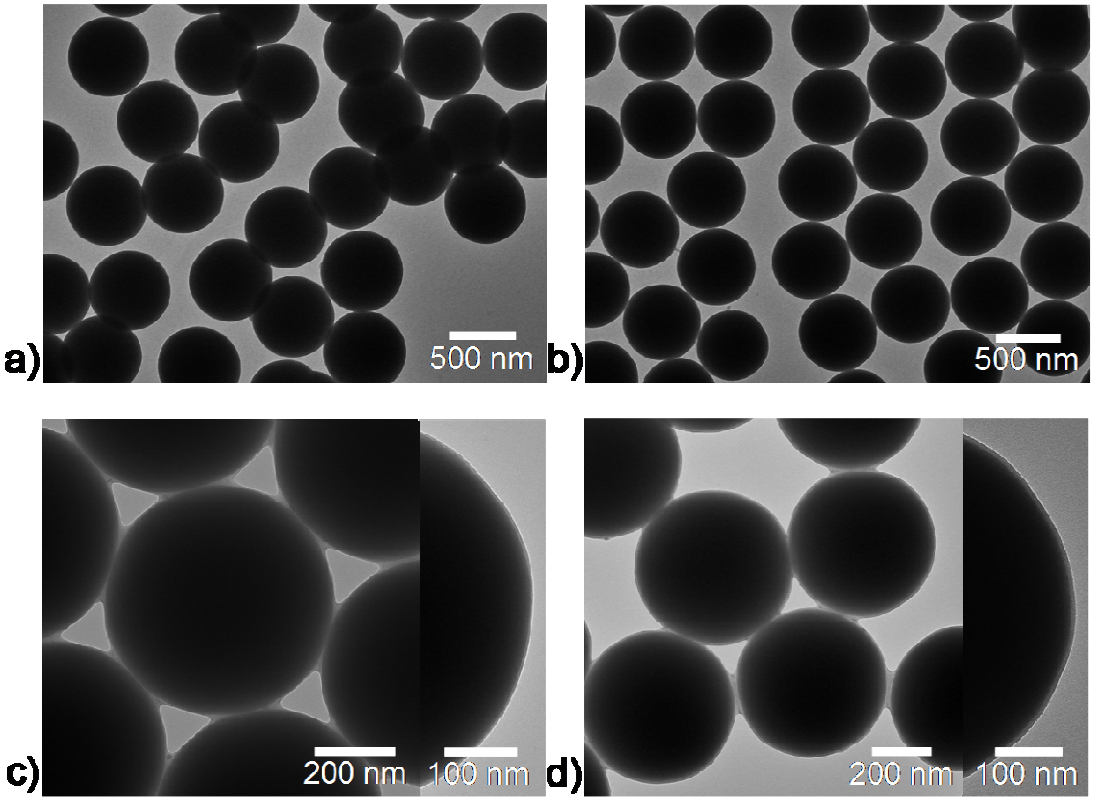


Fig. 3 TEM images of (a) bare SNP, (b) SNP-OH, (c) SNP-*g*-PCL and (d) SNP-*g*-PCL-*b*-cPDEAEMA.

Preparation of Janus hollow spheres

Polymeric JHSs are produced by etching out the silica nanoparticles (Figure 4a). The collapsed JHSs structures are observed due to the flexible thin polymeric JHSs are not robust enough to remain the hollow spherical structure in the dried state (Figure 4b). The LCSM image reveals that hydrophobic cavities of the polymeric JHSs can be selectively filled with the dyed toluene, while also can be dispersed well in water for their hydrophilic exterior surface (Figure 4c). It is a general method to fabricate JHSs.

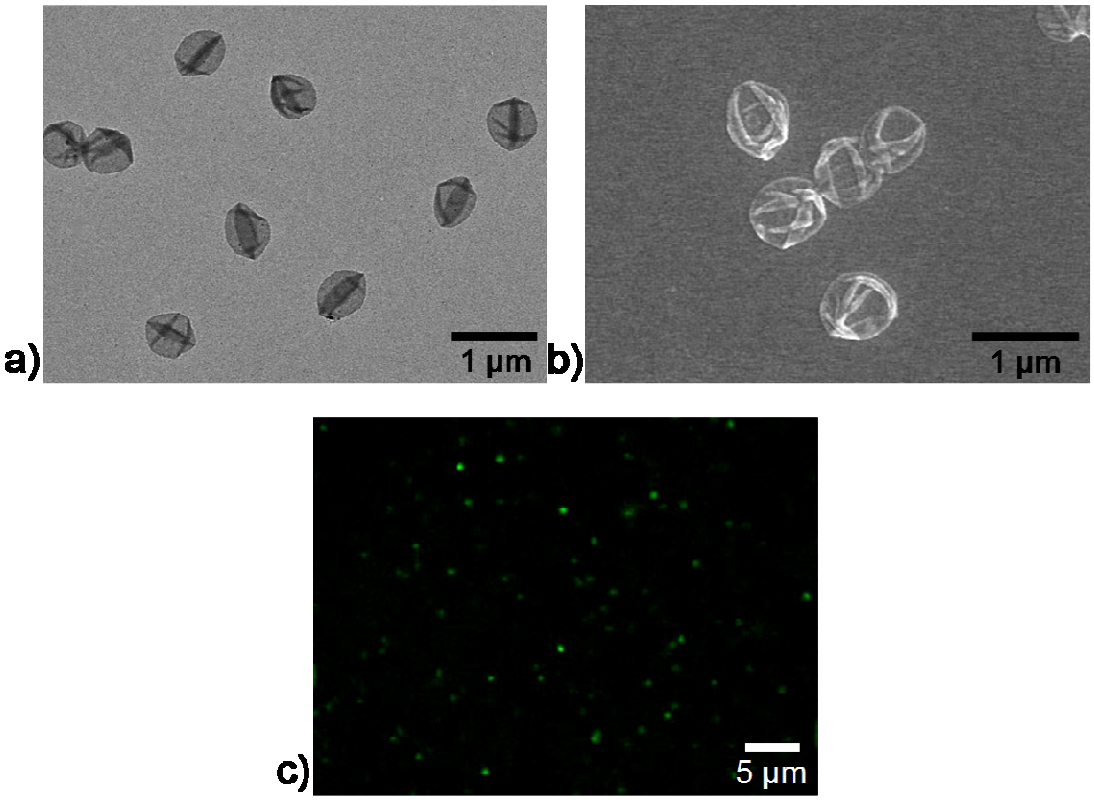


Fig. 4 The typically JHSs samples. (a) TEM and (b) SEM images of JHSs; (c) LCSM image of FITC-labeled JHSs.

The pH-responsive property of the JHSs

The JHSs (10 mg/mL) can be well dispersed in water at a low pH of 2 (Figure 5a, left). While at pH of 10, the JHSs are heavily aggregated (Figure 5a, right). It is because that the exterior surface is hydrophilic at pH of 2 and hydrophobic at pH of 10 for the pH response of the external cPDEAEMA. To further confirm the pH sensitivity of JHSs, the hydrodynamic diameters (d_H) of the JHSs (1.0 mg/mL) in aqueous media at 25 ^oC and different pH values from 3 to 12 are investigated via DLS (Figure 5b). Hydrodynamic diameters of JHSs decrease from 710 to 450 nm and with an increase in the solution pH values from 2 to 12. The pK_a of PDEAEMA is around 7.2. As the acidity of the solution increases, the PDEAEMA chain undergoes physicochemical changes going from an unprotonated, hydrophobic entangled state at a high pH value to a protonated, hydrated, repulsive state at a low pH value,[24] and then will become more and more extended. From the DLS data of JHSs, the variation in diameter of JHSs increases near pH of 7.2. Finally, to further confirm the pH-sensitivity of the JHSs, TEM is employed to observe the diameter change of the JHSs at pH 2.0 (Figure 5c) and pH 10.0 (Figure 5d). It is consistent with the result of DLS.

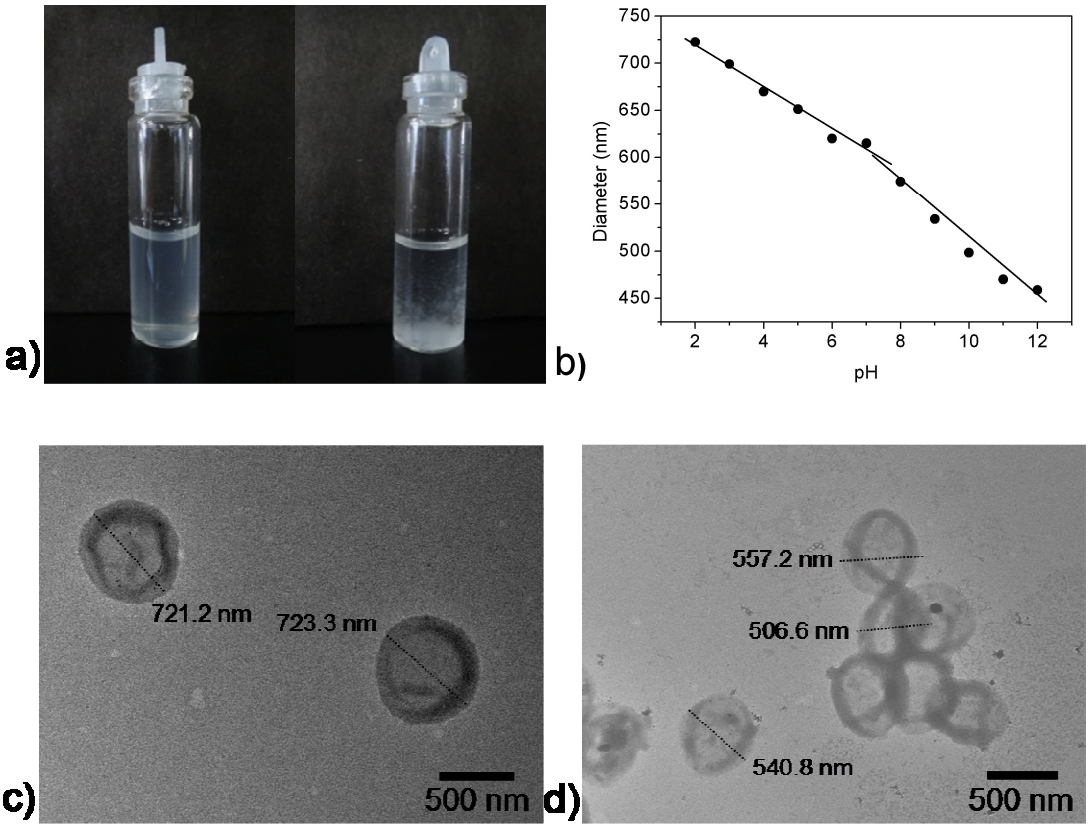


Fig. 5 pH response of the JHSs. (a) a dispersion of JHSs in water at a low pH of 2 (left) and at a high pH of 10 (right); (b) Hydrodynamic diameters of JHSs at different pH values from 2 to 12; (c, d) TEM images of JHSs in pH 2.0 (c) and pH 10.0 (d) solution.

Drug loading and release

Doxorubicin (DOX) is used as a model drug to evaluate the drug loading and controlled release behavior of JHSs. Drug-loading JHSs are prepared by diffusion method. The JHSs with polymeric mesh walls would be well able to be permeated by the small drug molecules like DOX.[25] The as-prepared JHSs is placed in a drug solution until it swells to equilibrium, thus allowing the drug to diffuse into the JHSs. DOX is loaded into the cavities of JHSs which are dispensed in DMF. In the partial crosslinked polymer shell, the crosslink density is low. The majority of polymer chain extended, allowing for the drug loading inside the JHSs via diffusion through the pores. The inner PCL layer serves as a microenvironment for incorporating hydrophobic drugs by physical entrapment and structure of JHSs should also help enhance the drug-loading capacity via swelling. The drug loading capacity of JHSs at different feed ratio of DOX/polymer JHSs is summarized in Table 2. The loading capacity of DOX increased with the increase of DOX/JHSs ratio and reached a saturate value of 39.7 wt.-% at DOX/polymer JHSs

ratio of 1. The loading limitation of JHSs might be resulted from the loaded DOX molecules. The loading drug is a process to reach the equilibrium state.

Table 2 DOX loading capacity of JHSs

Feed ratio of DOX to JHSs (w/w)	Drug loading capacity (%)
0.1	6.1
0.3	13.3
0.6	24.2
1.0	32.7
10.0	39.7

To evaluate the effects of pH-responsive behavior on controlled drug delivery, the in vitro drug release profiles of DOX-loaded JHSs are carried out under physiological condition (PBS, pH 7.4) and in a slightly acidic environment (pH 6.5 and 5.5) to simulate the pH of the endosomal and lysosomal microenvironments, as shown in Figure 6. DOX is used as a model drug, which is loaded into the JHSs. In the partial cross-linked polymeric shell, PDEAEMA is a weak cationic polyelectrolyte that shows pH-sensitive properties which exists the pendant tertiary amine groups.[26, 27] Thus it can be protonated or deprotonated when the pH below or above the pK_a of PDEAEMA. So the release of DOX is pH dependent, and the release rate obviously accelerates as the pH decreased from 7.4 to 5.5. Under neutral conditions (pH 7.4), Less than 30% of DOX is released in 50 h which demonstrates that the drug could be well protected and stayed stable with minimal DOX release from the JHSs under normal physiological condition. At pH 6.5, about 50% DOX are released after 48 h, and 83% released at pH 5.5. Decreasing the pH from 7.4 to 5.5, the protonated PDEAEMA chains becomes extent and electrostatic repulse each other, leading to the enlarged pore size, promoting efficiently the release behavior of DOX (Scheme 2). Secondly, DOX solubility in water was enhanced following protonation under acidic conditions. These results indicate that changing the external pH could effectively control the release behavior of the model drug molecules from the pH-responsive JHSs.

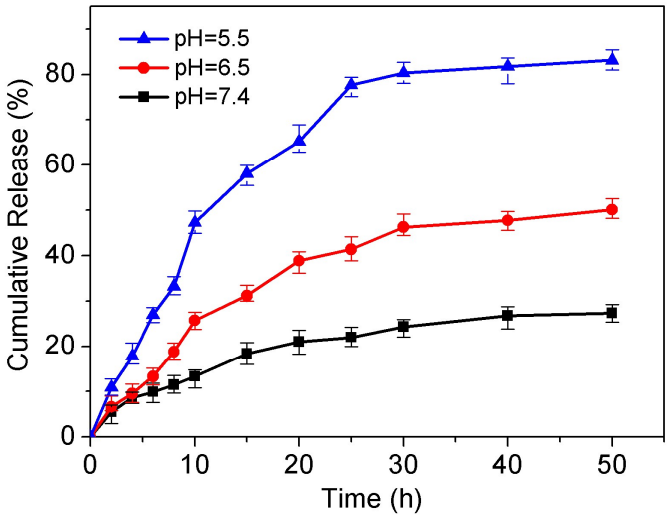
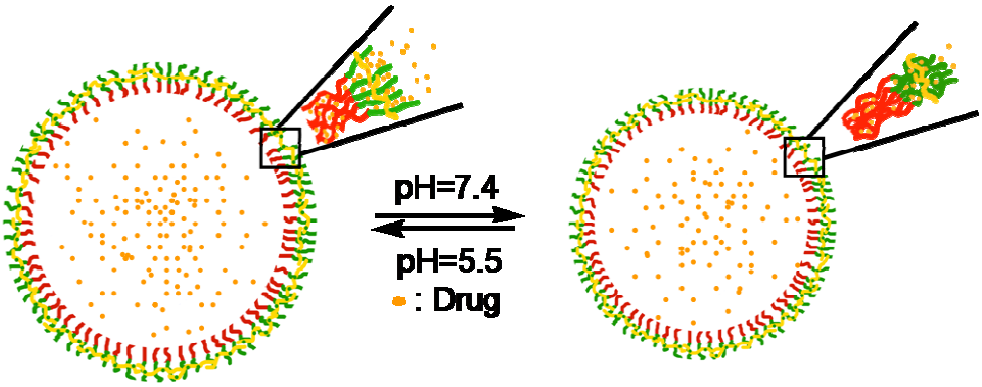


Fig. 6 In vitro drug release profiles of DOX-loaded JHSs under different pH conditions.



Scheme 2 Schematics of the drug release behavior of pH responsive polymeric JHSs at pH 7.4 and pH 5.5.

Intracellular uptake of JHSs

The uptake of JHSs is monitored by observing the L929 cells cultured with FITC-labeled JHSs using the CLSM. The nucleus stained with DAPI is dyed blue. Intracellular uptake of JHS is tested at pH 7.4 and pH 6.5. Contrasted with the neutral pH 7.4 of the normal physiological environment, the extracellular pH values in tumorous tissues are around 6.5-7.0.[28, 29] From the CLSM images (Figure 7), the JHSs enter cells faster and more at pH 6.5 than at pH 7.4 for 8 h and 24 h. The number of the uptake of JHSs (the green spot) at pH 6.5 is significantly larger than under the same conditions at pH 7.4 by the increase of positive charge. At the concentration of 1.0 mg/mL, the zeta potentials of JHSs are determined to be 21.8 mV at pH 6.5 and 5.93 mV at pH 7.4 (Figure SI2†). JHSs with the PDEAEMA outer layer are triggered by changing pH from acidity of tumorous tissues to the neutral of normal physiological environment. The pH-responsive PDEAEMA layer

with almost no charge at physiological pH 7.4 can prevent or weaken the cellular uptake of the JHSs effectively due to excluding negatively charged cell membranes, but becomes highly positively charged by protonation of the pendant tertiary amine groups[14] and would be adsorbed onto negatively charged cell membranes and subsequently endocytosed by tumor cells at tumor extracellular pH. No or weakened positive charged JHSs have little interaction with the normal cells.

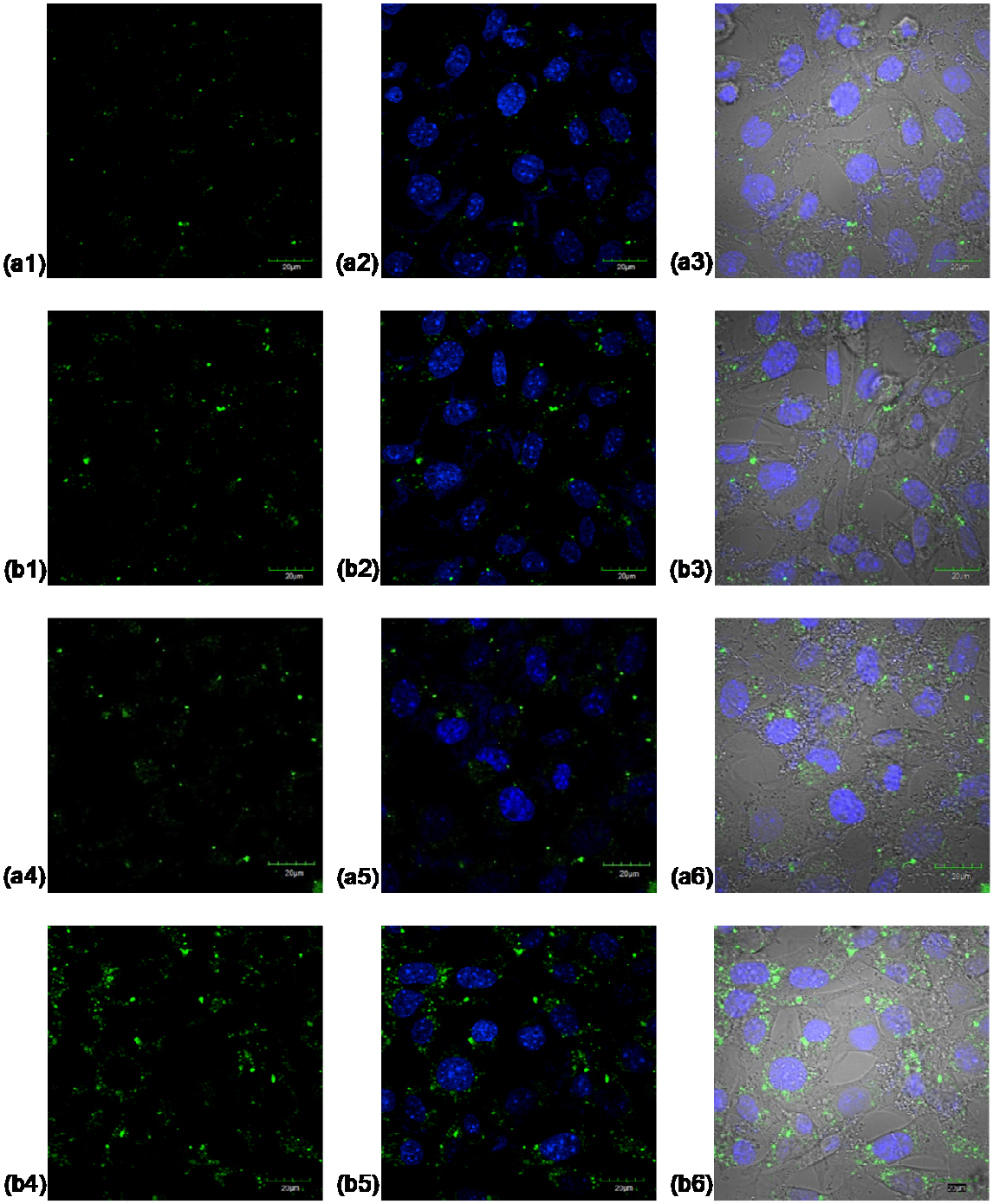


Fig. 7 CLSM images showing the uptake of JHSs by L929 cells for 8 h (a1-a3, b1-b3) and 24h (a4-a6, b4-b6). Intracellular uptake of JHSs is tested at pH 7.4 (a1-a6) and pH 6.5 (b1-b6). The images show the fluorescent signals arising from isothiocyanate (a1, a4, b1, b4), merged images of isothiocyanate and DAPI blue nucleus signals (a2, a5, b2, b5) and merged images of isothiocyanate, DAPI blue and phase contrast signals (a3, a6, b3, b6). The scale bars correspond to 20 μm.

Cytotoxicity of JHSs

Cytotoxic effects of the JHSs and DOX-loaded JHSs in U87MG cell line were determined by CCK-8 assay. As shown in Figure 8, while the neat JHS demonstrates desirable safety at both pH 7.4 and 6.5 with concentrations up to 200 μg/ml (Figure 8a), dose dependent cytotoxicity was revealed from the DOX loaded JHSs after a 24-hour incubation (Figure 8b). Meanwhile, it is noted that the toxicity caused by the formulation is pH dependent, since a treatment at tumor pH, i.e. 6.5, led to much less cell viability when compared to that at the physiological pH especially at high dose levels. The results are quite reasonable for the JHSs have shown properties of the enhanced cell uptake and faster drug release under mild acidic conditions. The cell viability test again suggests that the JHSs would be a promising vehicle for anti-tumor drug delivery.

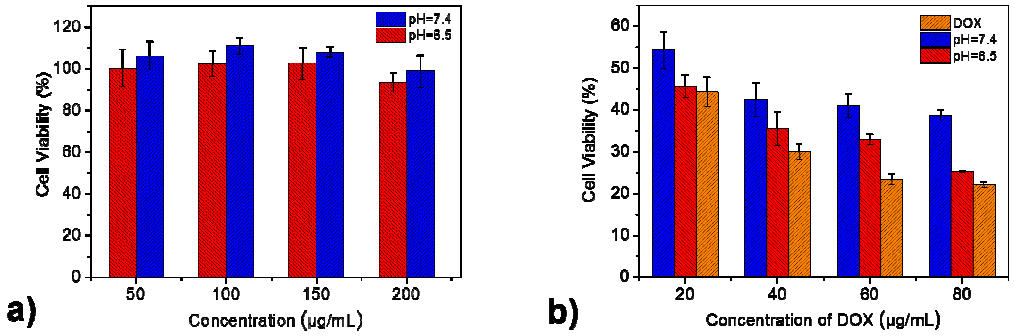


Fig. 8 (a) The cytotoxicity of JHSs at the different concentrations for 24 h; (b) The cytotoxicity of free DOX and DOX-loaded JHSs at the different DOX concentrations for 24 h.

Conclusions

In summary, we successfully synthesize JHSs by ROP and ATRP using silica nanoparticles as sacrificial templates. The biodegradable PCL brush is used as the inner layer and the partial crosslinked pH-responsive PDEAEMA is used as the outer layer and the bond of final JHSs. The chemical composition and morphology of the JHSs are confirmed well by FT-IR, TGA, EDX,

XPS, SEM and TEM. DLS analysis and TEM images demonstrate pH sensitivity of JHSs. For their pH-responsive property, the as-prepared JHSs can selectively loaded oil-soluble materials into their hydrophobic cavities and release them by adjusting the pH environment. DOX is loaded into the JHS as a model drug. The in vitro release exhibited pH sensitivity, showing faster rate at more acidic pHs within an interval between 7.4 and 5.5, due to the hydrophobic to hydrophilic transition of the PDEAEMA blocks and hence change of the permeability of the wall. The surface property change also led to the JHS being favorably taken up by cells under tumor acidic condition, i.e. pH 6.5. Here, considering these good virtues, a multi-responsively and biocompatible JHS is possible to reach in future via using the surface-initiated polymerization method. It will be a promising vehicle, especially considering that the release behavior can be controlled by both external stimuli such as temperature, pH, or magnetic and internal factors such as the thickness of the shell or the degree and character of cross-linking.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (51373073, 51233007, 51173191, 21274155).

Notes and references

1 F. X. Liang, C. L. Zhang and Z. Z. Yang, *Adv. Mater*. 2014, **26**, 6944-6949.

2 A. Walther and A. H. E. Müller, *Chem. Rev.* 2013, **113**, 5194-5261.

3 S. Jiang and S. Granick, Janus Particles Synthesis, Self-assembly and Applications, RSC, London, England 2012.

4 C. L. Zhang, W. Wei, F. X. Liang and Z. Z. Yang, *Scientia Sinica Chimica* 2012, **42**, 1616-1626.

5 L. F. Hu, M. Chen, X. S. Fang and L. M. Wu, *Chem. Soc. Rev.* 2012, **41**, 1350-1362.

6 J. Z. Du and R. K. O'Reilly, *Chem. Soc. Rev.* 2012, **40**, 2402-2416.

7 F. X. Liang, J. G. Liu, C. L. Zhang, X. Z. Qu, J. L. Li and Z. Z. Yang, *Chem. Commun.* 2011, **47**, 1231-1233.

8 K. Shen, F. X. Liang, J. G. Liu, X. Z. Qu, C. L. Zhang, J. L. Li, Q. Wang, W. Wei, Y. F. Lu and Z. Z. Yang, *Polymer* 2011, **52**, 4418-4422.

9 Y. Chen, F. X. Liang, H. L. Yang, C. L. Zhang, Q. Wang, X. Z. Qu, J. L. Li, Y. L. Cai, D. Qiu and Z. Z. Yang, *Macromolecules* 2012, **45**, 1460-1467.

10 L. L. Zhao, L. J. Zhu, Y. Chen, Q. Wang, J. L. Li, C. L. Zhang, F. X. Liang, X. Z. Qu and Z. Z. Yang, *Chem. Commun.* 2013, **49**, 6161-6163.

11 Y. Q. Yang, W. J. Lin, B. Zhao, X. F. Wen, X. D. Guo and L. J. Zhang, *Langmuir* 2012, **28**, 8251-8259.

12 Q. M. Liu, J. Chen and J. Z. Du, *Biomacromolecules* 2014, **15**, 3072-3082.

13 O. J. Cayre, N. Chagneux and S. Biggs, *Soft Matter*, 2011, **7**, 2211-2234.

14 Y. Q. Yang, B. Zhao, Z. D. Li, W. J. Lin, C. Y. Zhang, X. D. Guo, J. F. Wang and L. J. Zhang, *Acta Biomater.* 2013, **9**, 7679-7690.

15 J. V. M. Weaver and D. J. Adams, *Soft Matter*, 2010, **6**, 2575-2582.

16 W. J. Lin, S. Y. Nie, Q. Zhong, Y. Q. Yang, C. Z. Cai, J. F. Wang and L. J. Zhang, *J. Mater. Chem. B* 2014, **2**, 4008-4020.

17 C. Y. Zhang, W. S. Wu, N. Yao, B. Zhao and L. J. Zhang, *RSC Adv*. 2014, **4**, 40232-40240.

18 W. Stöber, A. Fink and E. Bohn, *Colloid Interface Sci*. 1968, **26**, 62-69.

19 H. Mori, A. H. E. Müller and J. E. Klee, *J. Am. Chem. Soc.* 2003, **125**, 3712-3713.

20 J. H. Moon, B. Ramaraj, S. M. Lee and K. R. Yoon, *J. Appl. Polym. Sci.* 2008, **107**, 2689-2694.

21 C. Z. Li, J. W. Han, C. Y. Ryu and B. C. Benicewicz, *Macromolecules* 2006, **39**, 3175-3183.

22 X. Huang, D. Appelhans, P. Formanek, F. Simon and B. Voit, *Macromolecules* 2011, **44**, 8351-8360.

23 B. Radhakrishnan, R. Ranjan and W. J. Brittain, *Soft Matter* 2006, **2**, 386-396.

24 X. Huang, D. Appelhans, P. Formanek, F. Simon and B. Voit, *ACS Nano* 2012, **6**, 9718-9826.

25 L. Cui, R. Wang, X. Q. Ji, M. Hu, B. Wang and J. Q. Liu. *Mater. Chem. Phys.* 2014, **148**, 87-95.

26 Y. Q. Shen, Y. H. Zhan, J. B. Tang, P. S. Xu, P. A. Johnson, M. Radosz, E. A. V. Kirk and W. J. Murdoch, *AIChE J*. 2008, **54**, 2979-2989.

27 H. Liu, C. H. Li, H. W. Liu and S. Y. Liu, *Langmuir* 2009, **25**, 4724-4734.

28 X. D. Guo, L. J. Zhang, Y. Chen, and Y. Qian, *AIChE J*. 2010, **56**, 1922-1931.

29 J. Chen, X. Z. Qiu, J. Ouyang, J. M. Kong, W. Zhong and M. M. Q. Xing, *Biomacromolecules* 2011, **12**, 3601-3611.