**Mesoporous silica nanoparticles combining Au particles as
Glutathione and pH dual-sensitive nanocarriers for doxorubicin**

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Abstract: Mesoporous silica nanoparticles (MSNs) combining gold particles (MSNs-Au) were synthesized as nanocarriers for glutathione (GSH) and pH dual-sensitive intracellular controlled release of the anti-cancer drug doxorubicin (DOX). The MSNs were used as an adsorbent for DOX, and the ultra-small gold nanospheres (Au NPs) partly operated as gatekeepers to control the release of DOX from the pores of MSNs and as the driver of drug release in the presence of GSH due to the association between GSH and Au particles. Under different pH conditions, DOX release changed due to different levels of dissociation between the –SH group on the MSNs and the Au particles. The composition, morphology, and properties of the as-prepared composites were characterized by elemental analysis, fluorescence spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, nitrogen adsorption-desorption, thermal gravimetric and UV-visible spectroscopy. The *in vitro* release experiments showed that these smart nanocarriers effectively avoided drug leakage in the neutral media. Cytotoxicity and imaging studies also indicated that DOX-loaded Au-MSNs (DOX@MSNs-Au) had a significant inhibitory effect on the growth of Tca8113 cells and sustained the release rate of DOX

Keywords: Gold nanoparticles; Mesoporous silica nanoparticles; pH Controlled release; Glutathione

1. Introduction

Doxorubicin (DOX) is regarded as a broad-spectrum anticancer drug, which can effectively inhibit the activity of topoisomerase II and is used in the treatment of leukemia, breast cancer and liver cancer. However, due to an organism's defense mechanism, it is very difficult for DOX to enter cancerous tissue through the blood circulation and to cause cancer cell apoptosis. With regard to the design of prodrugs, mesoporous silica nanoparticles (MSNs) are considered potential drug carriers due to their good biocompatibility, high surface area, tunable pore diameter and narrow particle size distribution [1–3]. MSNs offer both interior pore and exterior particle surfaces for loading different guest molecules [4]. Pan et al. [5] synthesized four types of MSNs with hexadecyl trimethyl ammonium chloride as the template reagent and triethanolamine as the catalyst for the nuclear-targeted drug delivery system. For amino-functionalized MSNs (50 nm), the DOX loading capacity (mg/g) can reach up to 40%.

For practical drug delivery applications, nanocarriers should not only have high drug loading capacity, but also “zero-premature release” and “stimuli-responsive controlled release” [6]. Under neutral pH 7.4, the premature release of DOX was reported to be 10%–20% [7,8], which produced serious side effects in normal human cells and metabolic organs. A number of researchers have attempted to solve this problem. Xu et al. synthesized matrix metalloproteinase degradable gelatin-coated MSNs (MSNs@Gel) for endogenous tumor microenvironment-triggered release systems for cancer treatment *in vivo* [9]. Premature release was only 3% at pH 7.4,

which was much lower than that of 8% under the same conditions without gelatin as the gatekeeper. Furthermore, Zheng et al. also reported polydopamine (PDA)-coated MSNs as pH-sensitive nanocarriers for controlled drug release [10]. Using PDA-coated MSNs as gatekeepers, the drug molecules were blocked at neutral pH and released at lower pH. Moreover, zinc oxide nanolids and cadmium sulfide nanoparticles were also used to block the premature release of drugs [11–12].

Ultra-small gold nanospheres (Au NPs) are stable and less susceptible to acidic or enzymatic degradation, and their surface ligands are easily replaced by a variety of thiol groups [13–15]. Moreover, Au NPs can act as good quenchers for many fluorescence donors due to the nanosurface energy transfer (NSET) effect [16–19], which is able to track drug release and take images of cancer cells. Au particles of suitable sizes can be used as gatekeepers for MSNs to control the release of drugs in the pores of MSNs [20].

In the present study, the DOX nanocarriers with MSNs as the adsorbent and Au NPs as the gatekeepers were constructed (DOX@MSNs-Au) with glutathione (GSH) and pH dual sensitivity. After loading DOX into the MSNs, the openings on MSNs were blocked by ultra-small Au NPs to control the release of DOX from pores in response to pH and GSH stimuli. The subsequent cytotoxicity and cell internalization behavior indicated that the resulting nanocarriers showed high anticancer efficacy *in vitro*.

2. Experimental

2.1. Reagents and materials

Hexadecyl trimethyl ammonium chloride (CTAC), tetrachloroauric acid, thioglycolic acid and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Doxorubicin hydrochloride (DOX), N-Hydroxysuccinimide (NHS), tetraethyl orthosilicate (TEOS), 3-aminopropyl trimethoxysilane (APTMS) and sodium borohydride (NaBH_4) were purchased from Aladdin (Shanghai, China). Triethanolamine (TEOA) was provided by the Zhengzhou chemical Reagent Plant (Zhengzhou, China). 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC • HCl) was purchased from GL Biochem (Shanghai, China). GSH was purchased from Sangon Biological Engineering Co., Ltd (Shanghai, China). Sodium chloride (NaCl) was provided by Guangfu chemical reagent Co., Ltd (Tianjin, China). Sodium hydroxide was purchased from Chengdu Kelong Chemical reagent Factory (Chengdu, China). Anhydrous ethanol and methanol were obtained from Lee coulombeau Medicinal Chemistry Co., Ltd (Tianjin, China). Deionized water prepared from Millipore (Bedford, MA, USA) was used in all experiments. All other reagents and solvents of analytical grade were used as received unless otherwise mentioned.

2.2. Characterization

Elemental analysis was performed on a VarioEL Elemental Analyzer (Elementar, Germany). The particle size was determined by DLS using a BI-200SM particle size analyzer (Brookhaven, USA). Thermogravimetric analysis (TGA) was conducted using a STAPT1600 thermal analyzer (Linseis, Germany). The samples were heated from room temperature to 900°C at a heating rate of $10^\circ\text{C}/\text{min}$ under nitrogen. The

nitrogen adsorption and desorption isotherms, surface area and median pore diameters were measured using a TriStar 3000 (Micromeritics, USA). Fourier transform infrared (FTIR) spectra were acquired on a Nicolet 20 NEXUS 670 FTIR spectrophotometer (Ramsey, MA, USA). The UV-Vis absorbance spectra were measured using a Puxi UV-1810 spectrophotometer (Beijing, China) with the samples diluted with water at pH 7.4. Transmission electron microscopy (TEM) observations were recorded using a Tecnai-G2-F30 (FEI, USA) and JEM-2100 (JEOL, Japan). TEM samples were prepared by depositing one drop of diluted solution onto a carbon-coated copper grid. Fluorescence spectra were recorded on a RF-5301PC fluorescence spectrophotometer (Shimadzu, Japan) at excitation and emission wavelength of 488 nm and 555 nm, respectively. Confocal microscopy images were obtained with a laser scanning confocal microscope (CLSM, Zeiss, LSM 510 Meta, Germany).

2.3. Synthesis of DOX@MSNs-Au

2.3.1. Preparation of mesoporous silica nanoparticles (MSNs)

Silica nanoparticles (SNs) with a diameter of 50–60 nm were obtained according to a previously published method [5]. Ultrapure water was heated to 95°C and refluxed for 10 min and then CTAC (2.0 g) and TEOA (60 mg) were added and dissolved under vigorous stirring. After 1 h, TEOS (1.5 mL) was added dropwise and the resulting mixture was stirred for another 1 h to obtain the milky products. The products were centrifuged at 12,000 rpm and washed three times with ethanol to remove the residual reactants.

In order to obtain the mesoporous structure of MSNs, the template CTAC was

washed out. 100 mL of CH₃OH and 0.8 g of solid NaCl were added and stirred constantly in a 250 mL round bottom flask. When NaCl was completely dissolved, SNs (130 mg) were added and the mixture was continue to be stirred for 1 h to make CTAC dissolve in CH₃OH, this was repeated several times. The products were centrifuged at 12,000 rpm for 10 min and washed several times with ethanol. The MSNs obtained were dried in a vacuum at 60°C for 24 h.

2.3.2 Modification of MSNs

MSNs were first functionalized with ATPMS to obtain MSNs-NH₂. MSNs (70 mg) were dispersed in 70 mL of ethanol by ultrasonic treatment for 1 h followed by the addition of APTMS (140 µL). After refluxing for 4 h, the products were centrifuged at 12,000 rpm for 10 min and washed several times with ethanol. The MSNs-NH₂ obtained were dried in a vacuum at 60°C for 24 h.

The MSNs-NH₂ were then functionalized with thioglycolic acid to obtain MSNs-SH. Thioglycolic acid was covalently conjugated onto MSNs-NH₂ through the -COOH and -NH₂ groups using the cross-linking reagents EDC • HCl and NHS. In this work, 40 µL of thioglycolic acid (0.56 mmol) was dissolved in 4 mL of phosphate buffered saline (PBS, pH 7.4) followed by the addition of 107 mg of EDC• HCl (0.56 mmol) and 160 mg NHS (0.56 mmol). After stirring for 1 h at room temperature, the mixture was added dropwise to 30 mg of MSNs-NH₂ (dispersed in 25 mL PBS) and then stirred for another 24 h at room temperature. The product MSNs-SH was obtained as a white powder after centrifugation and drying in a vacuum at 60°C.

2.3.3. Synthesis of DOX@MSNs

MSNs-SH (5.18 mg, dispersed uniformly in 9.0 mL of PBS) were mixed with DOX (2.13 mg, dissolved in 7.5 mL of PBS). The mixture was stirred at room temperature for 24 h in the dark, and then the DOX-loaded MSNs were collected by centrifugation.

2.3.4 Preparation of DOX@MSNs-Au

Ultra-small Au NPs with a diameter of 4 nm were obtained by a simple previously published method [1]. Chloroauric acid (25.0 mL, 2.5×10^{-4} M) was added to a round bottom flask with gentle stirring, followed by the addition of sodium citrate (25.0 mL, 2.5×10^{-4} M). After a few minutes, 0.75 mL of NaBH₄ (0.1 M) was quickly added to this solution and then stirred for another 4 h at room temperature to obtain a red solution. The red Au NPs were dialyzed for 24 h using a dialysis bag (molecular weight cut-off of 3 kDa).

DOX@MSNs (4.8 mg) were dispersed in 18 mL PBS, and then added to 50 mL of Au NPs solution. After stirring for 4 h in the dark, the dark red colored DOX@MSNs-Au were collected by centrifugation several times. To evaluate the DOX loading capacity, the supernatant solution was collected and the residual DOX content was measured by fluorescence spectroscopy.

The preparation of DOX@MSNs-Au is shown in Scheme 1.

Other related nanoparticles including MSNs-Au (without DOX) and DOX@MSNs (without Au NPs) were prepared according to the above procedures.

(Scheme 1)

2.4. *In vitro* drug release

The release studies were performed at the pH of normal body fluid and the endosome of cancer cells. DOX@MSNs-Au and DOX@MSNs (control) each with 315 µg DOX were first dispersed in 2 mL of buffer and transferred to a dialysis bag with a molecular weight cut-off of 3 kDa, respectively. The dialysis bag was then immersed in 28 mL of the release medium with gentle shaking at 37±0.5°C. Periodically, 2 mL of the release medium was removed and the same volume of fresh buffer was immediately added. The release experiments were performed in triplicate for each sample and the results are presented as the mean (± standard deviation). The following format was used to calculate the release of DOX:

$$m_t = C_t V + v \sum_{0}^{t-1} C_t$$

Where m_t represents the total release of DOX at time t . V is the total volume of the release medium. v is the volume of buffer removed each time at a predetermined time interval. C_t is the concentration of drug outside the dialysis bag measured at time t .

2.5. *In vitro* cytotoxicity study

The effects of MSNs-Au (control), DOX@MSNs-Au and free DOX on cells were determined using the sulforhodamine B (SRB) assay. Tca8113 cells (7×10^4 cells/well) were seeded into 96-well plates overnight. Then, the culture medium was replaced with fresh medium containing the above materials at different concentrations. Following incubation for 24 h, the cells were fixed with 100 µL cold trichloroacetic acid (10%, 4°C) for 1 h, gently washed five times with deionized water and then stained with 0.4% SRB solution (150 µL/well) for 30 min at room temperature. The unbound SRB was removed with 1% acetic acid. Aqueous Tris base [tris

(hydroxymethyl) aminomethane] (150 μ L, 10 mM) was added into each well to solubilize the SRB dye after the plate was air dried. The absorbance values of SRB were read on a micro-plate reader (Huake, Shanghai, China) at the wavelength of 570 nm. Each experiment was conducted in triplicate and results are presented as the mean ($\pm$ standard deviation).

2.6. Cellular uptake

The cellular uptake experiments were performed using a confocal laser scanning microscope (CLSM). Tca8113 cells (6×10^4 cells/well) were seeded in a six-well plate at 37°C for 24 h. The samples of free DOX (0.5 μ g/mL) and DOX@MSNs-Au (5 μ g/mL) were then added to the cell plates, respectively. After incubation for 6 or 12 h, these cells were washed with PBS three times to remove dead cells and then fixed with ethanol. The cell nucleus was stained with 4, 6-diamidino-2-phenylindole (DAPI, blue) for 10 min and then washed three times with PBS.

3. Results and Discussion

3.1. Preparation and characterization of DOX@MSNs-Au

The preparation of DOX@MSNs-Au was divided into four steps as follows:

In the first step, SNs were synthesized using positively charged CTAC as a template and TEOA as a catalyst. The elemental analysis results of SNs are summarized in Table 1. The content (%) of N, C and H was 0.55, 12.77 and 2.58, respectively. When the CTAC were removed by NaCl/CH₃OH solution, the content of N reduced to 0.34%.

In the second step, MSNs were first functionalized with ATPMS to give

MSNs-NH₂. The content (%) of N, C and H changed from 0.34, 8.05 and 1.74 to 0.80, 9.24 and 2.07, respectively, when MSNs were functionalized to MSNs-NH₂. For further modification of MSNs-SH, thioglycolic acid was covalently conjugated onto MSNs-NH₂ and the content of S was 0.60% (Table 1). The total weight of these compounds was 10% measured by TGA.

(Table 1)

In the third step, after DOX was adsorbed on MSNs, the color of DOX@MSN changed to red. Fluorescence spectra (Fig. 1) showed that before the addition of DOX, there was no fluorescence emission of MSNs-SH (Fig. 1a); however, a very strong fluorescence emission in the range of 500–700 nm appeared for DOX@MSNs (Fig. 1c), which was similar to the spectrum for free DOX (Fig. 1b).

In the final step, ultra-small Au NPs of appropriate diameters were used as the gatekeepers. Through the formation of a gold-thiol bond by thioglycolic acid, Au NPs were firmly locked on the surface of the MSNs. The color of DOX@MSNs-Au changed to purple. The fluorescence spectrum of DOX@MSNs-Au was a flat line (Fig. 1d). These emission changes from DOX@MSNs to DOX@MSNs-Au were due to the NSET effect between Au NPs and fluorescent DOX.

The synthesis of the as-prepared composites was also proved by FTIR spectroscopy (Appendices, Fig. A1).

(Fig.1)

3.2. Properties of the DOX@MSNs-Au

When the diameter of nanocarriers is smaller than 200 nm, the uptake of

nanocarriers by the reticuloendothelial system (RES) is reduced, and the permeability and retention effect enhanced [21]. Pan et al. reported that MSNs-TAT (nuclear-targeted peptide) with the diameter of 50 nm effectively entered into the nucleus [5]. The MSNs synthesized in the present study exhibited an average particle diameter of 58 nm with a honeycomb-like structure according to TEM and DLS (Fig. 2A). Fig. 2B shows an image of the MSNs-SH, which were dispersed well in water and had similar sizes to the MSNs. Fig. 2C shows the particle size distribution of Au NPs measured by HRTEM and DLS. As the average pore diameter of MSNs was 2.7 nm (Fig. A2), we synthesized ultra-small Au NPs with a diameter of 3.8–4.1 nm and their average particle diameter was 4.0 nm. From the HRTEM image, Au NPs had good dispersion in water and their crystal structure was very clear. Fig. 2D shows a TEM image of DOX@MSNs-Au. Au NPs were firmly fixed on the surface of the MSNs. The UV-Visible spectra of the products during the synthesis of DOX@MSNs-Au are shown in Appendices Fig. A3.

(Fig 2)

3.3. *In vitro* drug release

The drug loading efficiency of DOX was $27.4 \pm 0.6\%$, and the effective utilization ratio of DOX was as high as 96.2%. As the normal pH of blood is approximately 7.4, and both endosomes and lysosomes have a more acidic environment of pH 5.0–6.0 [22], drug release behavior was divided into three groups as follows: The first group was used as the control and the release behavior of DOX@MSNs was studied under pH 7.4. Fig. 3 shows that the cumulative release was 10.5% within 11 h at pH 7.4

without GSH. When the release time was extended, the cumulative release was up to 13.2%. At pH 7.4 in the presence of 10 mM GSH, the release behavior of DOX@MSNs changed significantly. The cumulative release continued to rise up to 41.2% within the first 46 h. These results showed that GSH was rapidly conjugated with DOX at this pH.

The second group was used to study the influence of pH on DOX@MSNs-Au drug release behavior. The drug release behavior was investigated at pH 7.4 (0.05 M PBS), pH 6.0 (0.05 M PBS) and pH 5.0 (0.05 M acetate buffer solution). At pH 7.4, the cumulative release was as low as 4.9% within 11 h. When the release time was extended to 51 h, the cumulative release was only 6.7%, which was only 50% of that released from DOX@MSNs and was mainly from the surface of MSNs (outside of the pores). Thus, DOX@MSNs-Au markedly reduced the premature release of DOX at normal physiological pH due to the presence of Au particles.

At pH 6.0, the cumulative release was 17.4% within 11 h. When the release time was extended, DOX release reached an equilibrium at 25.8% within 51 h. At pH 5.0, the release of DOX reached an equilibrium within 11 h and the highest cumulative release was 39.3%. These results indicated that the release of DOX from DOX@MSNs-Au was strongly dependent on the medium pH and showed an increased release ratio with increased acidity. The reason for this was that the association constant for the -SH groups and Au particles decreased with increased acidity. More Au particles left the gates of the pores at lower pH and more DOX was released from the pores. These results showed that the DOX@MSNs-Au were

pH-dependent. The release ratios were very similar with the results [23], in which polyelectrolyte as drug carrier was prepared to the control release of DOX. Compared with other drug carriers such as micelles based on poly(ethyleneglycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate-co-pyridyl disulfide carbonate) copolymers [24] and PEGylated poly(L-lysine) [25], pH had much more effect on control release of DOX in our work.

The combined effects of pH and 10 mM of GSH on DOX@MSNs-Au were studied in the third group. At pH 7.4 and in the presence of GSH 10 mM, the release ratio quickly increased to 30.1% within 11 h and to 39.2% within 46 h, which was similar to the data for the DOX@MSNs (41.2%). Compared with the second group without GSH (6.7%), it may be deduced that GSH played a greater role than pH.

At pH 6.0 plus GSH 10 mM, the release ratio was significantly faster, and reached 45.6% within 11 h and 56.2% within 51 h. At pH 5.0 plus GSH 10 mM, the release value was stable in the first 34 h with the highest cumulative release as high as 56.4%. These results indicated that under the dual conditions of pH and GSH, the release of DOX was less than that in the single conditions. The reason for this was that the acidic conditions were unfavorable for the conjugation of DOX and GSH [26], which hindered the release of DOX from MSNs. The release of DOX at lower pH and prevention of the conjugation between DOX and GSH was beneficial in the control and continued release of DOX. Besides the successful example of pH/redox effect on drug release in reference [24], another thiolatedcarboxymethyl chitosan nanoparticles [27] were used to control release the drug of methotrexate, in which GSH had a much

more influence on drug release.

(Fig. 3)

3.4. *In vitro* cytotoxicity

In vitro cytotoxicity of MSNs-Au, DOX@MSNs-Au and free DOX in Tca8113 cells was evaluated using the SRB assay. Fig. 4A shows that the MSNs-Au had low cytotoxicity to Tca8113 cells and are safe for future use in biomedical nanoplatforms. Fig. 4B shows that DOX@MSNs-Au and free DOX had strong cytotoxicity in Tca8113 cells. Cell viability decreased as the concentration of DOX@MSNs-Au increased. The half maximal inhibitory concentration (IC_{50}) value of DOX@MSNs-Au was 2.3 $\mu\text{g/mL}$ (represented as DOX content). When the concentration was increased to 5.0 $\mu\text{g/mL}$, cell viability decreased to 19.5% and remained at this level. Cell viability in the presence of free DOX decreased more quickly. The IC_{50} of free DOX was 0.3 $\mu\text{g/mL}$. When the concentration was increased to 1.25 $\mu\text{g/mL}$, cell viability was 9.9% and remained at this level. Fig. 4B indicates that the cytotoxicity of free DOX was higher than that of DOX@MSNs-Au. The reason for this may be that DOX uploaded in the DOX@MSNs-Au nanocarriers could not be released completely in a short time.

(Fig. 4)

3.5. Cellular uptake

Under the excitation wavelength of 488 nm, DOX@MSNs produced a red fluorescence; however, this fluorescence disappeared after the formation of DOX@MSNs-Au due to the NSET effect between Au NPs and fluorescent DOX.

When DOX@MSNs-Au were internalized by cancer cells, the connection between Au NPs and MSNs was broken and the fluorescence of DOX was restored. Due to this fluorescence quenching-restoring property, DOX@MSNs-Au was used for CLSM imaging.

CLSM images were divided into four groups. Fig. 5A1 and Fig. 5A2 are the images of free DOX incubated with cancer cells for 6 and 12 h, respectively. Fig. 5B1 and Fig. 5B2 are the images of DOX@MSNs-Au incubated with cancer cells for 6 and 12 h, respectively. For each panel, the images from (a) to (e) show a bright field image, the nucleus stained by DAPI (blue), DOX fluorescence in the cells (red), overlays of bright field, nucleus and DOX fluorescence, and overlays of nucleus and DOX fluorescence, respectively. Fig. 5A1 shows that the red fluorescence of DOX was weak and the impact of DOX on the overlaying images after incubating with free DOX for 6 h was barely seen. When the incubation time was extended to 12 h, the fluorescence of DOX increased significantly (Fig. 5A2). Strong red fluorescence was observed in the nucleus, and the nucleus turned to purple after overlapping with DAPI.

For DOX@MSNs-Au, some DOX was fixed in the pores of MSNs by Au NPs. Fig. 5 B1 shows that after incubation with DOX@MSNs-Au for 6 h, DOX appeared in both the cytoplasm and nucleus. The nucleus turned purple after overlapping and red color was seen around the nucleus. These results indicated that DOX was successfully transferred into the nucleus of cancer cells after release from DOX@MSNs-Au. When the incubation time was extended to 12 h, the red

fluorescence increased significantly and the color of the nucleus turned to pink, which indicated that the nanocarriers transferred more DOX into the nucleus of cancer cells than free DOX. These results indicated that DOX@MSNs-Au can be used as nanocarriers for intracellular controlled release of DOX.

(Fig. 5)

4. Conclusions

In order to reduce the premature release of DOX, a kind of pH/redox dual-sensitive nanocarriers was synthesized based on ultra-small Au NPs and MSNs. *In vitro* release experiments showed that these ultra-small Au NPs effectively reduced the premature release of DOX by more than 50% at pH 7.4 and the release was also lower than that at pH 5.0 plus 10 mM GSH. The cytotoxicity and imaging studies further indicated that DOX@MSNs-Au significantly inhibited the growth of Tca8113 cells. Based on these results, the MSNs-Au nanocarriers could be potentially applied to the biomedical devices for target-site controlled and stimulated release of small molecules.

Acknowledgements

The authors thank the financial support by the National Science Foundation (no. 21375052, 21575055).

Appendices

Fig A1 to Fig A3.

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Captions:

Scheme 1 Route for preparation of DOX@MSNs-Au

Fig.1 Fluorescence spectra of (a) MSNs-SH, (b) free DOX, (c) DOX-MSNs, and (d)

DOX@MSNs-Au

Fig.2 (A) Particle size and distribution of MSNs determined by TEM and DLS; (B) The image of

MSNs-SH determined by TEM; (C) The particle size and distribution of Au NPs

determined by HRTEM and DLS and (D) The TEM image of DOX@MSNs-Au

Fig.3 Release profiles of DOX from DOX@MSNs and DOX@MSNs-Au at different pH values

with or without 10 mM of GSH

Fig.4 Cell viabilities of Tca8113 cells incubated with different concentrations of (A) blank

MSNs-Au, (B) DOX@MSNs-Au and free DOX for 24 hrs, respectively

Fig.5 CLSM images of Tca8113 cells incubated with free DOX for 6 hrs (A1) and for 12 hrs (A2);

with DOX@MSNs-Au for 6 hrs (B1) and for 12 hrs (B2). For each panel, the images from

(a) to (e) showed bright field image, nucleus stained by DAPI (blue), DOX fluorescence in

cells (red), overlays of bright field, nucleus and DOX fluorescence, and overlays of nucleus

and DOX fluorescence

Figure1

Figure3

Figure4

Figure5

FigureA1

FigureA2

FigureA3

Figure2

Table 1. Elemental analysis of SNs, MSNs, MSNs- NH₂ and MSNs-SH

Sample	N	C	H	S
SNs	0.55	12.77	2.58	--
MSNs	0.34	8.05	1.74	--
MSNs-NH ₂	0.80	9.24	2.07	--
MSNs-SH	0.90	9.51	2.05	0.60