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PAPER

Light cross-linkable and pH de-cross-linkable drug nanocarriers for intracellular drug delivery

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Ideal drug delivery systems are required to be stable in circulation, while instable at targeted sites to induce a rapid drug release. To address this dilemma, novel light cross-linkable and pH de-cross-linkable micelles were developed as drug nanocarriers. Poly(ethylene glycol)-poly-(hydrazone umbelliferone)-aspartamide (mPEG-OMC) with light responsive coumarin moieties and pH responsive hydrazone groups in the side chains was synthesized. The polymers can self-assemble into micelles, which can be conveniently stabilized via UV-light induced cross-linking based on the dimerization of coumarin moieties. While under slight acidic conditions, the cross-linked micelles can also be de-cross-linked by the cleavage of hydrozone bonds in the cross-linking segments, accelerating the release of encapsulated drugs. Thus the benefits of pH and light stimuli-responsiveness can be combined and their disadvantages are avoided. The Doxorubincin-loaded micelles showed high mortality towards HepG2 cells. We demonstrated the UV light cross-linkable micelles with pH-triggered de-cross-linking property as a new approach of designing smart drug delivery systems.

Introduction

In the past decades, enormous efforts have been dedicated to developing micellar drug delivery system based on amphiphilic block polymers.[1-3] Because of their "stealthy" hydrophilic shells, amphiphilic polymeric micelles show effective resistance to both rapid renal clearance and nonspecific uptake by the reticuloendothelial system (RES).[4-8] Besides, passive targeting can be achieved through the enhanced permeability and retention (EPR) effects due to appropriate size of micelles. By integrated drugs inside hydrophobic cores, these micelles can tremendously reduce the side effect, enhance the aqueous solubility and improve the enriching efficiency of conventional anticancer drugs in tumor tissues.

Unfortunately, the lack of strong molecular interactions remains a great challenge for many polymer micelles. Upon intravenous administration, the body fluid may dilute the concentration of polymers to below critical micelle concentration, causing disintegration of polymer micelles or an adverse shift in the micelle-unimer equilibrium.[9-12] These effects will induce a premature release of loaded drugs, greatly increasing the amount of drug at undesired sites. So it is very important to obtain micelles with higher structural stability. Up to date, chemical cross-linking of micelles has proved a useful strategy to stabilize micelles, by providing integrated micellar structures.[13-15] However, within target cells, the cross-linked polymer chains may act as a diffusion barrier for the drugs, hindering a rapid on-site release. Therefore, a stimulus is still needed to render the cross-linked micelles de-cross-linkable.

Endogenous stimuli (pH, redox potential enzyme level) are especially advantageous in realizing the de-cross-linking of micelles in cells or in the body.[16-21] Changes of pH in the local microenvironment of cells make it ideal to achieve endogenous stimuli-triggered de-cross-linking of micelles. Krzysztof Matyjaszewski and co-workers succeeded in obtaining cross-linked micelles by introducing ketal containing (di(methacryloyloxy-1-ethoxy)isopropane into the main chain of the polymers via atom-transfer radical polymerization (ATRP).[22] Sang Cheon Lee and co-workers prepared stabilized micelles by adding ketal containing crosslinker 2,2'-(propane-2,2-diylbis(oxy))-diethanamine.[23] Under mild acidic condition, the ketal groups would be hydrolyzed, rendering the disintegration of micelles. However, pH sensitive cross-linking methods also have many drawbacks. The introduction of additional cross-linking agents may require further purification to eliminate the possibility of harmful residual chemical substances. And the lack of precise control of the cross-linking processes may make it undesirable in drug delivery applications.

On the contrary, light-cross-linking chemistry is very advantageous since no cross-linking agents are needed, and no byproducts are formed during the light-cross-linking procedure.[24] Light is an especially attractive stimulus because the wavelength, intensity, and irradiation time can be easily controlled.[25-27] Therefore, the cross-linking degree can be facilely adjusted. The most used reversible light-cross-linking is the photo-dimerization of coumarin groups under UV light irradiation. Zhao's group reported a series of reversible light-cross-linked micelles using coumarin chemistry.[28-30] In our previous study, we successfully fabricated core or shell reversibly light cross-linked micelles based on photo-dimerization of coumarin groups.[31] Under UV

light > 365 nm, two coumarin groups will dimerize, forming stable covalently cross-linked bonds. When exposed to UV light < 260 nm, the cross-linked bonds will be cleaved. However the short wavelength UV light cannot penetrate deeply into living body and is extremely harmful to healthy tissues.[32,33] What's more, the de-cross-linking of coumarin-based micelles is not complete under UV light < 260 nm[24,34], which may restrict the diffusion of payloads from the cross-linked micelles. These disadvantages, especially the former one, limit its further development in smart drug delivery system.

In order to reserve the advantages of light cross-linking and overcome the drawbacks of de-cross-linking of coumarin, endogenous pH-responsive de-cross-linking was introduced into the coumarin-based micelles in this research. By attaching 7-((4-oxopentyl)oxy)-4-methylcoumarin, a courmarin deritive, to the poly(ethylene-glycol)-poly(hydrazine)aspartamide block polymer via hydrazone bonds, we successfully synthesized a new responsive polymer with a novel cross-linker. It can be conveniently cross-linked by exposing to 365 nm UV-light and easily cleaved at pH 5.0. In this way, the use of detrimental 265 nm UV-light is avoided and new pH responsive property is integrated. As far as we know, this is the first time that a light cross-linkable and pH de-cross-linkable drug nanocarriers was reported.

Experimental Section

Materials

Hydrazine hydrate and trifluoroacetic acid (TFA) were purchased from Aladdin. L-Aspartic acid-4-benzyl ester and 4-methy-Umbelliferones were purchased from Shanghai Energy Chemical Co., Ltd. α-methoxy-ω-amino-poly(ethlene glycol) with a molecular weight of 5000 ($mPEG_{5000}$-NH_2) was purchased from Shanghai Xibao Biochemical Co., Ltd. Triphosgene and 5-chloropenan-2-one were purchased from Shanghai Darui Chemical Co., Ltd. Doxorubicin hydrochloride (DOX) was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd. Tetrahydrofuran (THF) from Sinopharm Chemical Reagent Co., Ltd was dried over sodium. BLA-NCA was synthesized according to published procedure32. Other reagents not listed here were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received.

Instruments and measurements

^{1}H NMR spectra were recorded on a Agilent DD2-600 instrument operating at 600 MHz. Number average molecular weights and molecular weights distributions of the block-polymers were measured with a gel permeation chromatography (GPC) setup comprising of PL gel 5 mm MIXED-C column and using a refractive index (RI) detector and a series of narrowly distributed polystyrene as standards for the calibration curve. The eluent was DMF with LiBr (0.05 mol L^{-1}), and the flow rate was 1.0 mL min^{-1} at 60 °C. The size and zeta-potential of the nanoparticles were measured using a Zetasizer Nano-ZS from Malvern Instruments equipped with a He-Ne laser with a wavelength of 633 nm at 25 °C using a detection angle of 173 °. UV-vis spectra were recorded with a UV-vis Shimadzu UV-2505 spectrometer. Transmission electron microscopy (TEM) measurements were carried out on a HT7700 instrument operating at 100 kV. The samples were prepared by drying a drop of dilute micellar solution onto a carbon-coated copper grid. Mass spectrum was performed on a Broker Esquire 3000^{plus} ion trap mass spectrometer (Bruker-Franzen Analytik GmbH, Germany) equipped with an ESI source. A low-intensity 8 W long wave UV light lamp (365 nm, Spectroline, Model NO. EN-180L/FE, USA) was used as UV light source. It offers a relatively low UV-A dose of 1600 μW cm^{-2}.

Synthesis of 7-((4-oxopentyl)oxy)-4-methylcoumarin (OMC)

7-(hydroxy)-4-methylcoumarin (5.28 g, 0.03 mol) and 5-chloropenan-2-one (6.07 g, 0.045 mol) were dissolved in 50 mL dry DMF. K_2CO_3(4.20 g, 0.03 mol) and a spoonful of KI were added sequentially. The solution was refluxed and stirred at 100 °C under nitrogen flow for 24 hours. 200 mL deionized water was then added to completely dissolve the salt and precipitate OMC. The solid was filtered and recrystallize. Yield 6.50 g (83%). The ^{1}H NMR, ^{13}C NMR, and MS results of OMC were presented in Supporting Information (Fig. S1, S2, and S3).

Synthesis of block-polymer mPEG-poly(OMC)aspartamide (mPEG-OMC)

Firstly, mPEG-poly(β-benzyl-L-aspartate) (mPEG-PBLA) was synthesized according to published procedure by ring-open polymerization of BLA-NCA using $mPEG_{5000}$-NH_2 as initiator.[35] Briefly, $mPEG_{5000}$-NH_2 (0.50 g, 0.1 mmol) and BLA-NCA (1.00 g, 3.3 mmol) was dissolved in 10 mL DMF. The solution was degassed and then stirred at 50 °C for 72 hours. mPEG-PBLA was precipitated in diethyl ether and dried under vacuum. Yield 0.73 g (80%).

Secondly, mPEG-PBLA (0.50 g, 0.055 mmol) was dissolved in 10 mL DMSO and hydrazine monohydrate (2 mL, 63 mmol) was then dropped. The solution was stirred at 45 °C for 24 h. mPEG-poly(hydrazine)aspartamide (mPEG-pAsp(DAZ)) was obtained after the solution was dialyzed against deionized water for 48 hours and freeze dried. Yield 0.37 g (88%).

Finally, mPEG-pAsp(DAZ) (0.5 g, 0.066 mmol) and OMC (0.5 g, 1.92 mmol) was dissolved in 10 mL DMSO. 15 μL TFA was added and the reaction was stirred at 50 °C, without light for 24 hours. The solution was dialyzed against deionized water for 48 hours and freeze dried. Yield 0.33 g (52%).

Preparation of blank and DOX-loaded micelles

mPEG-OMC (41.2 mg, 4.7 μmol) was dissolved in 4 mL DMSO and CH_3OH mixed solvent (volume ratio=1:1). Half of the solution was taken and dialyzed against deionized water for 48 hours. 6 mL solution of blank micelles was obtained. DOX (2.1 mg, 3.3 μmol) and TEA (500 μL, 0.28 mmol) were then dropped into the other half of solution. 2 mL deionized water was added as stirred vigorously. Then half of the drug loaded solution was exposed to 365 nm ultraviolet-light for 30 min to crosslink the micelles. Both cross-linked and uncross-linked micellar solution was dialyzed against deionized water for 48 hours. Each yielded 6.5 mL micelle solution. The calculated concentration of encapsulated DOX was 112.6 mg L^{-1}. And the loaded ratio of DOX in mPEG-OMC micelles is 7.0% (wt%) and the loading efficiency is 69.7%.

Characterization of cross-linking property of the micelles

5 mL blank micellar solution without cross-linking was placed under 365 nm UV-light. At different exposure time points, 500 μL of solution was taken and its absorbance at 320 nm was measured. The percentage of dimerization of OMC was calculated. Both cross-linked and uncross-linked micelles were then placed in water and water/DMSO (v:v=1:1) solutions, respectively. And the cross-linked micelles were also put into acidic environment at pH 5.0. The hydrodynamic diameters of the micelles under all conditions mentioned above were measured by DLS. The intensity-average hydrodynamic diameter (D_h) was adopted in this research.

In vitro drug release

The release of DOX from the cross-linked micelles was carried out in both neutral and acid conditions. Two dialysis bags (MWCO 3500) containing 2 mL DOX loaded micelle solution was sealed and immersed in 20 mL phosphate buffer solution (PBS) with pH of 7.4 and 5.0, respectively. Uncross-linked drug-loaded micelles solution with the same concentration of DOX under pH of 7.4 was used as control. The bags were shaken constantly (120 rpm) at 37 ºC. At different time point, 2 mL PBS was withdrawn for Fluorescence absorbance measurements. And 2 mL fresh corresponding PBS was added to replenish the solution.

Flow cytometry

HepG2 cells were cultured into 24 well plates (2×10^5 cells per well) in 1 mL DMEM medium each well for 24 h. Both cross-linded and uncross-linked DOX-loaded micelle solution with a concentration of 10 mg mL^{-1} was added, respectively. Then, the cells were incubated for 1, 3and 5 hours and washed three times with PBS. Cells were detached by 400 μL trypsin and centrifuged (1000 rpm, 5 min). Then the cells were dispersed in 2 mL PBS and analyzed by FACScan flow cytometer.

Cellular uptake

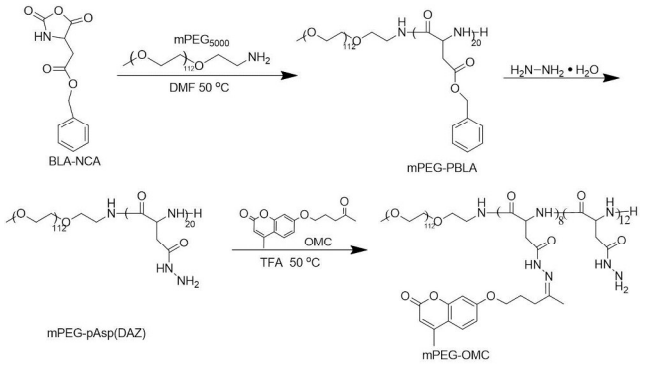
HepG2 cells were cultured in 24 well plates (5×10^4 cells per well) in 1 mL DMEM medium for 24 hours. 100 μL uncross-linked, cross-linked DOX-loaded micelles and free DOX solution with concentration of 10 mg mL^{-1} were added respectively. The cells were cultured for 1, 3 and 5 hours and washed three times with PBS. Then the cells were fixed with formaldehyde and stained with 400 μL 4-6-diamidino-2-phenylindole (DAPI) solution (0.1 μg mL^{-1}). Images were obtained using fluorescence microscopy.

Cell viability assays

The cell viability of DOX-loaded and blank micelles was evaluated by MTT assays. HepG2 cells were used as model cells and seeded into 96 well plates (6000 cells per well) with 180 μL DMEM culturing medium each well for 24 hours. 20 μL free DOX, uncross-linked and cross-linked DOX-loaded micelles solution with DOX concentrations from 1 to 100 μg ml^{-1} and blank micelle solution with mPEG-OMC concentration from 1 to 10 mg ml^{-1} were added to each well respectively. After incubated for 72 hours, 20 μL of MTT solution (0.1 mg mL^{-1}) was added and the cells were cultured for another 4 hours. Then the medium was removed and 150 μL DMSO was added to each well. The absorbance was recorded at 490 nm.

Results and Discussion

Synthesis of block copolymer mPEG-OMC



Scheme 1. Synthetic route of mPEG-OMC.

The synthetic route of mPEG-OMC was shown in Scheme 1. mPEG-PBLA, which acted as the backbone of the block-polymer, was successfully synthesized as proved by ^{1}H NMR measurement. The average degree of polymerization of BLA was calculated to be 20, based on signals at δ3.18 ppm belonged to the protons of methyl from α-methoxy-ω-amino- poly(ethlene glycol) and those at δ7.09 ppm and 7.14 ppm belonged to the protons of phenyl from BLA-NCA (Figure 1A). The number average molecular weight of mPEG-PBLA was determined by GPC as 12.5 k, with a PDI of 1.05 (ESI, Fig. S3). The narrow distribution was favourable for further synthesis of functional polymers with well-defined structures.

After mPEG-PBLA was treated with hydrazine monohydrate, the signals at δ 7.09 ppm and 7.14 ppm attributed to the protons of phenyl from BLA-NCA disappeared, indicating the complete removal of benzoyloxy groups from PBLA block (Figure 1B). OMC groups were then attached to the polymer chains via hydrazone bond. The average number of OMC units conjugated to each polymer chain was calculated to be 8, based on signals at δ3.18 ppm belonged to the protons of methyl from α-methoxy-ω-amino- poly(ethlene glycol) and those at δ2.24 ppm belonged to the protons of methyl from OMC units (Figure 1C).

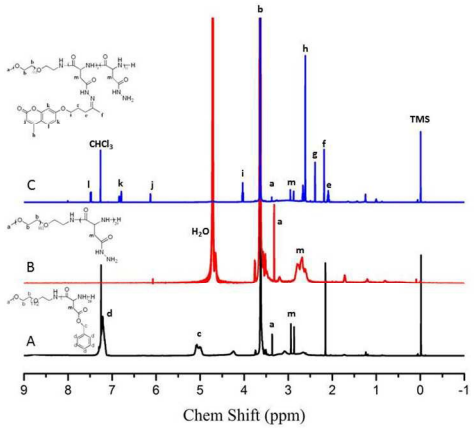


Figure 1. ^{1}H NMR results of A: mPEG-PBLA in $CDCl_3$; B: mPEG-pAsp(DAZ) in D_2O; C: mPEG-OMC in $CDCl_3$.

Self-assembly, cross-linking and pH-responsive de-cross-linking behaviours of blank micelles

The hydrodynamic diameter of the polymer micelles was measured by DLS. Results from DLS suggested the un-cross-linked micelles had a diameter of 126.8 nm with a PDI of 0.283. Spherical micelles with a diameter of 50 nm could be confirmed by TEM images, suggesting the self-assembling behaviour of mPEG-OMC polymer due to its amphiphilic properity (Figure 2A). The smaller size in TEM images could be attributed to the shrinkage of mPEG shell upon drying. After exposed to 365 nm UV-light for 30 minutes, the size of micelles decreased to 89.5 nm (PDI=0.221), indicating a light-induced cross-linking property. The decrease in size might be caused by a more compact structure of the micelles after cross-linking. In order to prove the cross-linking of micelles, DMSO, a good solvent of both mPEG block and OMC block, was added to the micelle solution after UV irradiation. Interestingly, the micelles didn't disassemble but swell to 231.1 nm (PDI=0.268), as shown in Figure S5. This suggested that the cross-linking method indeed improved the stability of the micelles even in extreme environment (below CMC or in good solvent). However, the size of the micelles increased to 500.6 nm with extremely high PDI (PDI=0.494) after the pH was adjusted to 5.0 (ESI, Fig. S5). The reason for this phenomenon might be the hydrolyzation of hydrazone bonds under acid condition, resulting in the disassembly of micelles, which was confirmed by TEM images (Figure 2C).

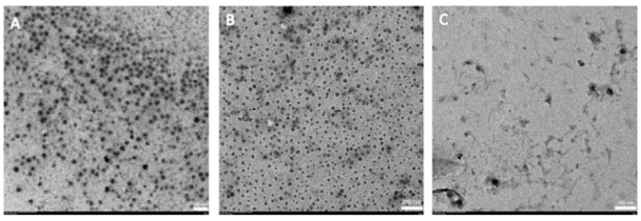


Figure 2. Representative TEM images of mPEG-OMC micelles under different conditions. (A) Uncross-linked micelles at pH 7.4; (B) Cross-linked micelles at pH 7.4; (C) De-cross-linked micelles at pH 5.0. (Scale bar: 200 nm)

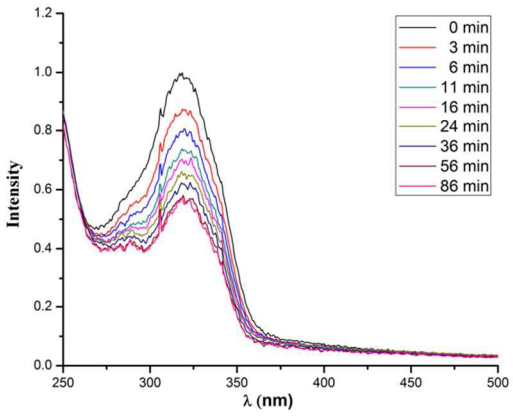


Figure 3. UV-Absorbance of micellar solution at different exposure time.

The cross-linking process of micelles was traced by measuring the UV-absorbance of micelle solution at different exposure time. As reaction time increased, the characteristic absorption of coumarin moieties at around 320 nm decreased, indicating the occurrence of dimerization and thus the cross-linking of the micelles (Figure 3). The dimerization degree of OMC was calculated based on the change of UV absorbance at 320 nm (ESI, Fig. S6). The dimerization degree increased with exposure time. After about 55 minutes, the cross-linking process reached equilibrium and the final dimerization degree was about 44%, lower than 100%. This was probably because the polymer chains inside micellar cores became more and more rigid with increasing cross-linking degree, which might limit further reactions between OMC groups.

***In vitro* pH-sensitive drug release**

The stimuli-responsive drug release property was investigated under different pH conditions, 7.4 and 5.0, respectively. As shown in Figure 4, less than 20% DOX was released after the cross-linked drug-loaded micelles were incubated at pH 7.4 for 72 hours, compared with a 30% release of un-cross-linked drug-loaded micelles. The 50% reduction of leaked drugs indicated the enhanced stability of cross-linked micelles. It is very important for drug nanocarriers because fewer drugs will be leaked during circulation and more drugs can be delivered to tumor site. However, more than 50% of DOX can be released within 72 h at pH 5.0. The cross-linking segment of the micelles contained pH-sensitive hydrazone bonds, which could be cleaved at pH 5.0 (endosomal pH) and accelerating the release of encapsulated drug. This result suggested that the cross-linked micelles could not only avoid the unfavourable premature release of loaded drugs during circulation, but also could be effectively de-cross-linked after internalized by cancer cells.

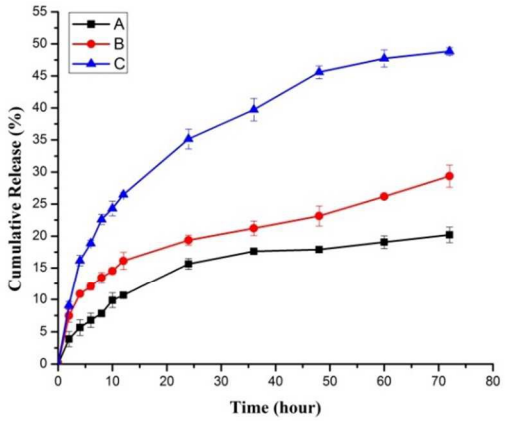


Figure 4. *In vitro* release of DOX from drug-loaded micelles in PBS under different pH conditions (A: cross-linked micelles in pH 7.4; B: uncross-linked micelles in pH 7.4; C: de-cross-linked micelles in pH 5.0)

Cellular uptake

Flow cytometry analysis was employed to investigate the cellular uptake efficiency of the drug-loaded micelles. The relative geometrical mean fluorescence intensities of cells cultured with cross-linked drug-loaded micelles increased with prolonged

incubation time from 30 minutes to 5 hours (Figure 5). The results indicated that the drug-loaded micelles could be conveniently internalized by HepG2 cells.

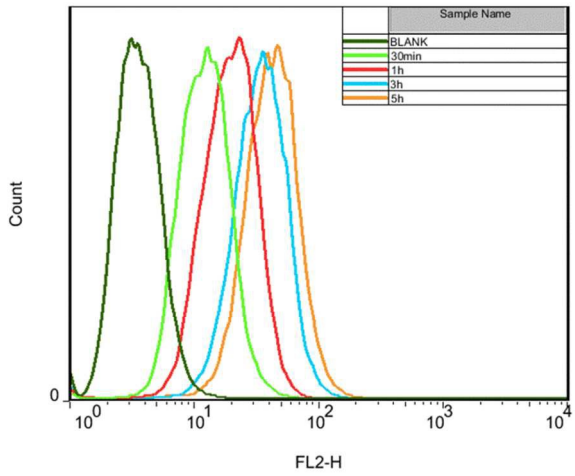


Figure 5. Flow cytometric profiles of HepG2 cells incubated with cross-linked drug-loaded micelles at different time intervals

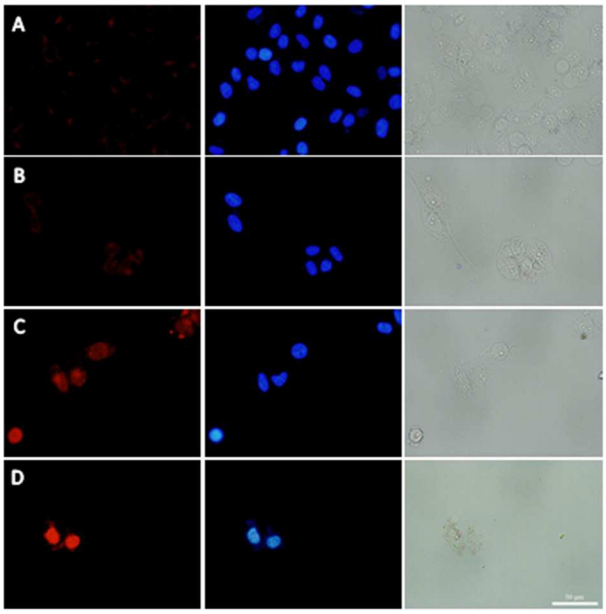


Figure 6. Fluorescence microscopy images of HepG2 incubated with the drug-loaded micelles and free DOX (10 μg mL^{-1}). (A: drug-loaded micelles for 1 hour; B: drug-loaded micelles for 3 hours; C: drug-loaded micelles for 5 hours; D: free DOX for 5 hours. Left: fluorescent images of DOX; Center: fluorescent images of DAPI; Right: bright field images of HepG2 cells. Scale bar: 50 μm)

Fluorescence microscopy was also applied to further study the internalization of the drug-loaded micelles. After being incubated with the same concentration of drug-loaded micelles for different time, HepG2 cells were stained and their fluorescent images were recorded, respectively (Figure 6). The red fluorescence of DOX could be observed in the cells after 1 hour of incubation, indicating the successful internalization of drug-loaded micelles. After being incubated for 5 h, stronger DOX fluorescence was observed in and around the nucleus. These phenomena suggested that the hydrazone bonds in the cross-linking segments of the micelles were cleaved as expected and the encapsulated DOX were released and escaped from the endosome. Since the rapid endosomal escape of DOX played a key role in inhibiting cancer cell proliferation, this pH responsive property of the cross-linking micelles would be important to improve the anti-cancer efficiency. Un-cross-linked micelles exhibited similar results with cross-linked micelles as indicated by Fig. S7 and S10. Images of free DOX were used as control (ESI, Fig. S9).

Cell viability assays

The zeta potential values of un-cross-linked micelles and cross-linked micelles were -9.24 mV and -7.97 mV, respectively. Both of the micelles showed negative zeta potential values, which is favorable as drug delivery systems. MTT assay was applied to evaluate the in vitro cytotoxicity of the blank polymer micelles and DOX-loaded micelles, using HepG2 cells as model. After incubated with the materials respectively for 48 hours, cells showed high cell viability (85% and above) in blank polymer micellar solutions at final concentrations up to 1000 μg L^{-1}, indicating the good biocompatibility and low toxicity of mPEG-OMC polymer micelles (Figure 7).

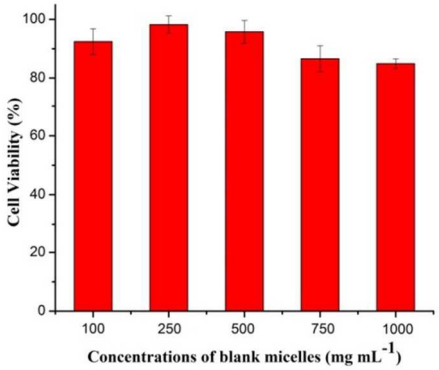


Figure 7. Cell viability of HepG2 cells incubated with various concentrations of mPEG-OMC blank micelles.

However, when cultured with DOX-loaded micelles, HepG2 cells showed high mortality. Cell viability decreased to 58% at a concentration of 1 mg L^{-1} of DOX for both kinds of micelles. As the concentration of DOX increased, the cell viability was further reduced. The DOX-loaded micelles dose required for 50% cellular growth inhibition (IC50) was 2.5 mg L^{-1} for cross-linked micelles and 3.0 mg L^{-1} for the uncross-linked micelles (the final DOX concentration). There was no significant difference in cell viability between the two kinds of micelles during the experiment (Figure 8). As shown in Figure 4, the release of DOX from un-cross-linked micelles at pH 7.4 was much faster than that of cross-linked micelles, which verified the improved stability of the cross-linked micelles. However, for the *in vitro* cellular experiments, the blood circulation procedure does not exist. Even if DOX was leaked from the micelles to cell culture medium, the free DOX in cell culture medium can be internalized by cells as well. That's why the in vitro intracellular results showed less difference between cross-linked micelles and uncross-linked micelles.

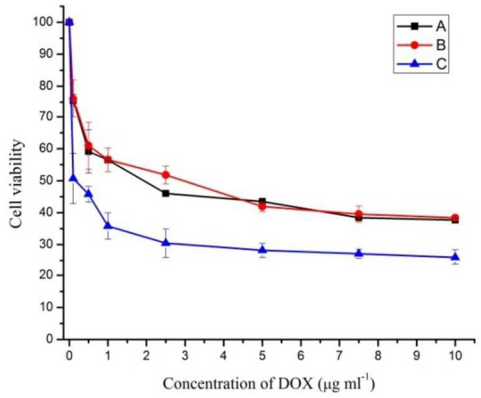


Figure 8. Cell viability of HepG2 cells incubated with various concentrations of the drug loaded micelles and free DOX for 72 hours. (A: uncross-linked drug-loaded micelles; B: cross-linked drug-loaded micelles; C: free DOX)

Conclusions

In summary, a novel coumarin-based pH-responsive polymer was successfully synthesized. Spherical micelles could be observed in TEM images. The dimerization process of courmarin moieties was monitored by ultraviolet-visible spectrophotometer. Results from DLS demonstrated the improved micellar stability after photo-induced cross-linking and the stimuli-resposiveness of the micelles under mild acidic condition. *In vitro* release of DOX indicated a much faster releasing rate at pH 5.0 than that under physiological conditions. The DOX-loaded micelles could be easily internalized by HepG2 cells through cell uptake and the encapsulated DOX could escape from endosome to the nucleus rapidly, as proved by flow cytometry and fluorescence microscopy results. The empty stabilized micelles showed good biocompatibility, while the drug loaded micelles demonstrated high toxicity to HepG2 cancer cells as indicated by MTT results. We are convinced that this new kind of UV light cross-linked micelles with pH-triggered de-cross-linking property would have a great potential in cancer therapy applications.

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Notes and references

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1. V. P. Torchilin. *J. Control. Release* 2001, **73**, 137-172.
2. K. Miyata, R. J. Christie and K. Kataoka, *React. Funct. Polym.* 2011, **71**, 227-234.
3. J. Liu, W. Huang, Y. Pang, X. Zhu, Y. Zhou and D. Yan, *Langmuir* 2010, **26**, 10585-10592.
4. J. Du, Y. Tang, A. L. Lewis and S. P. Armes, *J. Am. Chem. Soc.* 2005, 127, 17982-17983.
5. J. P. Salvage, S. F. Rose, G. J. Phillips, G. W. Hanlon, A. W. Lloyd, I. Y. Mab and S. P. Armes, *J. Control. Release* 2005, **104**, 259-270.
6. N. Nakabayashi and Y. Iwasaki, Bio-Med. *Mater. Eng.* 2004, **14**, 345-354.
7. H. Wang, F. Xu, D. Li, X. Liu, Q. Jin and J. Ji, *Polym. Chem*. 2013, **4**, 2004-2010.
8. H. Wang, F. Xu, Y. Wang, X. Liu, Q. Jin and J. Ji, *Polym. Chem*. 2013, **4**, 3012-3019.
9. C. Li and S. Wallace, *Adv. Drug Delivery Rev.* 2008, **60**, 886-898.
10. L. Zhou, R. Cheng, H. Tao, S. Ma, W. Guo, F. Meng, H. Liu, Z. Liu and Z. zhong, *Biomacromolecules* 2011, **12**, 1460-1467.
11. R. Duncan, *Nat. Rev. Cancer* 2006, **6**, 688-701.
12. X. Jiang, Z. Ge, J. Xu, H. Liu, and S. Liu, *Biomacromolecules* 2007, **8**, 3184-3192.
13. E. Read and S. P. Armes, *Chem Commun* 2007, **29**, 3021-3025.
14. Cornelus F. van Nostrum, *Soft Matter* 2011, **7**, 3246-3259.
15. Rachel K. O'Reilly, Craig J. Hawker and Karen L. Wooley, *Chem. Soc. Rev* 2006, **35**, 1068-1083
16. J. Du, X. Du, C. Mao and J. Wang, *J. Am. Chem. Soc.*, 2011, 133, 17560-17563.
17. S. Aryal, C. Hu and L. Zhang, *ACS Nano* 2010, **4**, 251-258.
18. X. Li, H. Wen, H. Dong, W. Xue, G. Pauletti, X. Cai, W. Xia, D. Shi and Y. Li, *Chem. Commun.* 2011, **47**, 8647-8649.
19. H. Xiao, L. Yan, Y. Zhang, R. Qi, W. Li, R. Wang, S. Liu, Y. Huang, Y. Li and X. Jing, *Chem. Commun.* 2012, **48**, 10730-10732.
20. Y. Wang, H. Wang, Y. Chen, X. Liu, Q. Jin and J. Ji, *Chem. Commun.* 2013, **49**, 7123-7125.
21. Y. Wang, H. Wang, Y. Chen, X. Liu, Q. Jin and J. Ji, *Colloids and Surfaces B: Biointerfaces*. 2014, **121**, 189-195.
22. W. Li, J. Yoon, and K. Matyjaszewski, *J. Am. Chem. Soc.* 2010, **132**, 7823-7825.
23. S. Lee, K. Min, H. Lee, A. Koo, H. Rim, B. Jeon, S. Jeong, J. Heo and S. Lee, *Biomacromolecules* 2011, **12**, 1224-1233.
24. J. Jiang, B. Qi, M. Lepage and Y. Zhao, *Macromolecules* 2007, **40**, 790-792.
25. C. J. Chen, G. Y. Liu, Y. T. Shi, C. S. Zhu, S. P. Pang, X. S. Liu, J. Ji, *Macromol. Rapid Commun.* 2011, **32**, 1077-1081.
26. Q. Jin, C. Luy, J. Ji and S. Agarwal, *J Polym Sci Part A: Polym Chem* 2012, **50**, 451-457.
27. H. Zhao, E. S. Sterner, E. B. Coughlin, and P. Theato, *Macromolecules* 2012, **45**, 1723-1736.
28. J. He, X. Tong and Y. Zhao, *Macromolecules* 2009, **42**, 4845-4852.
29. J. Babin, M. Lepage, and Y. Zhao, *Macromolecules* 2008, **41**, 1246-1253.
30. J. He, X. Tong, L. Tremblay and Y. Zhao, *Macromolecules* 2009, **42**, 7267-7270.
31. Q. Jin, X. Liu, G. Liu and J. Ji, *Polymer* 2010, **51**, 1311-1319.
32. S. Kumar, J. F. Allard, D. Morris, Y. L. Dory, M. Lepage and Y. Zhao, *J. Mater. Chem.* 2012, **15**, 7252-7257.
33. B. Yan, J. C. Boyer, D. Habault, N. R. Branda, and Y. Zhao, *J. Am. Chem. Soc.* 2012, **134**, 16558-16561.
34. Q. Jin, G. Liu, and J. Ji, *Euro. Polym. J.* 2010, **46**, 2120-2128.
35. S. Fukushima, K. Miyata, N. Nishiyama, N. Kanayama, Y. Yamasaki, and K. Kataoka, *J. Am. Chem. Soc.* 2005, **127**, 2810-2811.

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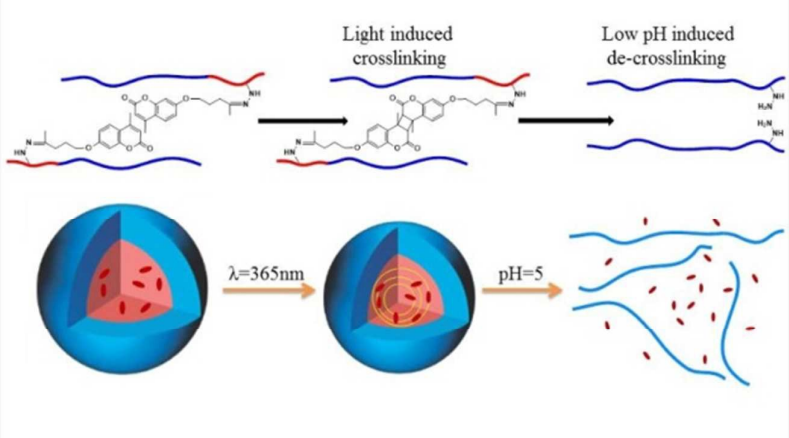

Novel light cross-linkable and pH de-cross-linkable micelles were developed as drug nanocarriers, based on the combination of light responsive coumarin moieties and pH responsive hydrazone groups in one cross-linking section.