Preparation and evaluation of reduction-responsive nano-micelles for miriplatin delivery

Ying Zhang¹, Dejian Hu², Shangcong Han¹, Guowen Yan¹, Chao Ma¹, Chen Wei¹, Miao Yu¹, Dongmei Li³ and Yong Sun¹

¹Department of pharmaceutics, School of Pharmacy, Qingdao University, Qingdao 266021, China; ²Weifang People's Hospital, Weifang 261041, China; ³Affiliated Hospital of Qingdao University, Qingdao 266071, China

The first two authors contributed equally to this work.

Co-corresponding authors: Dongmei Li. Email: ldmdrug@163.com; Yong Sun. Email: sunyongqdu@163.com

Abstract

A reduction-responsive amphiphilic core-shell micelle for miriplatin delivery was prepared and evaluated. A pyrene-terminated poly(2-(dimethylamino) ethyl acrylate) was synthesized through reversible addition-fragmentation chain transfer polymerization with 4-cyano-4-(ethylthiocarbonothioylthio) pentanoic acid as reversible addition-fragmentation chain transfer reagent and further modified by 2,2'-dithiodiethanol and 1-pyrenebutyric acid. Self-assembled blank micelles and drug-loaded micelles were obtained by dialysis method, and the particle size was proved to be about 40 nm with narrow dispersity by dynamic laser light scattering. Morphology results showed that blank micelles and drug-loaded micelles were spherical nanoparticles confirmed by transmission electron microscope, and the critical micelle concentration was as low as 6.09 $\mu\text{g/mL}$ via pyrene fluorescence probe method. The reductive sensitivity of disulfide bond in BMs was further verified by changes in particle size, pyrene fluorescence intensity ratio (I_{338}/I_{333}), and morphology after treatment by dithiothreitol. Moreover, drug release rate in vitro of drug-loaded micelles was evaluated and the results suggested that this amphiphilic pyrene-modified poly(2-(dimethylamino) ethyl acrylate) can be used as reduction-triggered controlled release drug delivery carrier for hydrophobic drug.

Keywords: Reduction-responsive, nano-micelle, drug delivery, miriplatin, passive targeting

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and chemotherapy is the major therapeutic tool for it.^{1,2} However, traditional chemical preparations show limited efficiency in hepatic tumor treatment, because these agents distribute evenly within human body through circulatory system. Consequently, the liver has a comparatively low drug concentration, leading to a low therapeutic effect, whereas other organs are likely to be impaired by the toxic effects of these anticancer drugs. Additionally, poor stability of some chemotherapy reagents may also reduce their anti-neoplastic effect. Therefore, a stable drug delivery system that can enhance the chemosensitivity of tumor cells but reduce the side effects to the normal cells or tissues is needed.

Miriplatin is a kind of the third generation of platinum-based anticancer drugs, with enhanced hydrophobicity and reduced adverse reaction compared to other platinum-based agents, and it has been applied in the transcatheter arterial chemoembolization (TACE) therapy of HCC in clinic.^{3–10} However, this administration route exists tremendous

defects, which limits its clinical application.¹¹ Consequently, targeting carriers of such agents have attracted increasing attention as efficient drug delivery systems, which could increase efficacy and decrease systemic side effects.

Nano-sized polymeric micelles are assemblies of synthetic polymers and have been actively studied as carriers of drugs and contrast agents since the 1980s.^{12–15} Polymeric micelles could provide many valuable characteristics such as biostability, biodegradability, biocompatibility, drug solubilization. Moreover, polymeric micelles with 10–100 nm could gather in the organization that has permeability of blood vessels, such as tumor, inflammation, or infarction areas, which was named enhanced permeability and retention effect (EPR). The so called EPR effect made polymer micelles to be nature passive targeting carrier.¹⁷ Therefore, polymeric micelles hold a great promising as novel anti-tumor drug carriers.

Radical addition-fragmentation chain transfer (RAFT) polymerization has been widely applied for defined polymer preparation.^{18,19} Compared with other controlled living radical polymerizations, RAFT-mediated polymerizations exhibit several advantages, including the tolerance to a variety of monomer structures and reaction conditions,

potential compatibility with aqueous solutions, and narrow molecular weight distribution of the desired polymeric products,^{20–22} particularly in biological applications as it can be performed at ambient temperature in the absence of metal catalysts.^{23,24} In this study, amphiphilic pyrene-modified poly(2-(dimethylamino) ethyl acrylate) (PDMAEA) was synthesized via RAFT polymerization and modified by pyrene with disulfide linkage via esterification, PDMAEA is a pH-sensitive biocompatible polymer ($pK_b = 6.5$)²⁵ and can self-catalyzed degrade to poly(acrylic acid), which is non-toxic.²⁶ Then, nano-sized blank micelles (BMs) and miriplatin-loaded micelles (DMs) were prepared by dialysis method. Characterizations of these micelles showed that BMs and DMs exhibited narrow dispersity around 40 nm and also reduction-sensitivity. Drug release properties of DMs were evaluated under simulated blood and intracellular environment *in vitro*, and results showed that DMs could maintain stability in blood environment but burst release drug in reduction environment. After 24 h, the accumulative rate could reach 80.18%.

Materials

Instruments

Electronic balance (BSI24S), Mettler Toledo; Magnetic stirring apparatus(RCT), IKA; Rotary evaporimeter(RE-2000A), Shanghai Yarong biochemical instrument factory; Vacuum drying oven, Shanghai Jinghong Experimental Equipment Co., Ltd; Nuclear Magnetic Resonance Spectrometer (JNM-ECP600), JEOL; Ultrasonic cell crusher (BILON92-2D), Shanghai Bilon Experiment Equipment Co., Ltd; Fluorescence Spectrophotometer (F-4500), HITACHI, Ltd; Transmission electronic microscope (JEM-1200EX), JEOL; Zetasizer Nano instrument (Zetasizer Nano-ZS90), Malvern Instruments Ltd; High Performance Liquid Chromatography(LC-2010C), Shimadzu; bag filter (MWCO = 1000 Da), Shanghai Bio Technology Co., Ltd.

Reagents

4-Cyano-4-(ethylthiocarbonothioylthio) pentanoic acid (CEPA), synthesized as reported²⁷; 2,2'-Dithiodiethanol (90%), Thermo Fisher Scientific Co., Ltd; 2-(Dimethylamino)

ethyl acrylate (DMAEA) (99%), 1-pyrenebutyric acid (99%), pyrene(98%), DL-dithiothreitol (DTT) (99%), Aladdin Reagent Co., Ltd; N,N'-dicyclohexyl carbodiimide (DCC) (99%), 4-dimethylamino pyridine (DMAP) (99%), Adamas Reagent Co., Ltd; tetrahydrofuran (THF) (AR), methyl cyanides(99.8%), tert-butyl alcohol (CP), methanol (GR), Chinese Medicine Group Chemical Reagents Co., Ltd; ethanol (GR), Tianjin Kermel Chemical Reagent Co., Ltd; n-hexane (AR), Tianjin Fuyu Fine Chemical Co.,Ltd; ethyl acetate(99.5%), Tianjin Yongda Chemical Reagent Co., Ltd; 2,2'-azobisisobutyronitrile (AIBN) (98%), 1,4-dioxane(AR), Tianjin Chemical Reagent Co., Ltd.

Experiments and discussions

Synthesis of amphiphilic PDMAEA

As shown in Figure 1.

Synthesis of hydroxy terminated RAFT agent (2)

2,2'-Dithiodiethanol (3.5450 g), DCC (1.6990 g), and DMAP (0.1003 g) were dissolved in THF (2 mL) in a round bottom flask, then CEPA (1.0880 g) in 5 mL THF was added dropwise. The mixture was stirred at room temperature for 48 h and filtered to remove the precipitate. Then, the filtrate was condensed by reduced pressure distillation. Final product was purified by silica gel chromatography using n-hexane/ethyl acetate (3:2) as the eluent and the ¹HNMR was shown in Figure 2. ¹HNMR (600 MHz, CDCl₃, δ): 1.36 (t, 3H, CH₂CH₃), 1.89 (s, 3H, CH₃C), 2.04 (s, 1H, OH), 2.30–2.60 (m, 2H, CH₂CO), 2.66 (t, 2H, CH₂CH₂CO), 2.89 (t, 2H, CH₂CH₂OH), 2.94 (t, 2H, CH₂CH₂O), 3.35 (q, 2H, CH₂CH₃), 3.89 (q, 2H, CH₂OH), 4.39 (t, 2H, CH₂O).

Synthesis of pyrene terminated RAFT agent (3)

RAFT agent (2) (0.4974 g), DCC (0.4992 g), and DMAP (0.0292 g) were dissolved with THF (4 mL) in a round bottom flask. 1-pyrenebutyric acid (0.4025 g) in 6 mL THF was added dropwise. Then, the mixture was stirred at room temperature for 58 h and filtered to remove the precipitate. The filtrate was condensed under reduced pressure and then subject to silica gel chromatography using n-hexane/ethyl acetate (5:2) as the eluent to obtain the expected

Figure 1 Synthesis route of amphiphilic PDMAEA

product. Chemical structure of RAFT agent (3) was confirmed by ^1H NMR (as shown in Figure 2). ^1H NMR (600 MHz, CDCl_3 , δ): 2.21 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.49 (t, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 3.40 (t, 2H, pyrene- CH_2), 7.86–8.31 (m, 9H, CH of pyrene).

Synthesis of pyrene-terminated PDMAEA

RAFT(3) (0.0925 g), DMAEA (2.0286 g), and AIBN (0.0080 g) in 1,4-dioxane (2.5 mL) were prepared followed by the deoxygenation with nitrogen for 30 min. Polymerization was carried out at 75°C for 15 h to afford PDMAEA, which was purified by repeated precipitation in n-hexane for three times and then dried under vacuum, as shown in Figure 2. ^1H NMR (600 MHz, CDCl_3 , δ): 3.70–4.15 (m, OCH_2CH_2 of DMAEA), 2.27–2.54 (m, OCH_2CH_2 of DMAEA), 1.50–1.92 (m, $\text{N}(\text{CH}_3)_2$), 7.80–8.40 (m, 9H, CH of pyrene). The molecular weight of pyrene-PDMAEA was calculated by ^1H NMR, $M_w = 14\text{ kDa}$ and the degree of polymerization of PDMAEA was about 92.

Preparation of BMs

A certain amount of PDMAEA (20 mg) was dispersed in 10 mL ultrapure water, then the solutions were treated with ultrasonic probe at 400 W for 30 min at 0°C (pulse on 2.0 s, pulse off 3.0 s) to obtain the BMs solutions (2 mg/mL), as shown in Figure 3. The micellar solution occurred "Tyndall effect".

Characterization of BMs

Critical micelle concentration of BMs. Pyrene fluorescence probe was the most common method to determine the critical micelle concentration (CMC).^{28–30} Briefly, 2 mL of pyrene solution ($2 \times 10^{-6}\text{ mol/L}$) with acetone as solvent was added into 12 test tubes, respectively. After the acetone was evaporated by water bath at 60°C , 10 mL of BMs solution at different concentrations (from $0.05\text{ }\mu\text{g/mL}$ to 3 mg/mL) was added into the test tubes orderly. The mixture was ultrasonicated for 30 min at room temperature. The fluorescence excitation spectrum of pyrene in the final solution was measured with fluorescence spectrophotometer after 24 h.

Figure 2 ^1H NMR spectrum of RAFT(2), RAFT(3) and pyrene-PDMAEA

Figure 3 Picture of BMs(a) and ultrapure water(b). (A color version of this figure is available in the online journal.)

The emission wavelength was set at 390 nm, and the slit of excitation and emission at 10 nm and 2.5 nm, respectively. Then, the intensity ratios of the excitation wavelength 338 nm and 333 nm in the pyrene emission spectra were calculated. The CMC was obtained from trend changing of the intensity ratios, as shown in Figure 4. The CMC of BMs was 6.09 $\mu\text{g/mL}$, which was obtained by tangent method.

The morphological observation of BMs. BMs solution (2 mg/mL) was dropped on a 300 mesh copper grid and kept for 3–5 min, the excess fluid was removed by absorbent paper. Negative staining was performed using 5% aqueous phosphotungstic acid for 30 min before observation with transmission electron microscope (TEM), as shown in Figure 5. The micelles were regular spherical particles, and their surfaces were smooth and not conglomerated.

Particle size and zeta potential determination of BMs. BMs solution (1 mg/mL) was prepared to measure the particle size and zeta potential by using Malvern Zetasizer Nano ZS90. The sample was measured thrice, as shown in Figure 6. The micelles have a unimodal size distribution and a small size (mean diameter was 39.68 nm) (a), as well as zeta potential (mean potential was 1.24 mV) (b).

The reductive sensitivity of the disulfide bond test. Preparation of BMs 20 mL (2 mg/mL) was divided

Figure 4 Fluorescence intensity ratio (I_{338}/I_{333}) against the concentration of BMs solution. (A color version of this figure is available in the online journal.)

into two groups evenly. The experimental group was treated with 10 mM DTT, and both the DTT treated group and untreated group were incubated at 25°C with slight vibration. Particle size and pyrene solubilizing were measured at given time point, and the morphology of the experimental group treated with DTT for 3 h was observed by TEM, as shown in Figure 7. As reported, DTT is a kind of reductant and 10 mM DTT was equivalent to the high reducing environment in the tumor cells.³¹ Results showed that after incubation with 10 mM DTT, fluorescence intensity of pyrene in BMs solution kept reducing, while the untreated group kept invariable, which indicated that BMs showed instability in reduction environment. This could owe to existence of disulfide bond between hydrophilic PDMAEA chains and hydrophobic pyrene core. When treated by 10 mM DTT, disulfide linkage broke and pyrene cores precipitated from aqueous solution, then the compatibilizing effect of pyrene probe disappeared. Morphology observation by TEM also proved that BMs lost their spherical shape and core-shell structure after treated by 10 mM DTT.

Preparation of drug-loaded micelles

Determination of encapsulation efficiency. We adopted the method of microporous membrane filter to determine the EE. DMs were broken by adding organic solvent (90% tert-butyl alcohol and 10% methyl cyanides) under ultrasonic.

$$\text{EE}(\%) = \frac{\text{Drug in micelles}}{\text{Drug in dose}} \times 100\%$$

After shifting by single factor experiment and orthogonal test by using EE as key index, the optimal prescription craft was obtained: 60 mg of pyrene-modified PDMAEA was dispersed in 15 mL ultrapure water by ultrasonication (400 W, pulse on 2 s, pulse off 3 s) for 30 min, to get the BMs solution (4 mg/mL); a certain amount of miriplatin (5.0 mg) was added to 1.875 mL tert-butyl alcohol: methyl cyanides (9:1) via ultrasonic dissolving, prepared the drug solution. Under the condition of electromagnetic stirring, drug solution was added into the BMs (v/v, 1:8) by drops with a certain speed (v, 1 d/2 s). The mixing solution was stirred for 10 min continuously. Then, it was placed in dialysis bag

Figure 5 Morphology and dispersity of BMs (2 mg/mL), observed by TEM. (A color version of this figure is available in the online journal.)

Figure 6 Particle size (a) and zeta potential (b) of BMs. (A color version of this figure is available in the online journal.)

(MWCO = 1000 Da) with 10 volumes of ultrapure water for dialysis medium and dialyzed 18 h under the condition of 37°C water bath. The water was refreshed at time intervals of 1 h. Eventually, DMs solution was obtained in the dialysis bags. As a result, the EE (%) of DMs solution was 25.28%.

The morphology observation of DMs. The method was same to BMs, as shown in Figure 8. The micelles were regular spherical particles, and their surfaces were smooth and not conglutinated.

Particle size and zeta potential determination of drug-loaded micelles. The method was same to BMs, as shown in Figure 9. The DMs have a unimodal size distribution and a small size (mean diameter was 51.5 nm) (a), as well as zeta potential (mean potential was 0.891 mV) (b).

Drug release characteristics of DMs in vitro

Dialysis was used to determine drug release in vitro.³² To simulate intracellular environment and blood environment, we have prepared 20 mL drug-loaded micellar solution and put it into two groups average. One group added into DTT

(a), made its final concentration 10 mM; the other group was untreated (b). Then, the mixture was placed in dialysis bag (MWCO=1000 Da) and the bag was put in 30 mL of ABS buffer (pH=5.0) and PBS buffer (pH=7.4) dialyzing after the seal, respectively. The Miriplatin contents in the dialysate at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0, and 24.0 h were determined by high performance liquid chromatography (HPLC), according to the established standard curve ($Y = 5833.1X - 1235.9$, $R^2 = 0.9999$), drawing the drug cumulative release amount of time curve, as shown in Figure 10. At 9 h, the drug accumulative rate reaches about 80.18%. In a sense, DMs have favorable drug-loading and reduction-responsive release property.

Conclusion

To achieve the systemic delivery of miriplatin, an amphiphilic pyrene-modified PDMAEA was synthesized with disulfide bond between hydrophilic PDMAEA moieties and hydrophobic pyrene. Then, nano-sized micelles, BMs, and drug-loaded micelles (DMs) were obtained by dialysis method. The mean diameter of BMs and DMs

Figure 7 Reducing test of disulfide bond (a) the change of particle size (b) the change of I_{338}/I_{333} (c) the picture of BMs with DTT 3 h. (A color version of this figure is available in the online journal.)

were around 40 nm, with a narrow size distribution. Such small size is helpful to avoid reticuloendothelial system (RES) capturing and to accumulate at tumor site via EPR effect. Due to the strong hydrophobicity of pyrene, BMs had a quite low CMC of 6.09 $\mu\text{g}/\text{mL}$ which indicated that BMs may have a stable structure in aqueous solution. However, owing to the existence of disulfide bond in BMs and DMs,

these micelles exhibited sensitivities to reducing environment. At last, drug release characteristics of DMs at simulative intracellular environment (10 mM DTT, pH = 5.0) were tested. It was remarkable that burst release of miriplatin was observed in the first 5 h and then it took about 9 h to reach the final release rate of miriplatin at 80.18%, comparing to 10.4% in PBS. In a sense, it indicated that DMs could

Figure 8 Picture of drug-loaded micelles. (A color version of this figure is available in the online journal.)

Figure 9 Particle size (a) and zeta potential (b) of DMs. (A color version of this figure is available in the online journal.)

Figure 10 In vitro release profiles of drug-loaded micelles in ABS (pH = 5.0) and PBS (pH = 7.4). (A color version of this figure is available in the online journal.)

exist stable in blood environment but rapid release miriplatin after endocytosis into tumor cells. From the above, we considered this reduction-responsive micelle formed by pyrene-PDMAEA as a potential drug carrier for sustained release of miriplatin.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; YZ conducted the experiments and wrote the manuscript, DH supplied critical reagents, SH, DL, YS wrote the manuscript, and GY, CM, CW and MY contributed review the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- Dufour J-F, Johnson P. Liver cancer: from molecular pathogenesis to new therapies: summary of the EASL single topic conference. *J Hepatol* 2010;**52**:296–304
- Ishikawa T, Abe S, Inoue R, Sugano T, Watanabe Y, Iwanaga A, Seki K, Honma T, Nemoto T, Takeda K, Yoshida T. Predictive factor of local recurrence after balloon-occluded TACE with miriplatin (MPT) in hepatocellular carcinoma. *PLoS One* 2014;**9**: e 103009
- Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y. Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. *Invest New Drugs* 2004;**22**:169–76
- Okusaka T, Kasugai H, Ishii H, Kudo M, Sata M, Tanaka K, Shioyama Y, Chayama K, Kumada H, Yoshikawa M. A randomized phase II trial of intra-arterial chemotherapy using SM-11355 (Miriaplatin) for hepatocellular carcinoma. *Invest New Drugs* 2012;**30**:2015–2025.
- Iwazawa J, Ohue S, Hashimoto N, Mitani T. Local tumor progression following lipiodol-based targeted chemoembolization of hepatocellular carcinoma: a retrospective comparison of miriplatin and epirubicin. *Cancer Manage Res* 2012;**4**:113–9
- Miyayama S, Yamashiro M, Shibata Y, Hashimoto M, Yoshida M, Tsuji K, Toshima F, Matsui O. Comparison of local control effects of superselective transcatheter arterial chemoembolization using epirubicin plus mitomycin C and miriplatin for hepatocellular carcinoma. *Jpn J Radiol* 2012;**30**:263–70
- Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T. Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. *Cancer Sci* 2009;**100**:189–94
- Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T, Chiba N, Nishikaku F. Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. *Cancer Chemother Pharmacol* 2009;**64**:473–83
- Oguro S, Hashimoto S, Tanaka T, Inoue M, Nakatsuka S, Kuribayashi S, Asakura K, Kawachi S, Tanabe M, Kitagawa Y. Short-term therapeutic effects of transcatheter arterial chemoembolization using miriplatin-lipiodol suspension for hepatocellular carcinoma. *Jpn J Radiol* 2012;**30**:735–42
- Okabe K, Beppu T, Haraoka K, Oh-Uchida Y, Yamamura S, Tomiyasu S, Yamanaka T, Sano O, Masuda T, Chikamoto A. Safety and short-term therapeutic effects of miriplatin-lipiodol suspension in transarterial chemoembolization (TACE) for hepatocellular carcinoma. *Anticancer Res* 2011;**31**:2983–8
- Facciorusso A, Licinio R, Muscatiello N, Alfredo DL, Barone M. Transarterial chemoembolization: evidence from the literature and applications in hepatocellular carcinoma patients. *World J Hepatol* 2015;**7**:2009–2019
- Kouichi S, Masayuki Y. Polymeric micelles possessing polyethyleneglycol as outer shell and their unique behaviors in accelerated blood clearance phenomenon. *Bio Pharm Bull* 2013;**36**:878–82
- Huang P, Wang W, Zhou J, Zhao F, Zhang Y, Liu J, Liu J, Dong A, Kong D, Zhang J. Amphiphilic polyelectrolyte/prodrug nanoparticles constructed by synergetic electrostatic and hydrophobic interactions with cooperate pH-sensitivity for controlled doxorubicin delivery. *ACS Appl Mater Interfaces* 2015;**7**:6340–50
- Ruiyuan L, Yan L, Zhenzhong Z, Xin Z. Drug carriers based on highly protein-resistant materials for prolonged in vivo circulation time. *Regen Biomater* 2015;**2**:125–33
- Botao S, Chengtie W, Jiang C. Ultrasound-triggered dual-drug release from poly(lactic-co-glycolic acid)/mesoporous silica nanoparticles electrospun composite fibers. *Regen Biomater* 2015. DOI: <http://dx.doi.org/10.1093/rb/rbv019>
- Liping A, Yapei W, Xiaomei L, Ning M, Haiying Du, Minghua J, Ying L, Long Z, Yanling X, Peili H, Zhiwei S. Block ionomer complex micelles based on the self-assembly of poly(ethylene glycol)-block-poly(acrylic acid) and CdCl₂ for anti-tumor drug delivery. *Chem Pharm Bull* 2011;**59**:559–63
- Maeda H, Greish K, Fang J. The EPR effect and polymeric drugs: a paradigm shift for cancer chemotherapy in the 21st century. *Adv Polym Sci* 2006;**193**:103–121
- Lian XM, Wu DX, Song XH, Zhao HY. Synthesis and self-assembly of amphiphilic asymmetric macromolecular brushes. *Macromolecules* 2010;**43**:7434–45
- Liu JX, Liu Z, Luo X, Zong XD, Liu JQ. RAFT controlled synthesis of biodegradable polymer brushes on graphene for DNA binding and release. *Macromolecular* 2013;**214**:2266–75
- Vazquez-Dorbatt V, Lee J, Lin EW, Maynard HD. Synthesis of glycopolymers by controlled radical polymerization techniques and their applications. *ChemBiochem* 2012;**13**:2478–87
- Wang W, Zhang J, Li C, Huang P, Gao S, Han S, Dong A, Kong D. Facile access to cyto-compatible multicompartments micelles with adjustable janus-cores from A-block-B-graft-C terpolymers prepared by combination of ROP and ATRP. *Colloids Surf B Biointerfaces* 2014;**115**:302–9
- Wang W, Li C, Zhang J, Dong A, Kong D. Tailor-made gemcitabine prodrug nanoparticles from well-defined drug-polymer amphiphiles prepared by controlled living radical polymerization for cancer chemotherapy. *J Mater Chem B* 2014;**3**:1891–1901
- Matyjaszewski K, Xia JH. Atom transfer radical polymerization. *Chem Rev* 2001;**101**:2921–90
- Tao L, Liu JQ, Tan BH, Davis TP. RAFT synthesis and DNA binding of biodegradable, hyperbranched poly(2-(dimethylamino)ethyl) methacrylate. *Macromolecules* 2009;**42**:4960–2
- Ye T, Aleksandra G, Wen Z, Thomas K, Malcolm X. Polymeric mesoporous silica nanoparticles as a pH-responsive switch to control doxorubicin intracellular delivery. *J Mater Chem B* 2013;**1**:5264–72
- Hartono SB, Phuoc NT, Yu MH, Jia ZF, Monteiro MJ, Qiao SH, Yu CZ. Functionalized large pore mesoporous silica nanoparticles for gene delivery featuring controlled release and co-delivery. *J Mater Chem B* 2014;**2**:718–26
- Tao L, Liu JQ, Davis TP. Branched polymer-protein conjugates made from mid-chain-functional p(HPMA). *Biomacromolecules* 2009;**10**:2847–51
- Hu FQ, Wu XL, Du YZ, Du YZ, You J, Yuan H. Cellular uptake and cytotoxicity of shell crosslinked stearic acid-grafted chitosan oligosaccharide micelles encapsulating doxorubicin. *Eur J Pharm Biopharm* 2008;**69**:117–25
- Qian C, Yong S, Jiangjun W, Guowen Y, Zhaoyuan C, Hongli Y, Haitian W. Preparation and characterization of glycyrrhetic acid-modified stearic acid-grafted chitosan micelles*. *Artif Cells Nanomed Biotechnol* 2013;**43**:217–23
- Lee KY, Kwon IC, Kim YH, Jo WH, Jeong SY. Preparation of chitosan self-aggregates as a gene delivery system. *J Control Release* 1998;**51**:213–20
- Pan YJ, Chen YY, Wang DR, Wei C, Guo J, Lu DR, Chu CC, Wang CC. Redox/pH dual stimuli-responsive biodegradable nanohydrogels with varying responses to dithiothreitol and glutathione for controlled drug release. *Biomaterials* 2012;**33**:6570–9
- Hostetter AA, Osborn MF, DeRose VJ. RNA-Pt adducts following cisplatin treatment of *saccharomyces cerevisiae*. *ACS Chem Biol* 2012;**7**:218–25

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