Block Ionomer Complex Micelles Based on the Self-Assembly of Poly(ethylene glycol)-block-poly(acrylic acid) and $CdCl_2$ for Anti-tumor Drug Delivery

Liping An,[a,d] Yapei Wang,[c] Xiaomei Liu,[a] Ning Ma,[c] Haiying Du,[a] Minghua Jin,[a] Ying Liu,[b] Long Zhang,[a] Yanling Xu,[a] Peili Huang,*[,b] and Zhiwei Sun*[,a,b]

[a] *School of Public Health, Jilin University; Changchun, Jilin 130021, P. R. China:* [b] *Department of Toxicology and Sanitary Chemistry, School of Public Health and Family Medicine, Capital Medical University; Beijing 100069, P. R.China:* [c] *Key Lab of Organic Optoelectronics and Molecular Engineering, Department of Chemistry, Tsinghua University; Beijing 100084, P. R. China: and* [d] *College of Pharmacy, Beihua University; Jilin, Jilin 132013, P. R. China.*

Received October 28, 2010; accepted February 4, 2011; published online February 15, 2011

A novel block ionomer complex micelles as drug carrier is developed utilizing self-assemble of poly(ethylene glycol)-block-poly(acrylic acid) (PEG-*b*-PAA) and cadmium chloride. This micelles are characterized to be have good bio-compatibility, hydrophilicity, passive targeting and sustained slow release property which shows great potential for liver cancer therapy. Block ionomer complex micelles based on PEG-*b*-PAA and cadmium chloride can self-assemble in distilled water, and Cd^{2+} agent is entrapped into the core stabilized by PEG shells. Results showed the block ionomer complex micelles to be spherically shaped. Cadmium was incorporated easily into the ionic core with remarkably high efficiency (34.25% weight (wt)/wt). The cadmium-loaded polymeric micelles exhibited sustained and slow release behavior of cadmium and a potent cytotoxicity against SMMC-7721 *in vitro*. This novel block ionomer complex micelles with cores of metal antitumor drug indicates to be potential carriers for effective drug delivery.

Key words poly(ethylene glycol)-block-poly(acrylic acid); cadmium chloride; drug delivery

Self-assembly of amphiphilic block copolymers induces polymeric micelles which have attracted significant attention as potential delivery vehicles for genes,[1,2] imaging agents,[3] and anticancer drugs.[4,5] Normally, these polymeric micelles are characterized by unique core-shell architectures which have nanoscale size (10 to 100 nm diameter) and a fairly narrow size distribution. These unique structures endow the formed assemblies with good loading capacity and releasing efficiency. However, the incorporation of metal ions is not successful by amphiphilic block copolymer aggregates, due to the poor compatibility between the hydrophobic cores and metal ions. Recently, double-hydrophilic block copolymers (DHBCs) were developed, which contain ionic and nonionic water-soluble segments (block ionomers), as novel functional materials for pharmaceutical applications.[6] Among the DHBCs, poly(ethylene glycol)-block-poly(acrylic acid), PEG-*b*-PAA, is one of the most popular polymers and has been employed to prepare the polymeric micelles by the electrostatic complexation with positive-charged functional species. The PAA core of the micelles is a loading space that accommodates various positive-charged therapeutic or diagnostic molecules. The hydrophilic PEG shell is a brush-like corona that stabilizes the nanostructure in aqueous dispersion. Meanwhile, PEG is biocompatible for these physicochemical properties such as hydrophilicity, anticoagulant, solubility, non-immunogenecity, and able to resist protein adsorption, thus, it has been approved by food and drug administration (FDA) for clinical using.[7] PEG shell on the surface of micelles increases the residence time of circulation in blood, preventing interactions with serum proteins and minimizes the detection by the immune system.[8] Therefore, it is frequently used as the hydrophilic block of amphiphilic block copolymers to create some polymersomes.

Chemotherapy has become an integral component of cancer treatment for most cancers. The metal anticancer drugs occupy the most promising space in the field of pharmaceutical chemotherapy. In 1991, Waalkes *et al.* found cadmium injection suppressed spontaneous liver tumor proliferation on the research for initiate or promote tumors on B6C3F1 mouse liver.[9] This result indicated cadmium might be used for tumor treatment. From then on, more and more attention has been paid about the effect of cadmium on antitumor. Our team has been some studies on effects of cadmium on human carcinoma cell. Likewise, our results showed that cadmium could significantly inhibit cellular proliferation, induce DNA damage, cause cell cycle arrest and apoptosis *in vitro*, and suppresse the growth of hepatocellular carcinoma cells xenografts *in vivo*.[10,11] Cadmium can induce metallothionein (MT), a non-specific protective protein which could combine with cadmium to reduce toxicity. Furthermore, due to the lower MT expression in hepatoma tissues, compared with normal liver tissue, free cadmium would preferably accumulate in hepatoma cells, which relatively raised its concentration on tumor location and efficiently killed tumor cells with minor toxic effect on normal liver cells.[12] Nevertheless, it is still unavoidable that high dose cadmium could cause acute and chronic defect for normal tissues and cells.[13,14] Hence, the free cadmium agent are restricted by dose-limiting toxicity and poor specificity in reaching tumor tissue. However, the limitation in conventional chemotherapy could be overcome by the drug delivery system.

The drug delivery systems may provide a more efficient and less harmful solution to achieving selective delivery of anti-cancer agents to cancer tissue at an effective concentration for the appropriate duration of time. In this regard, nanofabrication of polymeric micelles was significantly advanced by using DHBCs containing ionic and nonionic blocks. Furthermore, the polymeric micelles as drug delivery

* To whom correspondence should be addressed. e-mail: huangpl@ccmu.edu.cn; zwsun@ccmu.edu.cn

system have such excellent characteristics as structure stability, long circulation time, high drug loading capacity and safety.[15,16] Polymeric micelles are expected to increase the accumulation of drugs in tumor tissues utilizing "enhanced permeability and retention" (EPR) effect.[17] The EPR is a unique feature which allows drug delivery nanoparticles to extravasate through the leaky endothelial layer in tumor and subsequently remain there. Therefore, a reduced incidence of the adverse effects of the drugs may be occured.

In this study, we developed a novel type of block ionomer complex micelles encapsulated metal antitumor agents as drug delivery system for the first time. Self-assembly of PEG-*b*-PAA induces formation of block ionomer complex micelles through a combination of electrostatic interactions between Cd^{2+} and COO^- of PAA segment, encapsulating of Cd^{2+} agent into the core stabilized by PEG shells. One great advantage of this approach is that such assemblies are formed in water without introducing any organic solutions, which can eliminate the side-effect caused by residual solvents.[18] Resulting polymeric micelles were hydrophilic nanosphere, which combine two key structural features: hydrophilic PEG shells and nanoscaled size that make these systems very beneficial for effective drug delivery.

We attempt to examine the possibility of PEG-*b*-PAA-based block ionomer complex micelles used as potential carriers for cadmium agent. Towards this goal, the physicochemical properties of the micelles, the loading efficacy of cadmium in the micelle cores and the release rate were examined. Moreover, the cytotoxicity of the cadium-loaded micelles was also evaluated *in vitro*.

Experimental

Materials The block copolymer PEG43-PAA53 (M_w=5800) was synthesized according to the previous work.[19] The block lengths were 43 and 53 repeating units for PEG and PAA segments, respectively. The concentration of carboxylate groups in the copolymer samples was quantified by potentiometric titration. Cadmium chloride (analytical reagent grade, purity >99%) were purchased from Shanghai Tingxin Chemical Factory (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (Sigma, U.S.A.). All other chemicals were of analytical grade and used without any further purification.

Preparation of Cadmium-Loaded Micelles One milligram of polymer, PEG43-PAA53, was dissolved in 50 ml distilled water and simply mixed with 50 μl of 0.5 M cadmium chloride under stirring for 30 min. Then, the mixed solution was sonicated for 5 min. The obtained micellar solution was dialyzed against distilled water to remove the excess cadmium chloride and block copolymer by membrane with the molecular weight cutoff of 8000—14000 for 2 d (water changed twice every day). Finally, the PEG-PAA/Cd^{2+} complex micelles were obtained by lyophilization for further drug delivery experiments.

Characterization of Block Ionomer Complex Micelles by Dynamic Laser Scattering (DLS) and Transmission Electronic Microscopy (TEM) The average size and distribution of block ionomer complex micelles were evaluated by DLS at 25 °C, using a Malvern Zetasizer Nano-ZS90 apparatus. The measurements were carried out with a detection angle of 90°. Prior to measurements, the micelles solution (0.2 mg/ml) was passed through a 0.45 μm pore size filter.

A drop of freshly prepared micelles solution was placed on a carbon-coated copper grid and excess solution was blotted away after 2 min physical adsorption. The specimen on the copper grid was not stained. The morphology of PEG-PAA/Cd^{2+} micelles was observed by TEM (JEM 2010 electron microscope) under an accelerating voltage of 120 kV.

Determination of Drug Loading and Encapsulation Efficiency Accurately weighed lyophilized micelles were dissolved in 1 ml double distilled water. The amount of cadmium was measured using atom absorption spectrometry. The drug loading capacity=loaded cadmium mass/micelles mass×100%, encapsulation rate=loaded cadmium mass/input total cadmium chloride mass×100%.

***In Vitro* Drug Release** The release of cadmium from the block ionomer complex micelles was evaluated in cell culture medium-(Iscove's modified Dulbecco's media (IMDM), pH 7.2) by dialysis method using a dialysis tube with 8000—14000 Da cutoff. In the progress of dialysis, the drug-loaded micelle solutions in dialysis bags were directly immersed into 400 ml IMDM medium. Aliquots of 10.0 ml were withdrawn from the solution periodically and immediately replaced by an equal volume of corresponding solution after each sampling at every 2 h. The amount of cadmium release from micelles was analyzed by atom absorption spectrometry (AA240FS, Varian, U.S.A.).

Cell Culture The human hepatocellular carcinoma cell line, SMMC-7721, was purchased from Shanghai Cell Bank, Chinese Academy of Sciences. Cells were maintained in a humidified incubator with 5% CO_2 atmosphere at 37 °C, and cultured as monolayer in IMDM (Gibco, U.S.A.), supplemented with 10% fetal bovine serum, 31 U/ml penicillin G and 50 μg/ml streptomycin.

***In Vitro* Cytotoxicity Studies** Cytotoxicity of block ionomer complex micelles was assessed by MTT assay. Briefly, 96-well microtiter plates were seeded with 6000 cells/well and allowed to adhere for 12 h prior to the assay. Cells were exposed to various doses of cadmium chloride, PEG43-PAA53, block ionomer complex micelles for 48 h at 37 °C. The 20 μl of MTT indicator dye (5 mg/ml) was added to each well and the cells were incubated for 2 h in dark. Then, the supernatant was removed and 150 μl of dimethyl sulfoxide (DMSO) was added into each well and incubated for 10—15 min in order to dissolve formazan crystal. Absorption was measured at 490 nm using enzyme microplate reader (bio-rad, U.S.A.). The inhibitory rate (%)=(1−experimental A490/control A490)×100%. All experiments were repeated for eight times.

Statistical Analysis Values are expressed as mean±standard deviation (S.D.). All statistical analyses were performed using one way analysis of variance (ANOVA) with least significant difference (LSD) test by SPSS 14.0 software. $p<0.05$ was considered statistically significant.

Results and Discussion

Formation and Characteristic of Block Ionomer Complex Micelles The strategy employed to prepare block ionomer complex micelles is illustrated in Chart 1. PEG-PAA is hydrophilic and totally soluble, while the addition of positive cadmium ions can make the PAA segments to self-aggregate and form the micellar cores. Therefore, simply mixing PEG43-PAA53 with cadmium chloride in distilled water led to the spontaneous formation of block ionomer complex micelles. The micelles are assumed to have a spherical shape with core–shell architecture, in which the core of polyion complexes formed from negatively charged COO^- of PAA segments and positively charged cadmium is surrounded by the shell of nonionic and hydrophilic PEG segments, as shown in Chart 1.

Polymeric micelles were eventually obtained by lyophilization after the dialysis of the micelles solution against distilled water at room temperature. The block ionomer complex micelles were stabilized by an electrostatic interaction between

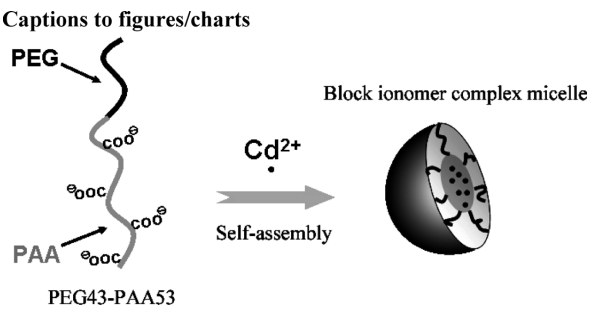

Chart 1. Schematic Illustration of the Self-Assembly of the Block Ionomer Complex Micelles

the carboxylic acid groups in the copolymers and divalent metal cations, Cd^{2+}. The re-dissolved micelles solution remained stable for long storage times and no precipitation was observed, suggesting the high stability of the block ionomer complex micelles. According the previous works, the electrostatic intereaction between divalent ions (Cd^{2+}) and COO^- has proven to be the major driving force for aggregation of the block copolymer, and result in the formation of core-shell micellar structure. The morphology of micelles was observed by TEM as shown in Fig. 1. The block ionomer complex micelles were well dispersed as individual nanoscale micelles with regularly spherical shape, with the size of around 50—70 nm. Figure 2 shows the size distributions of micelles determined by DLS. It was clear that the prepared block ionomer complex micelle had a mean diameter around 58 nm based on the intensity-averaged values, which agreed well with the TEM observations. It should be noted that the DLS experiments are performed in solution state while the TEM images are observed in a dry state, which may induce a slight, acceptable difference for the size of the block ionomer micelles.

Particle size and size distribution are key factors in the biodistribution of long-circulating nanoparticles and achieving therapeutic efficacy.[20] Numerous biological barriers exist to protect the human body from invasion by foreign particles include cellular and humoral arms of the immune system as well as mucosal barriers among others which must be overcome for polymeric micelles to reach their target. Particles might be cleared by phagocytic uptake as well as hepatic filtration in liver and spleen and excreted through kidneys. To improve circulation half-life, the particle in diameter should be below 100 nm to bypass filtration by interendothelial cell slits in the spleen and above 10 nm to avoid renal excretion.[21] Furthermore, this is especially true in the case of tumor tissue that smaller particle size increases accumulation and enhances diffusion through passive targeting.[22] It has been reported that the optimal nanoparticle size for tumor penetration should be less than 100 nm.[23]

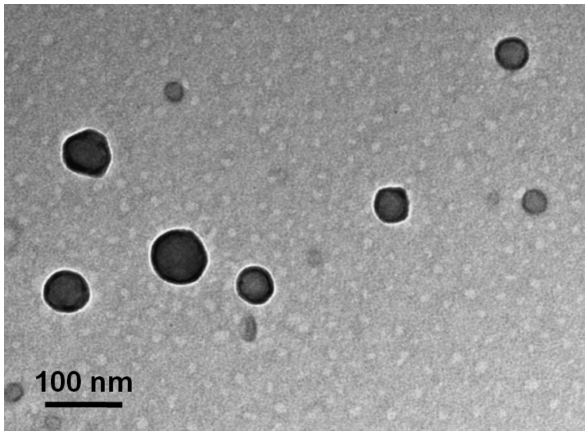

Fig. 1. TEM Images of Block Ionomer Complex Micelles

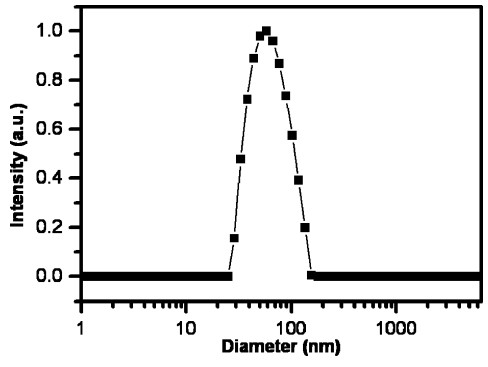

Fig. 2. The Distributions of Micelles as Determined by DLS

Additionally, small sized particles have relatively high cell uptake. Desai *et al.* reported that 100 nm nanoparticles had a 2.5-fold greater uptake rate than 1 μm microparticles, and a 6-fold greater uptake than 10 μm microparticles by Caco-2 cells.[24] Hence, nanosized polymeric micelles are available to powerful cellular and intracellular targets ability due to their small size and mobility.

In our work, the novel nanosized block ionomer complex micelles fulfill all the above mentioned requirement with these properties of hydrophilic surface, spherical shape and narrow size distribution with 100 nm or less in diameter which are able to reduce clearance by macrophages and repel pasma protein, and suitable for longer circulation times, greater ability to target to the tumor tissue.

***In Vitro* Release Experiments of Cadmium** The drug loading capacity still depends on the preparation method of polymeric micelles. The self-assembly of block copolymers was spontaneous and could be triggered in the presence of cadmium ion in water. Thus, precise concentrations of cadmium can be directly encapsulated within the supramolecular structure, offering a distinct advantage with respect to the loading of the drug. The cadmium-loading capacity and efficiency in the micelles were estimated to be 34.25% and 57.01%, respectively. Studies showed that the use of ionic interaction between drug and matrix material could be very effective in increasing drug-loading.[25] The high drug-loading capacity will reduce the quantity of matrix materials for administration. In addition, it is worth mentioning that the cell culture medium-(IMDM) was specifically used in the release test *in vitro* owing to its some of ingredients similar to body fluid. In this way, the cadmium-loaded micelles release *in vivo* could be simulated. The effect on the release behavior was clear. As shown in Fig. 3, it is remarkable that small burst was observed but slow release at initial 12 h. However, it takes about 24 h to reach the 78% release rate of cadmium, while the release rate of increase at 36 h was not significant.

The small burst effect and sustained release characteristic of the delivery system might be related to the drug loaded method. This drug loaded in the block ionomer complex micelles was relatively stable when compared with the conventional drug loaded methods.[26] The block ionomer complex

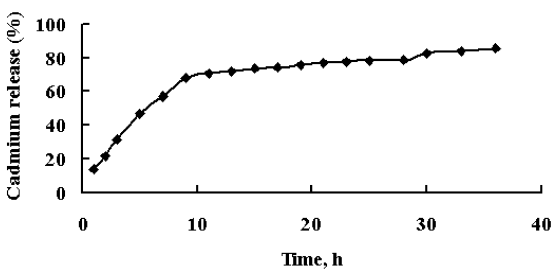

Fig. 3. Released Profile for Cadmium from Block Ionomer Complex Micelles

micelles were stabilized by an electrostatic interaction between the carboxylic acid groups in the copolymers and divalent metal cations (Cd^{2+}) to overcome the structural instability of the micelles in the blood stream.

In our study, no drug release occurred when the drug-loaded micelle was stored in water (data not shown). But in the cell culture medium, some of ingredients of the medium might penetrate the micelle and undergo a ligand exchange reaction with the carboxylates, thus regenerating cadmium and liberating it from the block ionomer complex micelles. Although the specific mechanism still needs further study, it implies that the cadmium-loaded polymer micelle would have an extended shelf life when stored in water, yet when administered and contacted with cellular component and the cell culture medium, become activated.

Greish *et al.* pointed out that the plasma concentration of the drug must remain sufficiently high for a prolonged period of time over 6 h, which could achieve EPR-mediated cancer targeting.[27] From our results of release experiments, the release rate was only 57% in the first 7 h. It can be assumed that the loaded polymeric micelles could have sufficient time to accumulate in the cancer target site before the cadmium is released *in vivo*. These data indicate that cadmium can be released from the polymeric micelles and the cadmium-loaded micelles might be useful as a slow release system for this anticancer drug.

The better drug release action might be acquired by the more thick shell of the polymeric micelles because the thick shell formed by long chain PEG acts as a cadmium release barrier. Various lengths of the PEG chains could be used in the copolymer which could regulate the sustained release stability in the future study. Additionally, high water solubilities are achieved for the surface of micelles which is an attempt to get a clearer picture of the protein repellence mechanisms.[28] The interactions between drug and protein molecules could be liable for rapid dissociation of the drug from the vector which results in the fate of the drug delivery system *in vivo*.[29]

***In Vitro* Cytotoxicity of Cadmium-Loaded Polymer Micelles** To examine whether cadmium incorporated in the block ionomer complex micelles can exhibit cytotoxic effect in cell culture by MTT assay, SMMC-7721 cells were exposed to PEG-*b*-PAA (0.125, 0.065 mg/ml), cadmium-loaded polymer micelles (10, 20 μM on cadmium basis), or cadmium chloride(10, 20 μM) for 48 h and blank (equal volume of cell culture medium) as control group. The results are presented in Fig. 4. The results showed that A490 value in the cadmium chloride and cadmium-loaded polymer micelles group had a significant reduction compared to that in control group ($p<0.01$) while there were no statistically significant difference between PEG-*b*-PAA and control group (Fig. 4). Cadmium incorporated in the micelles displayed no difference in cytotoxicity compared to free cadmium. As expected, both cadmium-loaded polymer micelles and cadmium chloride exhibited high cytotoxicity against SMMC-7721 cells with inhibitory concentration, 50% (IC_{50}) value of 14.99, 22.04 respectively. These data suggested that block copolymer alone were not toxic at concentrations used for the treatment by cadmium/polymeric micelle formulation. This indicates that cadmium released from block ionomer complex micelles play an essential role in the cytotoxic activity.

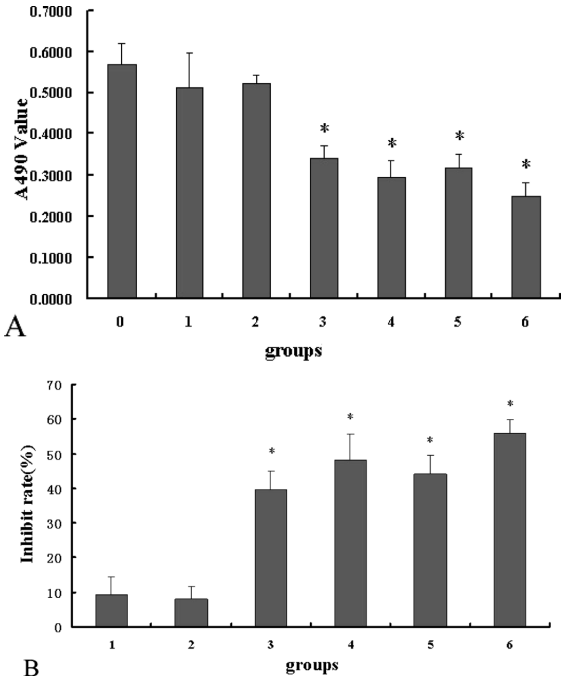

Fig. 4. Inhibitory Effect of $CdCl_2$ or Cadmium-Loaded Polymer Micelles in SMMC-7721 Cells (A: A490 Value; B: Inhibitory Rate (%) as Ordinate)

(0) Control; (1) 0.125 mg/ml of PEG-*b*-PAA; (2) 0.065 mg/ml of PEG-*b*-PAA; (3) 10 μM (on cadmium basis) cadmium-loaded polymer micelles; (4) 20 μM (on cadmium basis) cadmium-loaded polymer micelles; (5) 10 μM cadmium chloride; (6) 20 μM cadmium chloride; ±S.D., $n=6$, $*p<0.01$ *vs.* control.

As an effective anti-tumor agent, cadmium may induce cell death, at least in part, through apoptosis.[30,31] Induction of tumor cell apoptosis is a very important strategy for cancer chemotherapeutics.[32] Waalkes and Diwan proved that cadmiumn inhibited the formation of chemically induced by nitrosamine and spontaneously occurring tumors in liver and lung in mice, implying a unique sensitivity in certain tumor cells.[33] In our study, the SMMC-7721 as model cells was used to detect the anti-tumor property of the micelle-loaded Cd^{2+} ions, because liver is one of target organs of cadmium toxicity. In human and liver tumors there appears to be a down-regulation of MT expression, while surrounding normal tissue has much higher MT levels.[34] Furthermore, MT can protect normal liver cells from apoptosis induced by cadmium.[35] In this respect, cadmium as therapeutic agents can be used to selectively target liver tumor. In the present study, nanosized cadmium-loaded polymeric micelles can inhibit the growth of hepatocellular carcinoma cells *in vitro*. Therefore, the block ionomer complex micelles might be a passive targeting delivery system by virtue of their size and their surface properties for liver cancer treatment *in vivo*. In the near future active targeting delivery system for the polymeric micelles so called magic bullet could be developed by galactose-ASGPR recognition because of ASGPR overexpression on the liver surface, which is conjugated the galactose moiety from diamine-terminated PEG on the surface of nanosphere. The new polymeric micelles accompanying with this mechanism will be more selectively for obtaining higher drug concentration at the tumor site and causing less damage to normal tissues.

Conclusion

In summary, we demonstrate that block ionomer complex micelles can be obtained by electrostatic interaction between PEG–PAA and cadmium ion. The resulting micelles are characterized by unique core–shell architecture. It has an extended shelf life when stored in water. Slow release behavior is observed in sustained manner in the cell culture medium (IMDM). *In vitro* studies show that the cadmium-loaded micelles display potent cytotoxic activity to SMMC-7721 cells. The cadmium-loaded micelle might be a passive targeting delivery system by virtue of its nanosize and surface properties for liver cancer treatment. Further studies *in vivo* are needed to certify the possibility for the use of the block ionomer complex micelles as carriers for systemic delivery of cadmium ion in liver cancer.

Acknowledgements This work was financially supported by a grant from the Major State Basic Research Development Program of China (973 Program) (No. 2007CB808000).

We are grateful to Mr. Xi Zhang for his suggestion of this research and much appreciated diligent assistance in editing this manuscript.

References

1) Hubbell J. A., *Science*, **300**, 595—596 (2003).
2) Kakizawa Y., Kataoka K., *Adv. Drug Deliv. Rev.*, **54**, 203—222 (2002).
3) Torchilin V. P., *Adv. Drug Deliv. Rev.*, **54**, 235—252 (2002).
4) Kima J. O., Kabanova A. V., Bronicha T. K., *J. Controlled Release*, **138**, 197—204 (2009).
5) Bontha S., Kabanov A. V., Bronich T. K., *J. Controlled Release*, **114**, 163—174 (2006).
6) Sondjaja H. R., Hatton T. A., Tam K. C., *Langmuir*, **24**, 8501—8506 (2008).
7) Greenwald R. B., Choe Y. H., McGuire J., Conover C. D., *Adv. Drug Deliv. Rev.*, **55**, 217—250 (2003).
8) Branco M. C., Schneider J. P., *Acta Biomater.*, **5**, 817—883 (2009).
9) Waalkes M. P., Diwan B. A., Weghorst C. M., Bare R. M., Ward J. M., Rice J. M., *Appl. Pharmacol.*, **110**, 327—335 (1991).
10) Du H. Y., Liu X. M., Liu Y., Jin M. H., Huang W., Sun Z. W., *Chin. J. Public Health*, **22**, 194—195 (2006).
11) DuH Y., Jin M. H., Liu Y., Liu X. M., Wang W., Sun Z. W., *Modern Preventive Medicine*, **35**, 3763—3765 (2008).
12) Cherian M. G., Jayasurya A., Bay B. H., *Mutat. Res.*, **533**, 201—209 (2003).
13) Jin R., Chow V. T., Tan P. H., Dheen S. T., Duan W., Bay B. H., *Carcinogenesis*, **23**, 81—86 (2002).
14) Jayasurya A., Bay B. H., Yap W. M., Tan N. G., Tan B. K., *Carcinogenesis*, **21**, 1809—1812 (2000).
15) Kim J. E., Cha E. J., Ahn C. H., *Macromol. Chem. Phys.*, **211**, 956—961 (2010).
16) Luo Y. L., Wang A., Yuan J. F., Gao Q. Y., *Int. J. Pharm.*, **374**, 139—144 (2009).
17) Teicher B. A., *Drug Resist Update*, **3**, 67—73 (2000).
18) Wang Y. P., Han P., Xu H. P., Wang Z. Q., Zhang X., Kabanov A. V., *Langmuir*, **26**, 709—715 (2010).
19) Wang Y. P., Zhang M., Moers C., Chen S. L., Xu H. P., Wang Z. Q., Zhang X., Li Z. B., *Polymer*, **50**, 4821—4828 (2009).
20) Peppas L. B., Blanchette J. O., *Adv. Drug Deliv. Rev.*, **56**, 1649—1665 (2004).
21) Lamprecht A., "Nanotherapeutics: Drug Delivery Concepts in Nanoscience," Chap. 1, 1st ed., Pan Stanford Publishing, Singapore, 2009, p. 21.
22) Alexis F., Pridgen E., Molnar L. K., Farokhzad O. C., *Mol. Pharm.*, **5**, 505—551 (2008).
23) Xiao K., Luo J. T., Fowler W. L., Li Y. P., Lee J. S., Xing L., Cheng R. H., Wang L., Lam K. S., *Biomaterials*, **30**, 6006—6016 (2009).
24) Desai M. P., Labhasetwar V., Walter E., Levy R. J., Amidon G. L., *Pharm. Res.*, **14**, 1568—1573 (1997).
25) Singh R., Lillard J. W. Jr., *Exp. Mol. Pathol.*, **86**, 215—223 (2009).
26) Cha E. J., Kim J. E., Ahn C. H., *Eur. J. Pharm. Sci.*, **38**, 341—334 (2009).
27) Greish K., Fang J., Inutsuka T., Nagamitsu A., Maeda H., *Clin. Pharmacokinet.*, **42**, 1089—1105 (2003).
28) Wanga J. Z., del Rosarioa L. S., Demirdireka B., Baea A., Uhrich K. E., *Acta Biomater.*, **5**, 883—892 (2009).
29) Gaucher G., Dufresne M.-H., Sant V. P., Kang N., Maysinger D., Leroux J. C., *J. Controlled Release*, **109**, 169—118 (2005).
30) Ishido M., Homma-Takeda S., Tohyama C., Suzuki T. J., *Toxicol. Environ. Health*, **55**, 1—12 (1998).
31) Shimoda R., Nagamine T., Takagi H., Mori M., Waalkes M. P., *Toxicol. Sci.*, **64**, 208—215 (2001).
32) Martin S. J., Green D. R., *Curr. Opin. Oncol.*, **6**, 616—621 (1994).
33) Waalkes M. P., Diwan B. A., *Carcinogenesis*, **20**, 65—70 (1999).
34) Ghoshal K., Majumder S., Li Z., Dong X., Jacob S. T., *J. Biol. Chem.*, **275**, 539—547 (2000).
35) Shimoda R., Achanzar W. E., Qu W., Nagamine T., Takagi H., Mori M., Waalkes M. P., *Toxicol. Sci.*, **73**, 294—300 (2003).