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Degradable Thermoresponsive Core Cross-Linked Micelles: Fabrication, Surface Functionalization, and Biorecognition

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We report on the fabrication of core cross-linked (CCL) micelles possessing thermoresponsive and degradable cores and biocompatible coronas cofunctionalized with carbohydrate and biotin moieties. Well-defined poly(2-aminoethylmethacrylamide) (PAEMA) homopolymer was first synthesized in a controlled fashion via the reversible addition−fragmentation chain transfer (RAFT) process. CCL micelles comprising of well-solvated PAEMA coronas and thermoresponsive cores were then obtained in a one-pot manner via RAFT copolymerization of *N*-isopropylacrylamide (NIPAM) and bis(2-methacryloyloxyethyl) disulfide (DSDMA) difunctional monomers by employing PAEMA as the macro-RAFT agent. In the presence of dithiothreitol (DTT), the obtained CCL micelles can be disintegrated into unimers due to the cleavage of disulfide cross-linkers, whereas deswelling of micellar cores can be achieved via heating above the phase transition temperature of PNIPAM. Thus, the release profiles of this type of nanocarriers are expected to be triggered by temperature and thiols or a combination of both. Furthermore, primary amine residues located within coronas of CCL micelles have been further exploited for surface functionalization with biotin and carbohydrate moieties, rendering them biocompatible and bioactive. The availability of biotin within the coronas of CCL micelles was confirmed by HABA/avidin binding assay and Diffractive Optics Technology (DOT) biosensing instrument. After the micelles were immobilized on the surface of avidin-sensor chip, specific biorecognition of the available biotins and carbohydrate moieties on the CCL micelles was further confirmed. We expect that this novel type of bioactive and potentially biocompatible CCL micelles can be employed as smart nanocarriers for targeted drug delivery and controlled release.

Introduction

In the past decades, polymeric micelles comprising hydrophobic cores and hydrophilic shells have received increasing attention in terms of their biochemical and biomedical applications such as drug delivery and controlled release.[1−8] However, these self-assembled nanostructures tend to spontaneously dissociate under high dilution or high ionic strength,[9] which are typically encountered during intravenous administration.[10] Thus, the lack of sufficient stability results in decreased therapeutic efficiency and requires high drug loading for administration.

To solve this issue, a variety of strategies have been explored to enhance the structural integrity of polymeric micelles self-assembled from block copolymers.[11] Two representative approaches of covalent stabilization are core cross-linking[12−15] and shell cross-linking.[16−18] As regard to core cross-linked (CCL) micelles, the reported method usually includes three steps: the block copolymers containing polymerizable or cross-linkable moieties are first synthesized via living polymerization techniques, and then the micelles are formed by their self-assembly and the micellar structures are subsequently fixed using polymerization or other cross-linking methods by covalent stabilization of micellar cores. Although the uniform fixed micelles were obtained using this method, the procedure is time-consuming, and the CCL micelles are usually prepared at a very low concentration (0.1 wt %).

Recently, a one-pot synthetic approach was envisaged for the facile preparation process of CCL micelles, and two approaches have caught the attention of researchers. The first approach, reported by Pan and co-workers, relying directly on cross-linking one of the blocks during block copolymerization, was employed for the preparation of core-stabilized nanoparticles.[19,20] Another direct approach reported by Chen et al.[21] was used to prepare the

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(1) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200–1205.
(2) Zhang, L. F.; Eisenberg, A. *J. Am. Chem. Soc.* **1996**, *118*, 3168–3181.
(3) Brigger, I.; Dubernet, C.; Couvreur, P. *Adv. Drug Delivery Rev.* **2002**, *54*, 631–651.
(4) Rosler, A.; Vandermeulen, G. W. M.; Klok, H. A. *Adv. Drug Delivery Rev.* **2001**, *53*, 95–108.
(5) Gillies, E. R.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 14137–14146.
(6) Park, T. G.; Jeong, J. H.; Kim, S. W. *Adv. Drug Delivery Rev.* **2006**, *58*, 467–486.
(7) Schmaljohann, D. *Adv. Drug Delivery Rev.* **2006**, *58*, 1655–1670.
(8) Sokolsky-Papkov, M.; Agashi, K.; Olaye, A.; Shakesheff, K.; Domb, A. J. *Adv. Drug Delivery Rev.* **2007**, *59*, 187–206.
(9) Read, E. S.; Armes, S. P. *Chem. Commun.* **2007**, 3021–3035.
(10) Gaucher, G.; Dufresne, M.-H.; Sant, V. P.; Kang, N.; Maysinger, D.; Leroux, J.-C. *J. Controlled Release* **2005**, *109*, 169–188.
(11) Rodríguez-Hernández, J.; Chécot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691–724.
(12) Saito, R.; Ishizu, K.; Fukutomi, T. *Polymer* **1990**, *31*, 679–683.
(13) Stewart, S.; Liu, G. *Chem. Mater.* **1999**, *11*, 1048–1054.
(14) Loppinet, B.; Sigel, R.; Larsen, A.; Fytas, G.; Vlassopoulos, D.; Liu, G. *Langmuir* **2000**, *16*, 6480–6484.
(15) Menger, F. M.; Eliseev, A. V. *Langmuir* **1995**, *11*, 1855–1857.
(16) Henselwood, F.; Liu, G. J. *Macromolecules* **1997**, *30*, 488–493.
(17) Thurmond, K. B.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1996**, *118*, 7239–7240.
(18) Butun, V.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1998**, *120*, 12135–12136.
(19) Wan, W. M.; Pan, C. Y. *Macromolecules* **2007**, *40*, 8897–8905.
(20) Zheng, G. H.; Zheng, Q.; Pan, C. Y. *Macromol. Chem. Phys.* **2006**, *207*, 216–223.
(21) Chen, D. Y.; Peng, H. S.; Jiang, M. *Macromolecules* **2003**, *36*, 2576–2578.

CCL micelles by selectively cross-linking one of the blocks for block copolymer in a common organic solvent (DMF). Liu and co-workers[22,23] further extend this principle to fabricate the core-fixed micelles in aqueous solution by the direct addition of cross-linker into the polymer aqueous solution by avoiding the usage of organic solvent. The CCL micelles were fabricated via both approaches with high efficiency and high concentration (up to 10 wt %), which is suitable for large-scale production. However, the outer chains used are either poly(ethylene oxide) or poly-(4-vinylpyridine) chains, which simply increase the micellar water solubility with no possibility for further surface functionalization, and furthermore copper residues can potentially be present in the second approach. So an alternative approach for the synthesis of CCL micelles should be considered for their facile fabrication and for further surface functionalization.

In the previous reported systems, the CCL micelles were usually prepared from stimuli-responsive block copolymers aiming to eliminate the usage of organic solvents in the preparation process of CCL micelles,[22−25] and the stimuli-responsive properties of obtained CCL micelles also enhanced the smart release of the loaded drug at the specific position.[25] Recently, degradable structurally fixed micelles have received more attention because of their ability to degrade or dissociate under acidic[26] or reductive[27−32] conditions, especially for disulfide core cross-linked micelles. Kataoka et al.[29] were the first to report the fixation of polyion complex micelles prepared from the thiolated poly-(ethylene glycol)-*b*-poly(L-lysine) and oligonucleotide (ODN) through disulfide bonds. The fixed structures significantly increase the stability of ODN and can dissociate to release ODN in the presence of glutathione (GSH) at similar concentration as intracellular environment. Subsequently, the same group also reported a similar strategy for the enhanced siRNA delivery.[30] Li et al.[31,32] also reported the disulfide fixed micelles from poly-(ethylene oxide)-*b*-poly(*N*,*N*-dimethylacrylamide-*co*-*N*-acryloxysuccinimide)-*b*-poly(*N*-isopropylacrylamide), PEO-*b*-(DMA-*co*-NAS)-*b*-NIPAM, triblock copolymer. After the PNIPAM core micelles formed at elevated temperatures, the micellar structure could be easily fixed upon addition of a disulfide-containing difunctional primary amine, cysteamine. It is very interesting for the formation and cleavage of fixed micelles, although the system is shell cross-linked micelles and the rate of drug release can be easily controlled from these structurally fixed micelles. Stenzel's group[27] and Liu et al.[28] further reported this kind of fixed micelles by self-assembly of block copolymer and cross-linking to introduce the disulfide bond by the addition of cross-linker or polymerization of monomer bearing the disulfide bonds. With the stimuli-responsive and degradable disulfide cores, these micelles exhibit sharp changes in properties upon changes in environment and are promising for biomedical application.

Focused on functionalized micelles, the outer shell should also be considered, but the outer shells of CCL micelles are usually composed of water-soluble polymer chains to increase their water solubility. Few CCL micelles in the literatures deal with the surface functionalization, especially for the biorecognition. Li et al.[33] have reported the amino groups present on the surface of fixed micelles can further be functionalized with radiometal chelator diethyllentriamine pentaacetic acid (DTPA) and subsequently radio-labeled with indium-111 (^{111}ln) to study the biodistribution of core cross-linked polymer micelles (CCPMs). Wooley and co-workers have reported the preparation of fixed CCL micelles bearing azide or alkynyl groups on the surface of micelles to conjugate other fluorescence or functional groups onto the surface via click chemistry to extend their application.[34]

In light of the above discussion, three important issues should be addressed in order for an ideal CCL micelles system to have practical applications such as drug delivery and controlled release of loaded drug. First of all, the preparation of fixed micelles should be fairly simple, and organic solvents should be minimally used during the process; second, the prepared CCL micelles possessing the stimuli-responsive properties should be taken into consideration, which could trigger the smart release of loaded drug at specific location by small chemical or physical change of the environment; third, the CCL micelles should be easily functionalized to have bioactive molecules or targeting molecules on the surface, which could help to deliver drugs directly at diseased location with less potential toxicity to surrounding healthy cells or organism.

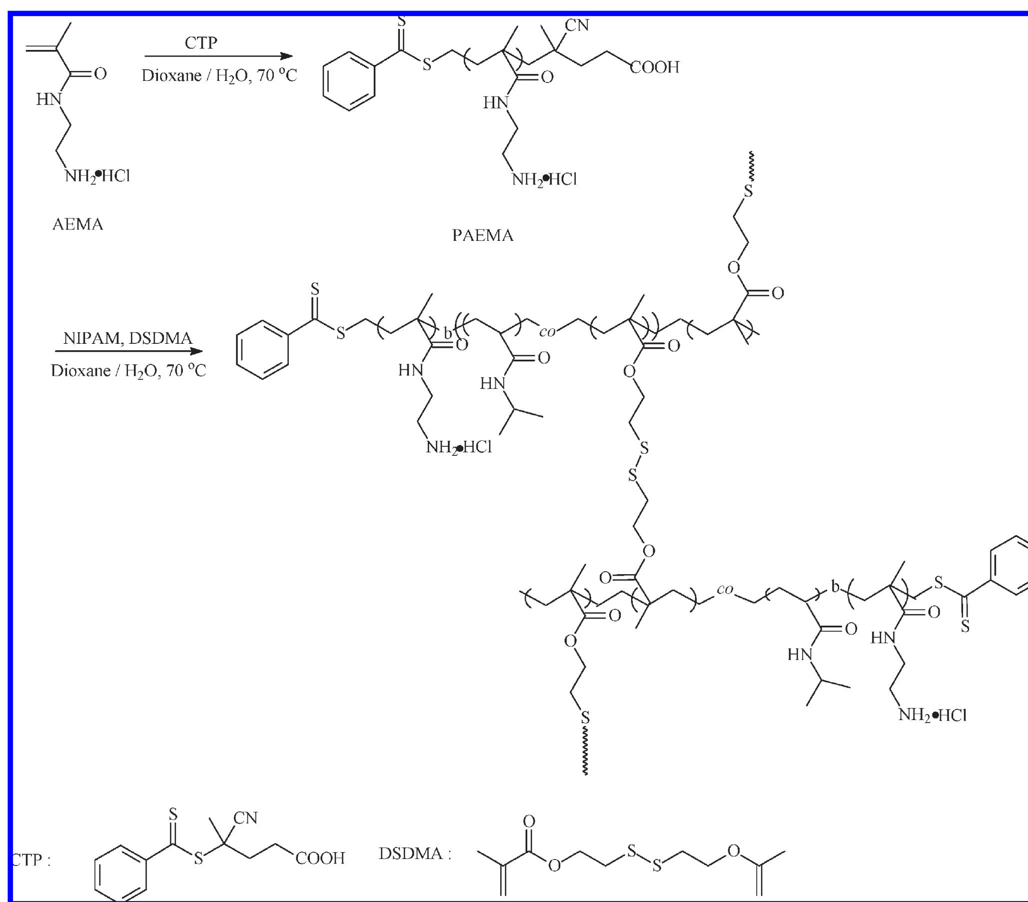
Herein, we report the synthesis of degradable and thermo-responsive CCL micelles via RAFT technique using a one-pot approach and PAEMA homopolymer as the macro-RAFT agent (shown in Schemes 1 and 2). These fixed micelles possessing stimuli-responsive properties and surface amino groups were further functionalized to conjugate with the biocompatible groups such as biotin and carbohydrate as the targeting moieties for the study of biomolecular recognition processes. This kind of nanoparticle possessing the biocompatible outer coronas (as the targeting molecules of drug-loaded carrier) and stimuli-responsive cores (as the drug-loaded carrier possessing the smart drug release properties) can potentially be employed as smart nanocarriers for targeted drug delivery and controlled release, and to the best of our knowledge, this kind of CCL micelle has not been reported yet.

Experimental Section

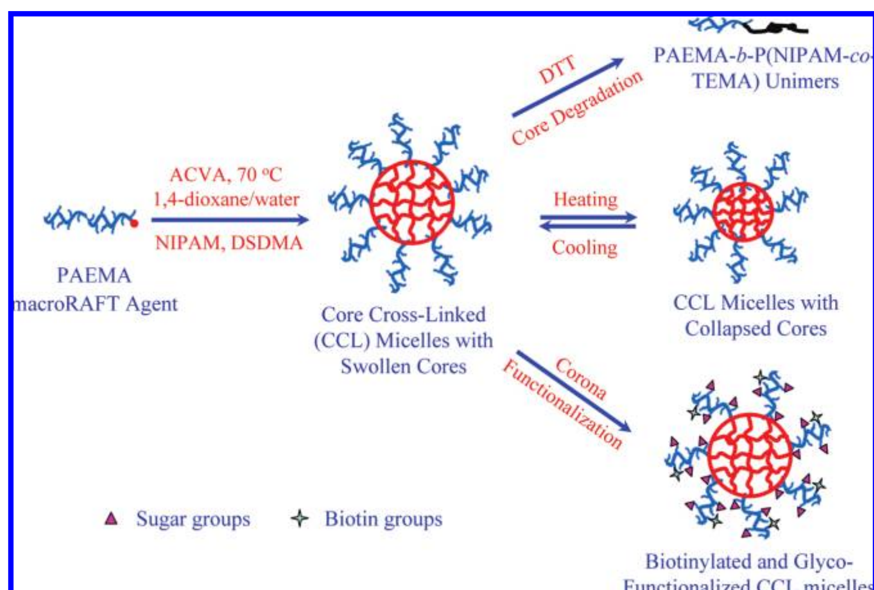
Materials. D-Biotin, lactobionic acid, ethylenediamine, biotinyl-*N*-hydroxylsuccinimide ester (Biotin-NHS), 1,3-dicyclohexylcarbodiimide (DCC), *N*-isopropylacrylamide (NIPAM), 2,2′-dithioldiethanol, 1,4-dithiothreitol (DTT), *Ricinus communis* agglutinin (RCA_{120}), and ethylenediamine dihydrochloride were purchased from Sigma- Aldrich and used as received except that the lactobionic acid was converted into the corresponding lactobionolactone. 2-Aminoethylmethacrylamide hydrochloride (AEMA),[35] bis(2-methacryloyloxyethyl) disulfide (DSDMA) cross-linker,[36] and 4-cyanopentanoic acid dithiobenzoate (CTP)[37] were synthesized as previously described. 4,4′-Azobis(4-cyanovaleric acid) (ACVA, 97%) was purchased from Acros Organics and

(22) Jiang, X. Z.; Zhang, J. Y.; Zhou, Y. M.; Xu, J.; Liu, S. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 860–871.
(23) Zhou, Y. M.; Jiang, K. Q.; Chen, Y. Q.; Liu, S. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6518–6531.
(24) Zhang, J. Y.; Zhou, Y. M.; Zhu, Z. Y.; Ge, Z. S.; Liu, S. L. *Macromolecules* **2008**, *41*, 1444–1454.
(25) Rijcken, C. J.; Snel, C. J.; Schiffelers, R. M.; Nostrum, C. F. V.; Hennink, W. E. *Biomaterials* **2007**, *28*, 5581–5593.
(26) Chan, Y.; Wong, T.; Byrne, F.; Kavallaris, M.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 1826–1836.
(27) Zhang, L.; Liu, W, G.; Chen, D. Y.; Stenzel, M. H. *Biomacromolecules* **2008**, *9*, 3321–3331.
(28) Zhang, J. Y.; Jiang, X. Z.; Zhang, Y. F.; Li, Y. T.; Liu, S. Y. *Macromolecules* **2007**, *40*, 9125–9132.
(29) Matsumoto, S.; Christie, R. J.; Nishiyama, N.; Miyata, K.; Ishii, A.; Oba, M.; Koyama, H.; Yamasaki, Y.; Kataoka, K. *Biomacromolecules* **2009**, *10*, 119–127.
(30) Kakizawa, Y.; Harada, A.; Kataoka, K. *Biomacromolecules* **2008**, *9*, 491–497.
(31) Li, Y. T.; Lokitz, B. S.; Armes, S. P.; McCormick, C. L. *Macromolecules* **2006**, *39*, 2726–2728.
(32) Li, Y. T.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81–89.
(33) Yang, Z.; Zheng, S. Y.; Harrison, W. J.; Harder, J.; Wen, X. X.; Gelovan, J. G.; Qiao, A.; Li, C. *Biomacromolecules* **2007**, *8*, 3422–3428.
(34) O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *New J. Chem.* **2007**, *31*, 718–724.
(35) Deng, Z. C.; Bouchékif, H.; Babooram, K.; Housni, A.; Choytun, N.; Narain, R. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4984–4996.
(36) Li, Y. T.; Armes, S. P. *Macromolecules* **2005**, *38*, 8155–8162.
(37) Benaglia, M.; Rizzardo, E.; Alberti, A.; Guerra, M. *Macromolecules* **2005**, *38*, 3129–3140.

Scheme 1. Synthetic Pathway of the Fabrication of the Core Cross-Linked (CCL) Micelles with Thermoresponsive and Degradable Cores



Scheme 2. Schematic Representation of the Fabrication and Surface Functionalization of the Core Cross-Linked (CCL) Micelles with Thermoresponsive and Degradable Cores



used as received. *N*,*N*′-Dimethylformamide (DMF), 1,4-dioxane, and other chemical solvents were purchased from Caledon Chemicals and used as received. Doubly distilled deionized water was used in all experiments.

Sample Preparation. *Preparation of Poly*(*2-aminoethylmethacrylamide Hydrochloride*) *PAEMA Homopolymer*. The RAFT polymerization of AEMA monomer was achieved at 70 °C, employing ACVA as the initiator and CTP as the chain transfer agent. A typical protocol is as follows. In a 10 mL flask, AEMA (1 g, 6.08 mmol) and CTP (21 mg, 0.074 mmol) were dissolved in distilled water (2 mL), and the dioxane solution (1.0 mL) of ACVA (4.3 mg, 0.015 mmol) was added. After degassing via three freeze−pump−thaw cycles, the flask was placed in an oil bath preheated at 70 °C to start the polymerization. After

6 h, the flask was put into liquid nitrogen to stop the polymerization. The mixture was diluted with water and precipitated into an excess of acetone. The red product was washed by methanol, and this purification cycle was repeated twice. The obtained red powder was dried in a vacuum oven overnight at room temperature. The yield was 78%.

Synthesis of Core Cross-Linked Micelles. The synthesis of CCL micelles was carried out at 70 °C employing ACVA as the initiator and $PAEMA_{61}$ homopolymer (M_n = 9800; M_w/M_n = 1.20) as macro-chain-transfer agent. A typical protocol is described as follows. In a 5 mL flask, NIPAM (0.565 g, 0.005 mol) and $PAEMA_{61}$ (0.494 g, 0.05 mmol) were dissolved in doubly distilled deionized water (3 mL); then ACVA (2.8 mg, 0.01 mmol) and DSDMA (58.0 mg, 0.2 mmol) were dissolved in 2 mL of dioxane and added into the above solution. After degassing via three freeze−pump−thaw cycles, the flask was immersed in an oil bath preheated at 70 °C for 18 h. The reaction was then quenched by cooling the reaction vessel in liquid nitrogen and by exposure to air. The product was purified by dialysis against distilled water for 2 days using dialysis membrane with a molecular weight cutoff of 12 000−14 000, and the colorless powder was obtained by lyophilization.

Cleavage of the Prepared Core Cross-Linked Micelles. The dried CCL micelles powder (0.1 g) was dissolved in 10 mL of water media in a 50 mL flask, and dithiothereitol (DTT, 0.1 g, 0.154 mmol) was added. After degassing via three freeze−pump−thaw cycles, the flask was immersed in an oil bath preheated at 40 °C, and the mixture was stirred overnight. The colloid solution became clear, which means that the cross-linked structures were degraded into diblock copolymer after the addition of DTT. After dialysis of the solution for 1 day using dialysis membrane with a molecular weight cutoff of 3500, the final degraded powder was obtained by lyophilization and characterized by the ^{1}H NMR spectrum.

Surface Functionalization of Core Cross-Linked Micelles. The pH of CCL micelles aqueous solution (10 mg/mL, 8.5 mL) was changed to pH 10 by the addition of concentrated NaOH solution; thus, the amino groups of the outer PAEMA block exist in the aqueous solution in the deprotonated form. Biotin-NHS (0.053 g, 0.155 mmol) (dissolved in 1 mL of DMF) and lactobionolactone (0.123 g, 0.362 mmol) (dissolved in 0.5 mL of H_2O) were added into above solution. The mixture was stirred at room temperature overnight. Finally, the mixture was purified by dialysis against distilled water for 2 days using dialysis membrane with a molecular weight cutoff of 12 000−14 000. The obtained biotinylated glyco-functionalized CCL micelles were freeze-dried under vacuum for 2 days.

Characterization. ^{1}H NMR spectra of the monomers and polymers were recorded on a Varian 200 MHz instrument using D_2O and DCl as the solvent. Aqueous gel permeation chromatography analysis was performed on a Viscotek Instrument using a 0.5 M sodium acetate/acetic acid buffer as eluent, two Waters WAT011545 columns, and a flow rate of 1.0 mL/min. Six near-monodisperse PEO standards (M_p = 1010−101 200 g mol^{-1}) were used for calibration.

Dynamic light scattering was performed using a Viscotek DLS instrument having He−Ne laser at a wavelength of 632 nm and Peltier temperature controller. The aqueous solutions of core cross-linked micelles were filtered through Millipore membranes (0.45 μm pore size). The data were recorded with OmniSize Software.

The HABA/avidin binding assay was performed as described by Green's group.[38,39] UV−vis absorption spectra were recorded on a Cary UV 100 spectrophotometer from the aqueous solution of biotinylated glyco-functionalized CCL micelles at room temperature. The absorbance values at 500 nm for the HABA/avidin reagent before and after addition of biotinylated glyco-functionalized CCL micelles were monitored. Before the samples were tested, the solutions were filtered through Millipore membranes (0.45 μm pore size) and then concentrated or diluted to appropriate concentration. The change of absorbance at 500 nm of HABA/avidin before and after the addition of biotinylated glyco-functionalized CCL micelles should be at 0.1−0.4.

DotLab data were measured on a Diffractive Optics Technology (DOT) system. A sensor was composed of polystyrene as the substrate and conjugated avidin groups on the surface.[40] The PBS buffer was used as the eluent. The sample containing biotin groups were loaded into the sensor according to the designed program, the binding events take place on the surface of the sensor by the interaction between the avidin and biotin, and the height of the diffractive pattern was increased. The increase in signals was detected synchronously using a laser-based optical system, and the real-time data generated are presented in dotLab Software.

Results and Discussion

Fabrication of Core Cross-Linked Micelles via RAFT Polymerization Using a One-Pot Method. Among the different core cross-linking strategies, the one-pot method reported by Pan et al.[20] is promising and may be used to prepare the structurally stable micelles on a large scale, which utilize the polymerization of monomer and cross-linker in selected solvent via the RAFT technique directly to get the CCL micelles. Therefore, our aim here is to synthesize fixed CCL micelles via a simple one-pot strategy with primary amino groups on the outer coronas for further surface functionalization.

The PAEMA homopolymer was first obtained via RAFT technique using CTP as RAFT agent and ACVA as the initiator. From the ^{1}H NMR spectrum of the sample withdrawn after 6 h, the conversion was calculated to be above 80%, and the GPC trace shows a monomodal with M_n = 9800 g/mol and M_w/M_n = 1.20. The obtained $PAEMA_{61}$ homopolymer was subsequently used to polymerize NIPAM and the cross-linker, DSDMA, in a mixture of dioxane and water media. In this RAFT process, the polymerization and cross-linking happened simultaneously; the PNIPAM and poly(bis(2-methacryloyloxyethyl) disulfide) (PDSDMA) block formed the cross-linked core and the PAEMA consisted of the solvated shell of micelles.

In order to prepare the uniform fixed micelles, it is important to add a suitable amount of cross-linker. If the amount of DSDMA is not enough, branched copolymers and/or the micelles with unstable structures were formed. If the excess amount of DSDMA is added, the macrogels would form as reported by Li et al.[36] To assess or confirm the successful cross-linking to obtain the stable structure of micelles, the added amounts of cross-linker were varied from 0.01, 0.02, 0.04, and 0.08 (molar ratio relative to the amount of NIPAM monomer) in the preliminary experiments. After polymerization, the macrogels were obtained for the high amount (0.08) cross-linker system. On the other hand, the other systems are the colloid solutions at room temperature; the dynamic laser light (DLS) instrument was used to measure these purified CCL micelle solutions to confirm their structural stability. The DLS results show that the CCL micelles have larger sizes after heating at high temperature (50 °C) compared with that at room temperature for low amounts (0.01 and 0.02) cross-linker system (data not shown), which indicated the structure was unsuccessfully locked and some PNIPAM chains aggregated at high temperature to form the nanoparticles with large sizes at the low amount cross-linker system. However, at the middle amount (0.04) of cross-linker system, the sizes of micelles decrease at high

(38) Green, N. M. *J. Biochem.* **1965**, *94*, 23c–24c.
(39) Green, N. M. *Methods Enzymol.* **1970**, *18*, 418–424.
(40) Borisenko, V.; Hu, W.; Tam, P.; Chen, I.; Houle, J.; Ausserer, W. *Clin. Chem.* **2006**, *52*, 2168–2170.

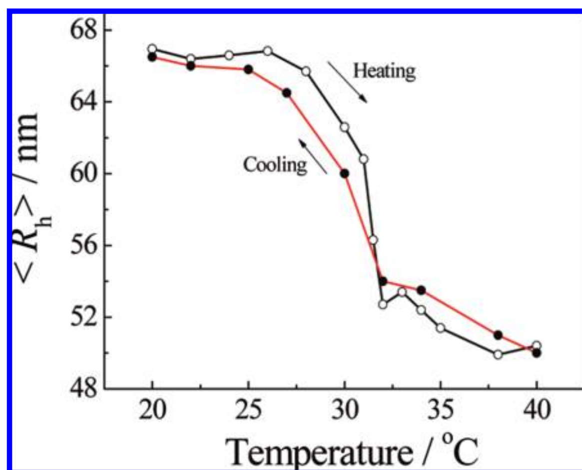


Figure 1. Variation of mass-average hydrodynamic radius, $\langle R_h \rangle$, with temperature obtained for 1.0 mg/mL aqueous original CCL solution at pH 4 during heating and cooling.

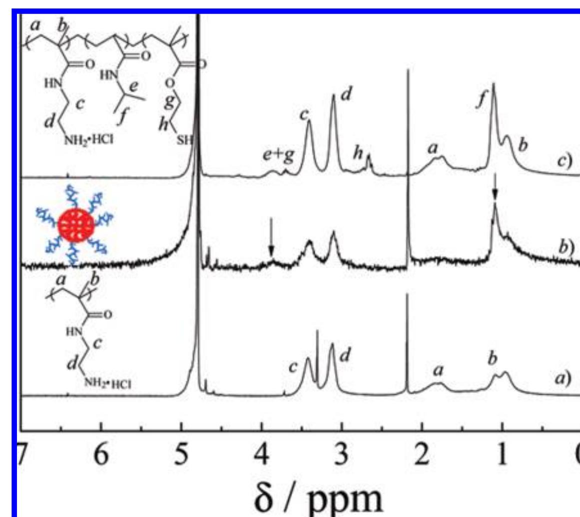


Figure 2. Assigned ^{1}H NMR spectra in D_2O solvent of (a) PAEMA homopolymer, (b) the synthesized core cross-linked (CCL) micelles with PNIPAM cores at 25 °C, and (c) purified degradable CCL micelles via dialysis against water after dissociation by the addition of DTT.

temperature (50 °C) and the polydispersity of micelles is very low (about 0.15), which means the cross-linking is successful. Therefore, the structurally stable micelles were synthesized via the RAFT technique using a one-pot approach by fixing the molar ratio of DSDMA and NIPAM at 4:100.

Core Cross-Linked Micelles with Degradable and Thermoresponsive Cores. After polymerization, the mixture is turbid, indicating the presence of colloidal particles at room temperature. On the basis of Pan's report,[20] the obtained fixed nanoparticles (micelles) should have the thermo- and redox-responsive NIPAM and DSDMA cores and PAEMA shells bearing primary amino groups. The micelles sizes could be tuned by changing the solution temperatures, the core can be degraded under reductive conditions, and the surface can be further functionalized.

To confirm the thermoresponsiveness of obtained CCL micelles, the size changes of obtained CCL micelles as a function of temperature were tested by DLS, and the temperature dependence of sizes for the obtained CCL micelles is illustrated in Figure 1. It can be seen that the most dramatic increases of micellar sizes from about 55 to 67 nm in radius occur within the temperature range of 28−32 °C, and the thermoresponsiveness behavior is reversible. The size increase of CCL micelles upon cooling is due to the solvation and swelling of PNIPAM cores, which is also confirmed by the peaks of PNIPAM from ^{1}H NMR spectra at room temperature and pH 4 shown in Figure 2b. The TEM image (shown in Figure 3) clearly shows the micelles have the fixed structures with sizes around 100−120 nm in diameter at room temperature and pH 4. Those results confirm that the core cross-linking is successful; otherwise, the CCL micelles would dissociate to become unimers with several nanometers for unsuccessful cross-linking, and the structure of micelles could not be observed via TEM measurement at room temperature. This type of CCL micelles can find potential applications as nanosized drug delivery vehicles because the changes in hydrophilicity of PNIPAM cores should also lead to the "triggered release" of encapsulated guest molecules.

After cross-linking, the micelles could be stored for up to two months at pH 4 and room temperature, which shows that the micelles is very stable at mild conditions. Recently, many types micelles bearing the disulfide bond were fabricated and studied for drug release at reductive conditions.[27,29,30] The disulfide bonds dissociate to thiol groups, thus leading to the disintegration of fixed nanoparticles under the presence of DTT. This disintegrated process is fast, and the yield is high.

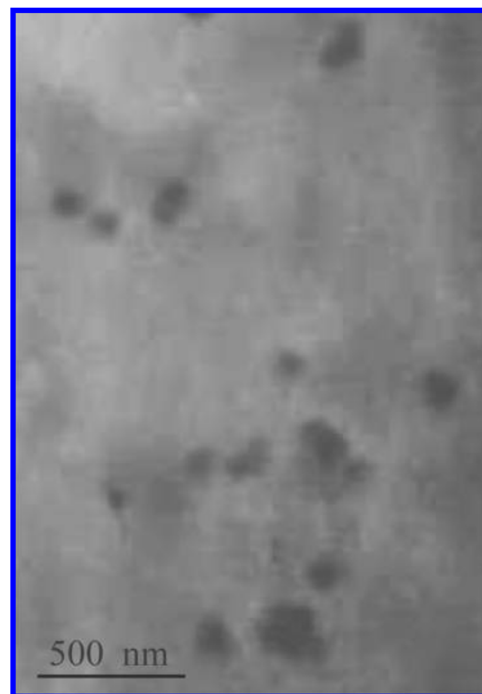


Figure 3. TEM image obtained for 10.0 g/L aqueous solutions of original CCL solution at pH 4 and room temperature.

In our case, the micelles undergo dissociation upon the addition of DTT. The fact that the colloid solution becomes clear at the reductive condition as shown in Figure 4 clearly shows the dissociation of CCL micelles. After dialysis of the resulting solution against water using dialysis membrane with a molecular weight cutoff of 3500 and freeze-dried, the obtained powder was further characterized by the ^{1}H NMR spectrum shown in Figure 2c. All of the assigned peaks of PNIPAM, poly(2-thioethyl methacrylate) (PTEMA) and PAEMA block could be observed, and the amounts of PNIPAM and PTEMA were calculated to be 25 and 2.5 from their characteristic signals at δ 1.0 ppm, δ 2.7 ppm, and 3.0 ppm for PNIPAM, PTEMA, and PAEMA, respectively.

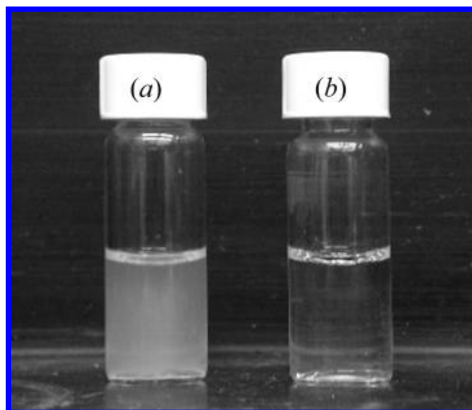


Figure 4. Digital photographs obtained for 10.0 g/L aqueous solutions of purified CCL micelles before (a) and after (b) dissociation by the addition of DTT at room temperature.

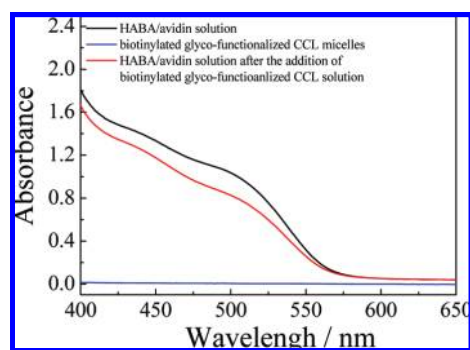


Figure 5. UV−vis spectra of biotinylated glyco-functionalized CCL micelles and HABA/avidin reagents before and after the addition of biotinylated glyco-functionalized CCL micelles, where the concentration of CCL micelles is at 0.005 mg/mL.

Surface Functionalization of Core Cross-Linked Micelles and Their Biorecognition. Although the literature contains several reports about micelles, only a few reports have focused on the surface functionalization of fixed nanoparticles.[33,34] The outer coronas are usually composed of water-soluble polymer chains (poly(ethylene oxide), PEO), which lacks the special functional groups to further conjugate with some biocompatible groups onto the surface of micelles. Recently, Wooley's groups[34,42] reported the surface clickable functionalized fixed micelles and biotin-decorated shell cross-linked nanoparticles. Li et al.[33] also reported the surface functionalization using the amino groups located the surface of fixed nanoparticles to synthesize dual nuclear/optical imaging.

For the surface functionalization, the PAEMA homopolymer bearing amino groups was chosen as the shell of micelles since the primary amino groups could be further modified with biocompatible groups such as biotin and carbohydrate moieties through the reactions with biotin-NHS and lactobionolactone to prepare the biotinylated glyco-functionalized CCL micelles. It should be noted that this kind of nanoparticle possesses the biocompatible outer coronas and stimuli-responsive cores, and to the best of our knowledge, this kind of fixed micelle is unprecedented.

In order to verify the availability of biotin groups on the surface, HABA/avidin binding assay was used to evaluate and quantify the amount of biotin groups. HABA is a dye that can bind to avidin, and when HABA complex with avidin in water, this aqueous solution has a maximum absorbance at 500 nm. Thus, biotin or biotinylated reagents are added to HABA/avidin solution; HABA is displaced quantitatively by available biotin as the avidin's affinity for biotin ($K_d = 10^{-15}$ M) is much stronger than that for HABA ($K_d = 10^{-6}$ M). This displacement can then be quantitatively monitored by the decrease in UV absorbance at 500 nm.

(41) Li, Y. T.; Lokitz, B. S.; McCormick, C. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 5792–5795.
(42) Qi, K.; Ma, Q.; Remsen, E. E., Jr.; C., G. C.; Wooley, K. L. *J. Am. Chem. Soc.* **2004**, *126*, 6599–6607.

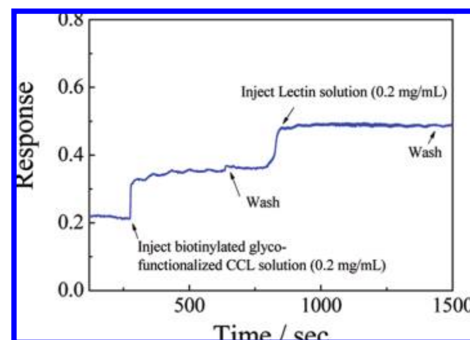


Figure 6. DotLab sensor response after the injection of biotinylated glyco- functionalized CCL micelles solutions and 0.2 mg/mL lectin (RCA_{120}) solution.

Upon addition of the functionalized CCL micelles, the absorbance value at 500 nm of HABA clearly decreases, suggesting the HABA/avidin complex is displaced by available biotin present on the CCL micelles surface. The results of these analyses are shown in the overlaid UV−vis spectra (Figure 5). The amount of biotin per mL of biotinylated glyconanoparticle solutions (1 mg/mL) was calculated to be 6.49 μmol according to the reported methods.[38]

Diffractive Optics Technology (DOT) instrument, named the dotLab biosensor, brings together two technologies: grating-based light diffraction and immobilized capture surfaces. The avidin-specific capture molecules are immobilized on the dotLab sensor surface in assay spots. The capture molecules (avidin) within each spot are not randomly distributed but are immobilized in a series of parallel lines that produces a specific diffraction pattern when illuminated with a laser. The sensor surface forms the base of a low-volume flow cell. When a flowing stream of sample bearing biotin moieties is introduced into the flow cell, target biotin molecules bind to the assay spots, resulting in an increased diffraction signal. The system uses the intensity of the diffraction signal to generate real-time binding curves. The illumination and detection beams never pass through the sample, which makes dotLab biosensor ideal for the detection of complex biological samples containing biotin molecules.

The dotLab biosensor is a cost-effective platform that has the format flexibility to provide a quick and simple solution to both assay development and routine analysis. It has been proved to be a sensitive and a very simple technique for the detection of molecular binding events without the use of fluorescent labels. So the bioavailability of biotin present on the surface of the biotinylated glyco-functionalized CCL micelles was confirmed using dotLab biosensor, and the result is shown in Figure 6. After loading the biotinylated CCL micelles, the response increases to reach a new step which does not decrease even after washing, which clearly shows the biotin groups were bound on the surface of sensor by the specific interactions of biotin and avidin, and the biotinylated CCL micelles were fixed on the surface of the sensor chip. This also shows the availability of the biotin groups located on the surface of fixed micelles. It should be noted that carbohy-

drate groups are now present on the surface of the sensor chip, and therefore their interactions with protein can also be assessed using dotLab biosensor.[43]

Carbohydrate–protein interactions play a key role in the cellular recognition process including cell growth regulation, differentiation, adhesion, cancer cell metastasis, cellular trafficking, inflammation by bacteria and viruses, and immune response.[44,45] However, individual carbohydrate–protein interactions are generally weak, and multivalent forms of carbohydrate ligands, such as glycopolymers[46,47] or glyconanoparticles,[48,49] have been used to demonstrate that inhibitory potencies of carbohydrates are enhanced through cooperative multiple interactions. Therefore, the functionalization of CCL micelles with carbohydrates is of interest for the study of carbohydrate–protein interactions. The incorporation of carbohydrates on the surface of the CCL micelles is also expected to improve the solubility and biocompatibility of CCL micelles.

Since the biotinylated glyco-functionalized CCL micelles are immobilized on the surface of sensor chip via biotin–avidin interactions, the dotLab biosensor could also be used to study the interactions of the carbohydrates on the surface of the CCL micelles with lectin. In order to confirm this, the Lectin, *Ricinus communis* agglutinin I (RCA_{120}), which has been usually used as a versatile tool to detect the galactose-base residues,[50] was injected, and a signal was recorded as shown in Figure 6. The change in response clearly indicates that the injected protein interacts with the carbohydrate moieties of the CCL micelles immobilized on the sensor chip. This result confirms the specific interactions of the carbohydrate groups immobilized on the surface of the nanoparticles with the protein and also shows the specific biorecognition between the carbohydrate groups and protein.

From the above results, the structurally fixed micelles were successfully conjugated with the biocompatible biotin and carbohydrate groups onto the surface of micelles, which obviously can increase the cell compatibility of synthesized micelles. This kind of CCL micelle could be used as a universal model to study the carbohydrate–protein interaction using dotLab biosensor and may realize the special application in biomedical applications.

Conclusion

We have successfully synthesized structurally stable micelles possessing thermoresponsive and degradable cores via the RAFT technique using PAEMA homopolymer as the macro-RAFT agent to polymerize the NIPAM and DSDMA monomers. The sizes of fixed micelles can be adjusted by the addition of reductive reagents (DTT) and/or change of solution temperatures, and furthermore this kind of micelle has the ability to be further surface functionalized with biocompatible moieties due to the presence of primary amine groups located on the outer coronas. The biotinylated CCL micelles were prepared by the reaction of amino groups present on the surface of fixed CCL micelles with BNHS and lactobionolactone. The biocompatible biotin groups were confirmed by the HABA/avidin binding assay and dotLab biosensor instrument. The availability of the galactose moieties located on the surface of the biotinylated CCL micelles was also detected by the dotLab biosensor via lectin interactions, which are of considerable interest in biomedicine. This augurs well for their application as smart nanocarriers for targeted drug delivery and controlled release.

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(43) Deng, Z. C.; Li, S. Q.; Jiang, X. Z.; Narain, R. *Macromolecules* **2009**, *42*, 6393–6405.
(44) Goldstein, J.; Iyer, R. N.; Smith, E. E.; So, L. L. *Biochemistry* **1967**, *6*, 2373–2377.
(45) Hong, C.-H. *Carbohydrate-Based Drug Discovery*; Wiley-VCH: Weinheim, 2003.
(46) Choi, S.-K.; Mammen, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 4103–4111.
(47) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, 413–421.
(48) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226–8230.
(49) Lin, C. C.; Yeh, Y. C.; Yang, C. Y.; Chen, C. L.; Chen, G. F.; Chen, C. C.; Wu, Y. C. *J. Am. Chem. Soc.* **2002**, *124*, 3508–3509.
(50) Itakura, Y.; Nakamura-Tsuruta, S.; Kominami, J.; Sharon, N.; Kasai, K.-I.; Hirabayashi, J. *J. Biochem.* **2007**, *142*, 459–469.