Polymeric nanocarriers possessing thermoresponsive coronas

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Polymeric micelles self-assembled from amphiphilic copolymers in aqueous solution have emerged as versatile drug nanocarriers in the past few decades. To enhance the bioavailability of drugs at the target disease site and upon cellular internalization, the use of stimuli-responsive nanocarriers with triggered release characteristics is highly desirable. This article highlights the recent developments in the field of polymeric nanocarriers possessing thermoresponsive coronas, focusing on the fabrication of structurally stable multi-responsive micelles *via* core or shell cross-linking, the phase transition behavior of thermoresponsive polymer brushes at the corona of unimolecular micelles, and their application as vehicles for targeted drug delivery and stimuli-responsive on-demand release.

1. Introduction

In aqueous solution, amphiphilic block copolymers spontaneously self-assemble into micelles with a hydrophilic outer corona and a closely packed hydrophobic core.[1,2] Polymeric micelles can serve as excellent nanocarriers in applications such as drug delivery, sensing, and image enhancement.[3] The hydrophobic inner cores can encapsulate hydrophobic drugs while still maintaining colloidal stability due to the presence of water-swollen outer coronas; moreover, the densely packed polymer brushes at the corona can dramatically reduce unfavorable interactions with serum proteins after intravenous administration. The passive targeting of drug nanocarriers to cancerous or inflamed tissues relies on the enhanced permeability and retention (EPR) effect, whereas active targeting can be accomplished by modifying the micelle surface with specific ligands and monoclonal antibodies, which are selective and complementary to tumor cell receptors and specific antigens over-expressed on the cancerous cell surface.[3–5]

After drug nanocarriers arrive at the target disease site and upon cellular internalization, a triggered release of encapsulated active drugs would be highly desirable. In this respect, stimuli-responsive nanocarriers with tunable core–corona hydrophilicity have found ever-increasing opportunities, endowing them with extra active targeting capability.[6,7] A variety of polymeric systems have been developed towards this goal, taking advantage of internal stimuli such as lower extracellular pH (~6.5) and glutathione (GSH) concentration gradients in tumor tissues.[8] As an external stimulus, temperature can be employed as another variable to achieve targeted delivery and triggered release. Recently, hyperthermia has been frequently employed as an adjunct to radiation or chemotherapy of various types of solid tumors. The local heating of tumors can be facilely achieved by various physical means such as microwave, radio-frequency, and ultrasound.[8–11]

In principle, thermoresponsive polymeric nanocarriers can be used in hyperthermia during cancer therapy due to following advantages: (1) upon local heating, tumor tissues will be ~5–10 °C higher than normal tissues due to slow heat dissipation in the former, and this can partially kill tumor cells;[12] (2) thermoresponsive polymeric nanocarriers

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possessing cores of low glass transition temperature (T_g) exhibit thermo-induced rapid release of active drugs due to structural distortion of the core upon collapsing of thermoresponsive coronas at elevated temperatures;[13] (3) upon passive entry into tumor tissues, the aggregation of thermoresponsive nanocarriers *via* selective local heating lead to the considerable increase of aggregate sizes, preventing their re-entry into circulation and leading to selective accumulation at the target disease site.[14] In this article, we highlight the recent developments in polymeric nanocarriers possessing thermoresponsive coronas from a fundamental point of view, and outlook the future directions in both academic research and practical applications in this rapidly developing field.

2. Nanocarriers self-assembled from amphiphilic block copolymers

Polymeric nanocarriers with thermoresponsive coronas can be fabricated from a variety of amphiphilic block copolymers.[15] Poly(*N*-isopropylacrylamide) (PNIPAM) has been extensively employed as a thermosensitive hydrophilic block, exhibiting a lower critical solution temperature (LCST) or cloud point (CP) at *ca.* 32 °C. The LCST of this thermosensitive block can be facilely tuned *via* copolymerization with hydrophilic or hydrophobic monomers. On the other hand, polystyrene (PS), poly(*n*-butyl methacrylate) (PBMA), poly(D,L-lactide) (PLA), and poly(ε-caprolactone) (PCL) can be used as the permanently hydrophobic building blocks.[16–19]

Okano and coworkers[13,20] reported that although PS-*b*-PNIPAM and PBMA-*b*-PNIPAM copolymer micelles formed in aqueous solution can encapsulate hydrophobic doxorubicin (DOX) below the LCST of PNIPAM coronas, the rapid release of DOX above the LCST can only be achieved for the latter. This is due to the fact that PBMA cores possess much lower T_g values than PS cores (~100 °C); thus PS cores embedded with DOX are insensitive to the collapse of PNIPAM coronas. As compared to that of PNIPAM homopolymer, random copolymerization of *N*-isopropylacryxlamide (NIPAM) with *N*,*N*-dimethylacrylamide (DMA) can elevate its LCST to body temperature.[15] In another example, they found that PCL-*b*-P(NIPAM-*co*-DMA) micelles did not exhibit rapid release of encapsulated DOX above the LCST of the thermoresponsive corona, whereas using a copolymer of D,L-lactide and ε-caprolactone as the hydrophobic block can solve this problem.[21] The above results indicate that the suitable choice of core-forming blocks is crucial for efficient thermo-induced on-demand release of drugs. Recently, the same research group prepared thermoresponsive micelles with hybrid coronas from two types of block copolymers, the water-soluble P(NIPAM-*co*-DMA) blocks of which were terminated with hydrophilic and hydrophobic groups, respectively. They found that the LCST of hybrid coronas can be facilely adjusted by varying the relative ratio of two copolymers.[22]

3. Nanocarriers self-assembled from double hydrophilic block copolymers

Double hydrophilic block copolymers (DHBCs) represent a new class of amphiphilic copolymers of rapidly increasing importance in nanobiotechnology.[23–30] They exhibit intriguing 'schizophrenic' micellization behavior, self-assembling into two or more types of micelles with invertible structures under a combination of external stimuli such as pH, temperature, and ionic strength. Past studies of DHBCs mainly focused on linear block copolymers, and we recently explored the effect of chain topology on the size and morphologies of self-assembled nanostructures. A series of non-linear (AB_4, A_2BA_2, and A_4BA_4) stimuli-responsive DHBCs were synthesized.[31,32] For example, the AB_4 miktoarm star copolymer of NIPAM and 2-(diethylamino)ethyl methacrylate (DEA), PNIPAM-*b*-$(PDEA)_4$, forms PNIPAM-core micelles in acidic solution at elevated temperature; whereas in slightly alkaline media at room temperature, PDEA-core micelles with thermosensitive PNIPAM coronas form due to the pH-responsive water-solubility of PDEA block. The chain architectural effects on the micellization properties and the underlying mechanisms were elucidated by comparison to that of the linear PNIPAM-*b*-PDEA copolymer.[31]

Polypeptide hybrid DHBC, PNIPAM-*b*-PLGA, also exhibits 'schizophrenic' micellization behavior where PLGA is poly(L-glutamic acid).[33] It molecularly dissolves in aqueous solution at alkaline pH and room temperature, but supramolecularly self-assembles into PNIPAM-core micelles at alkaline pH and elevated temperatures, and PLGA-core micelles at acidic pH and room temperature accompanied by a coil-to-helix transition for the PLGA sequence (Fig. 1). Moreover, the micellization process was investigated by a stopped-flow light scattering technique.

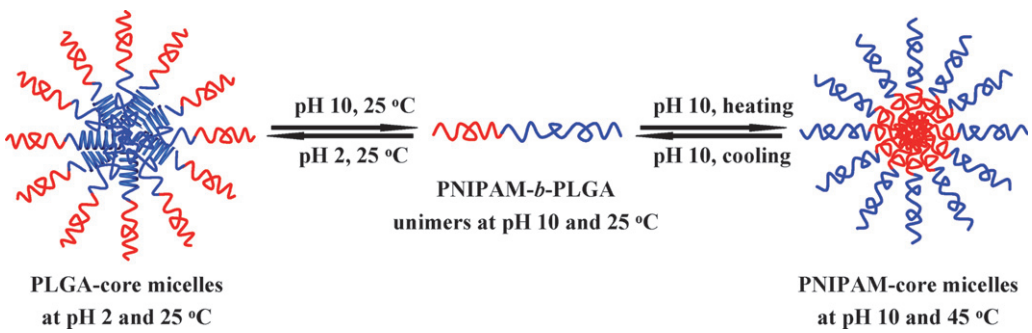

Fig. 1 Schematic illustration of thermo- and pH-responsive micellization of $PNIPAM_{65}$-*b*-$PLGA_{110}$ associated with coil-to-helix transitions. Reprinted with permission from ref. 33, copyright 2007, American Chemical Society.

The pH-induced growth of PLGA-core micelles exhibits drastically different kinetics compared to that of conventional pH-responsive DHBCs, probably due to the stabilization effects exerted by the formation of α-helix secondary structures.

In the above described examples, PNIPAM-core micelles of DHBCs formed at elevated temperatures can be in principle used as nanocarriers for hydrophobic drugs. However, the thermo-triggered release of drugs will require local cooling of disease sites to induce the micelle-to-unimer transition, which poses technical challenges in clinical practice. DHBCs with one building block exhibiting upper critical solution temperature (UCST) phase behavior can form micelles upon cooling and will disintegrate upon heating;[34] thus they should work better for triggered release during hyperthermia treatment. On the other hand, pH-induced micelles with thermoresponsive PNIPAM coronas might encounter the problem of structural stability if they are used as nanocarriers. It is well-known that most block copolymer micelles only exist above the critical micellization concentration (CMC); moreover, for DHBCs, the unimer exchange between micelles and bulk aqueous phase is much faster compared to that of amphiphilic block copolymers possessing highly hydrophobic blocks. Thus, after intravenous administration, the large dilution and shear forces during blood circulation will tend to disintegrate these self-assembled nanostructures.

To solve this problem, we fabricated multi-responsive stable micelles with thermoresponsive coronas *via* shell cross-linking (SCL).[35,36] As shown in Fig. 2, poly[2-(diethylamino)ethyl methacrylate]-*b*-poly[2-(dimethylamino)ethyl meth acrylate]-*b*-poly(*N*-isopropylacrylamide) (PDEA-*b*-PDMA-*b*-PNIPAM) triblock copolymer was synthesized *via* sequential atom transfer radical polymerization (ATRP).[37] At alkaline pH and room temperature, it self-assembles into three-layer 'onion-like' PDEA-core micelles with a PDMA inner shell and a PNIPAM outer corona. Novel shell cross-linked (SCL) micelles with pH-responsive PDEA cores and thermoresponsive PNIPAM coronas were then facilely fabricated by covalently cross-linking the PDMA inner shells with 1,2-bis(2-iodoethoxy)ethane (BIEE). As the obtained stable SCL micelles possess pH-controllable core swellability and thermo-tunable corona permeability, the release profile of a model hydrophobic drug, which is initially loaded within the hydrophobic PDEA-core, can be dually controlled by the combined stimuli of solution pH and temperature.

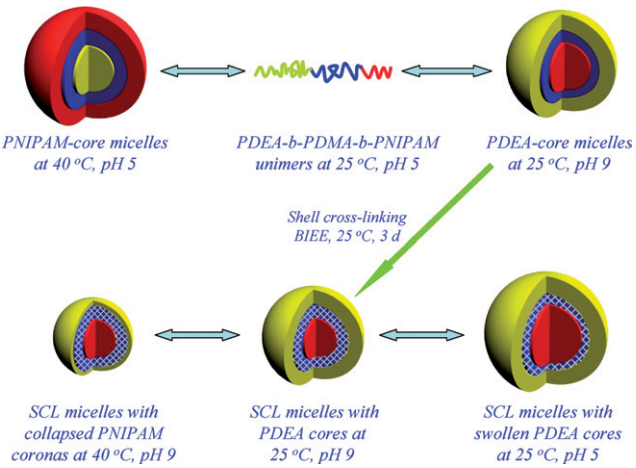

Fig. 2 Schematic illustration of 'schizophrenic' micellization of PDEA-*b*-PDMA-*b*- PNIPAM triblock copolymer in aqueous solution (top) and the subsequent fabrication of multi-responsive shell cross-linked (SCL) micelles (bottom). Reprinted with permission from ref. 37, copyright 2007, American Chemical Society.

Kataoka and coworkers[38] reported the preparation of polyion complex (PIC) micelles with a novel thermosensitive poly(2-isopropyl-2-oxazoline) shell *via* the complexation of oppositely charged block ionomers. The formation of PIC micelles can be employed to incorporate charged macromolecules of synthetic and biological origins (proteins and nucleic acids) into self-assembled nanostructures. The triggered release of these bioactives might be controlled *via* the thermal phase transition of thermoresponsive coronas. Recently, we synthesized two oppositely charged graft ionomers bearing oppositely charged backbones and thermoresponsive PNIPAM side chains.[39] In mixed aqueous solution, PIC micelles consisting of polyelectrolyte complex cores and PNIPAM coronas form due to electrostatic interactions between oppositely charged backbones. The obtained PIC micelles can be further core cross-linked *via* click chemistry due to the presence of azide moieties on the backbones of two graft ionomers.

4. Unimolecular micelles with thermoresponsive coronas

When hydrophilic polymer chains are tethered to a hydrophobic multifunctional core, such as dendritic macromolecules and multiarm star polymers, they can be considered as unimolecular micelles due to structural resemblance to block copolymer micelles. Moreover, polymeric unimolecular micelles generally possess well-defined chemical structures with pre-determined core size, controllable length and density of grafted chains, thus they could also serve as model systems for block copolymer micelles.

We recently reported the double phase transition behavior of thermoresponsive PNIPAM brushes at the surface of a spherical hydrophobic core.[40] Reversible addition–fragmentation transfer (RAFT) polymerization of NIPAM was conducted using a hyperbranched polyester (Boltorn H40)-based macro-RAFT agent. The resultant multiarm star polymer (H40-PNIPAM) exists as unimolecular micelles with hydrophobic H40 as the core and densely grafted PNIPAM brushes as the shell. The average grafting density of corona PNIPAM chains at the core surface is estimated to be 0.46 nm^2 per chain. Experimental results obtained from laser light scattering, transmittance measurements, micro-DSC, and excimer fluorescence measurements are self-complementary, and all of these four

techniques unambiguously support the conclusion that PNIPAM brushes densely grafted at the surface of hydrophobic dendritic cores exhibit double thermal phase transition behavior. The inner part of PNIPAM brushes collapse at lower temperatures (<30 °C); above 30 °C, the outer part of the PNIPAM brush starts to collapse. This provides the first direct evidence of the collapsing sequence of PNIPAM brushes tethered to a spherical core. It should be noted that the double phase transition behavior of the PNIPAM corona has also been observed for thermoresponsive PNIPAM coronas grafted at the surface of gold or silica nanoparticles.[41–44] Unimolecular micelles possessing two layers of thermoresponsive coronas with distinct LCSTs were also fabricated *via* successive RAFT polymerizations of NIPAM and 2-(dimethylamino)ethyl methacrylate (DMA) using a H40-based macroRAFT agent (Fig. 3). Upon continuously heating through the LCSTs of the PNIPAM inner shell and the PDMA outer corona, unimolecular micelles exhibit intriguing multi-stage thermo-induced collapse.[45]

Unimolecular micelles with thermoresponsive coronas can also serve as templates for the synthesis of nanoparticles. The thermoresponsive PNIPAM corona can be embedded with silver nanoparticles *via in situ* reduction.[46] On the other hand, the surface of thiol-functionalized thermosensitive unimolecular micelles can also be covalently attached with gold nanoparticles.[47] In both cases, heating the hybrid unimolecular micellar solution leads to the shrinkage of the PNIPAM shell and allows for finely tuning the relative spatial distances between embedded gold nanoparticles or surface attached gold nanoparticles.

5. Unresolved questions and future prospects

A major disadvantage of polymeric carriers is that thermal treatment (hyperthermia) is required for the triggered release of drugs, which is not always applicable during clinical practices in cancer therapy. The use of polymeric micelles with multi-responsive (pH, temperature) coronas might solve this problem. Shi and coworkers[48] recently reported the preparation of complex micelles possessing hybrid coronas of PNIPAM and poly(4-vinyl pyridine) (P4VP). The pH- and thermo-induced reversible hydration and dehydration of P4VP and PNIPAM corona chains can create tunable channels, the size and permeability of which can be adjusted.

Local heating at disease sites might also be achieved by secondary external triggers, such as near-infrared (NIR) light and magnetic field.[10,11] PNIPAM hydrogels embedded with gold nanoshells was prepared by Sershen *et al.*[10] The nanoshells can strongly adsorb NIR irradiation, thus the heating of hydrogels leads to their collapse and the concomitant release of embedded bioactives. NIR irradiation can penetrate the skin with lower risk of damaging normal cells and tissues in irradiated areas. Thus, after thermoresponsive micelles embedded with nanoshells and drugs have accumulated at the target disease site, NIR irradiation can facilely realize selective local heating and the triggered release of drugs. On the other hand, polymeric carriers bearing super-paramagnetic iron oxide nanoparticles might also achieve local heating under alternating magnetic fields.[11]

Finally, although there are numerous examples of polymeric micelles possessing thermoresponsive coronas, their *in vivo* applicability for controlled on-demand release of drugs remains to be explored. In principle, the performance of nanocarriers fabricated from amphiphilic or double hydrophilic block copolymers can be improved by attaching a tumor cell-specific recognizing moiety at the micelle surface; moreover, using poly [oligo(ethylene glycol)methyl ether methacrylate] (POEGMA, the degree of polymerization, DP, is 2 to 3) as the thermosensitive building block might reduce the toxicity of nanocarriers to normal cells as compared to that of PNIPAM.[49]

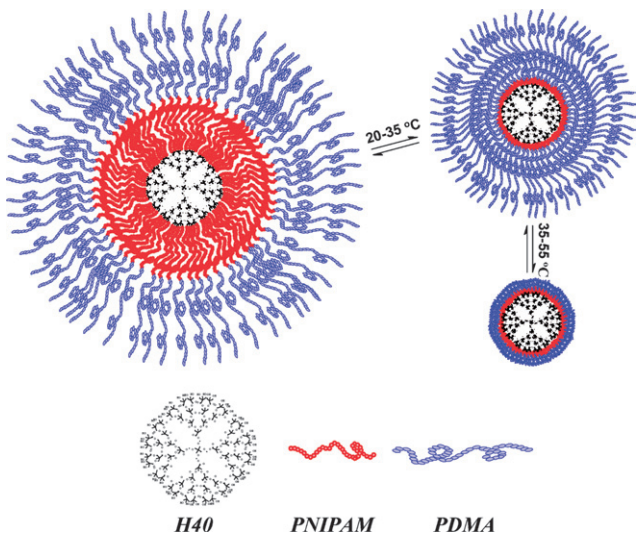

Fig. 3 Schematic illustration of thermo-induced collapse of H40-PNIPAM-PDMA upon heating through the LCSTs of the PNIPAM inner shell and the PDMA outer corona. Reprinted with permission from ref. 45, copyright 2006, American Chemical Society.

Acknowledgements

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