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Monodispersed Polymeric Nanocapsules: Spontaneous Evolution and Morphology Transition from Reducible Hetero-PEG PICmicelles by Controlled Degradation

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Polymeric nanocapsules offer significant potential applications as intracellular gene or drug delivery vehicles. However, preparation of uniform hollow nanocapsules still poses fabrication challenges.[1] To date, numerous approaches for nanocapsules preparation had been developed including the molecular self-assembly[1,2] and the sacrificed template method;[3] however further application is limited by major disadvantages such as large size distribution, time-consuming preparation, and harsh core-removal conditions, etc.

In this communication, a novel "self-templating" strategy will be introduced to prepare uniform and biocompatible nanocapsules. Highly monodispersed core−shell PEG-detachable PICmicelles (Figure 1), which rapidly detach a portion of the PEG chains in response to the thiol-reducing conditions via cleavage of disulfide bonds,[5] were utilized as "sacrificed templates". After reduction agents were injected into PICmicelle solution, the weight fraction of the PEG coronal shell decreased due to the release of PEG chains. Consequently, spontaneous and drastic morphology evolution from micelle to vesicle structures occurred when the PEG weight fraction decreases to a certain value.[6] Herein, we will demonstrate the reduction-induced morphology transition and formation of uniform nanocapsules with controllable capsule size by careful composition design of PICmicelles.

Proof-of-concept experiments were carried out using novel hetero-PEG-detachable PICmicelles, made by self-assembly of biocompatible PEG-*block*-polyions containing a disulfide linkage between PEG chains and charged segments in 10 mM PBS buffer (pH = 7.4), i.e., PICmicelle A of PEG-SS-P[Asp(DET)] (10K-100)/PEG-SS-P(Asp) (2K-91) or PICmicelle B of PEG-P[Asp-(DET)] (12K-68)/PEG-SS-P(Asp) (2K-91),[7] where PEG stands for poly(ethylene glycol) (M_n = 2000 (2K), 10 000 (10K), 12 000 (12K)), P[Asp(DET)] for poly([*N*-(2-aminoethyl)-2-aminoethyl]-α,β-aspartamide (DP = 68, 100), and PAsp for poly(α,β-aspartic acid) (DP = 91) (Figure 1). Advantages of hetero-PEG based PICmicelles include their highly monodispersed core−shell nanoparticles structure, a dense hydrophilic PEG palisade surrounding the core, fast response to the reduction conditions, and a tunable PEG weight fraction. The PEG weight fraction in hetero-based PICmicelles can easily be tuned from ∼6% to ∼30% without decreasing the PEG shell thickness, and thus their morphology transition windows can be easily explored. Structure changes were monitored by time-resolved transmission electron microscopy (TEM) and dynamic light scattering (DLS).[7]

PICmicelle A was formed by self-assembly of redox-sensitive block copolymers PEG-SS-P[Asp(DET)] and PEG-SS-P(Asp). The resulting micelles were monodispersed nanoparticles with an average size of 48.1 nm and a relatively low polydispersity (PDI) of 0.058 in 10 mM PBS buffer, pH = 7.4 (Figures 1 and S7[7]). The theoretical PEG weight fraction of as-formed micelles is ∼24.3%. After reduction by dithiothreitol (DTT), all PEG molecules are detached from block copolymers which lead to homopolymer

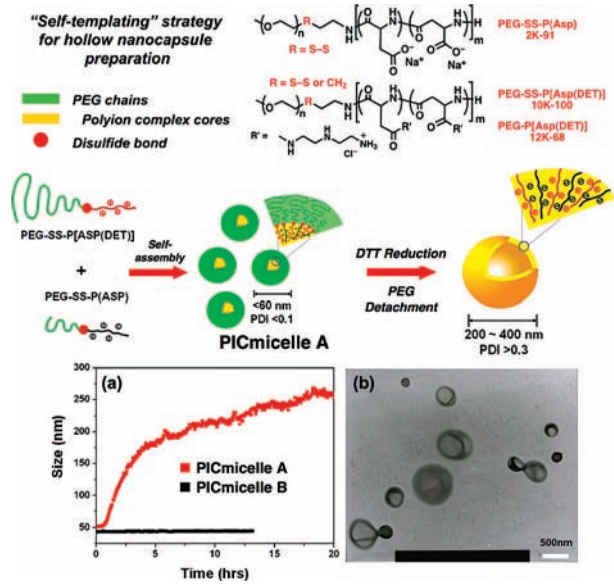

Figure 1. Schematic illustration of the preparation of hollow nanocapsules by self-templating strategy. Upon addition of reducing agent DTT into hetero-PEG-detachable PICmicelle A solution,[7] a morphology transition occurred. (a) Change in cumulant average diameter of PICmicelle A and B after the addition of DTT as a function of time. (b) TEM image of PICmicelle A deposited on copper grids 12 h after reduction. (Note: Chain lengths of polymer and PICmicelle or nanocapsule diameters are approximately to scale.)

complexes. Without a PEG shielding effect, the polymer assemblies will adopt a vesicular structure with a smaller curvature. A rapid increase in average diameter and changes in PDI value over time occurred spontaneously after reduction is clearly demonstrated by the red profiles in Figures 1a and S7.[7] After 12 h, the average particle size increased to ∼200 nm, while the PDI increased to higher than 0.3. Typically, hollow vesicles with varied sizes were observed in solution, consistent with the TEM image shown in Figure 1b and AFM results.[7] The formation of vesicle structure is mainly due to the decreased free energy of the corona following the loss of PEG chains from the polyion complex surface. However, the resulting nanocapsules are not monodispersed as indicated by their large PDI values. A similar phenomena also occurred in the case of the reduction-induced morphology transition of PICmicelles formed with PEG-P(Asp) (2K-75) and PEG-SS-P[Asp(DET)]) (10K-100). The average size increased from 70 to 127 nm, while the PEG weight fraction decreased from 25.3% to 6.66% and PDI increased from 0.14 to 0.33 (Figure S9).[7]

To maintain a narrow PDI, some hydrophilic PEG chains must be present in the nanocapsule surface. Therefore, PICmicelle B was prepared by replacing PEG-SS-P[Asp(DET)] with reduction-inert PEG-P[Asp(DET)] and complexing with PEG-SS-P(Asp) to form

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uniform PIC micelles, 40 nm in size with a PDI of 0.047. After reduction, the PEG weight fraction in PICmicelle B decreased from 37.0% to 34.3%. The average size increased slightly during the initial 4 h and then slowly reached 50 nm after 12 h (Figure 1a). Obviously, the size expansion behavior of PICmicelle B is strongly suppressed by the PEG remaining on the micelle surface. These structures are not nanocapsules but micelles. The PDI remained constant indicating that as-formed micelles retained homogeneity (Figure S7a[7]).

To achieve formation of monodispersed hollow nanocapsules, the PEG weight fraction was controlled by the addition of homo-P[Asp(DET)] into PICmicelle compositions, as shown in Figure 2a. Then new ternary system PICmicelle C was developed by self-assembly of PEG-P[Asp(DET)], PEG-SS-P(Asp), and homo-P[Asp(DET)]. Herein two different molecular weight homo-P[Asp(DET)]'s (DP = 30 or DP = 82) were added into the PICmicelle composition with different homopolymer molar concentrations. In Figure 2a, PICmicelle C containing homo-P[Asp-(DET)] (DP = 30) with varied molar ratios of ($[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP]) ranging from 0.1 to 0.8 showed similar size expansion phenomena. However, when $[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] > 0.7, large size expansions occurred. For instance, when $[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] = 0.8, the average size increased from 53 to 128 nm after 19 h of reduction, while the PDI remained less than 0.1 (Figure S10[7]). Hollow vesicle morphology was clearly observed in TEM images (Figure 2b). In these PICs, the PEG fraction will decrease from 13.5% to 8.76% upon reduction. This demonstrates that a low PEG fraction is sufficient to direct the formation of hollow nanocapsules. However, if $[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] < 0.7, for example 0.3, the average size increased from 41 to 56 nm (Figure 2a). The size corresponds to the micelle regime (Figure 2c). Size expansion was inhibited by PEG (25.8%) remaining on the particle surface after reduction, which is similar to observations with the PICmicelle B system (Figure 1). Such a high PEG fraction is not beneficial for vesicle formation. Therefore, the homopolymer ratio is a key parameter for morphology transition, as summarized in Figure 2c. It is noteworthy that such a low PEG fraction, ~10%, is required for the formation of vesicles, compared with conventional amphiphilic polymer systems where ~35% is the typical threshold value of the PEG fraction to form vesicles.[8] This implies that the longer chain length of the nondetached PEG, DP > 200, may be effective to suppress the increase of the aggregation number within PIC. On the other hand, the molecular weight of homo-P[Asp(DET)] is another crucial parameter to control nanocapsule size. If PICmicelle C contains high molecular weight homo-P[Asp(DET)] (i.e., DP = 82, $[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] = 0.8), the average size of reduced PICmicelles increased 42.9 to 51.8 nm although the PEG fraction decreased from 13.5% to 8.76% (Figures S11 and S12). The results are different from those for PICmicelle C containing low molecular weight homo-P[Asp(DET)] (DP = 30). The reason why the high molecular weight of homo-P[Asp(DET)] prevented capsule formation may be due to two effects: (1) the chain recognition effect,[4] since the block anion chain segment (DP = 91 of PEG-SS-P(Asp)) is pairing with homo-P[Asp(DET)] (DP = 82); and (2) the mobility effect, because high molecular weight polymers in the PIC membrane have a low mobility as compared with lower molecular weight species.

In summary, we have demonstrated a novel "self-templating" approach to produce highly monodispersed nanocapsules in a controlled and dynamic fashion. Reduction-induced micelle−vesicle transitions of hetero-PEG-detachable PICmicelles are strongly dominated by the residual PEG weight fraction following reduction. By careful control of the micelle composition and molecular weight of homo-P[Asp(DET)], the size expansion following reduction removal of a portion of PEG chains can be easily tuned with retention of monodispersity. Uniform and biocompatible nanocapsules with controlled size as described here have many potential applications in drug delivery and gene therapy.

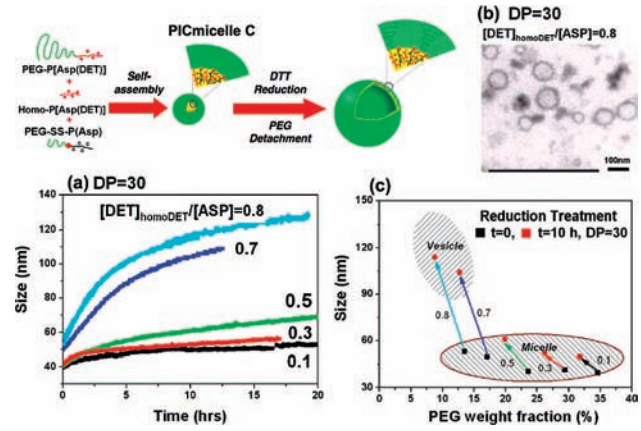

Figure 2. Schematic illustration of the preparation of PICmicelle C containing homo-P[Asp(DET)] (DP = 30) and reduction induced morphology transition. (a) Change in cumulant average diameter of PICmicelle C with different homo-P[Asp(DET)] composition ($[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] = 0.1 to 0.8) after the addition of DTT as a function of time. (b) TEM image of PICmicelle C ($[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] = 0.8) after 20 h. (c) Average sizes of PICmicelle C before and after reduction treatment as a function of PEG weight fraction, ($[DET]_{homo\text{-}P[Asp(DET)]}$/[ASP] = 0.1 to 0.8).

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Supporting Information Available: Schematic representation of PICmicelle B; synthesis, ^{1}H NMR spectra, and GPC results of polymers; TEM, DLS, and AFM results of PICmicelles before and after reduction treatment. These materials are available free of charge via the Internet at http://pubs.acs.org.

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