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Preparation of Redox-Sensitive Shell Cross-Linked Nanoparticles for Controlled Release of Bioactive Agents

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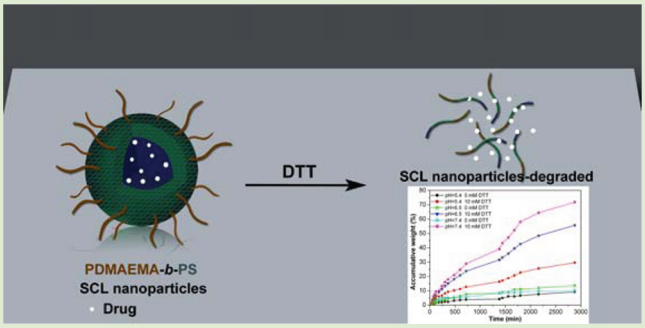
Supporting Information

ABSTRACT: Redox-sensitive shell cross-linked (SCL) poly(2-(dimethylamino)ethyl methacrylate)-*block*-polystyrene (PDMAEMA-*b*-PS) nanoparticles have been facilely fabricated by surfactant-free emulsion reversible addition−fragmentation chain transfer polymerization (SFE-RAFT), in which amphiphilic $C_{12}H_{25}$−PDMAEMA copolymers acted as stabilizers. ^{1}H NMR, dynamic light scattering (DLS), and transmission electron microscopy (TEM) were applied to investigate the compositions and the morphologies of the resultant nanoparticles. Then, the as-prepared nanoparticles were used as a carrier to encapsulate of hydrophobic drugs, and the release could be triggered by a redox reagent, dithiothreitol (DTT). The SCL nanoparticles had a good biocompatibility. These properties indicated that these nanoparticles would be used as promising drug delivery vehicles.



The synthesis of shell cross-linked (SCL) nanoparticles with environment-sensitive properties has been the focus of considerable research in recent years.[1−6] To date, several strategies have been developed to the formation of SCL nanoparticles, such as reacting with bifunctional cross-linking agents,[7−9] UV radiation,[10,11] and so on. An important consideration in fabrication process is that such multistep cross-linking procedures invariably turn out to be laborious and costly. Thus, it is urgent to apply the one-pot method for the preparation of SCL nanoparticles. At present, (inverse) miniemulsion polymerization combined with controlled radical polymerization (CRP) techniques has been widely used as a powerful tool for the precise construction of nanoscale carriers.[12−17] However, one drawback is that the target nanocarriers may be contaminated by surfactants, although their concentration is relatively low. Fortunately, surfactant-free emulsion reversible addition−fragmentation chain transfer (RAFT) polymerization (SFE-RAFT), in principle, can address these disadvantages to some extent. There are many examples of latex syntheses based on this approach. For instance, SFE-RAFT has been mediated to copolymerize styrene,[18−21] *n*-butyl methacrylate,[22] *n*-butyl acrylate,[23] and methyl methacrylate.[24] However, these examples all focus on the polymerization kinetics or the morphology control.

Most importantly, a majority of these above-mentioned nanocarriers cannot be cleaved under specific stimulus, which would hinder practical applications as well. Our previous studies showed that bis(acryloyloxyethyl) disulfide (BAEDS) was a kind of redox-sensitive cross-linker and can be cleaved at the presence of a redox-reagent, dithiothreitol (DTT).[15] Consequently, if this kind of cross-linker can be introduced into the target nanoparticles, it would be useful to regulate the release rate of the drugs. Furthermore, to the best of our knowledge, there is not yet a report on preparing redox-sensitive SCL nanoparticles by SFE-RAFT and their application in the drug control release system.

In the present work, SCL poly(2-(dimethylamino)ethyl methacrylate)-*block*-polystyrene (PDMAEMA-*b*-PS) nanoparticles and indomethacin-encapsulated SCL PDMAEMA-*b*-PS nanoparticles were prepared by the SFE-RAFT system, in which $C_{12}H_{25}$−PDMAEMA copolymers acted as stabilizers. Then indomethacin (IND) release behaviors at different pH conditions and their biocompatibility were investigated.

Our previous studies have showed that (inverse) miniemulsion polymerization combined with RAFT polymerization can be applied to prepare SCL nanocarriers.[14−16] However, the as-prepared nanocarriers can be contaminated by surfactants. Moreover, the diameters of nanocarriers can be controlled by the amount of cross-linker used in the emulsion system. When

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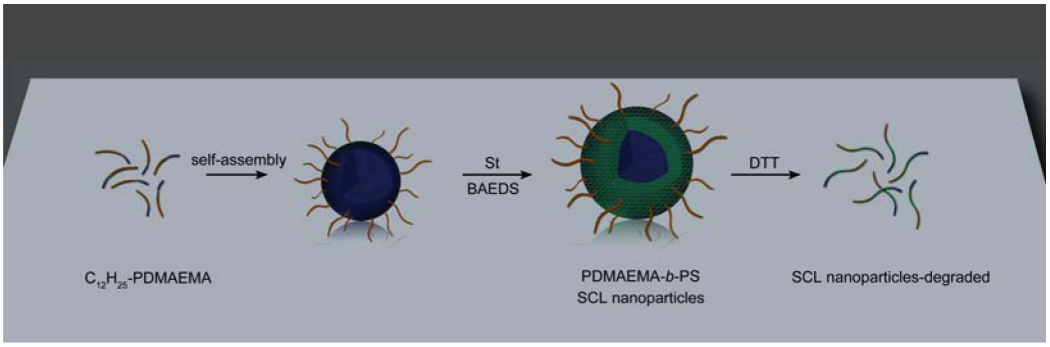
Scheme 1. Schematic Representation of the Fabrication of SCL PDMAEMA-*b*-PS Nanoparticles via SFE-RAFT Polymerization



the amount of cross-linker is over 0.2 mmol, the diameter of the as-prepared nanoparticles would be larger than 200 nm (Supporting Information, Figure S1), which is the threshold value of the enhanced permeability and retention (EPR) effect.[25,26] Thus in this work, the amount of BAEDS was set to be 0.1 mmol, and SCL nanoparticles were fabricated by SFE-RAFT. The final nanoparticles are expected to comprise of three distinct domains of the PDMAEMA outer corona, the cross-linked polystyrene (PS) middle shell, and the $C_{12}H_{25}$ inner core. The schematic representation for fabrication of SCL nanoparticles is illustrated in Scheme 1.

Figure 1 shows the ^{1}H NMR spectra of $C_{12}H_{25}$−PDMAEMA (A), PDMAEMA-*b*-PS (B), and SCL nanoparticles (C) in

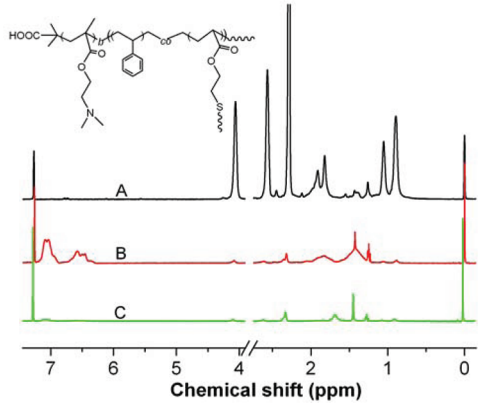


Figure 1. ^{1}H NMR spectra of $C_{12}H_{25}$−PDMAEMA (A), PDMAEMA-*b*-PS (B), and the SCL nanoparticles (C) in $CDCl_3$.

$CDCl_3$. The peaks at δ = 1.82 and 0.90 ppm are attributed to methylene and methyl groups of (S−CH_2−C−CH_3). Meanwhile, the proton peaks at δ = 4.06 and 2.56 ppm are assigned to methylene groups (−CH_2−CH_2−N). The signal at δ = 2.28 ppm is the proton peaks of methyl group connecting the nitrogen (N−CH_3), which indicates that $C_{12}H_{25}$−PDMAEMA copolymers have been successfully synthesized (Figure 1A). PDMAEMA-*b*-PS nanoparticles were then fabricated via SFE-RAFT in the presence of $C_{12}H_{25}$−PDMAEMA copolymers. PDMAEMA-*b*-PS can be dissolved in $CDCl_3$, so all the signals characteristic of PDMAEMA blocks and the PS blocks are observed as shown in Figure 1B. Compared to Figure 1A, the peak intensity attributed to PDMAEMA decreases significantly. Furthermore, it reveals the presence of new peaks at δ = 6.29−6.74 and δ = 6.87−7.21 ppm, which are ascribed to the PS blocks indicating a successful chain extension of $C_{12}H_{25}$−PDMAEMA. After the SCL reaction, the peaks pertinent to the PS blocks are almost invisible whose molecular mobility trapped by the cross-linked shells, while the PDMAEMA blocks are still well-solvated and can be detected. If the shell cross-linking was unsuccessful, dissociation into individual copolymer chains would be expected since $CDCl_3$ was a good solvent for both the PDMAEMA and the PS blocks, whose characteristic peaks could be both observed. All of the above clearly confirms that the PDMAEMA-*b*-PS has been successfully fixed by BAEDS to form SCL nanoparticles (Figure 1C).

Then, transmission electron microscopy (TEM) was employed to investigate the morphologies of nanoparticles, which revealed that the resultant samples consisted of spherical nanoparticles with definite core−shell structures. In case of PDMAEMA-*b*-PS, the size is about 194 nm, and the shell thickness is around 33 nm (Figure 2a,b). As for SCL

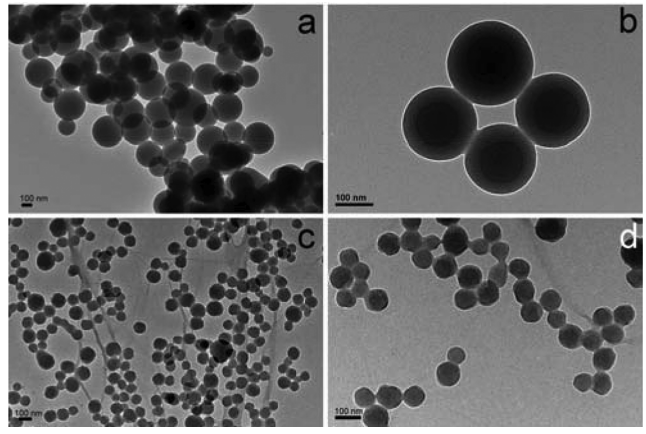


Figure 2. TEM images of PDMAEMA-*b*-PS (a, b) and the SCL nanoparticles (c, d). The concentration of the samples for measurements is 1 mg mL^{-1}. The scale bars for TEM images are 100 nm.

nanoparticles, the diameter and the shell thickness decrease to 95 and 10 nm, respectively (Figure 2c,d).

As reported, nanoparticles have extensively participated in drug delivery and control release system.[27−30] The release rate of guest molecules can be regulated by their cross-linked shells or cores.[31,32] For this purpose, BAEDS was chosen as the cross-linker, since the disulfide group can be cleaved to the corresponding thiols in the presence of reducing agents, such as tri(*n*-butyl) phosphine (Bu_3P),[33] dithiothreitol (DTT),[12,15,34] and glutathione.[35] To trace the degradation process of the SCL nanoparticles easily, the degradation experiments were carried out in DMF. If disulfide linkage was cleaved by DTT, the colloid solution would turn transparent. In

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our case, the colloid solution becomes transparent after a period of time, indicting that the SCL nanoparticles have been degraded into individual copolymer chains by DTT (Supporting Information, Figure S5).

The in vitro cytotoxicities of SCL nanoparticles to HeLa cells were evaluated using an MTT assay. As shown in Figure 3, it is observed that the viabilities of HeLa cells are around 100% at all test concentrations up to 25 μg mL^{-1}, indicating the low toxicity and good compatibility of the SCL nanoparticles to cells. According to our previous studies, SCL PDMAEMA-*b*-PS nanoparticles have an inherent fluorescent property without any fluorescence labeling treatment, which would simplify the preparation procedure of the nanocarriers when they are applied to in vivo experiments.[14] Consequently, these SCL nanoparticles can be directly used as drug carriers in vivo.

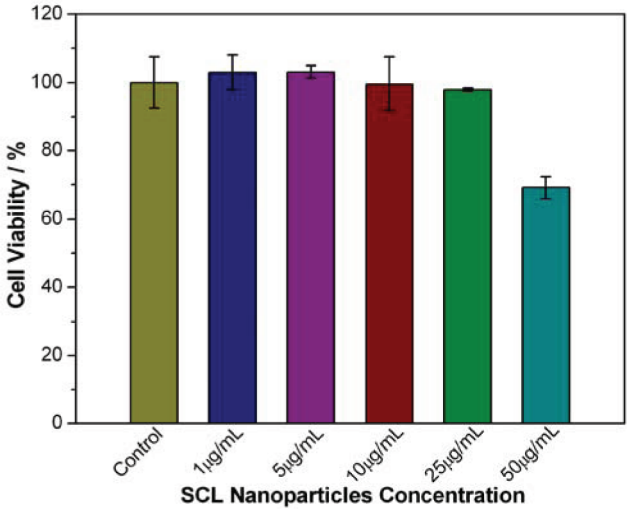

Figure 3. Cell viability of HeLa cells incubated with SCL nanoparticles at various concentrations. Data were presented as the average ± standard deviation (n = 5).

Up to now, various classes of SCL nanoparticles have been reported but, nevertheless, only a few studies concerning the drug release behaviors.[4,36−38] As discussed above, the disulfide groups can be cleaved by DTT and therefore can be expected to trigger the release of guest molecules. Then, indomethacin (IND) as a model for hydrophobic drugs was encapsulated into nanoparticles. The SCL nanoparticles containing IND were characterized by DLS and TEM. Both results revealed that the diameter of IND-encapsulated SCL nanoparticles was larger than that of pure SCL nanoparticles (Supporting Information, Figures S6 and S7).

To prove the rate of drug release can be triggered by DTT, the drug release profiles from the SCL nanoparticles were measured in phosphate-buffered saline (PBS), in which 10 mM DTT was present or absent, and the temperature was maintained at 37 °C. As can be seen from Figure 4, IND is released at a faster rate when DTT is added to the PBS solution. Moreover, the higher the pH is, the faster of the release rate is. More specifically, when the pH is 4.5, the total amount of IND released is around 30% after 48 h at the presence of 10 mM DTT while the figure only reached 9% without DTT. The phenomena are similar when the pH is increased to 6.5 and 7.4. As for the release experiments conducted in pH = 7.4, the maximum accumulative weight accounts to 72% in a DTT-containing environment after 48 h, compared to 10% without DTT. These results indicate that the SCL nanoparticles can be potentially used as a carrier for control release of hydrophobic guest molecules. Moreover, these

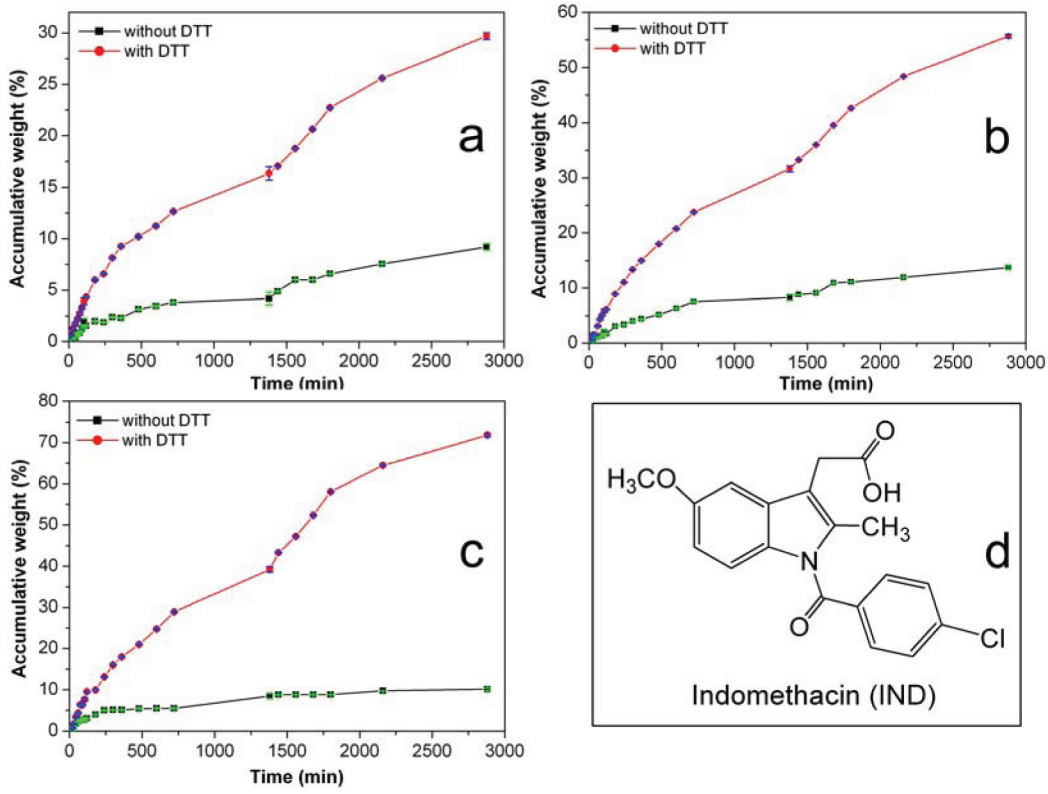

Figure 4. Cumulative release of indomethacin to PBS solution from SCL nanoparticles with or without DTT at 37 °C. The pH of PBS buffer is 5.4 (a), 6.5 (b), and 7.4 (c), respectively. The chemical structure of IND is also shown in d.

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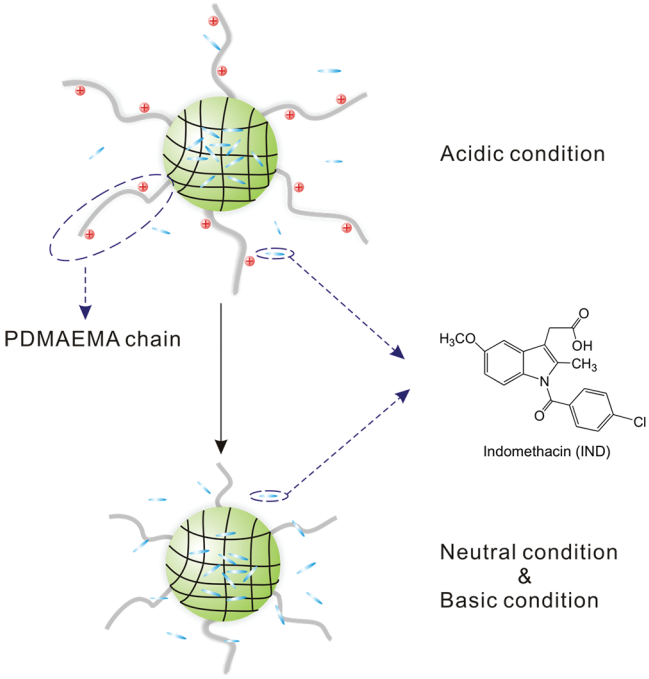
interesting phenomena can be attributed to the following reasons: (i) DTT can cause cleavage of the SCL nanoparticles; therefore, the diffusion of IND from the core to the environmental PBS buffer will be facile. (ii) Our previous study has shown that PDMAEMA blocks are pH-sensitive segments. Moreover, the diameter of nanoparticles in the acidic condition is larger than that of nanoparticles in neutral and basic conditions.[39] When the pH is lower than 7, the PDMAEMA is completely charged, so the chain of PDMAEMA would fully stretch to the PBS buffer due to the electric repulsion. That is, IND would be hard to diffuse from the polymer matrix since the diffusion distance is long. Whereas the pH reaches to 7.4, the extent of chain stretch decreases due to the deprotonation effect. It would cause a stress on the core of the nanoparticles, which gave rise to a core distortion inducing a leakage of loaded drugs and rapid drug release.[30,39−41] (iii) Besides, when pH of the buffer is over 7, the IND would have a better soluble state because of the carboxyl group, which would result in a fast diffusion rate as well.[42] The proposed release mechanism is shown in Scheme 2.

Scheme 2. Schematic Representation of the Release Mechanism Proposed for SCL PDMAEMA-*b*-PS Nanoparticles



In summary, a series of SCL PDMAEMA-*b*-PS nanoparticles were successfully synthesized via SFE-RAFT polymerization in a one-pot method for the first time. The as-prepared nanoparticles have a size of 177 nm and can be used as a carrier to encapsulate hydrophobic components such as IND. The release rate of encapsulated drug can be easily controlled from these SCL nanoparticles. The good compatibility of these nanoparticles would make them as promising nanoscale drug delivery vehicles.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis of SCL nanoparticles, experimental methods, and relevant figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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