Preparation and Redox-Controlled Reversible Response of Ferrocene-Modified Poly(allylamine hydrochloride) Microcapsules†

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Single-component microcapsules were fabricated by the in situ reaction of ferrocene-carboxaldehyde (Fc-CHO) with poly(allylamine hydrochloride) (PAH) doped inside CaCO3 microparticles, followed by core removal. The PAH-Fc microcapsules had very thick shells with remnant PAH-Fc inside, leading to a robust capsule structure that is less collapsed in the dry state. This single-component microcapsule is stabilized by the hydrophobic aggregation of Fc moieties and the protection of hydrophilic PAH backbones. Because of the excellent redox properties of Fc, the PAH-Fc microcapsules showed redox sensitivity to oxidation and reduction, as confirmed by UV–vis absorption spectroscopy and confocal laser scanning microscopy, resulting in reversible swelling and shrinking (11.7 vs 5.5 μm) in their size. Consequently, the permeability was also reversibly tuned, leading to the controlled loading and release of desired substances such as dextran.

Introduction

Hollow microcapsules (MCs) with well-defined structures and tailorable properties have attracted a great amount of interest in recent years because of their potential applications in drug and gene delivery, artificial cells and viruses, and catalysis. In particular, MCs that are environmentally sensitive to external stimuli such as pH, ionic strength, light, temperature, and redox are frequently required,1,2 enabling controllable/reversible alterations of terms in the MC size, shell morphology, interface properties, and permeability.3 Thus, the integration of different functional and responsive building blocks offers control over the response behavior of the functional capsule structure such as contraction and expansion and permeability change in a controlled fashion. For instance, several types of MCs assembled from weak polyelectrolytes via the layer-by-layer (LBL) technique show pH and ionic strength sensitivities because of their distinct physicochemical properties.2,4,6 Fukuhara and co-workers reported polymeric MCs having light-responsive properties for encapsulation and release.7 Chu et al. introduced a thermostressive core—shell MC consisting of poly(N-isopropylacrylamide), which exhibits controllable permeability around the lower critical solution temperature.8 These examples pave the way for exploring functional capsules with different stimuli responses in the pioneering stage but still with some limitations such as a low response efficiency and unreliable repeatability for further applications. The good sensitivity and reversibility are in great demand in many practical applications, for instance, drug delivery in vivo.

The redox reaction has a typical property of high sensitivity and stoichiometry, which is a common phenomenon in organisms.7 Typical applications of the redox sensitivity involve ferrocene, disulfide, and tetraethylenediamine as the redox centers.9 Ferrocene (Fc) is an organometallic compound that is well known for its redox properties, stability, and synthesis convenience.10 The redox properties of Fc have been utilized for electrochemical materials, sensors, and even molecular motors.11 Previous work concerning redox-sensitive MCs has been reported by Vanco and co-workers, who assembled redox-sensitive poly(ferrocenylenilsilane) (PFS) into LBL MCs, allowing dextran to penetrate the shell after oxidation.9 However, this kind of MC failed to complete the reversible redox process because of the unstable structure after oxidation.

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(b) Sukhishvili, S. A. Curr. Opin. Colloid Interface Sci. 2005, 10, 37.
Therefore, in this work we design an easy MC fabrication method consisting of a single polyelectrolyte and ferrocene derivative, which warrants a stable structure and reversibility during the redox process (Scheme 1).

The fabrication proceeds very easily under mild conditions. An −NH$_2$-containing polyelectrolyte, poly(allylamine hydrochloride) (PAH), is first doped into the CaCO$_3$ particles, which are reacted with ferrocenecarboxaldehyde (Fc-CHO) to form the covalent linkage after NaBH$_4$ reduction. Removal of the CaCO$_3$ template yields the hollow MCs composed of only Fc-modified PAH (PAH-Fc). Modification with Fc-CHO on the PAH-doped CaCO$_3$ particles stabilizes the PAH on the template and thereby maintains the complete structure after template removal. The PAH-Fc MC shows high size and permeability sensitivity to chemical redox agents. Controllable loading and release triggered by the redox potential is also demonstrated by using a polysaccharide, dextran.

**Experimental Section**

**Materials.** Poly(allylamine hydrochloride) (PAH, $M_n \approx 70$ kDa), fluorescein isothiocyanate-labeled PAH (FITC-PAH, $M_n \approx 70$ kDa), tetramethylrhodamine isothiocyanate-labeled dextran (4.4 kDa), ferrocene carboxaldehyde (Fc-CHO), calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O), sodium carbonate (Na$_2$CO$_3$), sodium borohydride (NaBH$_4$), ethylenediaminetetraacetic acid (EDTA), iron(III) chloride hexahydrate (FeCl$_3$·6H$_2$O), potassium iodide (KI), ammonium cerium(IV) nitrate ((NH$_4$)$_2$Ce(NO$_3$)$_6$), sodium thiosulfate (Na$_2$S$_2$O$_3$), pyrene, and glutaraldehyde (GA) were all purchased from Sigma-Aldrich. Other chemicals were used as purchased.

**Fabrication of PAH-Doped CaCO$_3$ Microparticles.** PAH or FITC-PAH was dissolved in 100 mL of a 0.33 M calcium nitrate solution in a beaker under magnetic agitation (∼600 rpm), into which an equal volume of 0.33 M sodium carbonate solution was rapidly poured at room temperature. The final PAH concentration was adjusted to between 0.4 and 8 mg/mL. After 20 min, the precipitated PAH-doped CaCO$_3$ particles were centrifuged and washed three times to get rid of the free PAH and salts.

**Fabrication of PAH-Fc MCs.** The as-prepared PAH-doped CaCO$_3$ microparticles were dispersed in ethanol and mixed with excess Fc-CHO, which was also dissolved in ethanol. After the mixture was maintained in a vessel under mild agitation for 2 h, centrifugation and washing by ethanol were conducted several times until the excess Fc-CHO was rinsed off. Excess NaBH$_4$ was added to reduce the Schiff base. Finally, the Fc-modified CaCO$_3$ microparticles were incubated in 0.2 M EDTA solution for 15 min under shaking to obtain the MCs (PAH-Fc MCs), which were further washed with fresh EDTA solution and water, each for three times, using the centrifugation protocol (2000 g, 3 min). The Fc degree of substitution was determined by atom absorption spectroscopy.

**PAH-Fc MC Oxidation and Reduction.** A 1 mL suspension of PAH-Fc MCs (the concentration of MC was about 5 × 10$^7$ MCs/mL) was mixed with a 0.2 mL solution of oxidizer such as FeCl$_3$ or (NH$_4$)$_2$Ce(NO$_3$)$_6$ with a concentration of 0.2–2 mM. The reduction treatment of MC was carried out by the same method with KI or Na$_2$S$_2$O$_3$ solution with a concentration of 0.2–2 mM. Other tests were carried out after 10 min of redox treatment.

**Redox-Controlled Loading and Release.** A 1 mL suspension of PAH-Fc MCs was first mixed with 2 mL of a 2 mg/mL TRITC-dextran solution. Then the suspension was incubated in (NH$_4$)$_2$Ce(NO$_3$)$_6$ solution for 3 h and reduced with a Na$_2$S$_2$O$_3$ solution, followed by three washings in water. The release was carried out using the same redox treatments as described above.

**Calculation of Dextran Concentration in MCs.** MCs with (5.2 ± 0.8) × 10$^5$ capsules were mixed with excess of TRITC-dextran solution to a final concentration of 2 mg/mL. After oxidation treatment, the MC suspension was centrifuged at 3500 rpm for 10 min. The supernatant (300 μL) was diluted to 3 mL with water and measured with a UV−vis spectrophotometer at a wavelength of 556 nm. The concentration of dextran in the supernatant was calculated according to $C = (V_i C_i - V_c C_c)/V_c$, where $C$, $C_i$, and $C_c$ represent the dextran concentration in MCs, the dextran feeding concentration, and the dextran concentration in supernatant after incubation, respectively. $V_i$, $V_c$, and $V_c$ refer to the volumes of feeding dextran, the supernatant, and the MCs, respectively. The size of the MCs was measured statistically under confocal laser scanning microscopy with at least 200 capsules, by which $V_i$ was calculated.

**Calculation of Diffusion Constant of Dextran in MCs.** After the loading of dextran as mentioned above, the MCs suspension was centrifuged at 3500 rpm for 10 min to remove the supernatant. The precipitated capsules were washed with water and then suspended in 1 mL water, to which 1 mL of an oxidant solution with a concentration of 0–2.5 mM was added. A 1 mL quantity of the supernatant was removed after incubation for a desired period of time, and the concentration was measured by UV−vis spectrophotometry at 556 nm by referring to a calibration curve. The cumulative released amount was integrated from each time point. Each value was averaged from 10 parallel experiments.

**Characterization.** Transmission electron microscopy (TEM) images were obtained with a Zeiss EM 912 Omega microscope at
Figure 1. (A–C) SEM and (D) CLSM images of PAH-doped CaCO₃ microparticles. C is a magnified image recorded at the place shown by the rectangular box in B. The inset in D is a higher-magnification image.

an acceleration voltage of 120 kV. Scanning electron microscopy (SEM) images were taken with a Gemini Leo 1550 microscope at an acceleration voltage of 3 kV. Confocal laser scanning microscopy (CLSM) images were taken on a LEICA TCS system (Aristoplan, Germany, 100× oil immersion using commercial software). The samples were prepared by casting the as-prepared suspension of CaCO₃ microparticles and MCs onto copper grids with a carbon film (for TEM), silicon wafers (for SEM), and glass slides (for CLSM) and air dried. The ultramicrotomy of PAH-Fc MCs was carried out by embedding an MC ethanol suspension in epoxy resin and cutting. UV-visible spectrophotometer. Steady-state fluorescence spectra were recorded using a Fluoromax 4 spectrophotometer.

Results and Discussion

The PAH-doped CaCO₃ particles were prepared by rapidly mixing and stirring the Ca(NO₃)₂ solution and Na₂CO₃ solution in the presence of PAH. After centrifugation and washing, the microparticles were characterized by SEM. As shown in Figure 1A–C, these particles had an average diameter of 5.5 μm. They were not completely round spheres but ellipsoids with some notches and cracks. This irregular morphology is caused by the incorporation of positively charged PAH, which influences the growth of CaCO₃ crystallites compared to that of spherical particles of negatively charged PSS-doped CaCO₃. The particle surface was so rough that uniformly distributed tiny pores can be clearly identified by the magnified SEM image (Figure 1C). Apparently, the microparticles are built from smaller, nearly spherical building crystallites with a typical diameter of 40 nm. To investigate the distribution of doped PAH, FITC-PAH was used to fabricate the CaCO₃ particles. The CLSM image (Figure 1D, transmission channel in Figure S1) shows clearly that the fluorescence emission was stronger from the periphery than inside the CaCO₃ particles, demonstrating the spatial inhomogeneity of the PAH.

The existence of pores is advantageous for the subsequent reaction. In this study, the doped PAH in the CaCO₃ particles (the weight ratio of PAH/CaCO₃ was 4.8% for this study) was reacted with Fe-CHO to form a Schiff base structure that is further reduced by NaBH₄. Alteration of the chemical structures was confirmed by IR spectra (Figure S2), showing that the aldehyde peak at 1678 cm⁻¹ of Fe-CHO disappeared and that the ferrocene peak at 1620 cm⁻¹ appeared in PAH-Fc MCs. The Fc degree of substitution of PAH in the MCs was calculated to be 16% by atomic absorption spectroscopy. However, the degree of substitution reached only 10.5% in PAH-Fc synthesized from solution (Figure S3). Hence, the higher Fc degree of substitution in the CaCO₃ particles indicates the higher efficiency of the Schiff base reaction, which should be attributed to the different status of the PAH molecules. In the porous particles, the PAH molecules anchored on the crystallite surface should be exposed to the microchannels between the crystallites, which facilitate the approach and reaction of the Fe molecules. Moreover, the PAH-Fc molecules precipitate in solution when the degree of substitution increases, which restricts further reaction. This phenomenon is remarkably alleviated in the CaCO₃ particles because PAH and its segments are in an anchorage state.

By removal of the sacrificial CaCO₃ particles with EDTA, the MCs were obtained as a result of the aggregation of hydrophobic ferrocene and the rearrangement of the hydrophilic PAH chains. The MCs collapsed only in the middle because of the very thick shells, leading to the concave morphology (Figure 2A). This phenomenon is very typical for soft polymeric capsules with a thick shell. EDX analysis detected iron but not calcium within the MCs, confirming the complete removal of the template (Figure S4). MC cross-sectional TEM images obtained by ultramicrotomy displayed distinguishable lumens and ultrathick shells (∼1.6 μm) (Figure 2B). Quantitative analysis showed that less than 5% of PAH was released from the MCs during the template-removal process, which explains the remnant thick shells. Some

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materials were also found inside the MCs, especially the concentric circles as indicated by the arrows (Figure 2B). This special distribution of PAH-Fc in the MCs could also be found in the CLSM (Figure 2C,D). The high efficiency of remained PAH-Fc within the shells and its special distribution in the MCs are useful in the self-enhancement of the capsule structure and the loading of desired substances to a larger extent.

The PAH-Fc MCs consist of only a single polyelectrolyte modified by hydrophobic Fc, which is different from the typical multilayer MCs known to be stabilized by electrostatic and/or other types of interactions. Although theoretically no covalent bonds are formed between the PAH chains, the MCs could be stably stored in water for more than 3 months without any change. Therefore, we assume that the stability of the PAH-Fc MCs results from the hydrophobic aggregation of Fc as the hydrophobic microphase (cross-linking point) under the protection of hydrophilic PAH chains. To confirm this special structure of the PAH-Fc MCs, fluorescence spectroscopy of pyrene was applied to investigate the polarity change in the MCs. As verified by hundreds of experiments, the intensity ratio of peak 3 and peak 1 (I$_3$/I$_1$) from pyrene monomer fluorescence emission peaks reflects the environment polarity. In our experiment (Figure 3A), with increasing volume of the PAH-Fc MC suspension, the absolute intensity decreased and the I$_3$/I$_1$ ratio increased as a result of the transition of the pyrene molecule from a polar solvent, water, to the hydrophobic Fc microphase in MCs. As a comparison, PAH-glutaraldehyde (PAH-GA) MCs in which PAH in the CaCO$_3$ particles was cross-linked by GA were also fabricated. Because this control sample did not have hydrophobic domains, the I$_3$/I$_1$ ratio showed no polarity change after pyrene was mixed with different quantities of PAH-GA MCs (Figure 3B).

This unique chemical constituent and the fabrication method ensure the peculiar redox-responsive properties of the PAH-Fc MCs. Fc, as a hydrophobic part, can maintain the MC structure in water. However, this hydrophobic interaction can be easily tuned because of the excellent redox properties of Fc so that the PAH-Fc MCs can display size and permeability changes. To study the response of the PAH-Fc MCs to the redox stimuli, ferric chloride (FeCl$_3$) and potassium iodide (KI) as the oxidant and reducing agent pairs were first used because of their highly efficient redox properties and water solubility. UV-vis spectroscopy was used to characterize the redox reaction (Figure 4). The light-yellow, pristine PAH-Fc MCs showed an absorption peak at 440 nm. When an aqueous FeCl$_3$ solution (1 mM, pH 4, adjusted by HCl) was added to the MC suspension, the oxidized Fc (ferrocenium cation, Fc$^{+}$) peak at 630 nm appeared with a slight color change from yellow to green (Figure 4A). When the concentration of FeCl$_3$ increased gradually to 2 mM, the Fc molecules in the MCs were completely oxidized, showing a strong peak at 630 nm and dark-green MCs. After the FeCl$_3$ solution was washed away and following the addition of KI solution to the MC suspension, the Fc$^{+}$ peak gradually decreased and the Fc peak at 440 nm increased again, accompanying by the color change from green to light yellow (Figure 4B). These results demonstrate the reversible redox sensitivity of the PAH-Fc MCs, which responds

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to even very low concentrations of chemicals. It is also worth mentioning that the redox color change could be repeated for at least three cycles.

The redox reversibility was characterized not only by the color change but also by the capsule swelling and shrinkage. At a first glance, we realized that the volume of the MCs was enlarged almost 2-fold in the oxidation state (digital camera pictures in the insets of Figure 4). While the Fe moieties form stable hydrophobic aggregates in the MCs in the reduction state, Fe\(^{+}\) with a positive charge decreases the hydrophobic interaction. The positive charge of the PAH backbone also introduces the repulsion into the MCs. With the combination of these two effects, the PAH-Fc MCs swell after oxidization. In contrast, reduction removes the positive charge of the Fe\(^{+}\), leading to re-forming of the hydrophobic aggregation of Fe and thereby the shrinkage of the PAH-Fc MCs. As shown in Figure 5, the swelling and shrinking process of the PAH-Fc MCs could be reversely repeated for at least three cycles regardless of the redox agent pairs. Taking the (NH\(_4\))\(_2\)Ce(NO\(_3\))\(_6\)/Na\(_2\)S\(_2\)O\(_3\) pair as a typical example, we found that the diameter of the PAH-Fc MCs changed from 5.5 to 11.7 μm. After three cycles of treatment, the MCs maintained their spherical morphology and good dispersion as representatively shown in Figure 5. In addition, the MC size could be tuned in small steps if the concentration of the redox agent increased gradually (Figure 6). In a control experiment, no apparent expansion of the PAH-Fc capsules in a solution of pH 4 (the pH for the capsule swelling is surely caused by oxidization but not by the charge repulsion of the shells at low pH. It is unusual that the structure of the PAH-Fc MCs could be maintained for at least 2 to 3 days even after the oxidation of Fe to a large extent. The intrinsic reason is not clear yet, but it is likely that Fe\(^{+}\) remains more or less hydrophobic and thereby holds the aggregation domains, which keep the macroscopic shape of the oxidized PAH-Fc MCs. These results reveal that the PAH-Fc MCs can not only respond to redox stimuli very quickly but also maintain the intact MC structure during the swelling and shrinking process, which is of great importance and convenience in drug loading and release.

By utilizing the redox-responsiveness of the PAH-Fc MCs, controllable loading and release of various substances such as biopolymers, enzymes, DNAs, and drugs can be conveniently realized. Taking rhodamine-labeled dextran (TRITC-dextran, 4.4 kDa) as a typical example, its loading and release are demonstrated in Figure 7. In the original reduced state, the MCs were in a shrunken state and did not permit the TRITC-dextran molecules to penetrate their interiors (Figure 7A). Hence, their interiors remained dark compared to the bulk solution. When the oxidant FeCl\(_3\) solution was added, as a result of the swelling the capsule walls were in an open state to permit the diffusion of the TRITC-dextran molecules (Figure 7B). By reducing this MC suspension in Na\(_2\)S\(_2\)O\(_3\) solution, the MCs shrunk once again to close the channels and thereby entrapped the TRITC-dextran molecules, as illustrated by the stronger emission from the capsule interiors (Figure 7C). Finally, the loaded TRITC-dextran could be released by incubating the MCs in FeCl\(_3\) solution (Figure 7D). After the final reduction, the MCs could be once again applied to the loading and release process. Controlled by permeation alteration of the MC walls, this loading and release could be versatile for various molecules.

It is known that the controlled release of medicine from containers in response to the intensity of stimuli is in high demand for in vivo applications. On the basis of Fe redox stoichiometry, different release rates from the PAH-Fc MCs could be achieved as shown in Figure 8. Here, TRITC-dextran (4.4 kDa) was loaded into the PAH-Fc MCs following the procedures shown in Figure 7. The concentration of TRITC-dextran in the MCs was quantified to be 67.2 mg/mL with an initial feeding concentration of 5 mg/mL. Assuming that all of the dextran inside the MCs is maintained during capsule shrinkage (from 11.7 to 5.5 μm), the maximum concentration inside the microcapsules is about 48 mg/mL. The still higher concentration of 67.2 mg/mL would imply that the additional physical adsorption of dextran by the capsule walls also makes a great contribution.

The release of TRITC-dextran was sensitively mediated by the concentration of oxidant (Figure 8). It shows that along with the increase in the oxidant concentration the release rate and amount were significantly improved. For example, the MCs leaked less than 5% of their dextran after 12 h, indicating good storage properties. In 0.4 mM FeCl\(_3\), about 20% of the dextran was released within the same period of time. With a still higher concentration of FeCl\(_3\) such as 1 mM, 40% of the dextran was released in 2 h. Figure 8 shows also that a linear simulation could be applied to all of the release profiles in the early stage regardless of the oxidant concentration, indicating the diffusion-controlled nature. According to Higuchi’s equation, the apparent diffusion constants D for dextran were found to be 1.16 × 10\(^{-11}\), 4.35 × 10\(^{-10}\), 5.44 × 10\(^{-9}\), and 5.08 × 10\(^{-8}\) cm\(^2\)/s at FeCl\(_3\) concentrations of 0, 0.4, 1, and 2.5 mM, respectively. The diffusion constant D of dextran from the poly(ethylene oxide) (PEG) hydrogel was

reported to be on the order of $10^{-8}$ cm$^2$/s, which is similar to our data when the MCs were fully swollen. The change in oxidant concentration could effectively control the release rate of dextran from the PAH-Fc MCs. However, there was still more than 40% of the dextran that could not be released within 1 h even at the highest FeCl$_3$ concentration. It is believed that the unreleased dextran should stably interact with the ultrathick shells of the PAH-Fc MCs via hydrogen bonding and other interactions, which is consistent with its excessively high concentration during loading.

Conclusions

Herein, PAH-Fc MCs were successfully fabricated by the reaction of Fc-CHO with PAH-doped CaCO$_3$ particles, followed by core removal with EDTA. The as-prepared MCs had ultrathick shells and a shell-in-shell structure. Their structure is stabilized by the hydrophobic microdomains of Fc (cross-linking points) and the protection of the hydrophilic PAH backbone. The PAH-Fc MCs could be oxidized and reduced by chemical redox agents as a result of the redox properties of Fc, even with a small trigger. During the redox process, the MCs could swell and shrink so that their permeability could be tuned accordingly. The reversible change in the size and permeability of the MCs was used for the controlled loading and release of dextran. The release rate could be precisely controlled by the stoichiometry of the Fc redox reaction. Moreover, the method can also be applied to the fabrication of other types of MCs with different properties and functionalities.

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Supporting Information Available: Optical image of PAH-doped CaCO$_3$. IR spectra of PAH-Fc MC in original, oxidized, and reduced states. EDX results of PAH-Fc MCs and other data. This material is available free of charge via the Internet at http://pubs.acs.org.