Photo-responsive polyethyleneimine microcapsules cross-linked by ortho-nitrobenzyl derivatives

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Abstract

Intelligent capsules are widely used as carriers for loading small molecules and particles for their capacity to respond to environmental stimuli. In this study, photo-responsive polyethyleneimine (PEI) microcapsules were fabricated using 4-bromomethyl-3-nitrobenzoic acid (BNBA) bearing a photodegradable ortho-nitrobenzyl group as a cross-linker. PEI-doped CaCO₃ particles were used as the sacrificial templates, in which the PEI molecules were cross-linked by BNBA molecules under the activation of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) and N,N-diisopropylethylamine (DIEA). After the removal of CaCO₃ particles by hydrochloric acid, the PEI–BNBA capsules were obtained. Since the C–N bond that formed via reaction of benzyl bromide and amine is photo-cleavable, the capsules could be decomposed under UV irradiation at 365 nm. The loaded macromolecules could be released upon UV irradiation, exhibiting the microcapsules' potential applications in the field of controlled release.

1. Introduction

Capsules are usually small sphere-like containers with core and shell regions. They are commonly used as carriers for drugs, proteins and peptides due to their suitable stability, tailored properties, and large inner space separated from the environment [1–3]. Capsules can be ingested naturally by a variety of mammalian cells and can be applied in fields such as drug delivery, biosensor and bioreactor [4–6]. Intelligent capsules are particularly important for controlled release by utilizing the alterations in terms of size, shell morphology and permeability [7]. In past decades, capsules that can respond to stimuli, such as pH, redox, carbohydrates, and enzymes [8–11], have been developed by incorporating various functional components into their structures. However, most of them are unable to be used in vivo given the limited change range of the pH, temperature, and ionic concentration of human body. Moreover, for most intrinsic stimuli such as hydrolysis and enzymolysis, the properties of the capsules cannot be regulated externally once they enter the cells. In comparison, light offers...
many advantages over other stimuli, as it can be used to achieve real-time spatiotemporal control with high precision in a minimally invasive manner when a suitable wavelength is used.

Photo-responsive materials based on different kinds of photolabile groups have been widely studied [12–15]. For example, Tyler [16] reported photodegradable polymers containing metal–metal bonds along the polymer backbones. The polymer ruptures upon the irradiation of visible light. Utilizing the photo-induced trans–cis isomerization of azobenzene, photo-switchable capsules based on the supramolecular interaction of azobenzene and α-cyclodextrin was reported by Zhang et al. [17]. As the result of the different binding ability of these two isomers to α-cyclodextrin, the capsules dissociated after 80 min of irradiation of UV light. Among the photolabile molecules, ortho-nitrobenzyl derivatives, which were first reported by Schofield and co-workers [18], have been widely used in designing photo-responsive systems [19]. The photolysis mechanism is an intra-molecular rearrangement process [20–22]. Hydrogels, micelles, nanoparticles as well as capsules [23–25] utilizing this chemical group are of considerable interest in the applications of drug delivery, biosensor research, and tissue engineering in the past decades. Recently, Prez et al. [26] prepared polyurea capsules with a photocleavable shell. A 6-nitro-veratroyloxycarbonyl based diisocyanate oligomer was synthesized and utilized in the preparation of capsules. The capsules could be dissociated under the trigger of UV light, leading to the release of encapsulated cargos.

Here we introduce a simple method to fabricate photo-responsive capsules by utilizing the ortho-nitrobenzyl derivative 4-bromomethyl-3-nitrobenzoic acid (BNBA) as a cross-linker. This differs from those previous methods that require complicated synthesis processes [24,26,27]. BNBA can diffuse into the polyethyleneimine (PEI)-doped CaCO₃ particles, allowing their carboxyl and benzyl bromide groups to react with the amine groups of PEI to form the cross-linking points. After core removal by hydrochloric acid, PEI–BNBA capsules were obtained. Irradiating the capsules with UV light (365 nm) cleaved the cross-linking points, leading to the decomposition of capsules (Scheme 1). During irradiation, the chemical and physical properties of the capsules were investigated. Photo controllable release was also demonstrated by using rhodamine B isothiocyanate labeled (RBITC)-dextran as a model cargo.

2. Experimental section

2.1. Materials

Polyethyleneimine (PEI, branched, Mw ~ 25 kDa), rhodamine B isothiocyanate (RBITC), and rhodamine B isothiocyanate-labeled dextran (RBITC-dextran, Mw ~ 55 kDa) were purchased from Sigma–Aldrich. 4-Bromomethyl-3-nitrobenzoic acid (BNBA) was purchased from Acros. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-me thylmorpholinium chloride (DMT-MM) and N,N-diisopropyl-ethylamine (DIEA) were obtained from Shanghai Aladdin Co., Ltd. Sodium carbonate anhydrous (Na₂CO₃), calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O), sodium bicarbonate (NaHCO₃) and hydrochloric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. The water used in all experiments was prepared via a Millipore Milli-Q purification system and had a resistivity higher than 18 MΩ cm⁻¹. PEI-doped CaCO₃ particles were prepared by mixing Na₂CO₃ and Ca(NO₃)₂·4H₂O solutions containing PEI according to literatures [28,29]. Briefly, PEI (200 mg) was dissolved in 50 mL Ca(NO₃)₂ solution and mixed with an equal volume of Na₂CO₃ solution under magnetic agitation (1200 rpm) for 1 min at room temperature. Then the particles were washed 3 times with water and ethanol, respectively.

2.2. Fabrication of PEI–BNBA microcapsules

The as-prepared PEI-doped CaCO₃ particles were reacted with BNBA to prepare microcapsules. The particles (150 mg) were dispersed in 5 mL BNBA (3.18 mg dissolved in methanol) solution, into which 2.2 μL DIEA was added to activate the reaction between BNBA and PEI. Due to the reaction between benzyl bromide and amine groups, BNBA was linked to PEI. After mild agitation for 12 h at room temperature, the sample was washed by methanol to remove free BNBA. The particles were dispersed in 5 mL DMT-MM (3.72 mg dissolved in methanol) solution for another 12 h to allow the reaction between carboxyl groups of BNBA and amine groups of PEI to form the cross-linking structure. The molar feeding ratio of the reagents (PEI:BNBA:DIEA:DMT-MM) was 8:1:1:1.1 [30]. Centrifugation and washing by methanol was conducted several times. For better observation, the capsules were labeled by

![Scheme 1. Fabrication and decomposition process of PEI–BNBA capsules.](image-url)
RB1TC. In brief, after cross-linked with BNBA, the particles were incubated in 1 mg/mL RB1TC Na2CO3/NaHCO3 buffer solution (pH 9.4), and the system was maintained in dark over night. Finally, the as-prepared particles were incubated in 0.1 M HCl solution for 15 min under shaking to remove the CaCO3 template. The obtained PEI–BNBA capsules were further washed with water until neutral, and dispersed in water in dark at room temperature.

2.3. Photodecomposition of PEI–BNBA microcapsules

The photodecomposition process of PEI–BNBA capsules was observed by fluorescence microscopy in situ. The capsules were dispersed in phosphate-buffered saline (PBS 0.01 M) with pH values ranging from 2 to 7 (105 capsules/mL). 20 μL capsules suspension was placed into the tiny well on a perforated glass slide, and then the photodecomposition was observed in situ by fluorescence microscopy (Zeiss Axiovert 200) equipped with a 100× oil immersion objective. During irradiation, the light of microscopy was cut off and the UV lamp (365 nm, UVEC-4 II system) was placed vertically above the sample with a distance of 40 mm (450 mW/cm²). The fluorescence images were captured under the excitation of 546 ± 12 nm after UV irradiation for different time.

2.4. Loading and photo-controlled release

Unlabeled PEI–BNBA capsules were incubated in 2 mg/mL RB1TC-dextran solution for at least 2 h. After being washed until free RB1TC-dextran was totally removed, the capsules were dispersed in 0.01 M PBS (pH 3.5). 300 μL capsules suspension (the concentration of capsules was about 6.0 × 107 capsules/mL) was placed in a well of a 96-well culture plate and irradiated for 5 min (450 mW/cm²). The control group was not irradiated. CLSM images of the capsules before and after irradiation for 5 min were obtained by TCS SP5 confocal laser scanning microscopy (Leica, Germany) at a heating rate of 20 °C/min in nitrogen atmosphere from 50 °C to 900 °C. Scanning force microscopy (SFM) images were obtained using a scanning force microscope from Seiko Instruments (SFM, SPI3800N Probe Station and SPA400 SPM Unit) at room temperature in a dynamic force mode. A drop of the capsules suspension was applied onto a freshly cleaned silicon slide and dried overnight at room temperature.

3. Results and discussion

3.1. Fabrication of PEI–BNBA microcapsules

The PEI-doped CaCO3 particles were used to react with BNBA and obtain photo-degradable capsules after core removal (Scheme 1). The particles were prepared by mixing the Ca(NO3)2 solution and Na2CO3 solution in the presence of PEI. After centrifuging and washing, the particles were characterized by SEM. As shown in Fig. 1(A), these particles were spherical with an average diameter of 5.4 ± 0.6 μm. Their surface was quite rough, and was built by smaller crystallites with a typical diameter of 40 nm (Fig. 1(A), inset). The weight ratio of the PEI doped in the particles was 2.8%, determined by thermal gravimetric analysis (Fig. 2(A)). The amine groups of PEI can react with the carboxyl and benzyl bromide groups of BNBA to form the cross-linking points. First, the benzyl bromide group of BNBA reacts with an amine group of PEI to form the photo-cleavable C–N bond. Then, under the activation of DMT-MM, carboxyl group of BNBA reacts with an amine group of PEI to form amide linkages. After the removal of CaCO3 particles by HCl, photo-responsive capsules were obtained. The capsules with a diameter of 4.7 ± 0.5 μm were smaller than the template (Fig. 1(B)). The capsules were not hollow, with more PEI molecules near the periphery and a loose network in the middle (Fig. 1(B), inset). This phenomenon may result from the inhomogeneous distribution of PEI inside the CaCO3 particles [28].

![Fig. 1](image_url) SEM images of PEI-doped CaCO3 particles (A) and PEI–BNBA capsules (B). The insets in A and B are the magnified SEM image of the particle surface, and the TEM image of the ultrathin section of capsules, respectively.
Alteration of the chemical structure was confirmed by FTIR. In Fig. 2(B), the strong band attributed to the N–H stretching vibration, influenced by hydrogen bonds [31] of PEI can be observed at 3248 cm\(^{-1}\). After BNBA cross-linking, this peak blue-shifted to 3428 cm\(^{-1}\), suggesting the disappearance of hydrogen bonding as steric hindrance increased. This may also account for the red-shift of the band at 1583 cm\(^{-1}\) as the hydrogen bonding has the effect to shift the N–H deformation bands to high frequencies [32,33], which depends on the strength of the hydrogen bond. A new absorbance peak appeared at 1635 cm\(^{-1}\), which can be attributed to the amide bonds and aromatic ring of PEI–BNBA capsules. This result indicates the successful cross-linking of the capsules. According to the result of elemental analysis, the C/N molar ratio of the capsule was 2.54. As the C/N molar ratio of PEI and BNBA is 2 and 8, respectively, the cross-linking ratio can be calculated as about 19.6%.

### 3.2. Photodecomposition of PEI–BNBA microcapsules

Since the C–N bond formed by benzyl bromide and amine groups is photo-cleavable [25], the cross-linking points are expected to be cleaved upon UV irradiation (365 nm), resulting

![Fig. 2] A Thermogravimetric analysis curve of PEI-doped CaCO\(_3\) particles recorded at a heating rate of 20 °C/min in nitrogen atmosphere. (B) FTIR spectra of PEI and PEI–BNBA capsules.

![Fig. 3] Snapshots of the photo-decomposition of PEI–BNBA capsules after UV irradiation for different time as noted in the figure in pH 3.5 buffer, scale bar, 10 µm (A–C). (D) TEM image of decomposition products of PEI–BNBA capsules.
in the decomposition of the capsules. The dissociative behavior of capsules was initially observed by microscopy in situ. However, the capsules did not show obvious change after irradiation in water. Then the photo-responsive behavior of the capsules was monitored in 0.01 M PBS with pH values ranging from 2 to 7. In the pH range of 2–4, the capsules were decomposed completely after UV irradiation within a short period of time (10 min). However, in the pH range of 4–6, the capsules did not show obvious change in the first a few minutes. When the irradiation time was prolonged to 30 min, about 70% of the capsules were decomposed. In the pH range of 6–7, almost no decomposition of capsules was observed.

Considering the mechanism of photolysis (Scheme 1) [20–22],
the aldehyde groups are generated after irradiation. In addition, they may further react with amino groups of PEI to form Schiff base bonds, which may serve as the new cross-linking points and prevent the decomposition of the capsules. Since the hydrolysis rate of Schiff base increases with the decrease of pH [34,35], the capsules can be decomposed more easily at a lower pH. Moreover, the protonation state of PEI may also affect the responsive behavior of the capsules. In acidic condition, electrostatic repulsion between protonated amino groups may also contribute to the expansion, and thereby facilitate the decomposition of capsules.

Due to the quick photo-response, the capsule suspension at pH 3.5 was selected for further study. As shown in Fig. 3, upon UV irradiation, the capsules began to swell. After 5 min, their size increased by about 30% (Fig. 3(B)). The capsules were decomposed completely after 10 min (Fig. 3(C)). During irradiation, the fluorescence intensity of the background increased as the decomposition of capsules resulted in re-dispersion of more and more RBITC-labeled PEI chains in the solution. Although the fluorescence microscopy showed that the capsules were decomposed thoroughly after irradiation for sufficiently long time (Fig. 3(C)), TEM observation of the decomposition products revealed some irregular aggregates with a size of tens of nanometers (Fig. 3(D)). The formation of these aggregates may be due to the incomplete cleavage of cross-linking points. Even though UV irradiation can cleave cross-linking points formed by benzyl bromide and amine groups thoroughly, the hydrophobic BNBA are still linked to the side chain of PEI by the amide groups, which may induce the aggregation of the BNBA-linked PEI in aqueous solution.

Although the capsules were not completely decomposed after 5 min irradiation, they indeed swelled and the structure of the capsules changed significantly. Before irradiation the capsules were not hollow, with a loose network-like structure in the cavity and a thicker shell (Fig. 4(A)). After irradiation, the cross-linking points are cleaved gradually and the amine groups are re-exposed, which may attract counter ions and increase the local osmotic pressure, resulting in the swelling and expansion of the capsules. Thus the shell of the capsules became looser, which may increase the permeability of the capsules. The cleaved PEI molecules could diffuse out and the capsules changed gradually from “non-hollow” to “hollow” (Fig. 4(B)). Expansion of capsules and release of loaded components also cause a thinner shell. The shell thickness decreased from 54.6 ± 4.6 nm to 12.3 ± 0.8 nm after irradiation (Fig. 4(C), (D)).

3.3. Photo-controlled release of loaded RBITC-dextran

The structure and shell thickness of the capsules can be changed by irradiation, which in turn would significantly influence the permeability of the capsules. Therefore, this kind of capsules is candidates for intelligent carriers. Here, using RBITC-dextran as a model, the loading and photo-controlled release capacity of the capsules was investigated. As shown in Fig. 5(A), after 2 h incubation, RBITC-dextran diffused into the capsules and was entrapped. The network-like structure inside capsules as well as the hydrogen bonding interaction between dextran and the network may contribute to the entrapment of RBITC-dextran. Before irradiation the fluorescence intensity inside the capsules was much higher than that of the background, indicating the accumulation of RBITC-dextran inside the capsules. Upon irradiation, the network-like structure inside capsules is cleaved. Due to the electrostatic repulsion, the detached PEI segments and RBITC-dextran diffuse out of the capsules through the thinner and looser capsule wall. Therefore, the fluorescence intensity inside the capsules decreased significantly after irradiation (Fig. 5(B)). The release of RBITC-dextran was further confirmed by the fluorescence spectra recorded for the supernatants, in which the irradiated one had significantly stronger emission than that of the virgin one (Fig. 5(C)).

4. Conclusion

Photo-responsive microcapsules were fabricated by utilizing an ortho-nitrobenzyl derivate BNBA as a cross-linker to cross-link PEI doped in CaCO₃ microparticles. After the removal of the CaCO₃ particles, photo-responsive capsules were obtained, which could be degraded under UV irradiation. During the irradiation, capsule swelling was found, which is caused by cleaving of the cross-linking points. The capsules changed gradually from “non-hollow” to “hollow” as confirmed by TEM, accompanying with the decrease of shell thickness. Finally, the capsule decomposed, with only small PEI fragments remained in solution. The swelling of the microcapsules in response to UV irradiation resulted in a faster release of RBITC-dextran. Compared with previous work of photo-responsive microcapsules, the method used here is much simpler but quite effective. And the physicochemical changes of the system during the response were thoroughly studied, which is important for understanding and using similar system to achieve their applications. Such a system can potentially be used as carriers to realize stimuli-responsive release of loaded cargos. In the further research the efficiency of response under physiological conditions should be further studied and improved to make the capsules more suitable for biomedical applications.

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References
