Communication

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Immobilization and Intracellular Delivery of an Anticancer Drug Using Mussel-Inspired Polydopamine Capsules

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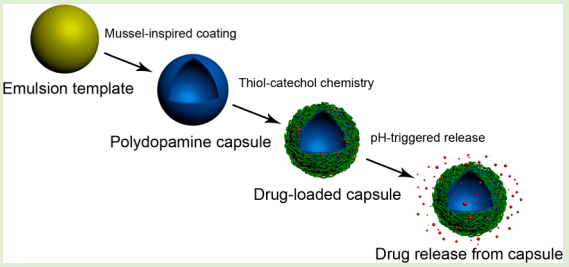
Supporting Information

ABSTRACT: We report a facile approach to immobilize pH-cleavable polymer-drug conjugates in mussel-inspired polydopamine (PDA) capsules for intracellular drug delivery. Our design takes advantage of the facile PDA coating to form capsules, the chemical reactivity of PDA films, and the acid-labile groups in polymer side chains for sustained pH-induced drug release. The anticancer drug doxorubicin (Dox) was conjugated to thiolated poly(methacrylic acid) (PMA_{SH}) with a pH-cleavable hydrazone bond, and then immobilized in PDA capsules via robust thiol-catechol reactions between the polymer-drug conjugate and capsule walls. The loaded Dox showed limited release at physiological pH but significant release (over 85%) at endosomal/lysosomal pH. Cell viability assays showed that Dox-loaded PDA capsules enhanced the efficacy of eradicating HeLa cancer cells compared with free drug under the same assay conditions. The reported method provides a new platform for the application of stimuli-responsive PDA capsules as drug delivery systems.



INTRODUCTION

Polymer capsules have attracted great interest for drug and gene delivery.[1−3] To achieve controlled and targeted drug release, polymer capsules should be functionalized with various functional moieties, such as targeting ligands,[4−6] and require triggered release mechanisms to respond to intracellular stimuli,[7,8] such as pH,[9] redox reactions,[10] and enzymes.[11] The pH-triggered release strategy is of particular interest for intracellular delivery, as carriers are subject to acidification when internalized from the slightly alkaline extracellular conditions into an acidic environment (pH 5−6) inside endosomal and lysosomal compartments.[12−15] However, the development of facile and robust strategies for the formation of pH-sensitive polymer capsules remains challenging.[16,17]

Recently, inspired by the adhesive properties of mussel proteins the Messersmith group introduced a facile approach to form polydopamine (PDA) films on a wide range of substrates.[18] The obtained PDA films can further undergo reactions with functional groups, including thiols and amines, via Michael addition or Schiff base formation. These films have been used for surface modification (e.g., PEGylation) and the immobilization of biomolecules (e.g., protein and DNA).[19−21] In addition, dopamine analogues and polymer-catechol conjugates have been widely investigated for surface modification and related applications.[22,23] The advantage of PDA modification lies in its ease, convenience, substrate-independence and chemical reactivity.[24−26] Furthermore, PDA shows excellent biocompatibility and low cytotoxicity, making it a versatile platform for bioapplications.[27−29] In our previous work, we applied the mussel-inspired chemistry on both solid and liquid templates to prepare PDA capsules with tunable diameters and wall thicknesses.[29−31] The PDA capsules did not significantly affect cell viability and were used to encapsulate anticancer drugs, which make PDA capsules promising candidates for drug delivery. However, for tailored drug delivery systems, a triggered response to biological stimuli is required. This is challenging in PDA systems as they are typically highly robust and nonresponsive. Although there has been some work on stimuli-responsive catechol polymers for delivery of anticancer drugs,[13] research on stimuli-responsive PDA-based drug delivery systems is limited. Therefore a strategy to combine the facile and robust assembly of PDA capsules with stimuli-responsive properties would improve the potential of these materials for drug delivery and related applications.

In this study, we report a facile approach to immobilize a pH-cleavable polymer-drug conjugate within PDA capsules for intracellular drug delivery (Scheme 1). The polymer−drug conjugate was synthesized via thiol-maleimide chemistry using thiolated poly(methacrylic acid) (PMA_{SH}) and a maleimide hydrazone derivative of doxorubicin (MAL-Dox). The hydrazone bond is suitable for use in such conjugates, as it is stable at physiological pH but readily degrades at acidic pH, such as those found in endosomal compartments within cells. The polymer−drug conjugate was loaded in PDA capsules via well-known thiol-catechol reactions between the polymer−drug

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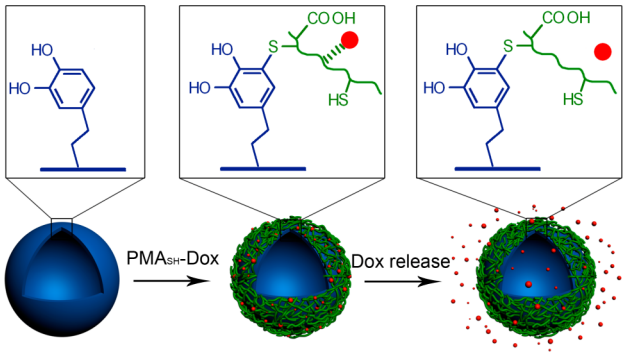
Scheme 1. Immobilization and pH-Responsive Release of Dox from PDA Capsules[a]



[a]The red dots represent Dox.

conjugate and capsule walls. These drug-loaded PDA capsules showed pH-responsive drug release behavior and enhanced anticancer efficacy compared with free drugs. Our strategy is based on the facile PDA coating to form capsules, the chemical reactivity of PDA films and the acid-labile groups in the polymer side chains to result in drug release. Furthermore, we have developed a method for the fluorescent labeling of PDA capsules to facilitate tracing the drug carrier systems in cancer cells, circumventing the broad-band absorption of PDA in the ultraviolet and visible light regions.[32,33] This approach is a general strategy to design pH-responsive PDA capsules that are expected to have potential in drug and gene delivery.

RESULTS AND DISCUSSION

For the immobilization of the anticancer drug, we designed a pH-cleavable polymer-drug conjugate that promotes drug release at acidic pH. First, MAL-Dox with a hydrazone bond and PMA_{SH} with 13.5% thiol-modification were synthesized (see Supporting Information). Next, MAL-Dox was conjugated to the PMA_{SH} via thiol-maleimide chemistry to form polymer–drug conjugates (PMA_{SH}-Dox, Figure S1). The degree of functionalization was characterized by using the absorbance (λ = 480 nm) of PMA_{SH}-Dox and a Dox standard curve, and corresponded to 4 mol % modification, leaving 9.5 mol % of free thiol groups on the polymer backbone (see Supporting Information).

Monodisperse PDA capsules with different sizes were prepared using emulsion droplets as templates according to our published method.[30] The mechanism of PDA formation is shown in Figure S2. It has been previously reported that PDA films exhibit high reactivity toward thiol groups at alkaline pH.[34,35] PDA capsules were incubated with the PMA_{SH}-Dox (9.5% of thiol modification) at pH 8, which resulted in the immobilization of the conjugate via Michael addition between thiol and quinone groups (Scheme 1). Both transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed PDA capsules remained intact with typical creases and folds after Dox loading (Figure S3). The average size of PDA capsules obtained from dynamic light scattering (DLS) measurements was 300 nm, which did not change significantly after Dox loading (Figure S3c).

Since Dox was conjugated to the PMA_{SH} by an acid-labile hydrazone bond, it should exhibit pH-responsive release. To investigate this, the release of Dox from PDA capsules (300 nm in diameter) was monitored in buffered solutions at pH 7.4, 6.0, and 5.0, mimicking the physiological pH in extracellular space, subcellular endosomes, and lysosomes, respectively. As shown in Figure 1, the fraction of Dox released from the PDA capsules

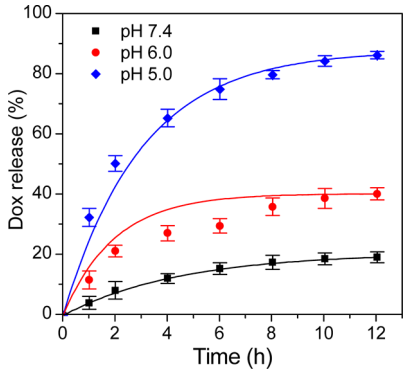


Figure 1. Time-dependent release of Dox from PDA capsules at pH 5.0, 6.0, and 7.4.

was below 20% at pH 7.4 over a 12 h period, while increased Dox release was observed at pH 6.0 over the same period of time. However, when the Dox-loaded PDA capsules were incubated at pH 5.0, approximately 85% of Dox was released over 12 h. This indicates that Dox release from PDA capsules is pH-dependent due to the pH-cleavable linker between the polymer and drug. This could potentially reduce side effects caused by premature release of drug during capsule circulation, and enhance intracellular drug delivery. In addition, the Dox release studies also demonstrate that the immobilization of PMA_{SH}-Dox is mainly based on Michael addition between the thiol and quinone groups, although there is a possibility of Michael addition/Schiff base reaction between the amine groups from conjugated Dox and quinone groups, in which case the expected release of Dox would be less than that observed.

The cytotoxicity of Dox-loaded PDA capsules to HeLa cells, a cervical cancer cell line that is sensitive to Dox treatment, was investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa cells were incubated with Dox-loaded PDA capsules (300 nm in diameter) at various capsule-to-cell ratios (from 1:1 to 1000:1) for 48 h at 37 °C. Using UV–vis spectrophotometry and flow cytometry, we deduced that the drug loading in each PDA capsule was approximately 6.45×10^{-16} g (see Supporting Information). The MTT assay results showed that the PMA_{SH}-loaded PDA capsules did not significantly affect cell viability (Figure S4). However, cell viability was reduced in a dose-responsive manner to Dox-loaded PDA capsules (Figure 2). As a control, HeLa cells were also treated with free Dox. Interestingly, Dox-loaded PDA capsules were found to be more effective than free drug at killing the cancer cells (for the same Dox concentrations) under the same assay conditions. This indicates that the Dox activity is not affected by polymer conjugation and immobilization and that the PDA capsules facilitate drug internalization, which enhances drug efficacy.

To visualize the cellular uptake of PDA capsules and the intracellular distribution of Dox, PDA capsules with a diameter of ca. 1.8 μm were used. This capsule size was used, as it can be more readily characterized by optical microscopy. The Dox-loaded PDA capsules showed excellent colloidal stability in aqueous and buffered solution (Figure 3a). Fluorescence microscopy images showed red fluorescence in the capsule walls, which indicated that the majority of loaded Dox was

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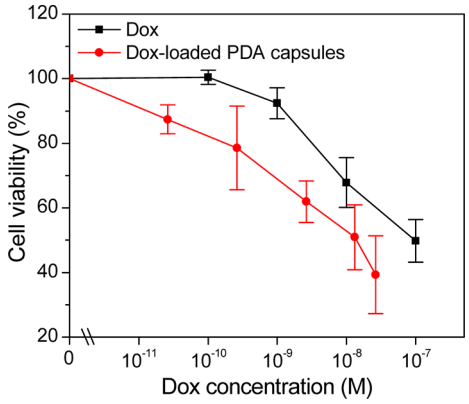

Figure 2. Cytotoxicity of free drug and drug-loaded PDA capsules as a function of drug concentration. The cell viability has been normalized by setting the viability of untreated cells to 100%. Dox-loaded PDA capsules in cell media with capsule-to-cell ratios of 1, 10, 100, 500, and 1000:1, respectively.

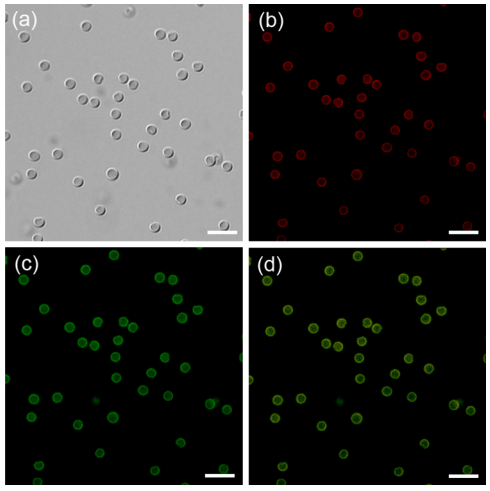

Figure 3. Fluorescence microscopy images of Dox-loaded AF488-labeled PDA capsules. (a) Differential interference contrast image. (b) Red fluorescence arises from the immobilized Dox. (c) Green fluorescence corresponds to the capsule wall labeled with AF488. (d) Overlay image. All scale bars are 5 μm.

associated with the capsule walls (Figure 3b). After immobilization of PMA_{SH}-Dox, the unreacted free thiols on the surface of the PDA capsules could be used for further modification. Here, we labeled the Dox-loaded PDA capsules with Alexa Fluor 488 C5 maleimide (AF488) based on thiol-maleimide chemistry (Figure 3c) to allow visualization of the capsule location after cellular uptake. The colocalization of green and red fluorescence in Figure 3d demonstrated the homogeneous distribution of the PMA_{SH}-Dox in the capsule walls.

AF488-labeled Dox-loaded PDA capsules (1.8 μm in diameter) were added to cell growth media and incubated with HeLa cells for 24 h at 37 °C. The intracellular distribution of Dox and the internalization of the PDA capsules were then visualized using deconvolution microscopy (Figure 4). It was shown that AF488-labeled PDA capsules with green fluorescence were internalized by HeLa cells (Figure 4c,d). The red fluorescence from Dox was distributed in both the cytoplasm and nuclei, which shows that Dox is released from

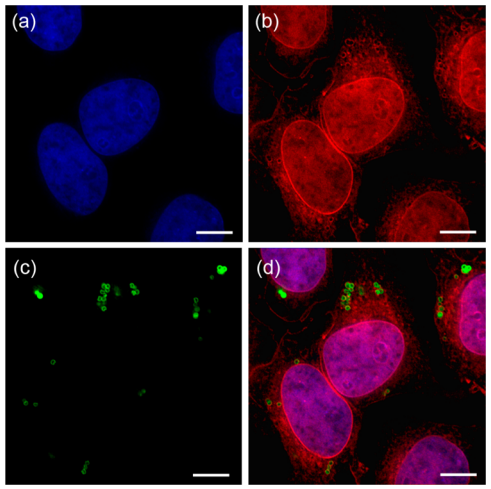

Figure 4. Representative deconvolution microscope images of HeLa cells treated with Dox-loaded capsules. (a) Nuclei were stained blue with Hoechst 33342. (b) Red fluorescence arises from Dox. (a) Green fluorescence represents internalized AF488-labeled PDA capsules. (d) Merged image of AF488, Hoechst and Dox signals. All scale bars are 10 μm.

the PDA capsules and accumulates in the nuclei after cell uptake.

CONCLUSIONS

In conclusion, we have reported the immobilization of Dox in monodisperse PDA capsules via robust thiol-catechol reactions between a polymer–drug conjugate and the PDA capsule walls. These Dox-loaded PDA capsules dispersed well in aqueous solution and showed pH-induced Dox release. Cell viability assays demonstrated the enhanced effectiveness of Dox-loaded PDA capsules in eradicating HeLa cancer cells, compared with free Dox under the same assay conditions. Furthermore, we overcame the labeling difficulties associated with broadband absorption of PDA (in the ultraviolet and visible light regions) and labeled PDA capsules to visualize them in cancer cells after internalization. The reported method provides a facile and robust strategy for the formation of polymer capsules containing a pH-triggered release mechanism, which may enhance therapeutic effectiveness and reduce systemic toxicity in drug delivery systems.

ASSOCIATED CONTENT

Supporting Information

Materials, synthesis of PMA_{PDA}, MAL-Dox, and PMA_{SH}-Dox, preparation of the DMDES emulsion templates and PDA capsules, immobilization of PMA_{SH}-Dox in PDA capsules, Dox release from PDA capsules, cell viability assay, and characterization methods. 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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