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Temperature-triggered on-demand drug release enabled by hydrogen-bonded multilayers of block copolymer micelles

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ABSTRACT

We report on hydrogen-bonded layer-by-layer (LbL) films as a robust, reusable platform for temperature-triggered "on-demand" release of drugs. Films with high drug loading capacity, temperature-controlled on–off drug release, and stability at physiological conditions were enabled by assembly of tannic acid (TA) with temperature-responsive block copolymer micelles (BCMs), which were pre-formed by heating solutions of a neutral diblock copolymer, poly(N-vinylpyrrolidone)-*b*-poly(N-isopropylacrylamide) (PVPON-*b*-PNIPAM), to a temperature above the lower critical solution temperature (LCST) of PNIPAM. The BCM/TA films exhibited temperature-triggered swelling/deswelling transitions at physiological conditions (swelling ratios of 1.75 and 1.2 at 37 °C and 20 °C, respectively). A model drug, doxorubicin (DOX) was incorporated into the film at a high drug-to-matrix ratio (~9.3 wt.% of DOX per film mass), with a total loading capacity controlled by the film thickness. At 37 °C, DOX was efficiently retained within the hydrophobic BCM cores of BCM/TA films, whereas exposure to a lower temperature (20 °C) triggered fast DOX release. While neither bare BCM-containing films nor films loaded with DOX showed cytotoxicity at 37 °C, drug released from films at lower temperature exhibited high potency against breast cancer cells. Repeated on/off drug release was demonstrated with 1.5-μm-thick DOX-loaded films, allowing at least three 30-min cooling cycles with consistent DOX (~12–16% of loaded DOX released for each cycle) released over a 4-day period. Despite significant stress associated with multiple swelling/deswelling cycles, films maintained their structural integrity in PBS, and each film could be repeatedly loaded with drug and used more than 15 times with only ~7% loss in film thickness and no obvious changes in reloading capacity or release profiles. This work presents the first proof-of-concept utility of temperature-responsive BCM-containing films for repeated on-demand release of a drug.

1. Introduction

On-demand drug release represents a promising strategy to potentially increase the therapeutic accuracy and minimize over-dosage induced side effects [1,2]. Ideally, the on-demand release system should release little or no drug in the "off" state, has fast stimuli response and reproducible drug release in each "on" state, and exhibits the ability to adjust drug dosage according to patient needs. More importantly, the system needs to maintain stability upon repeated switch to the "on" state without mechanical disruption.

Block copolymer micelles (BCMs) have been explored as promising vesicular drug carriers. The use of stimuli-responsive block copolymers for BCM assembly allows BCM carriers to release their load in response to environmental stimuli, such as pH, temperature, sound, or light [3–6]. Compared to other stimuli such as pH and ionic concentration alterations, tissue cells can better tolerate temperature changes especially in the region lower than physiological temperature. In this regard, temperature-triggered drug release is highly desirable. Temperature-responsive BCMs can retain and release drugs loaded within their micellar cores as a result of temperature-induced hydrophobic/hydrophilic transitions [7–10]. Often, this is realized through the inclusion of a BCM core of poly(N-isopropylacrylamide) (PNIPAM) — a widely used temperature-responsive polymer with phase transition occurring at close-to-physiological temperatures. Specifically, the lower critical solution temperature (LCST) of PNIPAM is ~32–33 °C [11,12], and this value can be further adjusted within a wide temperature range by using NIPAM-based copolymers. However, uncrosslinked micelles with responsive cores often lose their structural stability, and covalent stabilization through crosslinking of micellar cores or shells is necessary to improve structural stability and achieve tunable drug release [13–15].

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Unlike freely diffusible individual micelles that potentially accumulate in the body, film-embedded micelles can be permanently bound within coatings, preventing systemic distribution of micelles and providing localized therapeutic effects. In this study, we stabilize temperature-responsive drug-delivering micelles by binding them onto a surface using layer-by-layer (LbL) assembly with a branched polymer. The LbL technique affords non-line-of-sight deposition of conformal coatings at substrates of virtually any shape, and allows for control of film structure, composition and delivery of multiple therapeutic components from surfaces [16–18]. With non-micellar LbL films, release of functional compounds through diffusion [19] in response to environmental triggers [20–24], or through film biodegradation [25,26] has been previously demonstrated. However, little is known about the functionality of BCM-containing LbL films as drug delivery films, especially for repeated "on-demand" drug release. While demonstrating drug delivery from BCM-containing films through hydrolysis or biodegradation [27,28], serious concerns have been raised regarding the morphologic and structural integrity of responsive micelles upon external stimuli. For example, morphologic changes were observed with films containing pH responsive BCMs even after a single pH stimulation [29,30]. Our team demonstrated pH-regulated release of a model dye from LbL films with temperature-responsive diblock copolymer micelles [31], however, further work showed that the use of diblock copolymers as BCM components compromised film structural stability during long-term exposure to changing environmental conditions [32], and triblock copolymers were required to assure film stability [33,34]. Moreover, electrostatic pairing responsible for assembly of the above-described films is vulnerable to attack by small ions and cannot be used as a universal strategy for constructing functional films under high-salt physiological environments.

Here, we report on the use of hydrogen-bonded assembly to construct high-capacity, temperature-responsive, BCM-containing, reusable films for on-demand release of therapeutic compounds to cells. Previously, we demonstrated the ability of tannic acid (TA) to form stable hydrogen-bonded assemblies with a range of neutral homopolymers, including poly(N-vinylpyrrolidone) (PVPON) [35]. The assembled films were highly stable in a wide pH range and with tolerance to salt concentrations [36]. This all-homopolymer assembly was later used as a non-toxic, cytocompatible coating for encapsulation of yeast cells [37]. Following this strategy, here we report on novel films built *via* hydrogen-bonded assembly of TA with temperature-responsive micelles, formed from a neutral diblock copolymer, poly(N-vinylpyrrolidone)-*b*-poly(N-isopropylacrylamide) (PVPON-*b*-PNIPAM). Temperature-responsive micelles enable films with temperature-controlled swelling/deswelling transitions, as well as pulsed drug release, as a result of the LCST behavior of core-forming PNIPAM blocks. Unlike previous films constructed from similar BCMs but with another hydrogen donor, PMAA [31], the films described here remained highly stable at physiological conditions (PBS, 37 °C). At the same time, the films exhibited temperature-triggered swelling/deswelling transitions, which were used to "switch", on or off, the release of doxorubicin (DOX), a widely used hydrophobic anticancer drug. To our knowledge, this is the first report on pulsed, on-demand release of drugs from biocompatible hydrogen-bonded temperature-responsive LbL films containing BCMs. Due to the unique capacity of TA to effectively bridge micelles within the film *via* multisite hydrogen bonding with PVPON in the BCM coronas, the films were able to withstand at least 15 temperature-induced swelling/deswelling cycles and could be reused for repeated DOX loading and release. As a proof-of-concept experiment of functionality of the BCM/TA matrix, we show that pulsed release of DOX from these films effectively eradicated human breast cancer (MCF-7) cells. The robust, reversible, externally controlled swelling and drug release modes make these films promising candidates for on-demand drug release from surfaces.

2. Materials and methods

2.1. Materials

Benzyl chloride (Aldrich), elemental sulfur (Aldrich, St Louis, MO), and sodium methoxide (Fluka, St Louis, MO) were used as received. *N*, *N*-dimethylformamide (DMF) (99%), dioxane and tetrahydrofuran (THF) were received from Aldrich and distilled under reduced pressure prior to use. *N*-isopropylacrylamide (NIPAM) and azobis(isobutyronitrile) (AIBN) were received from Aldrich and recrystallized from benzene and methanol, respectively. Water with a resistivity of 18.2 MΩ cm^{-1} was supplied by a Millipore Milli-Q system. Branched polyethyleneimine (BPEI) with a weight-average molecular weight (M_w) of ~25 kDa, and $M_w/M_n = 2.5$, and PMAA with M_w ~ 150 kDa, were received from Sigma-Aldrich. DOX-HCl, hydrochloric acid, sodium hydroxide, sodium chloride, dibasic and monobasic sodium phosphate, as well as all other reagents were purchased from Sigma-Aldrich and used as received.

2.2. Synthesis of diblock copolymer

$PVPON_{165}$-*b*-$PNIPAM_{140}$ block copolymer was synthesized using reversible addition–fragmentation chain transfer (RAFT) polymerization and characterizations are described in our previous work [31].

2.3. Preparation and characterization of BCMs

2.3.1. Self-assembly of PVPON-b-PNIPAM BCMs

20 mg of block copolymer PVPON-*b*-PNIPAM was dissolved in 40 mL of 0.01 M phosphate buffer at pH 5.0, and the solution was gradually heated to 40 °C to enable the formation of BCMs. The presence of micelles in solution was confirmed by atomic force microscopy (AFM) and dynamic light scattering (DLS) as described below.

2.3.2. Dynamic light scattering (DLS)

Hydrodynamic sizes of block copolymer solutions were measured using Zetasizer Nano-ZS equipment (Malvern Instruments Ltd, Worcestershire, UK).

2.4. Preparation and characterization of BCM-containing multilayer films

2.4.1. Deposition of BCM/TA films

Silicon wafers or glass slides were cleaned as described elsewhere [38]. To enhance the adhesion of subsequently deposited multilayers to the surface, substrates were primed with a BPEI/PMAA bilayer as a precursor by alternately incubating with 0.2 mg/mL BPEI and PMAA solutions for 10 min with two 1-min rinsing cycles in buffer solution between polymer deposition. After rinsing with Milli-Q water, the substrates were then modified with neutral BCM/PMAA multilayers. All the deposition and rinsing steps were performed in 0.01 M pH 5.0 phosphate buffer at 40 °C. Hydrogen-bonded BCM/TA films were deposited by alternately exposing the substrate to respective solutions of 0.2 mg/mL BCM and 0.2 mg/mL TA solution for 10 min, with two 1-min intermediate rinsing steps with 0.01 M phosphate buffer. Deposition always started with BCM solution, and continued until the desired number of bilayers n was deposited. The produced $[BCM/TA]_n$ films were dried in air at 40 ºC.

2.4.2. AFM

AFM measurements were performed in air using NSCRIPTOR Dip Pen Nanolithography system (Nanoink Inc., Skokie, IL). For AFM imaging, micelles were deposited on silicon wafers by drying BCM-containing solution at 40 °C.