# Hydrogen-bonded multilayer of pH-responsive polymeric micelles with tannic acid for surface drug delivery†

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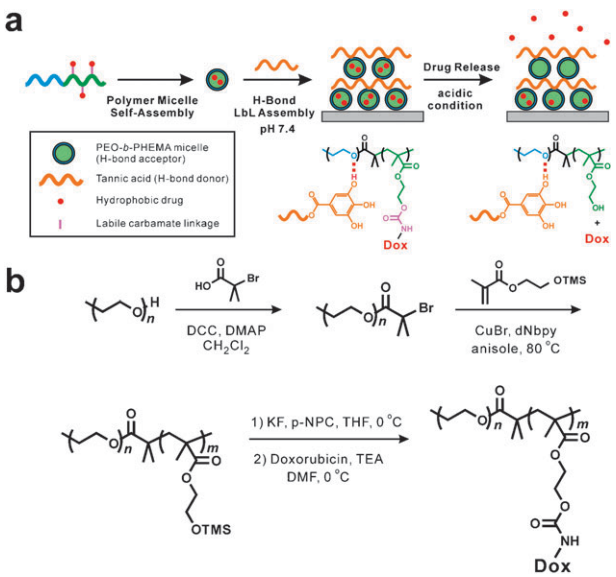
**We report the design of a platform for the delivery of hydrophobic drugs conjugated to block copolymer micelles *via* pH-responsive linkage that are assembled within hydrogen-bonded polymer multilayer thin films.**

A number of approaches have been developed to achieve site-specific and time-controlled delivery of therapeutics to improve therapeutic efficacy while minimizing undesired side effects.[1] In particular, polymeric nanostructures have emerged as promising vehicles for the delivery of therapeutically active materials. Amphiphilic polymer micelles have been extensively utilized in drug delivery applications not only due to their high capacity as drug carriers but also as a result of the tunability of drug pharmacokinetics in response to external stimuli such as pH, temperature, chemicals, and light.[2–5] For example, Kataoka and co-workers have demonstrated the development of intracellular pH-triggered polymeric micelles conjugated with the chemotherapeutic agent.[6,7]

As an alternative to traditional drug delivery by matrix or vesicular carriers, the development of a new thin film fabrication technique that allows for localized and precise controlled delivery of active therapeutics from a surface could offer important potential applications in biomedical surfaces and device coating. Layer-by-layer (LbL) assembly is particularly well-suited to these purposes as it can create highly tunable, conformal thin films on virtually any surface with nanometre-scale control over the film composition and structure.[8,9] LbL assembly can also allow the incorporation of diverse therapeutics, including polysaccharides, DNA, proteins, and hydrophobic drugs with a distinct concentration and release profiles, by controlling the number and sequence of layering.[10–15] However, relatively few reports have demonstrated the incorporation of highly biocompatible, nontoxic poly(ethylene oxide) (PEO) block copolymers within multilayers due to their limited functionality.[14,16] Moreover, given the widespread use of the PEO block as a key hydrophilic component in amphiphilic block copolymer micelles, it would be of great interest to develop a general means of integrating the PEO-containing micelles for functional biosurfaces.

Hydrogen bonding (H-bonding) is a unique means of generating alternating multilayer thin films[17–19] and recent work has indicated a range of pH values over which hydrogen-bonded multilayer films will remain stable.[20] In this communication, we present the integration of pH-responsive PEO block-containing polymeric micelles incorporated into LbL thin films based on H-bonding. Specifically, we have constructed LbL films employing H-bonding between biologically compatible tannic acid and a block copolymer micelle of poly(ethylene oxide)-*block*-poly(2-hydroxylethyl methacrylate) (PEO-*b*-PHEMA) conjugated with the chemotherapeutic agent, doxorubicin (Dox). Due to the pH-sensitive nature of the carbamate linkage between Dox and the polymer backbone, we demonstrate that the resulting LbL thin film can release Dox into the surrounding medium in a pH-controlled manner (Scheme 1).

By employing atom transfer radical polymerization (ATRP) using a PEO macroinitiator, we have polymerized a well-defined block copolymer of $PEO_{113}$-*b*-PHEMA-$TMS_{120}$ ($M_n$ = 5000 (PEO), $M_n$ = 31 000 (PHEMA), and polydispersity index (PDI) = 1.21). After the polymerization, trimethylsilyl (TMS) groups were deprotected in the presence of potassium fluoride and *in situ* functionalized with 4-nitrophenyl chloroformate (NPC) in THF, which subsequently reacted with Dox in the presence of triethylamine to afford the desired block copolymer conjugated with Dox (PEO-*b*-PHEMA-Dox) (see ESI†). The carbamate linkage employed in this study allows for hydrolysis and subsequent




**Scheme 1** (a) Schematic representation of hydrogen-bonding LbL assembly of doxorubicin-containing micelles with tannic acid. (b) Synthetic routes for PEO-*b*-PHEMA-Dox.

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drug release under slightly acidic conditions.[21–23] As determined by UV/Vis absorbance, the degree of Dox incorporation was 4 mol% with respect to PHEMA monomer units; moreover, we could easily increase the loading of Dox while maintaining solubility of the Dox-conjugate through the stable micelle formation that can solubilize the insoluble Dox in water. This is in clear contrast to the previous prodrug approach for multilayer buildup where the loading of hydrophobic drug needs to be kept low to preserve the solubility of the polymeric prodrug.[24]

Block copolymer micelles loaded with Dox, PEO-*b*-PHEMA-Dox (Dox-micelles), were prepared by dropwise addition of phosphate buffer (10 mM, pH 7.4) to a solution of polymers in *N*,*N*-dimethylformamide (DMF), and the resulting suspension was subjected to dialysis against phosphate buffer (10 mM, pH 7.4) for over 24 h to remove any residual solvent and unbound Dox (see ESI†). The Dox-micelle was found to have a hydrodynamic diameter of 192 ± 12 nm. Interestingly, we found that the addition of Millipore water instead of phosphate buffer during the formation of Dox-micelles leads to the leakage of the significant amount of Dox from the micelle core, which results in the formation of drug aggregates during the dialysis. This observation reveals the sensitive nature of the carbamate linkage in the pH condition of Millipore water (5.5–6.5) (see ESI†).

In order to create multilayers of Dox-micelles on the surface without special chemical modifications, we take the advantage of H-bonding; PEO is known to serve as an H-bond acceptor, while poly(carboxylic acid)s typically known to behave as H-bond donors.[14,17,19] In this H-bonding network of PEO polymers, however, the integrity of the multilayer is largely determined by the p$K_a$ of the H-bond donor, which can trigger a rapid disassembly of the multilayer when exposed to a pH condition above the p$K_a$ of H-bond donor. It would be desirable therefore to impart a higher pH-stability to the multilayer in these pH-sensitive polymeric micelle systems. As such, water-soluble, natural polyphenol, tannic acid (TA, pentagalloyl glucose), is an excellent choice of H-bond donor since it can form a strong H-bond network with PEO polymers even at physiological conditions, owing to its higher p$K_a$ of 8.5.[20,25] Moreover, TA's unique biological activities, including antitumor, antibacterial, and antioxidant properties, allow the introduction of surface coatings with bioactive properties. Sukhishvili and Erel-Unal have recently demonstrated the potential of TA in forming multilayers with PEO *via* H-bonding in a wide range of pH conditions (pH 2–7.5).[20]

Based on the H-bonding between the PEO block (corona of Dox-micelle) and TA, multilayer thin films were assembled by repeatedly layering Dox-micelles and TA under physiological conditions (pH 7.4) on a planar silicon wafer or glass slide to afford the multilayer in the architecture of (Dox-micelle/TA)$_n$. We monitored the growth of multilayers by the gradual increase of characteristic UV/Vis absorbance of TA ($\lambda_{max}$ = 280 nm, neutral). The growth of H-bonded films was shown to be linear with respect to the number of bilayers with an average bilayer thickness of 8.9 nm (Fig. 1), which is significantly smaller than the size of micelles in the solution measured by DLS. This discrepancy results from

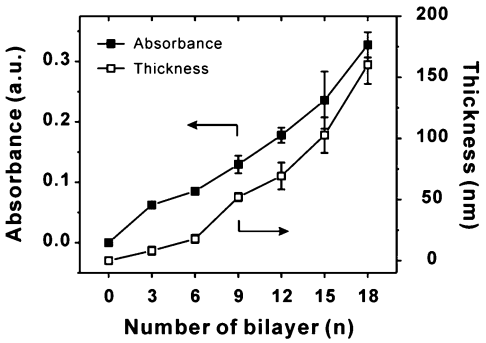


**Fig. 1** Growth curve of (Dox-micelle/TA)$_n$ multilayer prepared in phosphate buffer (10 mM, pH 7.4). Absorbance was measured at 280 nm and the film thickness was measured by surface profilometer ($n$ = 5).

the possibility of micelles being flattened within the multilayer as demonstrated in the case of micelles and dendrimers.[26] It could also indicate the low degree of packing density of micelles within the multilayer.

The (Dox-micelle/TA)$_n$ multilayer assembly was observed by atomic force microscopy (AFM). As illustrated in Fig. 2, AFM image of multilayer shows features characteristic of polymeric micelles with the appearance of globular objects that coalesce into larger aggregates with an average root-mean-squared roughness of 86 nm. This image is similar to that of plain micelles deposited on a clean silicon wafer (average size of 142 ± 23 nm), indicating that Dox-micelles retain their structural integrity during the LbL assembly process.

The release of Dox from a (Dox-micelle/TA)$_{40}$ multilayer was evaluated by exposing the construct to different pH conditions while measuring the fluorescence of released Dox (see ESI†).[27] As expected, the multilayer exhibited pH-responsive Dox release due to the acid-labile nature of the carbamate linkage between Dox and the polymer backbone. The film released a significant amount of Dox at pH 4 after 24 h, while only 20% of Dox is released at pH 7.4 (Fig. 3a). The accelerated release of Dox under acidic conditions can be a desirable feature for an effective cancer therapy, since local and endosomal pH is considerably lower than that of the normal tissue.[28] The stability of multilayers throughout the release experiments was also confirmed by measuring the thickness of films after exposing them to different pH conditions. We found that the films retained very good stability (*e.g.* thickness changes less than 10% for 48 h) in the pH conditions examined.

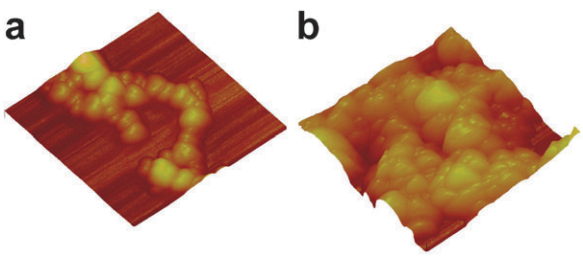


**Fig. 2** 3D height-mode AFM images of (a) Dox-micelle (b) (Dox-micelle/TA)$_{20}$ multilayer deposited on a silicon wafer. Scale of both images is 1 μm × 1 μm × 500 nm.

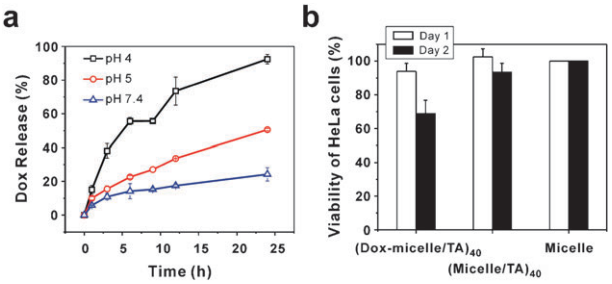


**Fig. 3** (a) Release profiles of $(Dox\text{-micelle/TA})_{40}$ multilayer at various pH conditions. (b) *In vitro* cell assay against HeLa cells with different film compositions. The release profile was normalized to the value of release of Dox at the pH 4 condition after 48 h, after which does not exhibit any signal of Dox release.

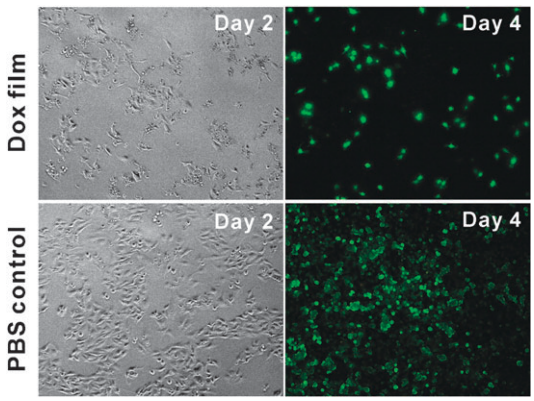


**Fig. 4** (Left) Bright-field and (right) fluorescence images of *in vitro* cell assay against HeLa cells with (top) Dox releasing film and (bottom) control PBS. Cells were plated at equal confluence on day 0 (25 K cells per well). Live cells are stained with calcein AM at day 4. All images are in 10× magnification.

*In vitro* efficacy of the $(Dox\text{-micelle/TA})_{40}$ multilayer was investigated with a cell viability assay using a human cervical cancer HeLa cell line (Fig. 3b). The assay was conducted by subjecting HeLa cells to release aliquots from the films to ascertain that Dox retained its activity. Based on an MTT assay, the percentages of cell survival were $68 \pm 7\%$ for HeLa cells treated with a release aliquot from the pH 7.4 condition in comparison to a control set of plain PEO-*b*-PHEMA micelle without Dox, which had no cytotoxic effect. It is also interesting to observe that the pure $(Micelle/TA)_{40}$ film exhibits an activity against HeLa cells possibly due to the released bioactive TA from the film. The cytotoxic effect of Dox released from our film constructs was confirmed with examinations of HeLa cells exposed to different release aliquots. As shown in Fig. 4, the population of confluent cells is significantly lower when the cells were exposed to aliquots from the Dox-micelle film.

In conclusion, we have demonstrated the design of a multilayered platform for the delivery of hydrophobic drugs from a surface assembled *via* the layer-by-layer technique. A PEO-*b*-PHEMA block copolymer conjugate of the chemotherapeutic drug doxorubicin through acid-labile carbamate has been synthesized and self-assembled to form a polymeric micelle. Through hydrogen-bonding interactions between the corona of the polymeric micelles and biologically active tannic acid, we have prepared bioactive multilayers. Release of the drug from the Dox-loaded multilayers upon hydrolysis of the pH-sensitive carbamate linkage resulted in a drastic cancer cell death, indicating that the cytotoxic effects of Dox is preserved in the film construct. The versatile nature of LbL assembly integrated with the smart polymeric micelle as a vehicle for controlled drug release could be of interest in the design of new therapeutic delivery platform for various biological and biomedical coatings.

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