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Multi-responsive drug release from hydrogen-bonding multilayers containing PEGylated nanoparticles and azobenzenes†

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Using PEGylated nanoparticles and light-sensitive azobenzenes, a multicolor fluorescence layer-by-layer film loading drug has been constructed based on hydrogen bonding. The multilayer film exhibited multi-responsive drug release properties.

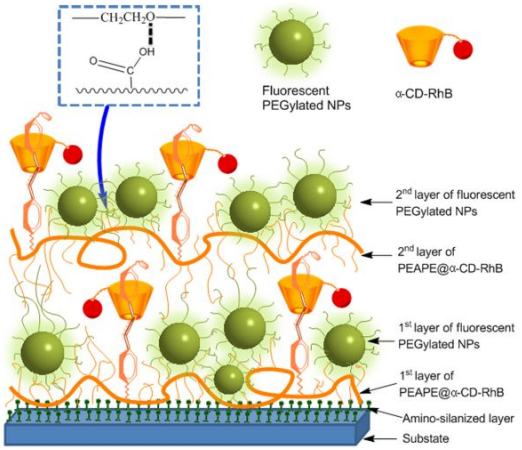
In recently decades, stimuli-responsive materials are finding a rising number of applications in surface biofunctional coatings, biological sensors, nanoreactors and drug delivery.[1] Stimuli-responsive ultrathin polymer films assembled ***via*** layer-by-layer (LbL) fashion are promising candidates for more complex tasks of loading, storage and release.[2] Remarkable reports on stimuli-responsive LbL multilayers for drug release have been presented,[3] however, their application in multi-responsive drug delivery is scarce and the studies in this direction move slowly.

Hydrogen-bonding (H-bonding) is a unique approach of fabricating LbL multilayer films, as this self-assembly method is reversible and the resulting multilayers could be selectively destroyed upon external pH changes.[4] Herein, we report a combined approach of photosensitivity and LbL platform to obtain multi-responsive H-bonding multilayers, which involves biologically compatible PEGylated nanoparticles (NPs) and azobenzenes (azo) polyelectrolytes loading α-cyclodextrin (α-CD) modified drug ***via*** host–guest interaction. Generally, Incorporation of NPs into LbL multilayers makes it possible to control film size, morphology, biocompatibility, permeability, and drug delivery.[5] Meanwhile, the obtained films can release drugs in a light-controlled manner due to the stimuli-sensitive nature of azo.[6] In additional, we demonstrate that the films can be rapidly deconstructed to simultaneously release drugs and NPs upon exposure to physiological conditions because of the pH-erasable characteristics of H-bonding multilayers.

Loading drugs on the multilayers ***via*** supramolecular interaction has been reported by Hammond,[7] and our studies on photo-controllable films loading drugs ***via*** host-guest interaction revealed that they are capable of loading or releasing cargo by converting light irradiation.[6] As well-known, ***trans***-azo could be well recognized by α-CD, while ***cis***-azo cannot.[8] Based on this principle, we could conjugate model compounds with α-CD through chemical bonding, thus enabling functional groups or drugs, such as antineoplastic doxorubicin, to incorporate within the LbL films. Herein, α-CD modified rhodamine B (α-CD-RhB) was selected as a drug model (see ESI†).

Azo-containing copolymer poly{2-[4-phenylazophenoxy]ethyl acrylate-co-acrylicacid} (PEAPE, Mn: ~19.5 kD determined by GPC measurement, PDI: 1.74) with an azo-modified degree of ~3% was synthesized (see ESI†) and used as light switch. The thiolated NPs were prepared through self-condensation of 3-mercaptopropyltrimethoxysilane with a z-potential of -38 mV, indicating that the surface of the NPs is full of hydrosulfide groups (Table S1, see ESI†). Next, they were labeled with Fluorescein 5(6)-isothiocyanate (FITC), a yellow-green dye. The amount of FITC conjugated was kept very small (0.68% w/w) so as to avoid any significant effect on the growth of the assembly. Then the methoxyethylene glycol maleimide (MePEG) was bound to the surface of thiolated NPs ***via*** the thiol–maleimide "click" reaction to afford the desired PEGylated NPs (see ESI†). The PEGylated NPs with a diameter of ~78 nm and low PDI (0.117) showed excellent dimensional stability over a wide range pH from 3.0 to 12.0 (Fig. S1 and S2, see ESI† for more details). The structure and morphology of the thiolated and PEGylated NPs were characterized using Raman spectroscopy, dynamic light scattering and transmission electron microscopy (Fig. S3 and S4, see ESI† for more details).

Due to the presence of the MePEG-based corona, the fluorescent PEGylated NPs are capable of forming H-bonding with PEAPE on solid substrate under acidic conditions. The alternating deposition of FITC-labeled PEGylated NPs and α-CD-RhB-loaded PEAPE (PEAPE@α-CD-RhB) ***via*** LbL approach leads to the formation of multilayered nanostructural films (Scheme 1). The multilayer assembly was observed by water contact angle measurements on the deposited layers. The results show that the contact angle decreases for the initial three layers, indicating the surface properties of the films have been altered (Fig. 1a). Since the fourth layer, however, it begins to alternate between 37° for PEGylated NPs and 25° for azo polymers, which is good agreement with the previous studies.[9] The buildup of the multilayers was also monitored with UV-vis spectra. The absorbance maximum of α-CD-RhB in the multilayers at 566 nm is taken as the reference for monitoring the film growth. As shown in Fig. 1b, the absorbance intensity increases as the layer number increases (Fig. S6, see ESI†). Furthermore, we observe exponential growth behavior consistent with previous report,[10] resulting from the interdiffusion of α-CD-RhB within the film during assembly. These results confirm that PEAPE@α-CD-RhB was well incorporated within the films. The resulting films were observed by atomic force microscopy (AFM). With NPs as the outermost layer, a



Scheme 1 Schematic expression of PEGylated NPs-azobenzenes LbL self-assembly at quartz wafer surfaces. The inset illustrates the H-bonding between the carboxylic groups of PEAPE and ether groups of MePEG.

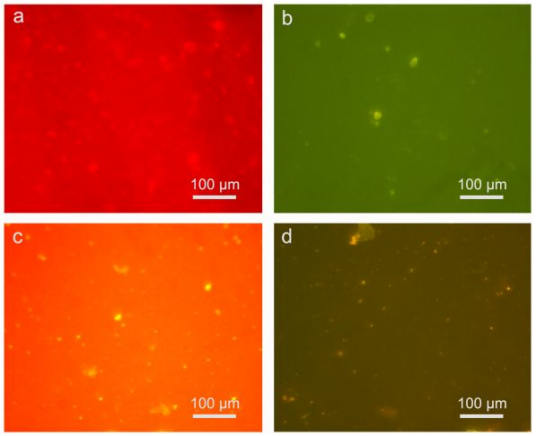


Fig. 2 Fluorescent microphotographs of PEAPE@*α*-CD-RhB/unlabeled NPs (a), PEAPE/labeled NPs (b) multilayers, multicolor fluorescence film before (c) and after (d) UV irradiation. The size bar is 100 μm.

rough surface morphology with ball-like structure is displayed. The boundaries are unclearly visible between globular objects (Fig. 1c), probably due to the nonrigid feature of the MePEG-based coronas resulting in strong diffusion. 3D height-mode AFM image of the film shows PEGylated NPs coalesce into larger aggregates with an average root-mean-squared roughness of 98 nm, indicating that they retain their structural integrity during the LbL assembly process (Fig. 1d).

Subsequently, to gain a better insight into the properties of PEAPE/NPs multilayers, we studied the LbL deposition of PEAPE/FITC-labeled NPs and PEAPE@*α*-CD-RhB/unlabeled NPs, respectively. As shown in Fig. 2a and 2b, both 15-bilayer films exhibit intense fluorescence, implying that the FITC-labeled PEGylated NPs and *α*-CD-RhB molecules were accordingly incorporated into the LbL films. Compared with the single-color fluorescent multilayer, the film assembled FITC-labeled NPs and PEAPE@*α*-CD-RhB also shows photoluminescence with a high intensity containing both red and yellow-green florescence (Fig. 2c). After irradiation with UV light for 78 min in a dark environment, the film turned yellow-green (Fig. 2d), indicating the release of red *α*-CD-RhB from the film. Compared with Fig. 2b, the yellow-green fluorescence intensity for released film is decreased due to the UV-sensitive of FITC.

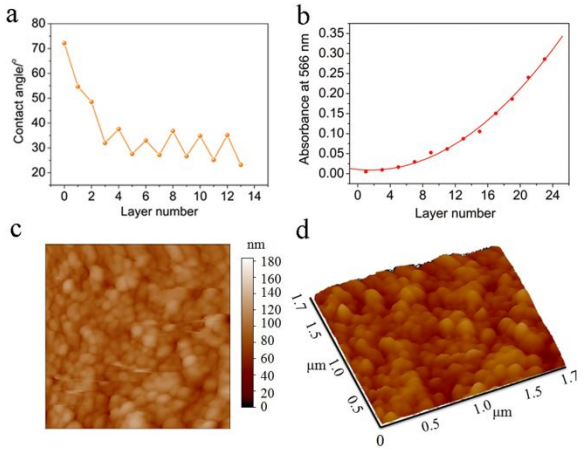


Fig. 1 Multilayer growth characterization. Water contact angles as a function of layer number (a) and absorbance at 566 nm vs. the layer number (b). AFM (c and d) images of 6-bilayer film deposited on a quartz wafer. Scan in 1.7 μm × 1.7 μm × 180 nm.

Next, in order to investigate the potential of the LbL multilayers as a light-controlled release system, the release percentage was measured as a function of time. Fig. 3a shows that 98 wt% of *α*-CD-RhB is released within 100 min from 20-bilayer film under UV irradiation, while 10-bilayer film takes 60 min to release ca. 99% drugs. For 20-bilayer film, the release percentage increases sharply in the first 20 min of UV light irradiation, which raises to ca. 80% in 60 min, and it reaches ca. 98% in 100 min. Meanwhile, the fluorescence intensity of the film is gradually declining during the process of UV irradiation (Fig. S8, see ESI†). These results confirm that UV irradiation could effectively regulate the release of cargo in the films. To further verify the ability of light-sensitive of the multilayers, we presented a fluorescent display of 'butterfly'-shaped pattern onto the LbL film surface ***via*** area-selective release (Fig. 3b, see ESI† for more detail). Furthermore, the photo-controllable release/loading process can be repeated at least 8 cycles, indicating the reversibility of the multilayers (Fig. S9, see ESI† for more details).

The pH-triggered dissolution of H-bonding multilayers has been recently utilized to release PEO-block-poly(caprolactone) (PEO-b-PCL) micelles ***via*** disintegration of PAA/PEO-b-PCL multilayers at physiological conditions.[11] To probe drug release behavior of our H-bonding films under physiological conditions, we incubated a 10-bilayer film to phosphate buffered saline (PBS) solution (pH = 7.4) at 25 °C. After the film is exposed to PBS for 3 min at pH 7.4, the deconstruction of the film proceeds through rapid bulk disintegration rather than gradual surface erosion, accompanying the originally smooth surface becomes significantly nonuniform and rough (Fig. 3c and Fig. S10, see ESI†). The thickness of the remained film is found to decrease greatly with respect to time of exposure to PBS, evidenced by profilometer measurements (Fig. 3d). With further increase of pH of the buffer solution, we find that the film displayed more serious destruction (e.g. thickness decreases more than almost 80% for 3 min exposure when the pH reaches 10.4). As expected, the disintegration of the LbL film leads to the rapidly release of drugs within 26 min (Fig. 3e). Furthermore, the release time is expected to be much shorter with the increase of pH value. The rapid co-release of drugs and these NPs under physiological conditions can be a desirable feature for cystic fibrosis therapy, since the PEGylated NPs may penetrate through mucus gel easily when excessive mucus appears in the airways, thereby strengthening drug

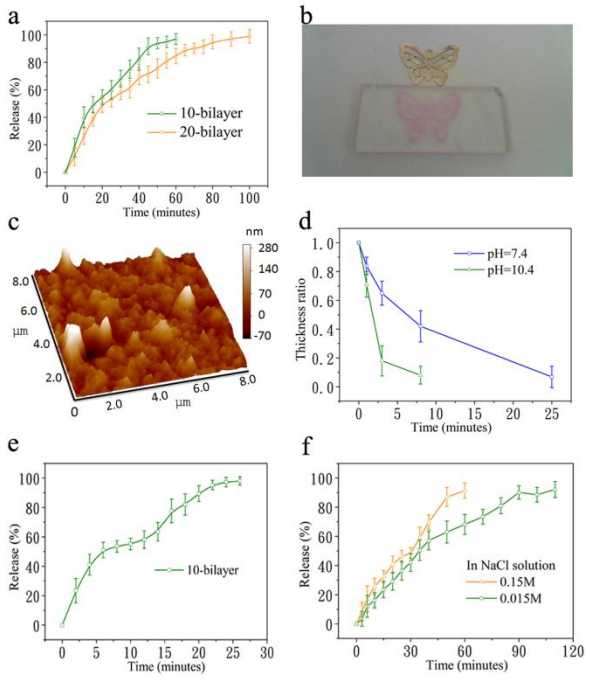


Fig. 3 (a) Release profiles of PEAPE@α-CD-RhB/unlabeled NPs film under UV irradiation. (b) The patterned photograph on the as-prepared film as a result of local release. (c) AFM images of the multicolor film surface after incubation in PBS solution for 3 min. Scale in 8.0 μm × 8.0 μm × 280 nm. (d) Change of the multicolor film thickness measured by profilometry. The measured thickness represent the average values from three different locations on the film. (e) Release profiles of the 10-bilayer multicolor film under PBS solution at pH 7.4. (f) Release profiles of the 10-bilayer multicolor film in NaCl aqueous solution.

penetration.[12] Apart from the disassembly of H-bonding, we suppose the quick-release of α-CD-RhB is also induced by the dissociation of host-guest system. To account for this, we immersed a 10-bilayer multilayer film in the NaCl aqueous solution at different concentrations. The results reveal α-CD-RhB can be largely released, resulting from the dissociation of the host-guest interaction between PEAPE and drugs (Fig. 3f). The data suggest the release of α-CD-RhB can be tuned by the ionic strength of solution. Compared with light-induced disassembly of host-guest interaction, the sensitivity to salt is unusual for multi-responsive drug release, which definitely deserves further investigation.[13]

In summary, a multi-responsive drug releasing film composed of PEGylated NPs and azo is successfully prepared ***via*** the combination of LbL assembly techniques and host-guest chemistry. The multilayers are able to respond to light, pH value and ionic strength for drug delivery. In general, the multi-responsiveness of this new films make them an interesting candidate to serve as multifunctional nano-carriers and coatings. Moreover, our system controlling of supramolecular architectures inherent in multilayers allows for synergistic effect between film components: the PEAPE can load and release multi-drug by converting light irradiation, while the mucus-penetrating PEGylated NPs can be released triggered by physiological conditions, and subsequently "slip" through the human mucus barrier, thereby enhancing drug permeability during their transfer. With further study of simulative release, the films appear promising for multi-responsive and multi-drug delivery to mucosal tissues.[14]

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Notes and references

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