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**Saline-Enabled Self-Healing**: Biocompatible polyelectrolyte multilayer films fabricated by Layer-by-Layer technique could undergo rapid healing of several tens of micrometers-sized cuts when exposed to normal saline, which shows highly promising as protective coating with self-healing properties for clinic applications.
Saline-Enabled Self-Healing of Polyelectrolyte Multilayer Films

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Self-healing materials have the capability of repairing or recovering themselves when suffering damages. But few of synthetic self-healing materials can keep their self-healing abilities under physiology conditions. In this work, Polyurethane (PU)/carboxymethyl cellulose (CMC) multilayer films were assembled on glass slides with/without poly(diallyldimethylammonium chloride) (PDDA) precoated through layer-by-layer technique. It was found that (CMC/PU)_n films assembled on glass slides with precoated PDDA can autonomically repair cuts of several tens of micrometers wide when contacted with normal saline. The mechanism of healing was studied using optical microscopy and SEM. Furthermore, a simple method was proposed by combining dispersion experiment and student t-Test to confirm the damaged films healed completely. The results suggest that the healing ability of certain films could be largely enhanced by introducing a third polyelectrolyte with relative high stiffness as the innermost layer. The results provided a new design route to fabricate new coating materials with self-healing ability.

Introduction

When biological materials such as bone, skin, and muscle are in healthy circumstances, they keep on undergoing in situ self-healing cycle to prevent the accumulation of defects due to tissue ageing and fatigue. Intrigued by the beauty and efficiency of natural healing processes, wide-spreading attentions have been paid on the preparation and application of synthetic self-healing materials over the last decade, covering different material classes, such as polymers, polymer composites, ceramics, concrete materials, and metals. A large variety of different approaches have also been developed, among which Layer-by-Layer (LBL) assembly technique with facile preparation and tunable properties was also used to fabricate self-healing films. However the preparation of biocompatible coating for clinic applications capable of healing under physiology conditions is still a challenge. The lack of success may be attributed to the deleterious effects of high salt concentration, which would cause the films to decompose.

Polyurethane (PU) is a large family of polymers with wide-ranging properties and uses, and it plays an important role in the development of many medical devices ranging from catheters to total artificial hearts. Based on its desired mechanical properties, exceptional biocompatibility, biodegradation and versatility, we have fabricated a series of PUs based multilayer films through LBL technique. It was observed that all these PU series multilayer films turned to be softened and swollen when immersed into water solution, which suggested PU based LbL multilayer films had the potential healing properties. In this study, PU and carboxymethyl cellulose (CMC, a natural macromolecule amyllose with high biocompatibility) were used to fabricate multilayer films through LBL technique. But the damaged PU/CMC multilayer film cannot be healed by normal saline. To enhance the self-healing ability of PU/CMC multilayer film, the multilayer film were assembled on the glass slide precoated with a layer of poly(diallyldimethyl-ammonium chloride) (PDDA). The as-prepared multilayer films showed significant saline-enabled self-healing ability, and can autonomically repair cuts of several tens of micrometers wide when contacted normal saline. The whole procedure was illustrated in Scheme 1.
Materials and methods

Materials
An aqueous dispersion of the water-soluble cationic polyurethane (PU, 18 wt.%, \(M_w\approx50\,000\)) was obtained from Guangzhou Imake Polymer Materials Co., Ltd. (Guangzhou, China). Carboxymethylcellulose sodium (CMC) was purchased from Tianjin Hengxing Chemical Reagent Co., Ltd. (Tianjin, China), and Poly(diallyldimethylammonium chloride) (PDDA, \(M_w\approx200\,000\)–350,000, 40 wt.%) was obtained from Luyue Chemical Reagent Company (Shandong, China). Methylene blue (MB) was obtained from Tianxin Chemical Company (Tianjin, China). Sodium chloride was purchased from Beijing Chemical Reagent Company (Beijing, China). 0.1 mol L\(^{-1}\) HCl and NaOH were used to adjust the pH values of solutions. Deionized water (DI) was used in all experiments, and all other chemicals and solvents were of analytical grade and used without any further purification. All experiments were carried out at room temperature.

Treatment of substrate
The LbL assembly was performed on glass slides, which were first cleaned with fresh Piranha solution (1:3 v/v mixtures of 30% H\(_2\)SO\(_4\) and 98% H\(_2\)O\(_2\)) for 40 min, followed by sonicating in DI water for 1 h, and then extensively rinsed with DI water. During the Piranha treatment, the residues of organic impurities were removed and the slides were completely hydrophilic at the same time. Finally, the cleaned slides were dried under air flow before use.

Assembly of LbL films
(\(\text{PU/CMC}_n\)) film. The Piranha-treated glass slide was first immersed into a 10.0 mg mL\(^{-1}\) solution of PU for 2 min, then rinsed with DI water for 1 min to remove the nonspecifically or weakly adsorbed PU and finally dried under air flow. Subsequently, the PU coated glass slide was immersed into a 10.0 mg mL\(^{-1}\) solution of CMC (pH adjusted to 3.0) for 2 min, followed by the same rinsing and drying cycle. The adsorption, rinsing and drying steps were repeated until the desired number of bilayers was obtained.

PDDA(CMC/PU)_n film. The Piranha-treated glass slide was immersed in a PDDA aqueous solution (5%) for 20 min to obtain a cationic ammonium-terminated surface, and was ready for CMC/PU multilayer deposition. The following steps were the same way as mentioned above.

Results and discussion
The films were fabricated by alternately depositing positively charged PU and negatively charged CMC directly on the slides through electrostatic interaction. Considering the fact that the polyelectrolyte of outmost layer had significant effect to the characteristic of the film, i.e. hydrophobicity, charge state and et al, two multilayer films were assembled with different outmost layer: (PU/CMC)\(_{20}\) containing 20 bilayer of PU/CMC with CMC as the outmost layer, and (PU/CMC)\(_{20.5}\) containing 20.5 bilayer with PU as the outmost layer. After the assembly, an incision of about 20 \(\mu\)m widths was made by a utility knife, and then 10 \(\mu\)L of normal saline was dropped on the films.

Procedures of healing were monitored by an optical microscopy (Olympus CH20, Japan) at 100× magnifications between certain time intervals.

From optical microscopy images (Figure 1a and 1b), it can be clearly seen an incision on the film after cut. After normal saline was dropped, the width of incision only narrowed a little. The results showed that the (PU/CMC)_n films did show some self-healing abilities but did not heal completely. However the swelling and wrinkling of the film that can be observed even by eyes. We assumed that it was the wrinkles hindered the film reaching perfect healing.

Based on this view-point, it will be beneficial to enhance the self-healing capability by introducing a third polyelectrolyte with low shrinkage rate as the innermost layer to decrease or diminish the wrinkles of the films. Here, PDDA (\(M_w\approx200\,000\)–350,000, 40 wt.%, positively charged) was chosen due to its stiff molecular skeleton endowed by five-membered nitrogen-containing heterocycles. After pre-coating glass slide with PDDA, negatively charged CMC and positively charged PU was alternately deposited to assemble multilayer films. Two multilayer films were also assembled with different outmost layer: PDDA(CMC/PU)\(_{20}\) with PU as the outmost layer and PDDA(CMC/PU)\(_{20.5}\) with CMC as the outmost layer.

The time-dependent self-healing processes of PDDA(CMC/PU) multilayer films were recorded using optical microscopy (Figure 1c and 1d). As expected, for both of PDDA(CMC/PU)\(_{20}\) and PDDA(CMC/PU)\(_{20.5}\) films, after normal saline dropped, no wrinkle can be observed. And the incisions vanished and surfaces of films became smooth in 5 seconds. The healed films were further investigated by a
Neoscope JCM-5000 bench-top scanning electron microscope (SEM, Jeol, Japan). SEM pictures illustrated the changes of surface morphologies of PDDA(CMC/PU)$_{20}$ (Figure 2 a-f) and PDDA(CMC/PU)$_{20.5}$ (Figure 2 a'-f') films in the stage of self-healing. These facts confirmed our presumption that the self-healing ability of PU/CMC multilayer films can be greatly increased by introducing PDDA as the innermost layer.

The optical microscopy and SEM images only provided surface morphologies of the films, but not able to confirm whether the films were healed homogeneously. Sun group used cyclic voltammetry (CV) to confirm the self-healing ability of the damaged film$^{10}$. In their study, the working electrode was firstly prepared by coating multilayer polymer on an Indium-Tin-Oxide (ITO) glass substrate. But unfortunately, CV cannot be applied into our system, because the assembled PDDA(CMC/PU)$_n$ film on the ITO slide would lose the ability of healing. Compared with the glass slides (mainly with -Si-O- groups), the charge density on the surface of the ITO slide was much higher. Strong electrostatic interaction between cationic quaternary amines of the PDDA and anionic oxygen ions of the SnO and InO on the surfaces of the ITO slide would limited the flowability of the PDDA$^{20,21}$. However, the flowability of the coatings and the interdiffusion of polyelectrolytes at the fractured surfaces are the key points of self-healing materials$^{22,23}$. The lower flowability of polyelectrolyte resulted in worse self-healing ability of the films.

In this study, a simple method combining dispersion experiment and student t-Test was proposed to investigate the healing level of the films. In principle, if one film was healed completely, the diffusion shape of the dye would be unchanged. Most importantly, there should not exist significant difference on the dispersion rate between original and healed films. In this work, Methylene blue (MB) was chosen as a model dye, because it can go into the inner of the film and the disperse not only on the surface but also in the inner of the film$^{24,25}$. After 5 µL of MB (0.3 mg/mL) was dropped onto the surface of films, the diffusion shape of MB was recorded by the optical microscopy 5 min later. For both of PDDA(CMC/PU)$_{20}$ and PDDA(CMC/PU)$_{20.5}$ films, the diffusion shapes of MB were all intact round on the original films (the first row in Figure 3). As a comparison, the diffusion circles formed clear bounds along the edges of the incisions in the cut films (the second row in Figure 3). While it turned to the films after healing, dispersion shapes of MB turned to be intact round again (the last row in Figure 3).

The dispersion diameters of MB on two films vs time were also determined by vernier caliper, and resulted data was analyzed with independent-sample t-Test (two-tailed) algorithm (Table S1). To overcome the effect of the poor uniformity of LbL assembled films, all tests were performed in triplicate. Interestingly, the results of statistical analysis showed there were no statistically significant difference between original and healed PDDA(CMC/PU)$_{20.5}$ film (the p value of independent-sample Students t-Test was less than 0.05). And statistically significant difference existed between PDDA(CMC/PU)$_{20}$ films before cutting and after healing. This result indicated that only the damaged PDDA(CMC/PU)$_{20.5}$ film, with the CMC as the outmost layer, can be reached the
perfect self-healing. Although the results of optical microscopy and SEM showed PDDA(CMC/PU)_{20} film was likely perfectly healed, but in fact it did not. This result showed the type of polyelectrolyte of outmost layer will play the key role on the self-healing ability of film. Only when CMC as the outmost layer, the perfect healing can be obtained. This is ascribed to the differences of the solubility and the flowability between PU and CMC in normal saline. Compared with CMC, the stronger hydrophobicity of PU shows the lower solubility and flowability in normal saline. This conclusion also can be drawn by the difference between diffusion diameters of high hydrophilic MB in the PDDA(CMC/PU)_{20} film with PU as outmost layer and PDDA(CMC/PU)_{20.5} film with CMC as outmost layer.

To further explore the mechanism of healing, water with various pH including 2.0, 5.0, 6.4 (pure water), 7.0 and 9.0 were dropped on the cut films. But the incisions still can be clearly observed even by naked eyes, and the pictures of optical microscopy showed the widths of the incisions were similar (Figure S1), which meant water with various pH were not enable films healing. For example, after pure water (pH 6.4) was dropped on the surface of PDDA(CMC/PU)_{20.5} film, the width of the incision only narrowed about 35% with a swelling ratio of 7.4% (Figure 2g'). Meanwhile perfect healing was obtained with a swelling ratio as much as 16.7% after normal saline dropped. The LbL-assembled PDDA(CMC/PU)_{n} coatings have an ionic cross-linking network structure and polyelectrolytes are hydrophilic in nature^{26}. Due to the electrostatic shielding induced by salt, the dissociation of weak polyelectrolytes would be reduced in normal saline^{27-29}. Under such conditions, the multilayer film would be easily swollen and exhibited a high tendency to heal the incision.

Conclusions
As the LbL assembled (PU/CMC)_{n} films exhibited limited defects healing abilities, a simple route to fabricate intrinsic self-healing biocompatible films was introduced. The glass slides was firstly coated a layer of PDDA as the innermost layer, and then deposited CMC and PU alternately through LbL technique. The resulted PDDA(CMC/PU)_{n} films showed significant saline-enabled self-healing ability. The cuts with widths of several tens of micrometers on PDDA(CMC/PU)_{20.5} film (with CMC as the outmost layer) can be perfectly healed under normal saline, which showed promise for application as protective films with the property of self-healing under the physical condition. This work also testified the enhancement of self-healing ability of multilayers can be achieved by introducing the third polyelectrolyte with relatively high stiffness. Besides, one simple method was introduced to investigate the healing level of the damaged films by combining the dispersion experiment and the statistical analysis method. We hope that our finding will serve as a helpful template to design new coatings with saline-enable self-healing ability.

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Notes and references
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† Electronic Supplementary Information (ESI) available: The effect of the outmost layer on the self-healing abilities of assembled multilayer films characterized by the dispersion experiment (n=3), and The healing properties of water with different pHs on a) PDDA(CMC/PU)_{20} and b) PDDA(CMC/PU)_{20.5} films. See DOI: 10.1039/b000000x/


