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Anticoagulant Surface Coating Using Composite Polysaccharides with Embedded Heparin-Releasing Mesoporous Silica

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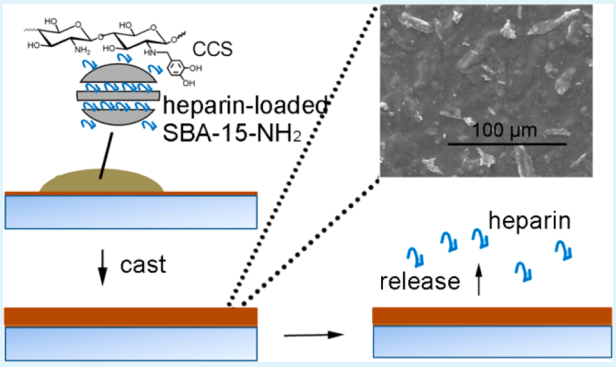
Supporting Information

ABSTRACT: Release of heparin from the surface of biomaterials is a feasible and efficient manner for preventing blood coagulation because of the high bioactivity of free heparin and a low application dosage compared to intravenous injection of heparin. Here we report a novel method featuring a blend of heparin-loaded SBA-15, catechol-modified chitosan (CCS), and heparin as a heparin-releasing film. The release of heparin was based on its leakage from heparin-loaded amino-functionalized mesoporous silica SBA-15 (SBA-15-NH_2), which was controlled by the amino density of the SBA-15-NH_2. Heparin-loaded SBA-15-NH_2, CCS, and heparin were mixed together, and the mixture was cast onto the surface of a polydopamine-modified substrate, forming a heparin-releasing film on the surface of the substrate. The polydopamine acted as an adhesive interlayer that stabilized the film coated on the substrate. The sustained release rates of heparin from the film ranged from 15.8 to 2.1 $\mu g/cm^2/h$ within 8 h. The heparin-releasing film showed low fibrinogen adsorption, platelet adhesion, and hemolysis rate, indicating that it has good blood compatibility. This new approach would be very useful for modifying the surface of versatile blood-contacting biomaterials and ultimately improve their anticoagulation performance.



KEYWORDS: *blood compatibility, heparin, polydopamine, SBA-15, surface modification*

1. INTRODUCTION

Extracorporeal circuits have long been used in blood purification, such as hemodialysis and hemoperfusion. However, the greatest concern of extracorporeal circuit is that once its surface is in contact with the blood, it can be regarded as a foreign surface by the internal environment and thus will induce blood coagulation and the thrombogenicity.[1] Heparin is a widely used anticoagulant drug, and it binds to antithrombin III, resulting in the suppression of thrombin formation when administered via intravenous injection. However, since the diffusion of heparin through the boundary layer near the circuit's surface is much slower than that through the axial convection,[2] most of the heparin in the circulating blood will pass over the circuit rather than contact the circuit's surface.[3] This means that a high dose of heparin needs to be administered to prevent the thrombotic events, and this may put the patients at high risk of bleeding or other severe side effects.[4]

Modification of the surfaces of these extracorporeal circuits, especially heparinized surfaces, has been proposed as one of the most popular strategies to prevent blood coagulation and improve blood compatibility.[5] Heparin coating on the surface by physical adsorption could maintain its bioactivity, but the quick leaching of heparin will leave the surface unprotected.[6] In contrast, covalent coupling could enhance the stability of the immobilized heparin but lower the bioactivity of heparin.[7] These problems can be resolved by the use of heparin-releasing coating in which bioactive heparin can be slowly released into the low-flow boundary layer over a period of time. In this way, heparin is administered at low-dose levels and be available at exactly where it is needed.[3] The most common method for preparing heparin-releasing films is by blending heparin with polymers,[3,8−13] such as chitosan/polyethylene vinyl acetate, *N,N,N*-trimethyl chitosan, zein, copolymers of *N*-vinylpyrrolidinone and *n*-butylmethacrylate, polyurethane, and poly(ε-caprolactone). Most of these films have drawbacks such as quick release rate at the very early stage,[14] and/or low release percentage,[9,11] which might be due to a chain entanglement effect.[15] Therefore, the sustained release of heparin from the film surface is crucial for its practical applications.

Mesoporous silica is nontoxic and chemically inert and has been widely used in drug delivery and release systems.[16] Very recently, two mesoporous silica materials, MCM-41 and SBA-15, have been studied for the loading and releasing of heparin.[17] Heparin adsorbed in the long and narrow channels of mesoporous silica will undergo a long diffusion distance to

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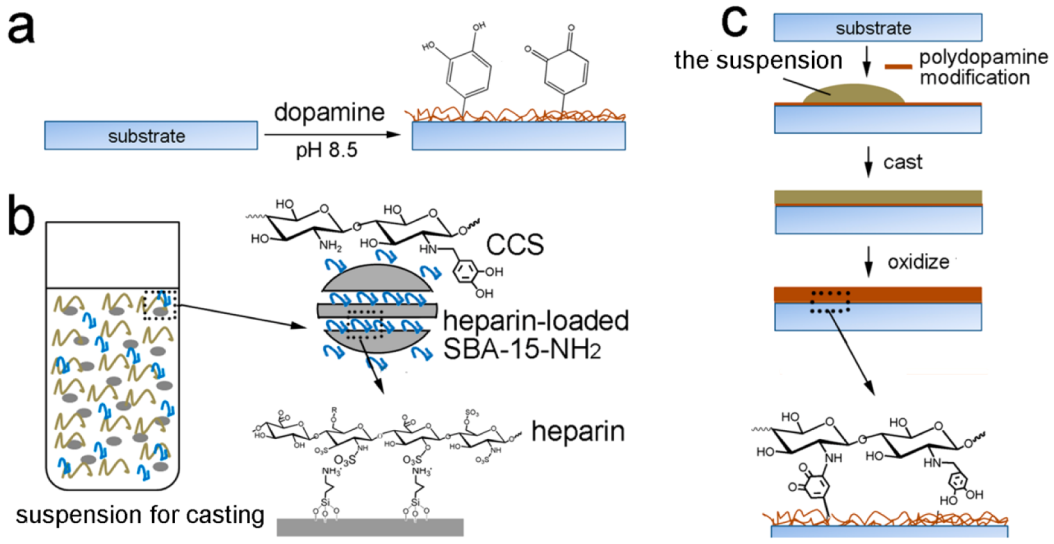

Figure 1. (a) Surface modification of a substrate with a polydopamine layer. (b) The cast solution containing heparin-loaded SBA-15, catechol-modified chitosan (CCS), and heparin and their interactions. (c) Schematic illustration of preparing heparin-releasing film coated on a polydopamine-modified substrate.

reach the pore entrances, which is beneficial for sustained release. Thus, immobilization of mesoporous materials onto blood-contacting substrates is a promising prospect to fabricate a heparin-releasing film. Mesoporous thin films are usually synthesized by sol-gel based rapid spin-coating or dip-coating onto a solid substrate.[18] Y. Zhou et al. recently prepared a mesoporous vascular prosthesis through epitaxial growth of mesoporous silica nanoparticles on the three-dimensional network of expanded polytetrafluoroethylene grafts (ePTFE) grafts for sustained release of heparin.[14,19] A large amount of heparin (exceeding 500 $\mu g/cm^2$) could be loaded onto the mesoporous vascular prosthesis, and the anticoagulant activity of the ePTFE grafts is greatly improved.[14] In spite of this significant progress, building up a heparin-releasing film (based on mesoporous silica materials) onto smooth substrates, which is significant to extend their potential biomedical applications, has yet to be realized. The main obstacle is that mesoporous silica films directly synthesized on a smooth solid surface by sol-gel process may be too fragile or unstable in water and biological media,[18,20] probably due to the discrepancy between the bulk properties of the substrates and films.

To meet these challenges, we derived a novel and facile approach to prepare a heparin-releasing film in which catechol-modified chitosan (CCS) and heparin were blended with heparin-loaded SBA-15 for coating onto a polydopamine-modified substrate. Polydopamine, inspired by mussel adhesive protein, could adhere to various surfaces via the self-polymerization of dopamine.[21,22] The polydopamine layer on the substrate surface could further react with amine-containing molecules.[23] Therefore, polydopamine might be able to act as an adhesive interlayer to allow the strong adhesion of the amine-containing heparin-releasing film onto a polydopamine-modified substrate. As for the preparation of heparin-releasing film, SBA-15 was used to load the heparin because of its low-cost and relative large mesopores (5−14 nm),[24] which is suitable for heparin loading and release since the width of heparin is about 1.5 nm.[14] Then, heparin-loaded SBA-15 was embedded into a blend of CCS and heparin. The film would be able to adhere strongly on the polydopamine-modified substrates because of the reaction between polydopamine and the amines of CCS. In detail, SBA-15 was first made functional with 3-aminopropyltriethoxysilane (APTES) to introduce amino groups, denoted as SBA-15-NH_2, and the influences of amino groups on the adsorption of heparin to SBA-15-NH_2 and the release of heparin from it were explored. Thereafter, heparin-loaded SBA-15-NH_2, CCS, and heparin were carefully blended together to form a homogeneous mixture and coated onto the surface of a polydopamine-modified substrate. The film was solidified by immersion in ethanol and then oxidized by $NaIO_4$ to make it cross-linked. We also performed an in vitro experiment to check whether the release of heparin from the surface could enhance the blood compatibility.

2. MATERIALS AND METHODS

2.1. Materials. SBA-15 was purchased from XFNano Materials Tech Co. Ltd. (Nanjing, China). Chitosan (M_w 100 kDa) was provided by Jinan Haidebei Marine Co. Ltd. (China). Heparin (sodium salt, 140 IU/mg) was obtained from Solarbio Co. Ltd. (Beijing, China). Human fibrinogen was bought from Shanghai Xinxing Medicine Co. Ltd. (China). 3-Aminopropyltriethoxysilane (APTES) was supplied by J&K Chemical (Beijing, China). Amino-functionalized SBA-15 (SBA-15-NH_2) was synthesized by silanization reaction with APTES. Catechol-modified chitosan (CCS) was synthesized by conjugating chitosan with 3,4-dihydroxy benzaldehyde (Figure S1 in the Supporting Information). The degree of conjugation was about 8% as calculated from UV-vis spectra (Figure S2 in the Supporting Information). The detailed synthesis procedures for SBA-15-NH_2 and CCS are presented in the Supporting Information. All other chemicals were of analytical grade.

2.2. Heparin Adsorption and Release on SBA-15 Samples. Before adsorption, all SBA-15 samples were equilibrated with 10 mM phosphate buffer (pH 3.0). Kinetic experiments were performed by incubating 100 mg of SBA-15 or SBA-15-NH_2 in 5 mL of 10 mM phosphate buffer (pH 3.0) containing 50 mg of heparin, and the mixture was then kept at 37 °C. At different time intervals, 100 μL of the mixture was sampled, and the concentration of heparin in the supernatant was determined by the toluidine blue method,[14] and the amounts of heparin adsorbed onto the adsorbents were calculated from the difference between this value and the original heparin concentration.

After heparin adsorption, the adsorbent was washed with phosphate buffer (pH 3.0) for three times, and then 50 mg of the adsorbent was put into 50 mL of 10 mM phosphate buffered saline (PBS, pH 7.4,

0.15 M NaCl) to assess the release of heparin. At predetermined intervals, 1.0 mL of the solution was taken out to determine the concentration of heparin and replaced with 1.0 mL of fresh PBS. The cumulative released amount of heparin at different times was determined. Adsorption isotherms were obtained by varying the initial heparin concentration while maintaining a constant SBA-15-NH_2 dosage of 20 mg/mL at 37 °C, pH 7.4. After 24 h of adsorption, the heparin concentration in the supernatant was determined by the toluidine blue method.

2.3. Preparation of Heparin-Releasing Film. The process of preparing the film is schematically illustrated in Figure 1. Substrates (silicon wafer, glass, and polyvinylchloride (PVC)) were immersed in dopamine solution (2.0 mg/mL) for 24 h at room temperature (Figure 1a). Thereafter, the modified substrates were rinsed in deionized water, further oxidized with $NaIO_4$, and then washed with 0.1 M HCl solution to stabilize the polydopamine coating.[25] Next, 45 mg of CCS, 6.0 mg of heparin-loaded SBA-15, and 1.5 mg of heparin were carefully mixed in 1.0 mL of 2% HAc (Figure 1b). The mixture was subjected to ultrasonic treatment until a homogeneous suspension was obtained, and 0.5 mL of the mixture was coated on the polydopamine-modified substrates (10 cm^2). The coated substrates were subsequently immersed in ethanol solution containing 1 wt % NaOH for 10 min to solidify the films. After that, the film-coated substrates were immersed in $NaIO_4$ solutions for 5 min (Figure 1c). This short time of oxidation may not oxidize the adjacent hydroxyls in heparin and affect the bioactivity of heparin, because our previous research has shown that it would take 4 h for $NaIO_4$ to activate the adjacent hydroxyls of β-cyclodextrin which is an oligosaccharide.[26] Finally, the film was washed with deionized water thoroughly.

2.4. Characterizations. Small-angle X-ray diffraction (XRD) patterns of powdered SBA-15-NH_2 were performed with D/MAX-2400. The amount of amino groups in the SBA-15-NH_2 was determined by elemental analysis (Vario EL III Elemental Analyser). Nitrogen adsorption-desorption isotherms were performed using AUTOSORB-1-MP at −196 °C. The pore size distribution curves were calculated from the analysis of the nitrogen adsorption isotherms using the Barrett-Joyner-Halenda (BJH) model. Fourier transform infrared (FTIR) spectra were taken with an EQUINOX55 FTIR spectrometer. Each spectrum was obtained with 32 scans at a resolution of 2 cm^{-1}. Transmission electron microscopy (TEM) images were captured with a JEOL JEM-2100 electron microscope. The sample was dispersed ultrasonically in ethanol and then spread onto a perforated carbon−copper microgrid. Scanning electron microscope (SEM) images of samples were obtained with a Quanta 450 microscope. The samples were coated with gold before observation.

2.5. Heparin Release from the Films. The release of heparin from the films coated on silicon wafer (50 cm^2) was performed in 50 mL of 10 mM PBS at 37 °C. At predetermined intervals, 1.0 mL of the buffer was removed to determine the concentration of heparin, and 1.0 mL of fresh PBS was added to preserve the volume of the solution. The amount of released heparin was determined from the concentration of heparin in the 1.0 mL buffer.

2.6. Blood Compatibility Tests. The surfaces of all the film samples (listed in Table 1) were washed with PBS for 10 min prior to the test. For protein adsorption experiments, the film samples were incubated with 1 mg/mL fibrinogen solutions (in PBS, pH 7.4) for 3 h at 37 °C.[27] They were subsequently rinsed slightly with PBS and distilled water. The adsorbed fibrinogen was then removed from the surface of the film by washing the film with 1 % SDS solution at 37 °C for 2 h. The protein concentration in the SDS solution was determined with a Micro BCA Protein Assay Reagent Kit (Shanghai Sangon Biotech, China), and the amount of adsorbed protein was calculated.

Fresh blood, obtained from a healthy rabbit (Laboratory Animal Center of Dalian Medical University), was mixed with anticoagulant (0.129 M citrate, anticoagulant to blood ratio 1:9).[28] The mixture was centrifuged at 200 × *g* for 10 min and 2500 × *g* for 15 min to obtain the platelet rich plasma (PRP) and platelet poor plasma (PPP), respectively. Platelet adhesion on the film surfaces was performed by mixing each film sample with PRP for 1 h at 37 °C. After washing with PBS, the platelet-adhered films were fixed with 2.5 wt % glutaraldehyde solution for 2 h and then immersed in 50, 70, 80, 90, and 100% (v/v) ethanol solution in sequence. The films were finally dried at room temperature, and the platelets that adhered to the film surface were examined with SEM.

The anticoagulant activity of the surfaces of the film samples was determined by the plasma recalcification time (PRT) assay. PPP and 25 mM $CaCl_2$ solutions were preheated at 37 °C for 5 min. The sample (0.5 × 0.5 cm^2) was placed into a tube, followed by addition of 0.1 mL of PPP. Then, 0.1 mL of 25 mM $CaCl_2$ solution was added, and the tube was shaken in a 37 °C water bath. The duration of time it took for the silky fibrin to appear in the solution was recorded as the PRT.

Hemolysis rate was evaluated by incubating the samples in diluted blood containing 5% fresh anticoagulant blood and 95% normal sodium chloride saline water at 37 °C for 1 h. Negative and positive controls were normal saline water and distilled water, respectively. After centrifugation at 1000 × *g* for 5 min, the absorbance of the supernatant at 541 nm was recorded. The hemolysis rate was calculated according to the following equation: hemolysis rate (%) = $(A1\text{-}A3)/(A2\text{-}A3) \times 100\%$ in which *A1*, *A2*, and *A3* are the absorbances of the sample, positive control, and negative control, respectively.[29]

Table 1. Fibrinogen Adsorption, PRTs, and Hemolysis Rates of Sample Surfaces[b]

sample surfaces	fibrinogen adsorption ($\mu g/cm^2$)	PRT (min)	hemolysis rate (%)
polydopamine-modified substrate	8.2 ± 1.3	6.8 ± 0.4	0.78 ± 0.18
C-SBA film	38.0 ± 3.1	5.7 ± 0.6	1.66 ± 0.53
CH-SBA film	18.1 ± 1.5	63.1 ± 4.1	0.78 ± 0.55
CH-hepSBA film	17.8 ± 1.9	>120	0.51 ± 0.20
CH-hepSBA film-8 h[a]	18.7 ±2.4	>120	0.52 ± 0.37

[a]The film was immersed in PBS for 8 h of heparin release before tests. [b]Data are presented as mean ± SD, $n = 3$.

3. RESULTS AND DISCUSSION

3.1. Characterization of Amino-Functionalized SBA-15. The density of amino groups on SBA-15-NH_2 is a key factor that affects the adsorption and release of heparin, since Zhu et. al. reported that positively charged amino groups can promote the adsorption of heparin and prolong the release time via electrostatic interaction.[17] Herein, three kinds of SBA-15-NH_2 were synthesized by regulating the molar ratio of APTES to SiO_2 (from 1:20, 1:10 to 1:5) to control the density of amine sites on the surface of SBA-15, denoted as SBA-15-NH_2(1), SBA-15-NH_2(2), and SBA-15-NH_2(3), respectively. FTIR spectra showed a peak at 2930 cm^{-1} for SBA-15-NH_2(1-3) which should be the asymmetric stretching of CH_2 from APTES, indicating that APTES had been modified on the SBA-15 matrix (Figure 2a). The reactive densities of amino groups were quantitatively determined by elemental analysis, and those of SBA-15-NH_2(1), SBA-15-NH_2(2), and SBA-15-NH_2(3) were 5.3 × 10^{-4}, 1.12 × 10^{-3}, and 1.80 × 10^{-3} mol/g, respectively (Figure 2b).

The macroscopic morphology and textural mesopores of SBA-15-NH_2 were investigated by SEM and TEM, respectively. SBA-15-NH_2 retained much of the (0.5−5 μm) rod-like shape of the untreated SBA-15 as seen from the SEM images (Figure 2c and Figure S3 in the Supporting Information). TEM images clearly showed the well-ordered pore channels of SBA-15 and SBA-15-NH_2(1-3) (Figure 2d and Figure S4 in the Supporting Information). The ordered mesoporous structure was further

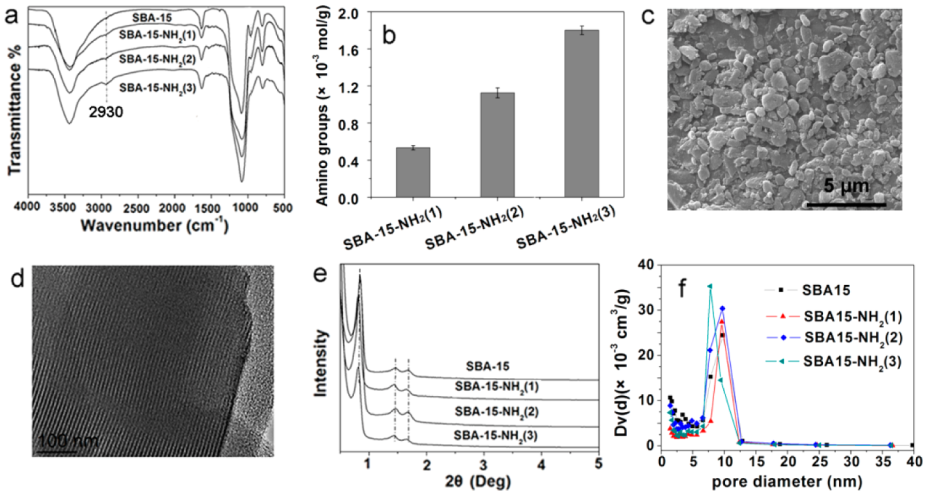

Figure 2. Physiochemical characterizations of SBA-15-NH_2. (a) FTIR spectra. (b) The density of amino groups on SBA-15-NH_2 (Data are presented as mean ± SD, n = 3). (c) SEM image of SBA-15-NH_2(1). (d) TEM image of SBA-15-NH_2(1). (e) Pore size distributions of SBA-15 and SBA-15-NH_2. (f) XRD patterns.

characterized by XRD. XRD analysis showed three characteristic diffraction peaks that could be assigned to (100), (110), and (200) diffractions, which associated with typical two-dimensional hexagonal symmetry (p 6mm) (Figure 2e),[24] which were in good agreement with the TEM results, implying that the chemical modification did not change the hexagonal structure of SBA-15-NH_2. The textural parameter was also detected by N_2 adsorption-desorption isotherms. The isotherms were of type IV and exhibited H1-type broad hysteresis loops typical of mesoporous solids in the partial pressure ranging from 0.60 to 0.90 (see Figure S5 in the Supporting Information).[24] Figure 2f shows that the pore size slightly decreased with increasing densities of amino groups. The above results suggested that the density of amino groups on SBA-15 matrix could be controlled by the ratio of APTES to SiO_2, and the uniform mesoporous characteristic of SBA-15 was preserved after the modification process.

3.2. Adsorption of Heparin by SBA-15. Figure 3a illustrates the kinetic curves for the adsorption of heparin onto SBA-15 and SBA-15-NH_2 at pH 3.0. The adsorption was rapid in the first hour and reached equilibrium at the end of 12 h. The time it took for the adsorption of heparin to SBA-15 or SBA-15-NH_2 to reach equilibrium was much shorter than that reported for MCM-41 (48 h).[17] This difference may be due to the larger pore sizes of SBA-15 and SBA-15-NH_2 (7−10 nm), compared to the pore size of MCM-41, which is around 4 nm.[17] In addition, the amino groups of SBA-15-NH_2 significantly promoted the adsorption of heparin, because more negatively charged heparin could be attracted to the protonated amino groups of SBA-15-NH_2 via electrostatic interaction at pH 3.0. The maximum adsorption of heparin to SBA-15-NH_2(1), SBA-15-NH_2(2), and SBA-15-NH_2(3) were 189.7 mg/g, 216.6 mg/g, and 216.3 mg/g, respectively. No significant difference was observed in the heparin adsorption capacity of SBA-15-NH_2(2) and SBA-15-NH_2(3) because the surface of SBA-15-NH_2(2) might be fully covered with heparin at this stage. As a result, further increase in amino groups had a negligible effect on the adsorption capacity.

3.3. Release of Heparin from SBA-15-NH_2. Sustained release of heparin from SBA-15-NH_2 was examined in PBS under pH 7.4. Figure 3b shows that an initial burst of release occurred in the first hour, because of the desorption of heparin

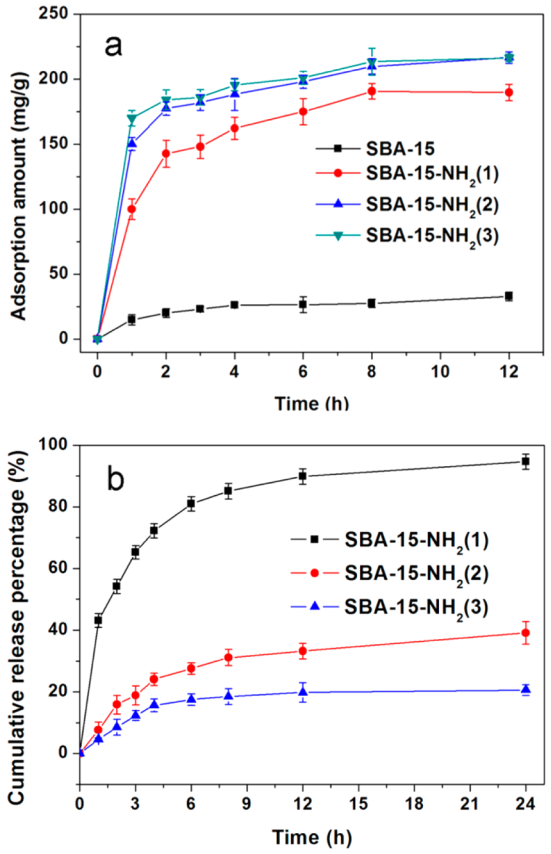

Figure 3. (a) Adsorption amount of heparin on SBA-15 and SBA-15-NH_2 as a function of adsorption time. (b) Kinetic release profiles of heparin from SBA-15-NH_2 (Data are presented as mean ± SD, n = 3).

located on the external surface and near the exit of the channels. After that, the release became relatively slow, which could be attributed to the long distance that the heparin needed to diffuse from the internal mesoporous channels. Besides, the cumulative release percentage of heparin released from SBA-15-NH_2(1) could reach 94.5% after 24 h, which was significantly higher than the value achieved by SBA-15-NH_2(2) and SBA-15-

NH_2(3). This phenomenon may be related to the binding affinity between SBA-15-NH_2 and heparin under pH 7.4.

3.4. Adsorption Isotherms. To evaluate the binding affinity between SBA-15-NH_2 and heparin at pH 7.4, we obtained the adsorption isotherms by varying the initial heparin concentration while maintaining a constant initial SBA-15-NH_2 concentration of 20 mg/mL and a constant temperature of 37 °C. As shown in Figure 4, the maximum heparin adsorption

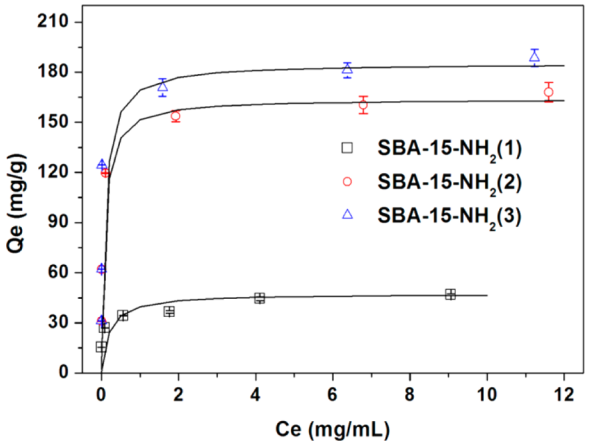

Figure 4. Adsorption isotherms of heparin on SBA-15-NH_2 (Data were presented as mean ± SD, n = 3).

capacities were 46.9, 168.0, and 188.6 mg/g for SBA-15-NH_2(1), SBA-15-NH_2(2), and SBA-15-NH_2(3), respectively. Compared with heparin adsorption capacity at pH 3.0 (Figure 3a), the adsorption capacity of SBA-15-NH_2(1) decreased by 75.2%, whereas those of SBA-15-NH_2(2) and SBA-15-NH_2(3) only decreased by 28.6 and 12.7%, respectively, at pH 7.4. Moreover, Figure 4 and Table S1 in the Supporting Information show that the equilibrium data fitted well with the Langmuir isotherm (R^2 > 0.99), indicating that the heparin monolayer did adsorb onto SBA-15-NH_2. The binding constants, calculated from the parameters of Langmuir isotherms at pH 7.4, significantly increased from 5.15 mL/mg for SBA-15-NH_2(1) to 17.67 mL/mg for SBA-15-NH_2(3) (see Table S1 in the Supporting Information). Although both SBA-15-NH_2(2) and SBA-15-NH_2(3) showed a high adsorption capacity for heparin at pH 3.0, the cumulative release mass of heparin in the case of SBA-15-NH_2(1) was highest at pH 7.4, due to its lowest binding affinity for heparin. Thus, SBA-15-NH_2(1) was selected for the preparation of heparin-releasing film in the subsequent study.

3.5. Preparation of Heparin-Releasing Film. As a natural biomacromolecule, chitosan has been used to fabricate biomedical materials because of its good biocompatibility and low toxicity.[15,30] Chitosan conjugated with catechol groups can be covalently cross-linked in situ to generate a robust chitosan-based hydrogel pad via the formation of cross-linking points between the catechol quinones and the excessive amines of chitosan.[31] However, the disadvantage of chitosan is its ability to trigger blood coagulation. Recently, a chitosan/heparin composite film with good blood compatibility was achieved by blending chitosan with heparin.[15] Therefore, in this study the prepared heparin-loaded SBA-15-NH_2(1) (Figure 1b) was embedded into the catechol-modified chitosan (CCS)/heparin blending film on polydopamine-modified substrates to fabricate a heparin-releasing film (CH-hepSBA film) (Figure 1c).

In a typical procedure, 45 mg of CCS, 6 mg of heparin-loaded SBA-15-NH_2, and 1.5 mg of heparin were mixed with 1.0 mL of 2% HAc solution, and the mixture was subjected to ultrasonic treatment until a homogeneous suspension was obtained that could be coated onto the polydopamine-modified substrates. The CCS concentration was fixed at 45 mg/mL because higher CCS concentration would make the mixture too sticky to form a homogeneous suspension. Besides, further increases in the amounts of heparin-loaded SBA-15-NH_2 and heparin in the mixture would lead to precipitation because of the strong electrostatic attraction between chitosan and heparin. After the polydopamine-modified substrate was coated with the mixture, it was immersed in ethanol to allow the coated film to solidify because chitosan, heparin, and SBA-15-NH_2 are insoluble in ethanol. The catechol in CCS was then oxidized to *o*-quinone by $NaIO_4$ (see Figure S2 in the Supporting Information),[31] causing the color of the film to change from light yellow to dark brown (Figure 5a).[32] During

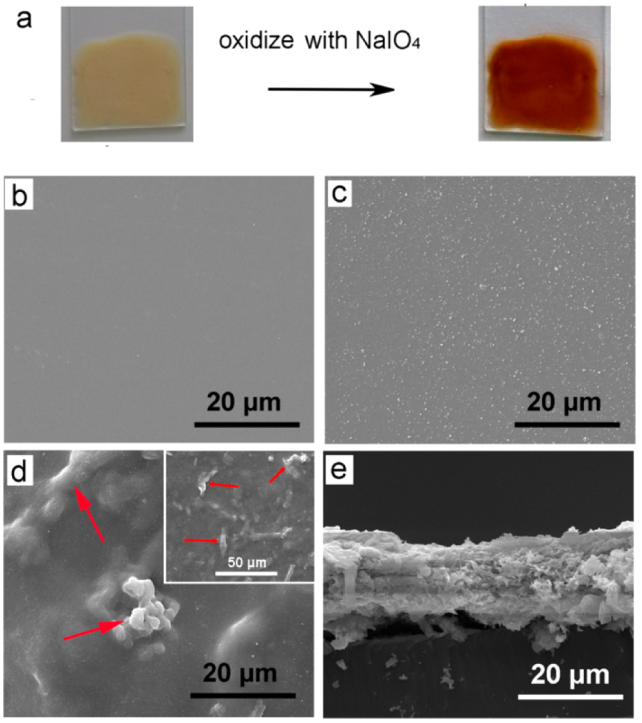

Figure 5. (a) Digital images of the mixture of heparin-loaded SBA-15-NH_2, CCS, and heparin coated on the polydopamine-modified substrates before and after $NaIO_4$ oxidation. SEM images of (b) silicon substrate, (c) polydopamine-modified silicon substrate, (d) top surface (inset is the image with lower magnification), and (e) cross-section of the heparin-releasing film. The arrows in (d) show the embedded heparin-loaded SBA-15-NH_2.

this process, part of the excessive amines in CCS could react with *o*-quinones in CCS to induce cross-linking and make it more stable, while other amines could react with the *o*-quinones of the polydopamine deposited on the substrate to maintain the complete anchorage of the films (see Figure S7 in the Supporting Information).[23] By contrast, the films coated on the bare surfaces of the substrates (such as glass and PVC) clearly cracked and abscised after it was dried at room temperature (see Figure S7 in the Supporting Information). Therefore, the pretreatment of the substrates with polydopamine could stabilize the heparin-releasing film, and polydop-

amine acted as an adhesive layer that bonded the CH-hepSBA film to the substrate. Besides, the wet film was flexible and could be completely coated on the substrate after it was bent with tweezers for 25 bending cycles and immersed in 10 mM PBS for 24 h (see Figure S8 in the Supporting Information).

Figure 5b-d presents the SEM images of the sample surfaces. The silicon substrate showed a smooth surface (Figure 5b) but turned rough after modification with polydopamine (Figure 5c). After coated with CH-hepSBA film, the surface became much rougher, and some SBA-15-NH_2 particles with several micrometers in size were observed on the outer surface and the inner part of the film (Figure 5d, arrow indicated), which were consistent with the diameter (0.5−5 μm) of SBA-15-NH_2 (Figure 2c). The thickness of the CH-hepSBA film was 15 μm, as estimated from the cross-sectional image (Figure 5e). The polydopamine layer was hard to observe in the cross-sectional image since it was about 0.1 μm in thickness as reported by many investigators[21] and was negligible compared to the thickness of the CH-hepSBA film.

3.6. Release of Heparin from the Films. The effects of the cross-linking on the release of heparin were firstly investigated (see Figure S9 in the Supporting Information). Then, the profiles of the release of heparin from CH-hepSBA film and the film prepared by blending CCS/heparin and SBA-15-NH_2 (CH-SBA film) are shown in Figure 6. The cumulative

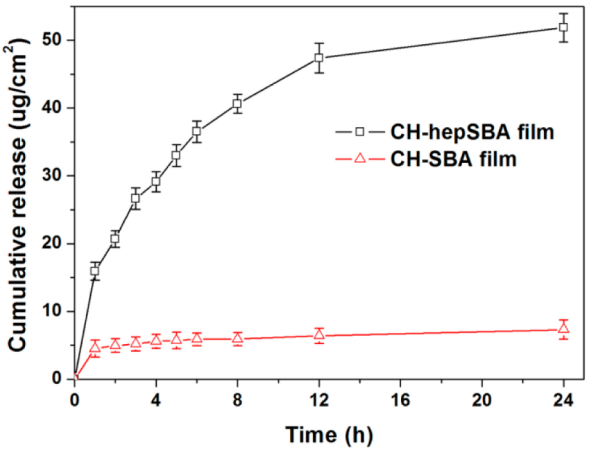

Figure 6. Release profiles of heparin from CH-SBA film and CH-hepSBA film (Data are presented as mean ± SD, n = 3).

release was quite low for CH-SBA film and almost stopped after 2 h as the majority of the heparin chains might be entangled with the CCS chains via electrostatic attraction, forming the polyelectrolyte complex. Consequently, the released heparin might be those that were weakly bound to CCS on the surface. However, heparin in the CH-hepSBA film showed a fast release within the first hour and gradually released up to 24 h. The cumulative release of heparin that was loaded on SBA-15-NH_2 in CH-hepSBA film (not including the heparin blended with CCS in the bulk film) was 34.7 μg/cm^2 within 8 h. Compared to the theoretical amount of heparin loaded on SBA-15-NH_2 in CH-hepSBA film (56.9 μg/cm^2), 61.0% of the heparin entrapped in the channel of SBA-15-NH_2 was released to the film surface within 8 h. Idezuki et al. reported that a minimal heparin release rate of 1.7 μg/cm^2/h was needed to render the thrombus free.[33] In our work, the rates for the continuous release of heparin from CH-hepSBA film ranged from 15.8 to 2.1 μg/cm^2/h within 8 h (see Table S2 in the Supporting Information), which was higher than the minimal value. Therefore, the sustained release of heparin from CH-hepSBA film seems promising for the prevention of blood coagulation in short-term applications, such as blood purification.

3.7. Blood Compatibility. The fouling process usually starts with protein adsorption on the surface of biomaterials, once contacting with blood and/or body fluid.[34] The adsorption of fibrinogen to the surfaces of the different film samples (listed in Table 1) in PBS was investigated because fibrinogen has been identified as one of the most important types of adsorbed proteins in blood-material interaction that induces coagulation and platelet adhesion.[1] CH-SBA film and film prepared by blending CCS with SBA-15-NH_2 (C-SBA film) were used as reference. The results showed that 38.0 μg of fibrinogen adsorbed to every cm^2 of the C-SBA film, which was much higher than that achieved for polydopamine surface (8.2 μg/cm^2). For CH-SBA and CH-hepSBA films, the values of fibrinogen adsorption were significantly reduced down to 18.1 μg/cm^2 and 17.8 μg/cm^2, respectively, illustrating that the heparin-containing film was resistant to fibrinogen adsorption. This could be explained by the electrostatic repulsion between the negatively charged heparin on the surface of the film and the negatively charged fibrinogen.[15] In addition, the adsorbed masses of fibrinogen on both CH-SBA film and CH-hepSBA film were higher than on polydopamine surface, and this might be attributed to the rougher surfaces of CH-SBA and CH-hepSBA (Figure 5d).

It has been known that the adsorbed fibrinogen must be converted to fibrin to further induce blood coagulation which is catalyzed by thrombin. Thus, the PRTs of the sample surfaces were measured to evaluate the anticoagulant activity. The PRTs of polydopamine surface and C-SBA film were 6.8 min and 5.7 min, respectively, while it was 63.1 min for CH-SBA film (Table 1). Therefore, blending heparin with chitosan could significantly improve the anticoagulant activity of the film, which is in agreement with previous report.[15] When the heparin-loaded SBA-15-NH_2 was used to prepare the CH-hepSBA film, the anticoagulant activity was further improved, and no clotting was observed within 120 min. The high affinity between heparin and antithrombin III can impair the formation of thrombin.[3] For this reason, the formation of fibrin from fibrinogen was greatly suppressed in the presence of heparin released from the surface of the film, and thus the anticoagulant activity was significantly improved.

Adhesion of platelets on biomaterials is a key event in blood coagulation, because platelets can adhere, aggregate, and release the contents of their granules to promote blood coagulation.[35] A polydopamine-modified substrate and C-SBA film were used as positive controls, because it has been reported polydopamine and chitosan surfaces can induce platelets adhesion.[15,36] Figure 7a shows that a polydopamine surface was covered with shape-changed platelets, and platelets underwent aggregation, implying these platelets were activated. For C-SBA film, the number of adhered platelets was enhanced and the silky fibrin could be observed (Figure 7b). The adhesion of platelets and fibrin on the surface of CH-SBA film was significantly reduced (Figure 7c). By contrast, almost no platelet adhesion and fibrin formation occurred on the surface of CH-hepSBA film (Figure 7d). The formation of fibrin observed by SEM was consistent with that determined by PRTs, and these results showed that CH-hepSBA film containing heparin-loaded SBA-15-NH_2 had better resistance against the formation of fibrin and platelet adhesion than polydopamine-modified surface, C-SBA film, and

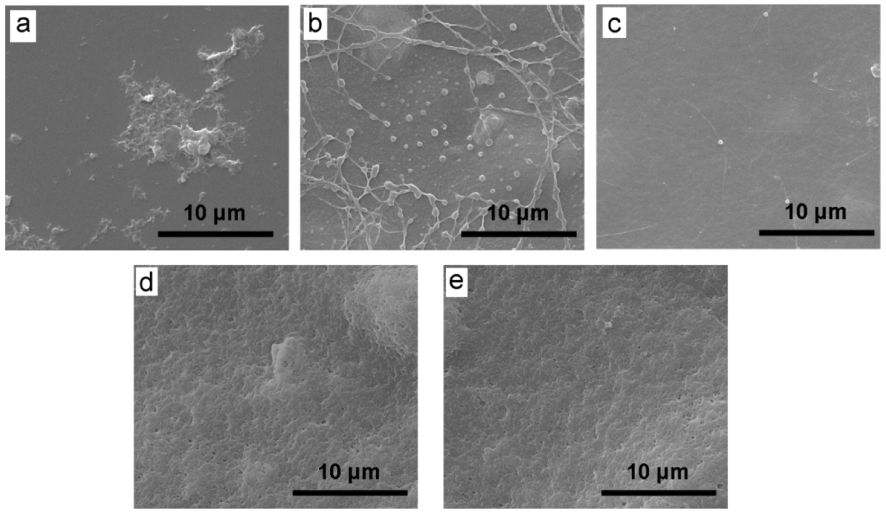

Figure 7. SEM images of platelets adhesion on (a) polydopamine surface, (b) C-SBA film, (c) CH-SBA film, (d) CH-heparin film, and (e) CH-hepSBA film after immersion in PBS for 8 h.

CH-SBA film. Receptors of thrombin and fibrin on the surface of platelet can mediate the adhesion and activation of the platelet.[37] Resistance against platelet adhesion on CH-hepSBA film might be due to the fact that CH-hepSBA film could suppress the formation of thrombin and fibrin on its surface.

Hemolysis rate is another important factor for characterizing blood compatibility. Table 1 shows that the hemolysis rate of C-SBA film was 1.66%, which corresponded to the highest hemolytic activity. However, the values for the hemolysis rates of CH-SBA and CH-hepSBA film were 0.78% and 0.51%, respectively, and no apparent hemolytic activity was observed. Therefore, CH-hepSBA film exhibited the best blood compatibility, compared with polydopamine-modified surface, C-SBA film, and CH-SBA film.

The durability of CH-hepSBA film was evaluated. After CH-hepSBA film was immersed in PBS for 8 h to allow the release of heparin, fibrinogen adsorption showed minimal changes (Table 1), and no platelet adhesion and fibrin formation occurred on the surface (Figure 7e). PRT showed that no clotting was observed within 120 min (Table 1). In addition, the hemolysis rate did not change either. In this case, it appeared that CH-hepSBA film exhibited good durability, which could be due to the continuous release of heparin within 8 h (Figure 6). Besides, an in vitro experiment was further carried out to simulate the extracorporeal circuit, and the anticoagulant activity of CH-hepSBA film was tested when it contacted the whole blood. In detail, after the heparin-releasing film was coated on the inner surface of a centrifugal tub, 1.0 mL of the fresh whole blood was added. The result showed that the whole blood in the functionalized tube could not coagulate after at least 3 h (see Figure S10 in the Supporting Information), which may be promising to meet some clinical demands, such as hemoperfusion which normally needs 2−3 h of extracorporeal circulation of blood.[38] These results demonstrated that the CH-hepSBA film could maintain its anticoagulant activity in the short-term, which is very promising for improving the blood compatibility of the extracorporeal circuits.

4. CONCLUSIONS

We report a novel method for developing an anticoagulant film in which CCS and heparin is blended with heparin-loaded SBA-15-NH_2 for a sustained release of heparin. The film is coated on the polydopamine-modified substrate, and polydopamine could act as an adhesive interlayer to stabilize the heparin-releasing film. The release of heparin is based on its leakage from heparin-loaded SBA-15-NH_2. The amino-functionalization of SBA-15 is beneficial for the adsorption and release of heparin when the density of amino groups is 5.3×10^{-4} mol/g, and the release percentage of heparin decreases with increasing the density of amino groups. The sustained release rates of heparin from the film range from 15.8 to 2.1 $\mu g/cm^2/h$ within 8 h. The film exhibits low fibrinogen adsorption, platelet adhesion, and hemolysis rate. Besides, no clotting of plasma is observed on the film within 120 min even after the film is immersed in PBS for 8 h. Therefore, this study demonstrates the potential of the heparin-releasing film based on heparin-loaded SBA-15-NH_2 as a coating material to prevent blood coagulation on the surfaces of versatile biomaterials.

■ ASSOCIATED CONTENT

Supporting Information

Detailed preparation processes of SBA-15-NH_2 and catechol-modified chitosan (Figure S1), UV-vis spectra of catechol-modified chitosan (Figure S2), SEM images and TEM images of SBA-15-NH_2(2) and SBA-15-NH_2(3) (Figure S3 and Figure S4), nitrogen adsorption-desorption isotherms of SBA-15 and SBA-15-NH_2 (Figure S5), FTIR spectra of films coated on a silicon substrate (Figure S6), effects of pretreatment of the substrates with polydopamine layer (Figure S7), stability test of the film (Figure S8), effect of cross-linking on the heparin release (Figure S9), the whole blood coagulation test (Figure S10), parameters of Langmuir isotherms (Table S1), and heparin release rate from the film surface (Table S2). 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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