Micelle-embedded coating with ebselen for nitric oxide generation

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Abstract

Nitric oxide generation is considered to be a key factor to mimic endothelial function in terms of anti-coagulation and anti-hyperplasia. Herein, ebselen which could play the similar role as glutathione peroxidase-like was loaded into micelles and was further assembled into a layer-by-layer coating. The ability of nitric oxide generation and corresponding biological effect were investigated. Endothelial-mimetic surface has now attracted huge attention in blood-contacting materials, due to its inherent ability of secreting nitric oxide. Among those categories, nitric oxide generation surface is considered to be safe and tunable in the modification of vascular biomedical devices. How to adsorb or immobilize glutathione peroxidase-like catalyst and maintain sustained/safe nitric oxide generation is full of interest. This study aimed at developing a functional coating constructed via layer-by-layer assembly to introduce the catalyst into the coating by pre-loading ebselen in micelles. We firstly introduced phenylboronic acid moiety into the micelle molecule backbone and grafted catechol moiety to chitosan backbone. Then, chitosan, micelles (containing ebselen) and heparin were adopted as polyelectrolytes and then alternatively assembled onto the substrate via layer-by-layer protocol. The catechol was conjugated to the amine groups of chitosan by Schiff base reaction to synthesize chitosan-catechol. The hydrophobic cholesterol was conjugated to the one end of the hydrophilic hyaluronic acid, and the hydroxymethylphenylboronic acid was conjugated to the other end via the esterification of carboxyl (–COOH) and hydroxyl (–OH). The modified hyaluronic acid could spontaneously form micelles in aqueous solution. Ebselen was the loaded into the as-prepared micelles. Chitosan-catechol, heparin, and micelles were alternatively assembled onto the substrate layer by layer to form a micelle-embedded coating. The micelle-embedded coating with ebselen was successfully obtained and the nitric oxide generation ability was in a safe level which was close to healthy endothelial cells. The coating could effectively inhibit platelet adhesion and smooth muscle cell proliferation. The use of ebselen preloaded into micelles could provide a sustained release of catalyst for in situ nitric oxide generation. Besides, this method could also be used to load diverse drugs and regulate desired properties. The study was approved by the Institutional Review Board of the West China Hospital in Sichuan University on March 3, 2018, with approval No. K2018044.

Key words: micelle; ebselen; nitric oxide; layer-by-layer; cardiovascular implants; anti-coagulation; surface modification; endothelialization

INTRODUCTION

Cardiovascular disease has become the most fatal killer in threatening human health.1,2 Coronary stents are devices designed to prevent elastic recoils and intimal hyperplasia associated with percutaneous transluminal coronary angioplasty.3,4 With decades of clinical application, coronary stents have already demonstrated the effectiveness and saved millions of lives. Though successful, clinical failures like late stent thrombosis has been treated as a significant risk when using drug-eluting stents, mainly due to the non-selective inhibition of the proliferation of both endothelial cells and smooth muscle cells (SMCs), affected by the released anti-hyperplasia drug, like sirolimus and paclitaxel.5 Moreover, delayed re-endothelialization would possibly cause late/very late stent thrombosis. Rainier6 discussed the new drug-eluting stent concepts and concluded that the drugs applied should inhibit SMCs proliferation and migration without affecting endothelial regeneration, and reduce the risk of stent thrombosis and anti-inflammation. Besides searching for drugs or a combination of drugs that can provide multifunction, surface coatings which potently address the enhanced antithrombotic and anti-hyperplasia while do no harm to endothelialization are also of special interests.7,8

Nitric oxide (NO) plays a pivotal role as a messenger and signaling molecule in maintaining vascular microenvironment in terms of mediating vascular endothelial function, inhibiting the adhesion of platelets and leukocytes, down-regulating vascular SMC proliferation, and their synthesis of protein and collagen.9,10 Healthy endothelial cells could secret a NO flux of $0.5-4 \times 10^{-10} \text{ mol/(cm}^2\text{ min)}$.11

Stent implantation usually causes acute blood vessel damage and thus stents fabricated with a coating that mimic the basic function (NO releasing) of endothelial cells would protect the endothelialization process while inhibiting SMCs proliferation and antithrombosis formation. This approached is now well known as “NO-generating” and has been fully developed...
by researchers, especially represented by Prof. Meyerhoff’s team.\textsuperscript{12,13} Polymers or coatings contain an immobilized catalyst such as glutathione peroxidase-like catalytic mimics (i.e., selenocystamine, cystamine) and copper nanoparticles or copper (II) ion/ligand complex have been reported by decomposing circulating S-nitrosothiols (RSNO) to release NO at the blood/material interface.\textsuperscript{14} On this basis, Yang et al.\textsuperscript{15,16} had immobilized the NO generation catalyst like copper and selenocystamine on the surface of 316L stainless steels stents and found that due to the potent catalytic effect of \textit{in situ} generation of NO \textit{in vivo}, both the thrombosis formation and intimal hyperplasia were impressively inhibited. Luo et al.\textsuperscript{17} also grafted cystamine onto heparin backbone and then further prepared a heparin-cystamine/polyethyleneimine nanoparticles functionalized coating to produce the continuous generation of NO. However, some \textit{in vivo} toxicity studies revealed that there might be the reaction between reduced selenium species and oxygen, which was too fast to produce a significant amount of superoxide that could react with NO to produce peroxynitrite, a toxic species.\textsuperscript{17} And among those selenium catalysts, aromatic organoselenium species have been found to be far less toxic (for example, ebselen (Ebs)).\textsuperscript{18}

As commonly accepted, tuning surface properties is a convenient method for controlling interactions between materials and surrounding microenvironment. Layer-by-layer (LBL) assembly has been treated as a versatile and easy technique for fabrication of multifunctional coatings in biomedical applications. With alternative assembly of selected polyelectrolytes, alone or in combination with bioactive components packaging, desired surface properties are simply achieved.\textsuperscript{19-21} Though widely been investigated, most LBL components are water soluble, which means it is not an ideal platform for loading hydrophobic drug, such as clinically used anti-hyperplasia drug rapamycin.\textsuperscript{22,23} In order to enforce the local drug administration in the multifunctional LBL coating, the pre-loading of drug into a carrier is feasible. In our previous study, we fabricated a micelle (MIC)-embedded LBL coating with catechol and phenylboronic acid for tunable drug loading and sustained release.\textsuperscript{24} Briefly, a self-healing sandwiched LBL coating was constructed by using chitosan/heparin as the adopted polyelectrolytes with embedding of MICs, in which chitosan backbone was grafted with catechol and the MIC was modified with exposed phenylboronic acid. Moreover, rapamycin and atorvastatin calcium were selected as drug candidates and loaded into MICs, following by drug releasing behavior study. We found that using such coating protocol, the introduction of catechols and boronic acids would endow the coating with enhanced stability by abundant interactions among each coating component (e.g., boric acid ester bond formation, weak intermolecular cross-linking, π-π interactions and H-bonding).\textsuperscript{24}

Based on above considerations, herein, we used the similar coating approach, and merely changed the preloaded drug to Ebs, a low toxic aromatic organoselenium that has been used as anti-inflammatory drug.\textsuperscript{18,19} Ebs had also been demonstrated as a glutathione peroxidase-like catalyst to decompose RSNOs to release NO \textit{in vivo}. In this work, we also tested the release profile of Ebs in the MIC-embedded LBL coating, along with the anti-platelet adhesion test and SMCs proliferation test.\textsuperscript{18,25}

**Materials and Methods**

**MIC preparation and drug loading**

The amphiphilic block MIC molecule is synthesized with three parts, using hyaluronic acid (HA; 10 kDa; Sigma-Aldrich, St. Louis, MO, USA) as the backbone, modified with 2-hydroxymethylphenylboronic acid (2-HMPBA; Sigma-Aldrich), and using cholesterol to form the hydrophobic core. In brief, HA (1 g), cholesterol (700 mg; Sigma-Aldrich), 2-HMPBA (500 mg) were entirely dissolved in dimethyl sulfoxide (30 mL) at 80°C, following with the adding of N,N’-dicyclohexylcarbodiimide (500 mg; Sigma-Aldrich) and 4-dimethylaminopyridine (200 mg; Sigma-Aldrich) with continuously stirring for 24 hours at 80°C. Thereafter, the mixture was dialyzed in deionized (DI) water (dialysis membrane bag MWCO = 3000 Da) for 2 days, and the solid phase was separated from the reaction solution by vacuum filtering under vacuum to remove the excessive cholesterol. Finally, the mixture was dialyzed in DI water (dialysis membrane bag MWCO = 3000 Da) for 1 more day. The final product was lyophilized and kept in a moisture-free desiccator for further use. The degree of catechol substitution on the surface of 316L stainless steels stents was measured by proton nuclear magnetic resonance (Bruker Avance, London, UK, 400 MHz, D$_2$O). The hydrophobic cholesterol was conjugated to the one end of the hydrophilic HA, and the 2-HMPBA was conjugated to the other end via the esterification of carboxyl (-COOH) and hydroxyl (-OH). MIC molecules (40 mg) were completely dissolved into dimethyl sulfoxide (10 mL) at 80°C, and then Ebs was added into the solution. After that, DI water (10 mL) was slowly added into above solution drop by drop under vigorous stirring. Finally, the mixture was dialyzed in DI water for 2 days (dialysis membrane bag MWCO = 500 Da). The concentration of the mixture solution was adjusted to 1 mg/mL by evaporation using rotary evaporators and attenuation using DI water, the pH was adjusted to 6.5 by 1 M hydrogen chloride. Ebs (98%; BioChemPartner, Shanghai, China) loaded MICs were denoted as micelles (MIC)-Ebs and the MICs without drug loaded were labeled as MIC. After that, the size and morphology of MIC and MIC-Ebs were evaluated using transmission electron microscopy (TEM, Hitachi H-600, Tokyo, Japan).

**Synthesis of catechol-modified chitosan**

Catechol-conjugated chitosan (CS-C) was synthesized using chitosan ($M_w$ 100 kDa, 80% deacetylated; Sigma-Aldrich) and 3,4-dihydroxybenzaldehyde (3,4-DHB; Sigma-Aldrich) following previously published method by Clifford et al.\textsuperscript{26} The catechol was conjugated to the amine groups of chitosan by Schiff base reaction. Methanol was used to dissolve 3,4-DHB, NaBH$_4$ (Sigma-Aldrich) as a reductant was used to reduce the C=O to C-N, and the degree of catechol substitution on the backbone of chitosan was determined by proton nuclear magnetic resonance. Stock solutions of CS-C (2 mg/mL, pH 6.5) were prepared for further use.

**LBL coating construction and characterization**

316L stainless steels with the size of 10 mm × 10 mm were mirror-polished as the substrate for biocompatibility test. The MIC-embedded LBL coating fabrication process was shown in Figure 1. Briefly, the CS-C, MIC and heparin (185 U/mg;...
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Catalytic ability of NO generation

The NO release catalyzed by sample was examined using Saville-Griess reagent reported before.27,28 Briefly, the coated substrate (1 cm × 1 cm) was placed in a 24 well plate and interacted with 1 mL testing solution. The testing solution contained two parts: (1) 0.5 mL donor solution (200 μM ethylenediaminetetraacetic acid, 65 μM S-nitroso-N-acetyl-DL-pencillamine (SNAP; Sigma-Aldrich) and 30 μM L-ethylenediaminetetraacetic acid, 65 μM S-nitroso-N-acetyl-DL-pencillamine (SNAP; Sigma-Aldrich)); and (2) 0.5 mL Saville-Griess reagent (2.5 μM). Once interacted with the coating surface with glutathione (Sigma-Aldrich)), the released NO would quickly form NO reagent (2.5 μM). The NO release catalyzed by sample with Ebs loading was labeled as LBL10@Ebs. Particularly, ultraviolet-visible light spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) on a quartz plate and the ultraviolet adsorption between 180 and 600 nm was recorded. X-ray photoelectron spectroscopy (XSAM800, Kratos Ltd., Manchester, UK) was used for further investigations of the surface chemical compositions (Al Kα X-ray source, 1486.6 eV). Coatings prepared on silicon wafer were examined by scanning electron microscopy (S-3400N; Hitachi) to study the surface morphology. The Ebs loading capacity, encapsulation efficiency and Ebs release profile were also obtained following the similar protocol. The amount of the released Ebs in the collected medium solution was measured via high performance liquid chromatography (Waters 1525 chromatography, Milford, MA, USA; equipped with a C18 (250 mm × 4.6 mm, 5 μm)).

Platelet adhesion

More than five samples were used for statistical count, and each test was done for more than three times. The whole blood was collected from rabbit in negative pressure tubes containing sodium citrate as the anticoagulant. The platelet plasma was obtained by centrifuging the obtained whole blood at 1500 r/min for 15 minutes. Briefly, samples were incubated with platelet rich plasma (SNAP containing solution that contained 65 μM SNAP and 30 μM L-glutathione) for 30 minutes at 37°C, and after fixing and dehydration treatment, the platelets morphologies were viewed using scanning electron microscopy.

Smooth muscle cell proliferation

Human umbilical artery SMCs were isolated from newborn umbilical cord (ethics approval was obtained by the Institutional Review Board of the West China Hospital in Sichuan University, approval No. K2018044 on March 3, 2018. The enrolled subjects signed the informed consent.) by the explant method as described previously.30 SMCs were cultured using Dulbecco’s modified Eagle medium containing 1% antibiotic penicillin-streptomycin and 10% fetal bovine serum). Before seeding, the samples were sterilized by ultraviolet for 1 hour. The samples were then incubated with 1 mL of SMCs suspension solution (with a density of 3 × 10^4 cells/mL) at 37°C under 5% CO2 for 4 hours, 1 day and 3 days. The cells interacting with NO donors were also carried following our previous study.14 The cell viability was tested following the cell counting kit-8 (Dojindo, Kumamoto, Japan) assay. Besides, samples cultured with SMCs were washed completely with saline solution thrice and soaked in 2.5% glutaraldehyde solution for 12 hours, and then stained with rhodamine for 20 minutes. After fully washing, the cells were observed via fluorescence microscope (TE2000; Nikon, Tokyo, Japan).

Statistical analysis

The date was obtained and analyzed using SPSS 11.5 (SPSS, Chicago, IL, USA) and expressed as the mean ± standard deviation (SD). The statistical significance between and within groups was determined using a one-way analysis of variance.

RESULTS

Synthesis of chitosan-catechol and phenylboronic acid modified MICs

It is well known that catechol modified biomolecules will make contribution in strengthening coating stability due to the inherent adhesive properties and enhanced intermolecular interactions.31-33 Herein, the catechols were conjugated to chitosan backbones as shown in Figure 2A. The value of the catechol proton peaks which appeared from 6.5 to 7.0 ppm indicated successful preparation, and the degree of catechol conjugation was about 31.4%. Besides, the phenylboronic acid modified micellar molecule was also successfully prepared. As shown in Figure 2B, the 2-HMPBA peaks appeared from 7.4 to 7.5 ppm, the proton peaks of acetyl group from 7.9 to 8.1 ppm and the cholesterol from 5.5 to 5.75. The value of the degree of 2-HMPBA was 16%, and the value of the degree of cholesterol substitution was 6%. These data indicated successful synthesis of modified molecules.

MICs and MIC-embedded coatings

With the successful synthesis of MIC molecules, which consisted of hydrophilic HA backbone, hydrophobic cholesterol and terminated phenylboronic acid, we further tested the micellar structure and property. The transmission electron microscope results of MIC and MIC-Ebs were shown in Figure 3A. The particle size of MIC was around 200 nm, and after drug loading, the size was bigger due to the additional contribution in the hydrophobic core of MICs which resulted in the increasing of particle size (Figure 3B). The ultraviolet-visible light absorbance at 280 nm indicated the catechol moieties and the absorbance at 325 nm represented the successful loading of Ebs into the MICs (Figure 3C). The averaged particle size, polydispersity index, zeta-potential, drug loading and encapsulation ratio of these MICs are shown in Table 1. The polydispersity index value of MIC was 0.125, indicating that
Figure 1: The schematic diagram of MIC-embedded layer-by-layer coating construction and the potential intermolecular interactions. Note: MIC: Micelles.

Figure 2: The illustration of synthesis of catechol modified chitosan (A) and phenylboronic acid modified MIC molecule (B) with their corresponding proton nuclear magnetic resonance results. Note: MIC: Micelles.

Figure 3: The micellular structure and property of MIC and MIC-Ebs. Note: (A) The transmission electron microscope of MIC and MIC-Ebs. (B) The particle size of micelles. (C) The ultraviolet-visible light result of the micelles. MIC: Micelles; Ebs: ebselen.
the particle size distribution was more concentrated than the drug loaded MICs. Moreover, the zeta-potential value of these MICs was under −20 mV which derived from the carboxyl of HA that were the hydrophilic ends of MIC, which also strongly implied that it could be applied as an anionic polyelectrolyte in constructing LBL coatings. The drug loading efficiency of MIC-Ebs was about 6%, and the encapsulation ratio was more than 30%. The zeta potential value (−27.33 mV) indicated that MICs could be well applied as negatively charged polyelectrolytes, endowing the potential to be applied into the LBL coatings and functionalized as a drug eluting model.

As shown in Figure 1, the existence of catechols and phenylboronic acids would provide more intermolecular interactions among each component. The borate ester bond formation would possibly happen when the pH is higher than 6.44 Besides, weak intermolecular crosslinking between adjacent catechols, along with the nucleophilic addition between oxidized quinone and primary amines will also enhance the coating stability.31,33 Prior to LBL coating construction, we tested the potential interactions between MICs and catechol-modified chitosan at different pH value. As seen in Figure 4, CS-C and MIC were mixed under pH 5.0 and pH 7.0, and the mixture was viewed again after 24 hours. The solution of CS-C and MIC were transparent at pH 5.0. The color of the CS-C solution at pH 7.0 turned to pale yellow, due to the slight oxidation of catechols to quinones. Once mixed together for 24 hours, a faint haze like solution and few precipitates were observed, suggesting weak intermolecular interactions between CS-C and MIC at pH 5.0, mainly are electrostatic interactions. However, at pH 7.0, more epinephelos yellow precipitates were observed, which might be ascribed to the boric acid ester formation between catechols and phenylboronic acid. Thus, more interaction force between CS-C and MIC make the complex denser. On the basis of above phenomenon, the CS-C and MIC would also form similar interactions on the substrate-liquid interface and thus maintain a tunable LBL coating construction process.

We have constructed the MIC embedded LBL coating in our previous work and found that due to the modular assembly of each coating component, the introduced bionic acid ester bond formation and intermolecular crosslinking would help to tuneable the coating growth.24 In this work, the surface morphology of LBL10 and LBL10@Ebs was shown in Figure 5A. Obviously, the porosity was maintained and the basic micellar structure with multi-spherical particles near 200 nm in diameter was performed. The coating became denser in the Ebs loaded coatings which might be ascribed to the enriched π–π stacking interactions. Such step-by-step steadily coating growth might make potential contribution in tunable functionality introduction or drug loading. The X-ray photoelectron spectroscopy wide scan indicated successful loading of Ebs with the facile assembly of MICs (Figure 5B). The total amount of loaded Ebs on LBLn@Ebs samples was about 52.50 μg/cm² (Figure 5C).

With the enriched interactions, such coating also presented an inhibition of burst release of Ebs (only 18.6% of released Ebs for the first day), compared to commercial used drug-eluting stents (usually more than 30%).36 The coating could also support sustained release of Ebs (more than 1 month) and heparin (more than 20 days).

**NO generation and biological effect**

As mentioned before, in the presence of reductant L-glutathione, organoselenium would function as a catalyst to in situ decompose RSNOs to release NO.18 In this work, the *in vitro* catalytic degradation of SNAP was evaluated using Saville-Griess reagent (Figure 6). It could be seen that the LBLn@Ebs coating could decompose considerable NO generation level at a speed about 2.0 × 10⁻¹⁰ mol/(cm²•min), which was within the level that healthy endothelial cell could produce (0.5–4 × 10⁻¹⁰ mol/(cm²•min)). Considering the release profile of Ebs, the sustained release of Ebs would also be expected and give the basic requirement for long-term catalytic effect in NO generation, thus providing anti-thrombotic ability (to be fulfilled in further study).

Following the NO generation ability test, the platelet adhesion to different samples was also tested. As seen in Figure 7A and B, the platelets adhered on stainless steel surface were activated with extended pseudopods, suggesting severe platelets activation, which was frequently reported in other

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**Table 1: The properties of micelles**

| Sample IDs | Diameters (nm) | Polydispersity index | Zeta potential (mV) | Drug loading efficiency (%) | Encapsulation efficiency (%) |
|------------|----------------|----------------------|---------------------|-----------------------------|----------------------------|
| MIC        | 194.82±5.34    | 0.146±0.03           | −25.52±2.53         | 0                           | 0                          |
| MIC-Ebs    | 206.53±11.50   | 0.292±0.05           | −27.33±3.04         | 6.15±1.84                   | 30.77±4.57                 |

Note: Data are expressed as the mean ± SD. MIC: Micelles; Ebs: ebselen.
Figure 5: The surface morphology and Ebs release of LBL_{10} and LBL_{10}@Ebs.
Note: (A) The morphology of micelles-embedded coatings; (B) the X-ray photoelectron spectroscopy wide scan; (C) the release profile of Ebs from the coating. Data are expressed as the mean ± SD. LBL: Layer-by-layer; Ebs: ebselen; LBL_{10}: polydopamine coating sample with 10 cycles of LBL; LBL_{10}@Ebs: micelles embedded sample with 10 cycles of LBL and Ebs.

Figure 6: The nitric oxide (NO) generation ability of the ebselen loaded coating.
Note: Data are expressed as the mean ± SD. ***P < 0.001. LBL: Layer-by-layer; Ebs: ebselen; LBL_{10}: polydopamine coating sample with 10 cycles of LBL; LBL_{10}@Ebs: micelles embedded sample with 10 cycles of LBL and Ebs.

study.\textsuperscript{15,16,37} As the coating constructed in our study contains the anti-coagulant heparin, the LBL_{10} coating presented a certain improved anti-platelet adhesion and activation effect. For the catalyst Ebs loaded samples, the LBL_{10}@Ebs could effectively suppress platelet adhesion and activation with the addition of NO donor solution (SNAP containing solution) to mimic the endogenous RSNOs environment in blood. Interestingly, the LBL_{10}@Ebs also showed a slight improved hemocompatibility than LBL_{10} sample, which still needed investigation. The drug Ebs was also found to present to upregulate the level of CD62P on platelet membrane.\textsuperscript{38} Briefly, the synergistic effect of heparin and NO generation ability of Ebs could effectively enhance the coating hemocompatibility and thus could be well allied as a safe blood-contacting material.

Excessive SMCs proliferation after stents implantation has been treated as a key factor for in-stent restenosis.\textsuperscript{1} And at that condition, without endothelial cell protection, the SMCs proliferation could also further cause thrombus deposition and microphages invasion.\textsuperscript{2} Thus, fabricating a surface that functions well to suppress SMCs proliferation is of special significance. As reported, NO could inhibit SMC proliferation through the cyclic guanosine monophosphate-dependent pathway.\textsuperscript{10} We have also evaluated the SMCs proliferation behavior on different samples, which were presented in Figure 8A and B. The controlled stainless steel samples presented nice affinity for SMCs proliferation, which agreed with other studies.\textsuperscript{7,15,16} The introduced heparin and the generated NO indicate a synergistic effect to suppress the growth of SMCs. Heparin could suppress SMCs proliferation in a dose dependent manner. Based on our previous findings, heparin release content at the early stage could reach up to 10 μg/cm,\textsuperscript{24} which is higher than the reported inhibitory density (3.5–5 μg/cm).\textsuperscript{39} The strongest inhibitory effect could be seen on LBL_{10}@Ebs samples with the addition of SNAP, due to the additional effect of \textit{in situ} releasing of NO. Though we have demonstrated the effect of MIC-embedded LBL coating with Ebs loading on inhibit platelet activation and SMCs proliferation, the anti-inflammatory ability of Ebs was not sufficiently evaluated. Besides, further \textit{in vivo} implantation of such coating is also needed to better verify their biological function, especially the subcutaneous experiment (to evaluate inflammation response) and stent implantation (to assess the in-stent restenosis and thrombus deposition). Nevertheless, the current study has already proved the potential of MIC-embedded coating with...
Ebs loading for modifying cardiovascular implants.

**Discussion**

The key function of native endothelium is to secrete functional agents, including nitric oxide (NO), prostacyclin (PGI2), antithrombin III and membrane-bound species (heparan sulfate). These functional components can effectively inhibit thrombus formation, and suppress excessive intima hyperplasia. Thus, developing multifunctional coatings that can mimic the endothelium function would make sense in the whole endothelialization process. In this study, heparin, a clinically used anti-coagulant was adopted as a derivate of endothelium secreted heparan sulfate. The nitric oxide generation, which also means the in situ continuous release of nitric oxide, was simulated. How to combine those two functional mimics are also the main purpose of this work.

As LBL assembly is a versatile and facile technique to address desired surfaces properties, it can be recommended to fabricate multifunctional coatings, with the assembly of polyelectrolytes, alone or in combination with drug loading. Herein, a sandwich-like LBL coating was prepared to achieve the combination of functional polyelectrolytes and loaded drugs. The polyelectrolytes were consisted of catechol-modified chitosan, phenylboronic acid modified MICs and heparin. The drug Ebs was pre-loaded into a MIC, which was functionalized with phenylboronic acid groups. The phenylboronic acid can form covalent bonding with catechol-modified chitosan at pH 7.4, so as to stabilize the LBL coating, resulting sustained release of loaded components. Within the abundant interactions inside the coating components, the loaded Ebs could present inhibited burst release and maintain sustained release till 1 month. The stability of the coating would be a potential barrier for protecting the loaded components. Therefore, the Ebs in the coating could in situ catalyze and decompose RSNOs to NO in the blood/material interface. The Ebs loaded coating could effectively suppress platelet adhesion/activation and SMCs proliferation, due to the synergetic effect of both heparin and in situ generation of safe level NO. With the sustained release of Ebs, the catalytic effect of Ebs on decomposing RSNOs to continuous release NO could be expected and such MIC-embedded coating with Ebs loading showed potential for vascular material modification.

**Author contributions**

Concepts, design, funding support, manuscript review and guarantor: RFL, JQP and YBW; definition of intellectual content, data analysis, manuscript editing, literature research: LHL and LJ; experimental studies: LY, LHL, LJ; data acquisition: LHL, LJ; statistical analysis: LJ; manuscript preparation: LY, LJ and RFL. All authors read and approved the final version of manuscript for publication.

**Conflicts of interest**

None declared.

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