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Original Article

Preparation of sustained-release composite coating formed by dexamethasone and oxidated sodium alginate

Wenqing Gao[1*], Tong Li[1], Meili Yu[2*], Xiaomin Hu[1], Dawei Duan[1], Tingting Lin[3*]

[1]Department of Heart Center, The Third Central Hospital, Tianjin, China; [2]Key Laboratory of Artificial Cells, The Third Central Hospital, Tianjin, China; [3]Tianjin Medical University Eye Hospital, Tianjin, China. [*]Equal contributors.

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Abstract: Inflammatory reaction and thrombosis are the unsolved main problems of non-coated biomaterials applied in cardiac surgery. In the present study, a series of sustained composite coating was prepared and characterized, such as in the chemical modification of polyvinyl chloride (PVC) for applications in cardiac surgery and the assessment of the biological property of modified PVC. The composite coatings were mainly formed by dexamethasone (DXM) and oxidated sodium alginate (OSA) through ionic and covalent bond methods. The biocompatibility and hemocompatibility of the coating surface were evaluated. Scanning electron microscopy analysis of the surface morphologies of the thrombus and platelets revealed that DXM-OSA coating improved the antithrombogenicity and biocompatibility of PVC circuits, which were essential for cardiac pulmonary bypass surgery. Evaluation of in vitro release revealed that the DXM on group PPC was gradually released in 8 h. Thus, DXM that covalently combined on the PVC surface showed sustained release. By contrast, DXM on groups PPI and PPD was quickly or shortly released, suggesting that groups PPI and PPD did not have sustained-release property. Overall, results indicated that the DXM-OSA composite coating may be a promising coating for the sustained delivery of DXM.

Keywords: Dexamethasone (DXM), oxidated sodium alginate (OSA), hemocompatibility, biocompatibility, sustained release

Introduction

Cardiac surgery can be performed with the aid of cardiac pulmonary bypass (CPB) by partially replacing the cardiopulmonary function. Biomaterials that come in contact with whole blood can motivate host-defense mechanism, especially the blood stability mechanism, and cause thrombosis and embolism in cascade thus endangering the life of the patient [1-3]. Systemic anticoagulation, as well as the inhibition of inflammatory reaction activating, are essential for CPB circuits to improve the hemocompatibility and biocompatibility of the non-endothelial surface.

Glucocorticosteroids are used extensively to minimize and prevent post-operative oedema [4], myocardial injury, and inflammatory reaction [5, 6]. DXM seems to be the most suitable because it has the highest anti-inflammatory activity, no mineralocorticoid activity, and the longest available half-life of 36 h to 54 h [7]. Besides their anti-inflammatory effect, corticosteroids have a wide range of side effects, including hyperlipidemia, hypertension, hyperglycemia, and effects on body composition and bones [8, 9]. Drug delivery systems that provide sustained release may revolutionize treatment in a number of indications [10]. A wide range of materials have been pursued for the preparation of drug delivery systems, and the most common materials are polyesters and polysaccharides [11-14]. Sodium alginate (SA) has also been pursued for depot delivery applications because of its biocompatibility, biodegradability, and ability to provide sustained drug release. SA contains mannuronic (M) and glucuronic (G) groups [15], with prothrombic G groups and anticoagulant M groups [16]. SA has been used to develop skin substitutes and wound dressing materials [17] as a matrix to immobilize enzymes and cells [18], as well as a vehicle for drug and gene delivery [19, 20].

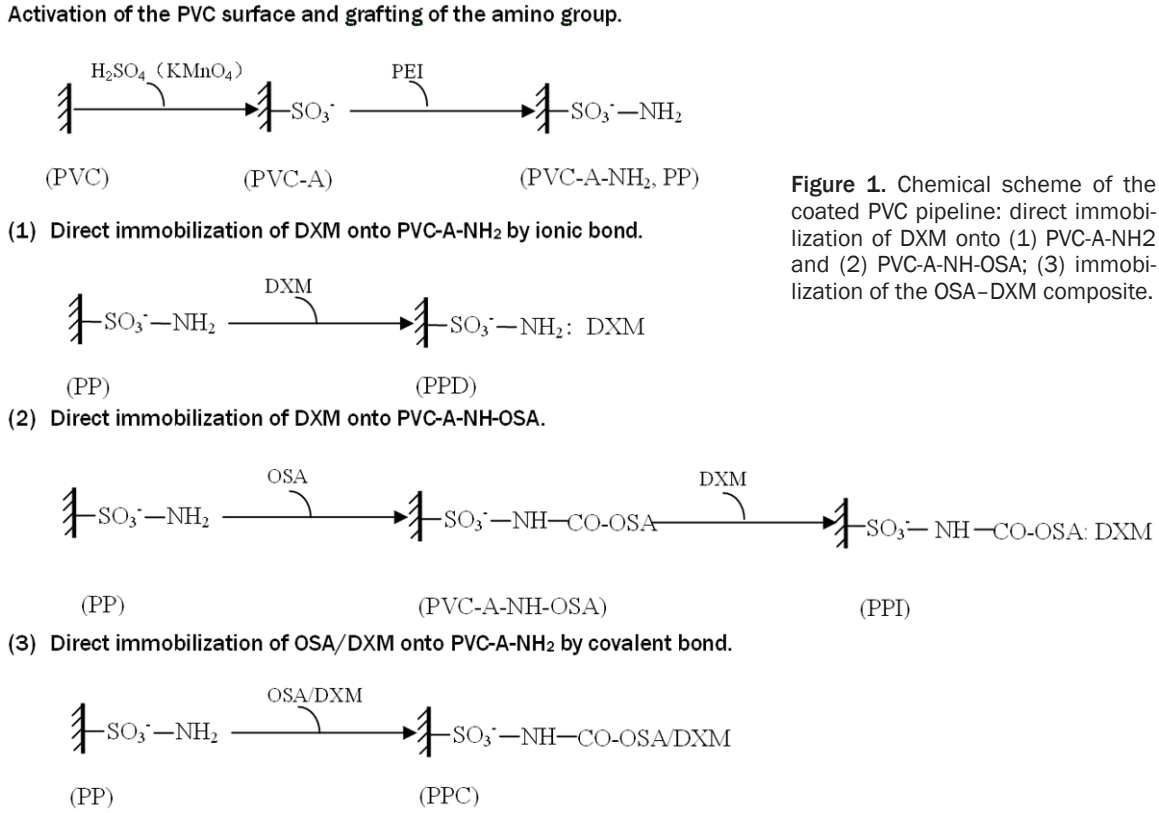

Figure 1. Chemical scheme of the coated PVC pipeline: direct immobilization of DXM onto (1) PVC-A-NH2 and (2) PVC-A-NH-OSA; (3) immobilization of the OSA–DXM composite.

In this study, we modified PVC circuits to behave sustained drug release and antithrombotic activity. The surface of PVC circuits was immobilized with a DXM-OSA composite. The study revealed that chemical modification provides PVCs with an important clinical value.

Materials and methods

Reagents

PVC circuits (10 cm^2) were obtained from KE-Wei (Dong Guan, China). SA, polyethyleneimine (PEI), and sodium periodate were procured from Sigma (St. Louis, USA). DXM was supplied by DA-Hua (Wuhan, China). Human serum albumin (HAS) was obtained from CSL Behring GmbH (Marburg, Germany), whereas human fibrinogen (HPF) was obtained from EMD Chemicals (San Diego, Germany). All other reagents were of analytical grade.

Surface modification

The PVC circuits were cut into 10 cm^2 pieces and acidized with concentrated sulfuric acid (with 2 g/l potassium permanganate) to form new carboxyl groups on the PVC surface. The acidized PVC samples were rinsed with double-distilled water three times to remove the unreacted sulfuric acid. The carboxyl-bearing PVCs were immersed in PEI with multi-amino groups. The process of PPI is described in the chemical scheme in **Figure 1**. (1). The process of PPD is described in the chemical scheme in **Figure 1**. (2). The DXM-OSA-immobilized PVC surfaces (PPC) were prepared with a different method according to the scheme in **Figure 1**. (3). Amino-bearing PVC samples were immersed in a DXM-OSA solution (pH 3.5) that contains $NaBH_3CN$ at 40°C for 3 h. $NaBH_3CN$ was used to couple the polysaccharides with the amino-bearing PVC surfaces.

Determination of surface grafting density

The surface density of the OSA and DXM was measured by the phenol sulfuric acid procedure and high-performance liquid chromatography (HPLC), respectively. The dye concentration was determined with an ultraviolet spectrometer (UV-2800; Hitachi, Japan).

Modified PVC characterization

The functional groups on modified PVC circuits were analyzed with Fourier-transform infrared spectroscopy (FTIR; NICOLET 6700; Thermo,

USA). The OSA and DXM standards were determined with the potassium bromide tablet mode (detector: DTGs KBr; beam splitter: KBr; wavelength range: 650-4000 cm^{-1}).

Protein adsorption measurements

Human whole blood from a healthy volunteer was collected and centrifuged at 800×g for 10 min at 4°C to separate blood corpuscles. The resulting platelet-rich plasma (PRP) was centrifuged at 3000×g for 10 min at 4°C to obtain the platelet-poor plasma (PPP) for the test of protein adsorption. The protein adsorption was measured for human serum albumin (HSA) and human plasma fibrinogen (HPF) with the bicinchoninic acid assay, as described by Ishihara et al. [16].

Blood coagulation time

Human whole blood from a healthy volunteer was collected and centrifuged at 800×g for 10 min at 4°C to separate blood corpuscles. The resulting PRP was used for the platelet adhesion experiment. A sample of 10 cm^2 PVC circuits was incubated in 0.5 ml of PPP at 37°C for 1 h. The activated partial thrombin time (APTT), thrombin time (TT), prothrombin time (PT), and fibrinogen time (FT) of PPP were then determined with an automated blood coagulation analyzer (STA-R Evolution®; Diagnostica Stago, France).

Evaluation of platelet adhesion

The concentration of human PRP before adhesion (PLT_1) was determined with a hemocytometer (ADVIA2120; Siemens, Germany). The PVC circuit samples (0.5 cm × 0.5 cm; 10 cm^2) were washed three times with double-distilled water and placed into 24-well culture plates incubated with human PRP (750 μl/well) in 5% CO_2 at 37°C for 60 min. The concentration of human PRP after adhesion (PLT_2) was obtained. Adhesion quantity = PLT_1 - PLT_2, Adhesion rate = (PLT_1 - PLT_2)/PLT_1.

The PVC circuit samples were washed three times after incubation with PRP and then fixed with 3% (w/v) GA solution in PBS for 2 h. The fixed samples were dehydrated with graded ethanol (i.e., 50%, 60%, 70%, 80%, and 90%, v/v; absolute alcohol) and dried by the critical-point procedure with CO_2. The platelet adhesion morphology was observed with scanning electron microscopy after metal spraying.

Thrombus formation

The PVC circuit samples were washed three times with double-distilled water and placed into 24-well culture plates with 1.5 ml of human whole blood per well. The samples were incubated in 5% CO_2 at 37°C for 60 min and then washed three times with PBS. The formed thrombus was fixed with 3% (w/v) GA solution in PBS for 1 h. The samples were dehydrated with graded ethanol and dried by the critical-point procedure with CO_2. The degree of thrombosis (DT) of the PVC pipeline at a given time was defined as follows:

DT = (W_2 - W_1)/W_1, W_1 and W_2 are the weight of the dry PVC and blood coagulation samples, respectively.

Sustained release test

The coated samples of PPD, PPI, and PPC were cut into pieces and immersed in distilled water. The beakers with coated samples were closed and oscillated in 37°C water bath. The oscillation reaction liquid from PPD, PPI, and PPC was collected at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, and 8 h after oscillation. The DSP quantity was measured by HPLC.

Statistical methods

Statistical analysis was performed with SPSS (version 17.0). All quantitative data were expressed as the mean ± standard deviation. Comparisons among groups were performed by ANOVA (analysis of variance), whereas pairwise comparisons were conducted with the Student-Newman-Kuels q test. Differences with $p < 0.05$ were considered statistically significant.

Results

Surface characterization

The DXM/OSA-coated PVC surface was provided with a characteristic peak. A strong and wide adsorption peak appeared at 2500-4000 cm^{-1} in the fluorescein isothiocyanate (FITC) labeling of the DXM tablet and DXM-coated surface (**Figure 2**).

Surface grafting density

The DXM grafting densities were measured by HPLC. DXM was bound to the PPD-coated surface more than to the PPC- and PPI-coated

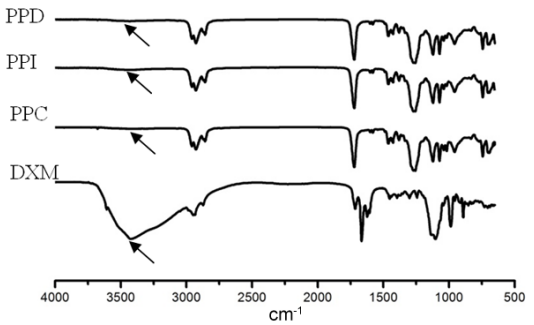

Figure 2. Qualitative analysis of immobilized DXM. FTIR was used to detect the characteristic peak of PPD-, PPI- and PPC-coated PVC, as well as the KBr tablets of DXM. A strong and wide O–H adsorption peak appeared at 2500-3000 cm^{-1} in the FITC of DXM, as well as the PPD-, PPI-, and PPC-coated surfaces.

Table 1. Platelet adhesion of different coating groups ($\times 10^9$)

Group	*n*	Adhesive capacity	Adherence rate%
C	8	41.38 ± 3.20	16.82 ± 1.30
PPD	8	26.00 ± 2.73	10.57 ± 4.41
PPI	8	13.88 ± 1.89	5.64 ± 0.76
PPC	8	19.13 ± 3.40	7.77 ± 1.38

Table 2. Thrombosis of different coating groups

Group	*n*	Adhesive capacity/mg
C	8	0.99 ± 0.12
PPD	8	0.92 ± 0.03
PPI	8	0.83 ± 0.16
PPC	8	0.77 ± 0.05

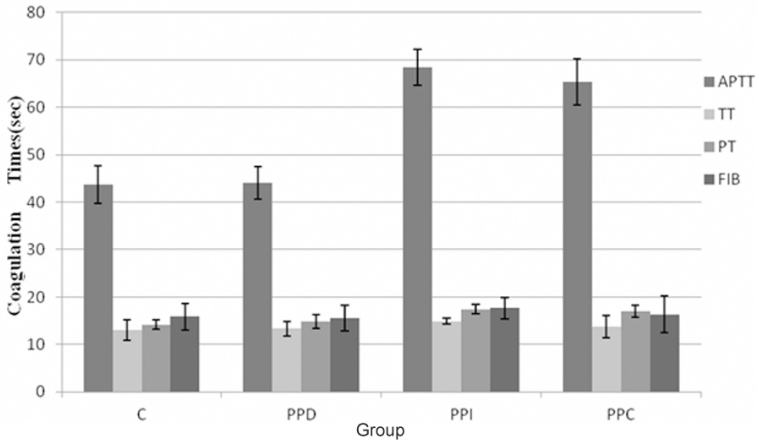

Figure 3. Comparison of anticoagulation time of uncoated (C), DXM-coated (PPD), OSA-coated and DXM ionic bond (PPI), and OSA–DXM composite-coated (PPC) surfaces. The coagulation time of APTT, TT, PT, and FT (n = 6) were evaluated by an automated blood coagulation analyzer. The APTT of PPI and PPC surfaces were significantly longer than those of C and PPD surfaces. OSA demonstrated their anticoagulant properties by prolonging APTT.

ones. However, no significant differences were observed in the DXM density of the PPC- (2.06 ± 0.68 μg/cm^2 DXM) and PPI-coated surfaces (1.63 ± 0.76 μg/cm^2 DXM).

Protein adsorption

Protein adsorption was measured by ultraviolet spectrometry from solutions that contain various concentrations of purified HSA and HPF. The amount of protein adsorbed by the uncoated, PPD-coated, PPI-coated, and PPC-coated surfaces was depicted in **Table 2**, as a function of their concentration in the free solution. The two proteins that were adsorbed by the PPI- and PPC-coated surface were lower (HAS 29.86 ± 3.57, HPF 34.99 ± 3.52; HAS 46.67 ± 3.20, HPF 45.67 ± 3.79) than those adsorbed by the non-coated surface (HAS 75.34 ± 3.23, HPF 85.08 ± 1.26) with a significant difference. The non-coated surface adsorbed slightly more proteins than the PPD-coated surface. The PPD-coated surface adsorbed higher amounts of HAS and HPF than the PPI- and PPC-coated surface.

Coagulation time

The anticoagulant effects of OSA can be observed by comparing the APTT, TT, PT, and FT of the uncoated surface with those of the PPI, PPD, and PPC surfaces, as shown in **Figure 3**. The results showed the different clotting times of different surface types. The clotting times of the PPD surface were nearly the same as those of the uncoated surface, whereas those of the PPI- and PPC-coated surfaces were longer than those of the uncoated surfaces. The OSA-coated surfaces (PPI and PPC) prolonged the APTT, such that the APTT of the OSA-coated surfaces were almost twice that of the uncoated and PPD surface.

Platelet adhesion

The equilibrium platelet adhesion and activation on the surface was related to the protein

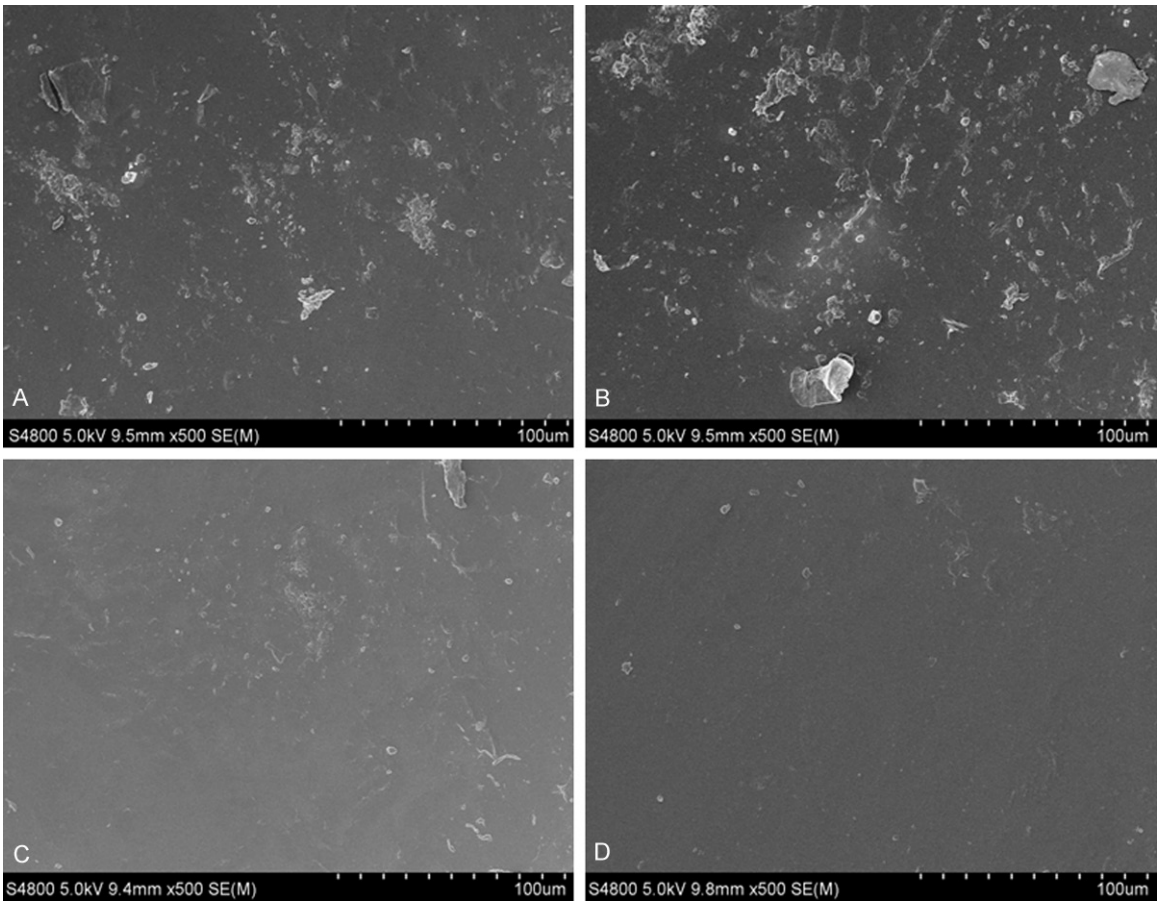

Figure 4. Platelet adhesion of different groups. A-C; B-PPD; C-PPI; D-PPC. Platelet adhesion on C, PPD, PPI, and PPC surfaces. SEM (×500) was used to detect the morphology of the PVC surface after platelet adhesion. Numerous platelets adhered to the uncoated surface (A) and PPD surface (B), but few platelets adhered to the PPI and PPC surfaces (C and D). OSA improved the hemocompatibility of the PVC surface. The OSA–DXM coating can significantly inhibit platelet adhesion.

adhesion. The maximum number of adhering platelets on the PVC surfaces after the 2-h incubation appeared on the uncoated surface. The platelet adhesion on the PPD, PPI, and PPC surfaces significantly decreased after the 2-h incubation. The PPI and PPC surfaces adsorbed lower platelet (13.88 ± 1.89, 19.13 ± 3.40) than the PPD surface (26.00 ± 2.73) with a significant difference. The PPC surface adsorbed slightly more platelet than the PPI surface, as shown in **Table 1** and **Figure 4**.

Thrombin formation

The effect of surface modification on thrombin formation is shown in **Table 2** and **Figure 5**. Unlike the uncoated and PPD surfaces, the PPI and PPC surfaces can significantly decrease thrombin formation. The PPC surface has slightly less thrombin formation than the PPI surface.

Sustained release test

The release of DSP on PPD surface appeared with quick release and rapidly reaching plateau. The sustained release time is short. The DSP on PPI surface appeared with full release shortly. The DSP on PPC surface appeared with sustained release, as shown in **Figure 6**.

Discussion

Extracorporeal life support (ECLS), including CPB and ECMO, causes an acute inflammatory response and was fraught with problems related to coagulation, bleeding, and other organ dysfunction. During ECLS, a continuous contact occurs between circulating blood and the foreign surface of the extracorporeal circuit (ECC). The hemostatic balance is shifted to hypercoagulability with patients and ECC and components at risk for thrombosis. The administration

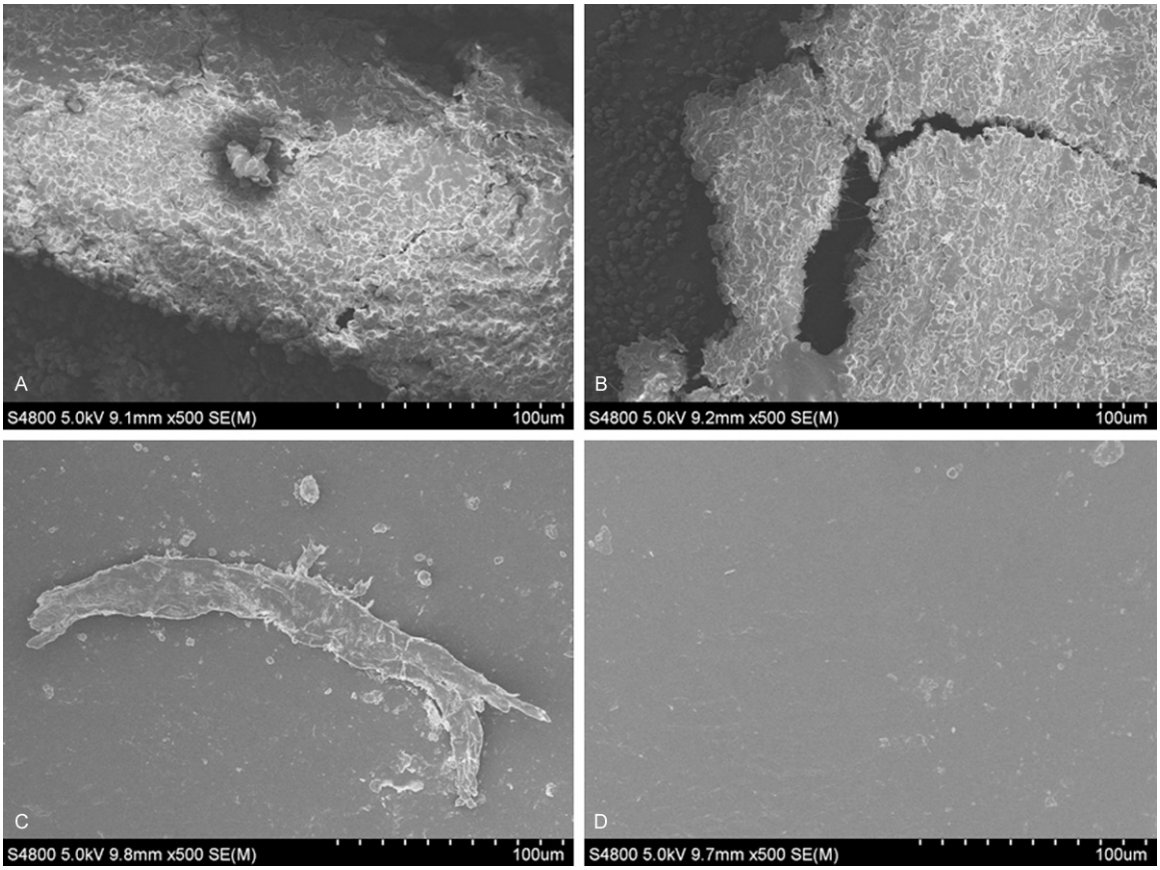

Figure 5. Surface thrombosis of different coating surface. A-C; B-PPD; C-PPI; D-PPC. Thrombin formation of C, PPD, PPI, and PPC surfaces. SEM (×5000) was used to detect the morphology of PVC surfaces after 2-h ncubation in fresh whole blood cells. The thrombin complex appeared on uncoated PVC (A) and PPD surface (B), but that appeared on the SA-coated surface (B) was less. Much less thrombin appeared on the PPI and PPC surfaces (C and D) than on previous ones. The PPC surface has slightly less thrombin formation than the PPI surface. OSA was antithrombogenic with the same trends as that of the platelet adhesion test.

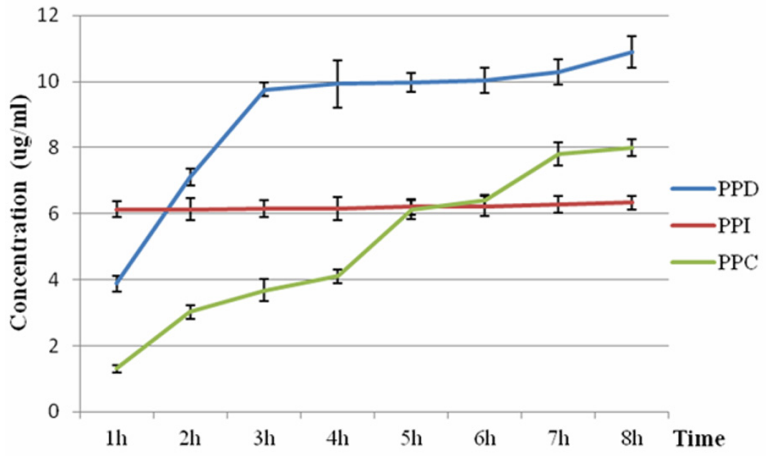

Figure 6. Sustained release test of PPD, PPI, and PPC surfaces. The release of DSP on PPD surface appeared with quick release and reached plateau rapidly. The sustained release time is short. The DSP on the PPI surface appeared with full release shortly. The DSP on the PPC surface also appeared with sustained release.

of antithrombotic therapy is necessary to regain the loss of hemostatic balance and prevent thrombosis. The most widely used antithrombotic therapy for the provision of ECLS is the anticoagulant heparin (HEP). Unfortunately, the use of HEP can result in bleeding in the systemic circulation and, despite its use, clotting in the ECC. The bleeding and thrombosis that occur regularly during ECLS can ultimately result in significant clinical complications, including death. Systemic inflammatory response can be induced by several ECC factors, including

blood contact with ECLS non-endothelial surface, non-physiologic perfusion, direct tissue trauma of surgery, ischemic-reperfusion injury, endotoxemia, and temperature variation. When blood is exposed to the non-biologic surfaces of ECCs, a complex inflammatory response is initiated involving both the coagulation and inflammatory response pathway [21]. This complex response leads to capillary leak, which can cause a temporary dysfunction of every organ. In fact, the response to extracorporeal circulation is remarkably similar, clinically and biochemically, to that seen in the systemic inflammatory response syndrome and the acute respiratory distress syndrome [22]. The therapeutic treatments for the suppression of the inflammatory reaction include non-ECC cardiac surgery, HEP-coated ECC circuits, leukocyte filter, hyperfiltration, and drug therapy. The drug therapy includes glucocorticoid, complement inhibitor, monoclonal antibody, and aprotinin.

The artificial steroid DXM is known for its potent glucocorticoid effects, such as anti-inflammatory and immunosuppressant effects, as well as remarkable inhibition of fibroblast growth. However, most application methods, such as one-shot injections via syringe or cannula, are based on short-term release without the possibility of affecting the long-term tissue reactions, such as fibrosis. As a polyanionic macromolecule, SA is natural and non-toxic and has high biocompatibility and anticoagulant effects [23-25]. The results of this study revealed that DXM and OSA are constantly immobilized on the PVC surface by surface modification. The partial degradation of SA [26, 27] with sodium periodate gives rise to the fraction of molecules with highly reactive aldehyde terminal groups. The end-point bond is established between the aldehyde terminal groups and primary amino functions by reductive amination [28-30]. DXM was bonded on PVC surface by electrostatic bonding force, ionic bond, or covalent bond. The protein adhesion was significantly decreased in the SA-coated surfaces (PPI and PPC) more than in the non-SA coated surfaces (C and PPD). Hence, the SA coating can improve the hydrophilicity of the PVC surface. Hemocompatibility is an important property of modified PVCs. The partial degradation and covalent binding of SA can inevitably lead to the complete loss of antithrombin binding. The ideal concept of SA binding includes an inherently stable covalent endpoint bond of the molecules, which leave the other parts of the molecule intact to react with the blood constituents [31]. The blood coagulation cascade includes intrinsic, extrinsic, and common pathways. APTT and PT were used to examine the intrinsic and common pathways, whereas FT was used to measure the time for transferring fibrinogen into fibrin [32, 33]. The results showed that the SA coating can significantly prolong the coagulation time of APTT by inhibiting the intrinsic coagulation pathway. Moreover, the composite coating demonstrated its anticoagulant property by inhibiting the intrinsic coagulation pathway. Furthermore, the fragmentation of SA did not destroy their functional molecules, and the binding procedure did not shield the anticoagulant molecules. The SA-DSP composite coating can decrease platelet adhesion and thrombosis with the same trend as that of the coagulation time. The release of DSP on the PPD surface appeared with quick release and rapidly reaching plateau. Moreover, the sustained release time is short, which indicates that the DSP ionic bond on the PPD surface is unable to tolerate long-time dynamic washing with mass flow. The DSP on the PPI surface appeared with full release shortly, which indicates that the OSA that covalently bonded on PPI surface is unable to supply adequate ionic binding sites for DSP. In addition, the physics winding of OSA for DSP is poor. The DSP on the PPC surface appeared with sustained release. The DSP and OSA were crosslinked by ionic bond and physics winding, after which the OSA-DSP composite were covalently bonded on the PP surface. DSP dual fixed by ionic bond and physics winding can tolerate dynamic washing by exerting a sustained release effect.

Conclusion

The DXM-OSA composite coating may be promising for the sustained delivery of DXM. The immobilized DXM-OSA composite can improve the hemocompatibility and biocompatibility of the PVC surface. Intelligent CPB materials with near-complete physiological surfaces will solve the main problem of CPB, including the inflammatory reaction and thrombosis.

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Address correspondence to: Dr. Tong Li, Department of Heart Center, The Third Central Hospital, 83 Jintang Road, Hedong District, Tianjin, China. Tel: 86-22-8411-2006; Fax: 22-2431-5132; E-mail: litong3zx@sina.com

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