RSC Advances

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

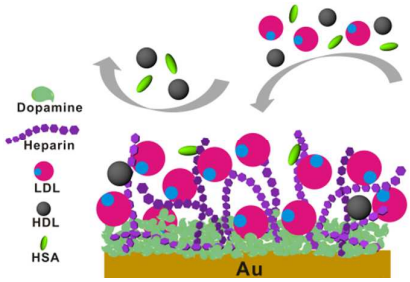
You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Table of Contents



RSC Advances Accepted Manuscript

Polydopamine-Assisted Deposition of Heparin for Selective Adsorption of Low-Density Lipoprotein†

Yang Liu, Wen-Ze Qiu, Hao-Cheng Yang, Yue-Cheng Qian, Xiao-Jun Huang, Zhi-Kang Xu*

MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

*Corresponding author, e-mail: hxjzxh@zju.edu.cn

†Electronic supplementary information available.

RSC Advances Accepted Manuscript

Abstract

Low-density lipoprotein (LDL) is the main carrier of blood cholesterol, with elevated levels of LDL increasing the risk of atherosclerosis. Blood purification therapy is an option for serious cardiovascular diseases and familial hyperlipidemia, and the therapeutic effect is largely determined by the LDL adsorbent used. In this study, a facile method is proposed to prepare LDL capture surfaces. Coatings rich in heparin were prepared by co-depositing heparin and dopamine. The mixed coatings were thoroughly characterized by X-ray photoelectron spectroscopy, ellipsometry, atomic force microscopy and water contact angle measurements. Protein adsorption was analyzed by surface plasmon resonance and quartz crystal microbalance with dissipation monitoring, the results indicate that the heparin-incorporated coatings have great adsorption capacity and selectivity for LDL. In addition, the mixed coatings exhibit favorable blood compatibility in platelet adhesion assay. The described polydopamine-assisted heparin deposition process is a simple and universal method for LDL adsorbent preparation.

Keywords: polydopamine, low-density lipoprotein (LDL), heparin, selective adsorption

RSC Advances Accepted Manuscript

Introduction

Cardiovascular disease, which is mainly caused by atherosclerosis, is a leading cause of mortality worldwide.[1] Atherosclerosis is a complicated syndrome and the underlying pathogenesis has not been clarified completely yet. However, high levels of low-density lipoprotein (LDL) has always been regarded as the major risk factor of atherosclerosis although later research revealed that other factors such as oxidized low-density lipoprotein (oxLDL), lipoprotein(a) (LP(a)), apolipoprotein E4 and its variants promote atherosclerosis as well.[1-8] LDL is a type of lipoprotein with hydrated density of 1.019~1.063 mg/mL, and it transports ~70% of the total cholesterol in plasma.[9] The accumulation of LDL particles in the intima of arteries is an important process in early atherosclerosis.[10] Elevated levels of LDL are considered to be a major cause of atherosclerosis with LDL-lowering therapy shown to reduce the risk of coronary heart disease (CHD).[11] A variety of LDL-lowering therapies can be used based on a person's risk status. Generally, lifestyle changes and drug therapy are the most common LDL-lowering treatments.[11] However, these methods do not always work well, especially for people suffering from severe hyperlipidemia and familial hypercholesterolemia (FH). FH is an inherited autosomal codominant disorder of lipoprotein metabolism characterized by very high LDL levels in the blood.[12,13] LDL-apheresis (LDL-A) is a procedure considered when the above-mentioned therapies are inefficient. LDL-A is based on plasma exchange and can effectively and selectively remove LDL from blood. Currently, there are six LDL-A systems available worldwide, and four of these are based on the principle of adsorption: immunoadsorption, dextran sulfate adsorption (Liposorber LA-15 system), polyacrylate-coated polyacrylamide direct perfusion, and dextran sulfate direct perfusion (Liposorber D).[14,15]

RSC Advances Accepted Manuscript

A lot of basic research has been carried out on model surfaces to understand the mechanisms involved in LDL adsorption.[16-23] Another main branch of research involves in the development of LDL adsorbents due to their important roles in LDL apheresis systems.[24-38] Generally speaking, the choice of ligand and the preparation method are highly important factors in the manufacture of an effective adsorbent. Under physiological conditions, ligands that are rich in acidic groups have been extensively investigated, as electrostatic interactions between basic amino acid regions of apolipoprotein B-100 (apoB-100) in LDL, and negatively charged groups in LDL ligands, is the predominant force driving LDL adsorption.[39] Heparin is a sulfated polysaccharide that can interact with a protein through the combination of ionic interactions, hydrogen bonding, and hydrophobic forces.[40] Moreover, heparin has been proven to have specific interactions with LDL through seven binding-sites on apoB-100.[41-43] For this reason, heparin is one of the most wildly used LDL ligands,[24-31] although other ligands have also been investigated, such as antibodies,[32-34] cholesterol,[35,36] and polyanions.[37,38] To use heparin as the LDL ligand, various preparation methods have been investigated to immobilize heparin on substrates. Generally, materials containing hydrophilic functional groups such as Poly(vinyl alcohol) (PVA) are optimal substrates,[25] otherwise the material surfaces first have to be activated. For example, polysulfone membranes can be activated with plasma treatment in atmospheric ammonia to generate amine,[30] while graft polymerization of acrylic acid can be used to activate a polypropylene non-woven fabric membrane.[31] Heparin is then covalently linked to the activated substrate with the aid of glutaraldehyde,[24,25] toluence-2,4-diisocyanate,[28] or 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride.[26,30] These chemical methods provide a stable linkage although the procedures are somewhat tedious.

RSC Advances Accepted Manuscript

Catecholic compound dopamine (DA), a mussel adhesive protein inspired molecule, can adhere to virtually any materials in an alkaline environment. Self-polymerization of dopamine has served as an important strategy for surface modification because of the ease in forming polydopamine (PDA) coating on a wide array of substrates.[44,45] Inspired by this phenomenon, PDA-based methods have been introduced for heparin immobilization. One method involves depositing a layer of PDA onto the designated substrates followed by direct covalent coupling of heparin onto the reactive polydopamine layer.[46,47] Another method, first conjugates heparin with dopamine, and then oxidizes the heparin conjugated dopamine to adhere to substrates.[48-50] Compared to the traditional heparin immobilization strategies, these PDA-assisted methods are more convenient and universal. To further simplify the experimental protocol, one-step methods have been designed, where material surfaces can be functionalized directly by co-depositing dopamine with other chemicals.[51-56] This one-pot immobilization method can be used to functionalize diverse surfaces without chemical reaction. At present, a wild range of chemicals like PEI,[51] 2-(Dimethylamino)ethyl methacrylate (DMAEMA),[52] PEG,[53] Poly(N−isopropylacrylamide),[55] and dextran[56] have been co-deposited with dopamine to produce functional surfaces. In the present work, we use this PDA-assisted co-deposition as a simple and versatile method to immobilize heparin on solid surfaces for LDL adsorption. Surfaces containing different amounts of heparin were easily prepared by regulating the concentration of heparin in the reaction mixture. The mixed coatings were analyzed using surface plasmon resonance (SPR) and a quartz crystal microbalance with dissipation monitoring (QCM-D) to test their adsorption capacity and selectivity for LDL. Additionally, blood biocompatibility was also examined using a platelet adhesion assay.

RSC Advances Accepted Manuscript

Experimental

Materials

Dopamine (DA) hydrochloride and heparin (Mw=6000-20000, 185 USP units/mg) were purchased from Aladdin Industrial Corporation (Shanghai, China). Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were provided by Millipore (Massachusetts, USA), human serum albumin (HSA) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Amresco (Ohio, USA). Platelet rich plasma was provided by the Second Affiliated Hospital of Zhejiang University School of Medicine. All of the products were used as received. Commercial LDL was examined by dynamic light scattering (DLS) and aggregation was considered negligible (see supporting information Fig. S1 and Table S1). Ultrapure water (18.2 MΩ·cm) produced from the ELGA Classic UF system (Veolia Water Systems, France) was used throughout the experiment.

Preparation of PDA/heparin mixed coatings

Prior to the deposition of PDA/heparin coatings, the substrates (Si chips for ellipsometry, Au chips for other tests) were thoroughly cleaned to remove possible contaminants. The chips were first sonicated in acetone for 10 min followed by rinsing with deionized water. Afterwards, the substrates were treated with piranha solution (H_2SO_4:H_2O_2 = 7:3) for about 10 min (Au chips) or 30 min (Si chips). Finally, the chips were rinsed with copious amounts of deionized water and blown dry under argon before being immediately immersed in the different dopamine/heparin solutions.

Specific amounts of dopamine and heparin were dissolved in 10 mM Tris-HCl buffer (5 mL, pH = 8.5) separately. Equal volumes of dopamine and heparin solutions were then mixed to obtain the final concentrations. Dopamine was kept at 2 mg/mL among all the mixture solutions while heparin was 0, 2, 5 or 10 mg/mL in different samples, and the corresponding coating surfaces was named as PDA-Hep(0), PDA-Hep(2), PDA-Hep(5), PDA-Hep(10), respectively. Clean Au or Si chips were

RSC Advances Accepted Manuscript

immediately immersed in the mixture, which was shaken and then incubated at 25 ℃ for 4 h. The obtained coatings were thoroughly rinsed with water and ethanol alternatively three times, and finally blown dry under argon gas before being analyzed.

Characterization of coated surfaces

X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was carried out on an RBD upgraded PHI-5000C ESCA system (Perkin Elmer, Massachusetts, USA) with Al Kα radiation (hν = 1486.6 eV). The X-ray anode was run at 250 W and the high voltage was kept at 14.0 kV with a detection angle of 54°. The base pressure of the analyzer chamber was ~5 × 10^{-8} Pa. The complete spectrum and narrow spectrum of all elements were both recorded with high resolution using an RBD 147 interface (RBD Enterprises, USA) with the AugerScan 3.21 software. Binding energies were calibrated by using the containment carbon (C 1s = 284.6 eV).

Ellipsometry

In the ellipsometry (ELM) experiment, silicon was used as the substrate. Thickness of the PDA/heparin coating was determined using variable-angle spectroscopic ellipsometry spectra collected on an MD-2000I spectroscopic ellipsometer (J. A. Woollam Co. Inc., Lincoln, NE, USA). The measurements were performed in air and in phosphate buffer (PBS, pH = 7.4, containing 137 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4) respectively. The dried samples were measured at incident angles of 60°, 65° and 75° in the wavelength range 300~1700 nm. The samples immersed in PBS, however, were measured at a fixed incident angle of 75°. The thickness was calculated from the ellipsometric parameters using a Cauchy model.

Atomic Force Microscopy

Morphological features of the PDA/heparin coatings in air and in PBS solution were analyzed using

RSC Advances Accepted Manuscript

atomic force microscopy (AFM, PicoSPM2100, Molecular Imaging Corp., USA) at room temperature in contacting mode. All of the images were taken at a scanning speed of 2 Hz. The roughness (root-mean-squared, RMS) was analyzed from a 4.5 × 4.5 μm^2 image using Gwyddion software.

Static water contact angle

Static water contact angle (WCA) measurements were performed by a CTS-200 contact angle system (MAIST Vision Inspection & Measurement CO. Ltd., Ningbo, China) at room temperature using the sessile drop method. In brief, a droplet of 2 μL ultrapure water was placed on the sample with a micro-syringe, and then digital images of the water droplet were recorded and the WCA was calculated with DropMeter software. Final results were the average of five independent measurements.

Protein adsorption measurements

SPR was used to test the LDL binding affinity of the PDA/heparin coatings. Firstly, 10 μg/mL single protein (LDL, HDL or HSA) solutions were used to test the adsorption capacity of the coatings. Secondly, HDL or HSA were mixed with LDL to examine the selectivity of the coatings. In the binary protein solutions, HDL or HSA were fixed at 10 μg/mL while LDL was either 10 or 30 μg/mL. Despite of the competition of HDL or HSA, the adsorption would increase when LDL was increased from 10 μg/mL to 30 μg/mL, and the adsorption increment was regarded as an indicator of selectivity for LDL. All the SPR measurements were performed on a dual channel instrument (Reichert SR7000DC, Reichert Inc., Depew, NY, USA). The flow rate of the analyte solution and PBS through the flow cell was set to 30 μL/min. After the base line was steady, protein solution was injected and allowed to flow through the channel for 30 min. One channel was used for

RSC Advances Accepted Manuscript

measurements, while the other channel was used as a control to obtain the background noise from the machine and environment, which was subtracted from the measurements.

Protein competitive adsorption was further monitored by QCM-D (Q-Sense E1 system, Biolin Scientific, Sweden). The sensor crystals used were 5 MHz, AT-cut, polished quartz discs (chips) with electrodes deposited on both sides (Q-Sense). In this procedure, HDL or HSA solution (10 μg/mL) was first injected for 30 min, followed by flushing with PBS for another 30 min. Subsequently, LDL solution (10 μg/mL) was injected for a further 60 min to compete with the adsorbed protein. Finally, PBS was used to flush away any loosely bounded protein. The flow rate was maintained at 30 μL/min and the working temperature was fixed at 25 ℃ during all tests. Resonance frequency and dissipation shifts were simultaneously measured at the 3rd, 5th, 7th, 9th and 11th overtones, and the values reported in the results were measured at the chosen third harmonics.

Platelet adhesion experiments

Blood biocompatibility of the PDA/heparin mixed coatings were tested using a platelet adhesion essay. Platelet rich plasma was added (500 μL per well) to deposited gold chips in 24-well plates. After 30 min of incubation at 37 ℃, the gold chips were gently rinsed with PBS several times. Then platelets adhered on the surface were fixed with 2.5 wt% glutaraldehyde in PBS for 30 min. Finally, the samples were washed with PBS and dehydrated with a series of ethanol/water mixtures of increasing ethanol concentration (40, 50, 60, 70, 80, 90 and 100 vol% ethanol) for 30 min at each concentration. The gold chips were air-dried, coated with gold and imaged by scanning electron microscopy (SEM, Hitachi S4800, Japan).

RSC Advances Accepted Manuscript

Results and discussion

Characterization of coated surfaces

XPS was used to investigate whether heparin was successfully incorporated into PDA coatings. Sulfur, which is not found in dopamine, is the characteristic element of heparin. The appearance of sulfur means that heparin has been mixed with polydopamine successfully. In the complete spectra (Fig. 1A), however, no S(2p) peak can be observed in the mixed coatings no matter what the heparin concentration was. Further, narrow high-resolution spectra of the region containing the S(2p) signal were recorded to confirm the success of co-deposition (Fig. 1B). Signals of S(2p) at 169.6 eV were observed in samples made from dopamine/heparin mixture solutions while no signal was detected in the PDA-Hep(0) sample. This confirms that heparin is successfully incorporated into the PDA/heparin coatings. The narrow spectra also clearly show that the intensity of S(2p) increases with the concentration of heparin in the solutions, hence the heparin content in the coating could be controlled by the composition of dopamine/heparin mixture solutions. Although the narrow high-resolution spectra have proved that heparin is successfully mixed in the PDA coatings, the amount of heparin incorporated seems to be low considering that there is not a visible S(2p) peak in the complete spectra. Similar results about heparin immobilization have been reported in some other work as well.[46-49] Another phenomenon worthy of note is that the peak of Au also changed with the heparin concentration used. It is well known that the depth XPS can detect is limited (usually to less than 10 nm), therefore the change of Au content implies that the thickness of the coatings varied.

--Fig. 1--

In order to gain a better understanding of the co-deposition process, ELM was used to figure out the exact thickness of the different coatings. In this experiment, silicon was used as the substrate to detect the coating's thickness in PBS solution. The results in Fig. 2 indicate that the introduction of

RSC Advances Accepted Manuscript

heparin can decrease the thickness of PDA coating. PDA-Hep(0) in the air was as thick as 12.7 nm, which was in-line with previous results,[44,57] but it fell to 2.7 nm immediately after 2 mg/mL heparin was added to the solution. Then the thickness returned with increasing heparin concentration, and the thickness of PDA-Hep(10) reached 10.7 nm. The thickness of the samples in PBS displayed a similar trend whereby it drops drastically before gradually going back. Compared with values of samples measured in air, however, all the wet samples were thicker and more swollen. Moreover, wet samples containing heparin displayed greater swelling and their thickness increased significantly. This may be attributed to embedded or interpenetrated heparin molecules, where these coiled macromolecules likely promoted the dilation of the packed PDA particles.

--Fig. 2--

The process of protein adsorption involves the interaction between protein particles and interface surface morphology and chemical composition. The influence of heparin on the topography of the PDA/heparin coatings was investigated by AFM. The surface morphologies of different samples in PBS are shown in Fig. 3 and the calculated roughness are displayed in Table 1. Some large protrusions could be easily detected on sample PDA-Hep(0) and it had a rough surface. Dopamine and its oxidation products, such as 5,6-dihydroxyindole and 5,6-indolequinone have a strong tendency to form aggregates and black precipitation particles via covalent or non-covalent interactions.[45] It would be reasonable to assume that coatings made from these large PDA particles has a rough surface. However, the roughness immediately fell from 10.7 nm to 5.8 nm when 2 mg/mL heparin was added, with PDA-Hep(2) having a fairly smooth surface. The presence of heparin greatly influenced the behavior of the PDA particles. During the co-deposition process, the precipitation decreased and the color of the solution became lighter. A similar phenomenon has also been observed for some nonionic polymers.[53,56] When mixed with dopamine solution, heparin

RSC Advances Accepted Manuscript

molecular chains are thought to be attracted to and trapped in PDA aggregates through ionic interactions and hydrogen bonding. Yet chain movements and electrostatic repulsion of the trapped heparin molecules prevents the approach of other dopamine oligomers, inhibiting formation of larger particles. Coatings packed with relatively small particles usually have a lesser degree of roughness when compared to coatings made from large particles. However, surface roughness increased when heparin concentration was further increased. A large quantity of protrusions can be found in PDA-Hep(5) and PDA-Hep(10), and more importantly, the protrusions were evenly distributed in contrast with PDA-Hep(0). The surface roughness of PDA-Hep(2), PDA-Hep(5) and PDA-Hep(10) increased with the increasing of heparin concentration. A relatively large number of heparin molecules were incorporated into PDA particles during the co-deposition process, some of these macromolecules were entirely embedded under the PDA coatings, while some macromolecules interpenetrated the coating, which means part of the molecule chain was trapped in the coating and other parts were exposed on the surface. While part of heparin molecules may anchor their chain end at the surface and worked like a brush molecule, which can also enhance roughness. When the coating samples were immersed in PBS solution, macromolecule chains that were buried in the coatings mainly contributed to the swelling behavior described above, while chains that were exposed on the surface altered the surface morphology. This effect was limited on sample PDA-Hep(2) because the quantity of heparin molecules that incorporated into the mixed coating was small, with the phenomena being magnified with increase heparin concentrations (PDA-Hep(0), PDA-Hep(10)). This presumption is supported by comparing the roughness of the samples measured in air and in PBS. The change in roughness followed the same trend in both air and PBS (AFM images measured in air are shown in supporting information Fig. S2). Sample PDA-Hep(0) and PDA-Hep(2) have greater roughness in PBS than in air, although the difference was relatively small,

RSC Advances Accepted Manuscript

and likely due to swelling. In contrast, PDA-Hep(5) and PDA-Hep(10) have much larger roughness in PBS than in air. PDA-Hep(10) had a roughness of 27.9 nm in PBS which is three times of the value in air. Moreover, the roughness was even higher than the thickness which means it could not be simply caused by swelling. For these reasons, we concluded that a large number of heparin molecules have been successfully immobilized on the coating surface, with more being immobilized the higher the heparin concentration.

--Fig. 3--

--Table 1--

The incorporation of heparin changed both the chemical composition and the surface morphology of the PDA coatings. WCA is a simple and effective indicator of surface properties, and is particularly important when protein adsorption phenomena are investigated. The water contact angles of the different PDA coated surfaces are shown in Fig. 4. The WCA of the PDA coating is around 34°, with the observed hydrophilicity attributable to the presences of hydrophilic groups, such as hydroxyl and amine groups. The WCA further decreased when heparin molecules were added to the surfaces, although the changes were small. As a sulfated polysaccharide, heparin has great hydrophilicity, too. Therefore, when heparin was incorporated to the hydrophilic PDA coating, the improvement of hydrophilicity was limited just like the previous reported results.[46]

--Fig. 4--

Protein adsorption and analysis

A basic requirement of blood purification is to remove undesirable substrates, yet retain beneficial substances. Although excess LDL is harmful, HDL is required for healthy physiological functions. HDL plays an important role in reverse cholesterol transport, which can remove excess cholesterol from peripheral tissue. Low HDL levels have been recognized as one of the major risk factors for

RSC Advances Accepted Manuscript

CHD.[11]. For the application of adsorbents, both the adsorption capacity and selectivity are important. SPR and QCM-D were used to measure the affinity of LDL for different coatings. In a typical SPR profile (supporting information Fig. S3), LDL had high adsorption values usually with a SPR response higher than 8100 μRIU. HDL displayed weaker adsorption, with a SPR response less than 3500 μRIU. Compared with LDL and HDL, HSA showed the lowest adsorption rate and adsorption ability. The PDA/heparin coatings showed much stronger adsorption for LDL, even though it is easier for small proteins (HDL and HSA) to penetrate into the swollen coatings. Besides electrostatic interaction, the soft structure is also helpful for LDL to stick on the rough coating surfaces. Despite the different adsorption properties of LDL, HDL, and HSA, the complex constituents of the PDA coating generally bound well to proteins. Polymerization of dopamine is a complex process, with a fair number of chemicals involved in the deposition and creation of diverse functional groups exposed at the coating surface.[45,58] PDA is a zwitterion with an isoelectric point around 4.[59,60] At pH = 7.4, PDA will be negatively charged because of the deprotonation of the phenolic groups, which makes it attractive for many proteins. Moreover, catechol in the PDA matrix can be oxidized to the corresponding quinine, which can react with proteins containing amine or thiol groups.[61] Hydrophobic groups, such as phenyl and indolyl, probably also take part in the protein adsorption process.

The single protein adsorption results for different PDA/heparin coatings are shown in Fig. 5. Interactions between the mixed coatings and proteins are the result of the combined effects of PDA and heparin. The adsorption results clearly show that the PDA/heparin mixed coatings have better recognition for LDL. The amount of adsorbed LDL increased with increasing heparin concentration, and PDA-Hep(5) adsorbed 20% more LDL than PDA-Hep(0). PDA-Hep(10), however, adsorbed slightly less LDL than PDA-Hep(5), although it still had higher adsorption capacity than

RSC Advances Accepted Manuscript

PDA-Hep(0). This indicates that appropriate proportions of PDA and heparin give the best adsorption of LDL. Compared with LDL, HDL and HSA adsorption remarkably decreased when heparin was incorporated, the greater heparin concentration the lower adsorption. Compared with PDA-Hep(0), PDA-Hep(10) adsorbed 30% and 67% less HDL and HSA respectively.

--Fig. 5--

Single protein adsorption has fully demonstrated that the PDA/heparin mixed coatings have great adsorption capacity for LDL, but selectivity is equally important in selecting an adsorbent as there are a variety of proteins in the plasma. As such, competitive adsorption experiments were performed to test the selectivity of the mixed coatings. It is well known that protein adsorption is highly dependent on the protein concentration used. Regardless of the competition of HDL or HSA, protein adsorption increased in the binary protein tests when LDL increased from 10 μg/mL to 30 μg/mL. The adsorption increment could be regarded as an indicator of preference for LDL because it was resulted from the concentration growth of LDL. Fig. 6 shows that the adsorption increment was smaller when LDL was mixed with HDL than with HSA, in other words, HDL has greater interference with LDL adsorption. Nonetheless, protein adsorption increased with an increase in heparin concentration whether HDL or HSA worked as the competitive protein. The adsorption increment of PDA-Hep(0) was 23.9% for HDL and 35.0% for HSA, the values increased to 57.7% and 70.1% respectively for sample PDA-Hep(10). Therefore, heparin endows the PDA/heparin coating surfaces with greater selectivity for LDL.

--Fig. 6--

Fig. 7 shows another strategy to test adsorption selectivity for LDL. In this method, competitive protein (HDL or HSA) was first adsorbed to form a protein layer. Firstly, different coating surfaces displayed similar Δf for HDL or HSA which is disagree with SPR results. This can be explained by

RSC Advances Accepted Manuscript

the fact that SPR is based on the change of refractive index at the interface, while QCM relies on the change of adsorbed mass including the mass of any solvent that is either entrained or coupled with the adsorbed layer.[62,63] More and more macromolecule chains would be exposed on the coating surfaces as heparin concentration increased from sample PDA-Hep(0) to PDA-Hep(10), and these dangling molecule chains would couple and trap a lot of water at the interfaces and lead to larger mass than the adsorbed protein. The presumption is also favored by ΔD values, which are indicators of film viscoelasticity. The results (see supporting information Fig. S4) illustrate that ΔD decreased with increasing heparin concentration. As the different samples with differing heparin quantities had similar Δf, this signifies that samples containing more heparin become softer and more dissipative after protein adsorption. After the first stage of protein adsorption, LDL was used to substitute the adsorbed competitive proteins. Little change in frequency of sample PDA-Hep(0) was observed after LDL was injected, irrespective of the competitive protein used. This result indicates that HDL or HSA was firmly attached on PDA coating surface and can not be easily substituted by LDL. This rules out the possibility that LDL could adhere to the protein layer directly. The QCM results (Fig. 8 and Table 2) clearly indicate that more and more HDL or HSA can be substituted by LDL, which means the mixed coatings have greater affinity for LDL as the heparin content is increased. In summation, two separate methods determined that the incorporation of heparin improves the PDA/heparin coatings' LDL selectivity.

--Fig. 7--

--Table 2--

Hemocompatibility of coated surfaces

Biocompatibility is essential for biomedical materials, and as such LDL adsorbents for use in hemodialysis require biocompatibility with blood. Generally, the quantity and morphology of

RSC Advances Accepted Manuscript

adhered platelets are considered to be early indicators of the biocompatibility of blood-contacting biomaterials. The results from platelet adhesion assays using the different PDA/heparin coatings are shown in Fig. 8. The platelets adhesion clearly changed with increasing heparin content. A number of platelets can be observed on sample PDA-Hep(0), with a strong tendency to aggregate. With an increase in heparin content, the quantity of adhered platelets decreased drastically, coupled with the aggregates gradually dispersing. Compared with PDA-Hep(0), much fewer platelets were adhered on sample PDA-Hep(10), and almost no aggregates were visible. The results of the adhesion assay suggest that the incorporation of heparin has significantly improved the compatibility of PDA with blood.

--Fig. 8--

Conclusions

In conclusion, this study proposed a simple method to prepare LDL capture surface. Heparin was easily deposited onto substrates by co-depositing with dopamine, the composition and surface properties of the coating can be easily controlled by adjusting the heparin concentration in the mixture solution. The PDA/heparin mixed coatings were thoroughly characterized, and they exhibited excellent adsorption capacity and selectivity for LDL when the heparin concentration is high enough. Furthermore, platelet adhesion experiment shows that appropriate heparin could give the PDA/heparin coating surface great hemocompatibility which is promising for biomedical application. The proposed co-deposition strategy combines the biological characteristics of heparin and the versatility of PDA coating and shows great potential to prepare LDL adsorbents.

RSC Advances Accepted Manuscript

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 51473143), National "Twelfth Five-Year" Plan for Science & Technology Support of China (No. 2012BAI08B01).

RSC Advances Accepted Manuscript

References

1 D. Lloyd-Jones, R. J. Adams, T. M. Brown, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee, *Circulation*, 2010, **121**, 948-954.

2 C. Weber, H. Noels, *Nat. Med.*, 2011, **17**, 1410-1422.

3 A. E. Fraley, S. Tsimikas, *Curr. Opin. Lipidol.*, 2006, **17**, 502-509.

4 Y. Ishigaki, Y. Oka, H. Katagiri, *Opin. Lipidol.*, 2009, **20**, 363-369.

5 S. Erqou, S. Kaptoge, P. L. Perry, et al. Emerging Risk Factors Collaboration, *J. Am. Med. Assoc.*, 2009, **302**, 412-423.

6 A. P. Cai, L. W. Li, Y. Zhang, Y. J. Mo, W. Y. Mai, Y. L. Zhou, *Dis. Markers*, 2013, **35**, 551-559.

7 R. W. Mahley, K. H. Weisgraber, Y. D. Huang, *J. Lipid Res.*, 2009, **50**, S183-S188.

8 H. Li, P. Dhanasekaran, E. T. Alexander, D. J. Rader, M. C. Philips, S. Lund-Katz, *Arterioscl. Throm. Vas.*, 2013, **33**, 687-693.

9 T. Alonzi, C. Mancone, L. Amicone, M. Tripodi, *Expert Rev. Proteomics*, 2008, **5**, 91-104.

10 K. Skålén, M. Gustafsson, E. K. Rydberg, L. M. Hultén, O. Wiklund, T. L. Innerarity, J. Borén, *Nature*, 2002, **417**, 750-754.

11 Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, *J. Am. Med. Assoc.*, 2001, **19**, 2486-2497.

12 D. Marks, M. Thorogood, H. A. W. Neil, S. E. Humphries, *Atherosclerosis*, 2003, **168**, 1-14.

13 F. Civeira, International Panel on Management of Familial Hypercholesterolemia, *Atherosclerosis*, 2004, **173**, 55-68.

14 J. L. Winters, *J. Clin. Apheresis*, 2011, **26**, 269-275.

15 U. Julius, S. Fischer, U. Schatz, B. Hohenstein, S. R. Bornstein, *Clin. Lipidol.*, 2013, **6**, 693-705.

RSC Advances Accepted Manuscript

16 G. Siegela, M. Malmsten, D. Klüßendorf, W. Leonhardt, *Atherosclerosis*, 1999, **144**, 59-67.

17 M. Malmsten, G. Siegel, W. G. Wood, *J. Colloid Interf. Sci.*, 2000, **224**, 338-346.

18 M. Rodríguez, L. Ringstad, P. Schäfer, S. Just, H. W. Hofer, M. Malmsten, G. Siegel, *Atherosclerosis*, 2007, **192**, 438-444.

19 G. Siegel, E. Ermilov, A. R. Pries, K. Winklerc, A. Schmidt, L. Ringstad, M. Malmstenb, B. Lindman, *Colloid. Surface. A*, 2014, **442**, 173-180.

20 L. D'Ulivo, J. Saint-Guirons, B. Ingemarsson, M. L. Riekkola, *Anal. Bioanal. Chem.*, 2010, **396**, 1373-1380.

21 K. Lipponen, Y. Liu, P. W. Stege, K. Öörni, P. T. Kovanen, M. L. Riekkola, *Anal. Biochem.*, 2012, **424**, 71-78.

22 A. Lookene, R. Savonen, G. Olivecrona, *Biochemistry*, 1997, **36**, 5267-5275.

23 K. Gaus, E. A. H. Hall, *J. Colloid Interf. Sci.*, 1999, **217**, 111-118.

24 F. Li, G. X. Cheng, S. P. Liang, *Polym. Bull.*, 2006, **57**, 261-267.

25 K. W. Ma, L. Ma, S. X. Cai, X. Wang, B. Liu, Z. L. Xu, X. Z. Dai, J. Y. Yang, A. H. Jing, W. J. Lei, *J. Mater. Sci.: Mater. Med.*, 2008, **19**, 3255-3261.

26 X. J. Huang, D. Guduru, Z. K. Xu, J. Vienken, T. Groth, *Acta Biomater.*, 2010, **6**, 1099-1106.

27 Ye. Cao, H. Wang, C. Yang, R. Zhong, Y. Lei, K. Sun, J. X. Liu, *Appl. Surf. Sci.*, 2011, **257**, 7521-7528.

28 K. W. Ma, X. Z. Dai, S. Y. Feng, A. H. Jing, J. Y. Yang, *Transfus. Apher. Sci.*, 2011, **44**, 3-9.

29 X. J. Huang, D. Guduru, Z. K. Xu, J. Vienken, T. Groth, *Macromol. Biosci.*, 2011, **11**, 131-140.

30 J. Li, X. J. Huang, J. Ji, P. Lan, J. Vienken, T. Groth, Z. K. Xu, *Macromol. Biosci.*, 2011, **11**, 1218-1226.

31 X. D. Hou, T. Zhang, A. M. Cao, Polym. *Adv. Technol.*, 2013, **24**, 660-667.

RSC Advances Accepted Manuscript

32 H. Yavuz, A. Denizli, *J. Biomat. Sci. Polym. E.*, 2003, **14**, 395-409.

33 H. Yavuz, A. Denizli, *Macromol. Biosci.*, 2005, **5**, 39-48.

34 N. Bereli, G. Şener, H. Yavuz, A. Denizli, *Mat. Sci. Eng., C*, 2011, **31**, 1078-1083.

35 S. Q. Wang, Y. T. Yu, T. Cui, Y. Cheng, *Biomaterials*, 2003, **24**, 2799-2802.

36 Y. Cheng, S. Q. Wang, Y. T. Yu, Y. Yuan, *Biomaterials*, 2003, **24**, 2189-2194.

37 G. Q. Fu, H. Y. Li, H. F. Yu, L. Liu, Z. Yuan, B. L. He, *React. Funct. Polym.*, 2006, **66**, 239-246.

38 H. F. Yu, G. Q. Fu, B. L. He, *Cellulose*, 2007, **14**, 99-107.

39 R. Prassl, P. Laggner, *Eur. Biophys. J. Biophy.*, 2009, **38**, 145-158.

40 I. Capila, R. J. Linhardt, *Angew. Chem. Int. Ed.*, 2002, **41**, 390-412.

41 K. H. Weisgraber, S. C. Rall, *J. Biol. Chem.*, 1987, **262**, 11097-11103.

42 A. D. Cardin, C. J. Randall, N. Hirose, R. L. Jackson, *Biochemistry*, 1987, **26**, 5513-5518.

43 M. Gigli, A. Consonni, G. Ghiselli, V. Rizzo, A. Naggi, G. Torri, *Biochemistry*, 1992, **31**, 5996-6003.

44 H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science*, 2007, **318**, 426-430.

45 Y. L. Liu, K. L. Ai, L. H. Lu, *Chem. Rev.*, 2014, **9**, 5057-5115.

46 L. P. Zhu, J. Z. Yu, Y. Y. Xu, Z. Y. Xi, B. K. Zhu, *Colloids Surf., B*, 2009, **69**, 152-155.

47 J. H. Jiang, L. P. Zhu, X. L. Li, Y. Y. Xu, B. K. Zhu, *J. Membrane Sci.*, 2010, **364**, 194-202.

48 T. G. Kim, H. Lee, Y. Jang, T. G. Park, *Biomacromolecules*, 2009, **10**, 1532-1539.

49 I. You, S. M. Kang, Y. Byun, H. Lee, *Bioconjugate Chem.*, 2011, **22**, 1264-1269.

50 C. Cheng, S. Li, S. Q. Nie, W. F. Zhao, H. Yang, S. D. Sun, C. S. Zhao, *Biomacromolecules*, 2012, **13**, 4236-4246.

51 W. B. Tsai, C. Y. Chien, H. Thissen, J. Y. Lai, *Acta Biomater.*, 2011, **7**, 2518-2525.

RSC Advances Accepted Manuscript

52 S. M. Kang, N. S. Hwang, J. Yeom, S. Y. Park, P. B. Messersmith, I. S. Choi, R. Langer, D. G. Anderson, H. Lee, *Adv. Funct. Mater.*, 2012, **22**, 2949-2955.

53 Y. Zhang, B. Thingholm, K. N. Goldie, R. Ogaki, B. Städler, *Langmuir*, 2012, **28**, 17585-17592.

54 C. Y. Chien, W. B. Tsai, *ACS Appl. Mater. Interfaces*, 2013, **5**, 6975-6983.

55 Y. Zhang, K. Panneerselvam, R. Ogaki, L. Hosta-Rigau, R. van der Westen, B. E. B. Jensen, B. M. Teo, M. F. Zhu, B. Städler, *Langmuir*, 2013, **29**, 10213-10222.

56 Y. X. Liu, C. P. Chang, T. Sun, *Langmuir*, 2014, **11**, 3118-3126.

57 O. Pop-Georgievski, C. Rodriguez-Emmenegger, AD. Pereira, V. Proks, E. Brynda, F. Rypáček, *J. Mater. Chem. B*, 2013, **1**, 2859-2867.

58 N. F. Della Vecchia, R. Avolio, M. Alfé, M. E. Errico, A. Napolitano, M. d'lschia, *Adv. Funct. Mater.*, 2013, **23**, 1331-1340.

59 B. Yu, J. X. Liu, S. J. Liu, F. Zhou, *Chem. Commun.*, 2010, **46**, 5900-5902.

60 Q. Z. Liu, B. Yu, W. C. Ye, F. Zhou, *Macromol. Biosci.*, 2011, **11**, 1227-1234.

61 H. Lee, J. Rho, P. B. Messersmith, *Adv. Mater.*, 2009, **21**, 431-434.

62 J. Homola, *Chem. Rev.*, 2008, **108**, 462-493.

63 K. A. Marx, *Biomacromolecules*, 2003, **4**, 1099-1120.

RSC Advances Accepted Manuscript

Figure Captions

Fig. 1 XPS survey spectra (A) and high resolution S 2p spectra (B) of PDA/heparin coatings.

Fig. 2 Ellipsometric thickness of PDA/heparin coatings.

Fig. 3 AFM images (4.5 × 4.5 μm^2) of PDA/heparin coatings in PBS: (A) PDA-Hep(0), (B) PDA-Hep(2), (C) PDA-Hep(5), (D) PDA-Hep(10).

Fig. 4 Water contact angles of PDA/heparin coatings.

Fig. 5 Protein adsorption capacities of different PDA/heparin coatings.

Fig. 6 Binary protein adsorption by PDA/heparin coatings.

Fig. 7 QCM Frequency changes when HDL (A) or HSA (B) was used as competitive protein.

Fig. 8 SEM of platelet adhesion on PDA/heparin coatings: (A) PDA-Hep(0), (B) PDA-Hep(2), (C) PDA-Hep(5), (D) PDA-Hep(10).

RSC Advances Accepted Manuscript

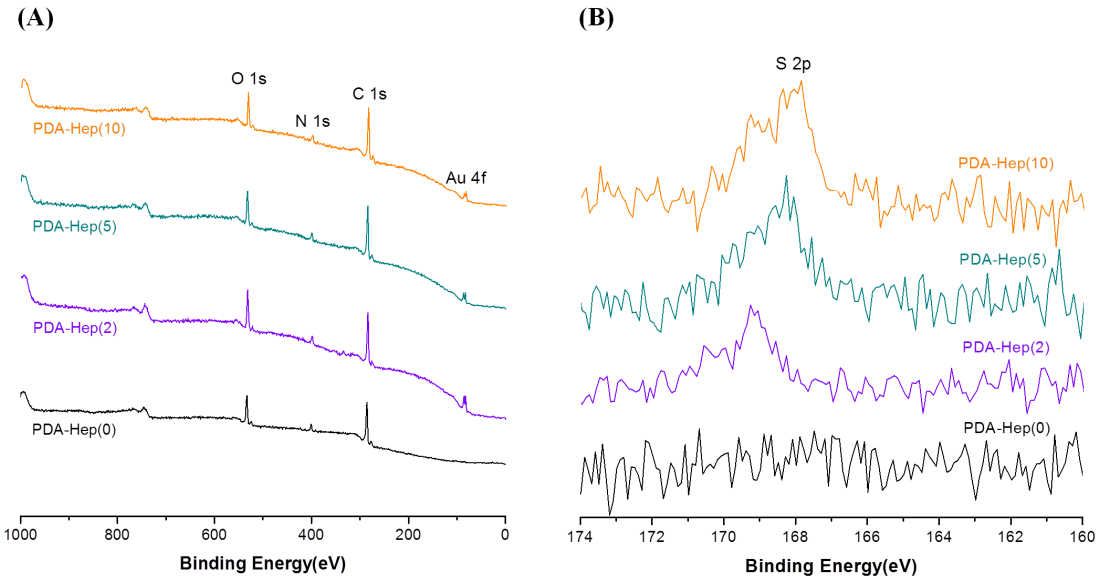


Fig. 1 XPS survey spectra (A) and high resolution S 2p spectra (B) of PDA/heparin coatings.

RSC Advances Accepted Manuscript

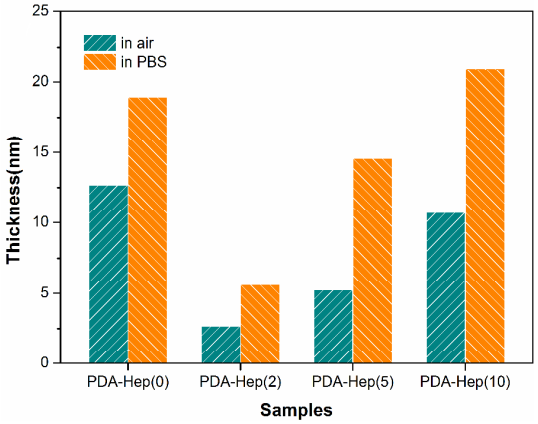


Fig. 2 Ellipsometric thickness of PDA/heparin coatings.

RSC Advances Accepted Manuscript

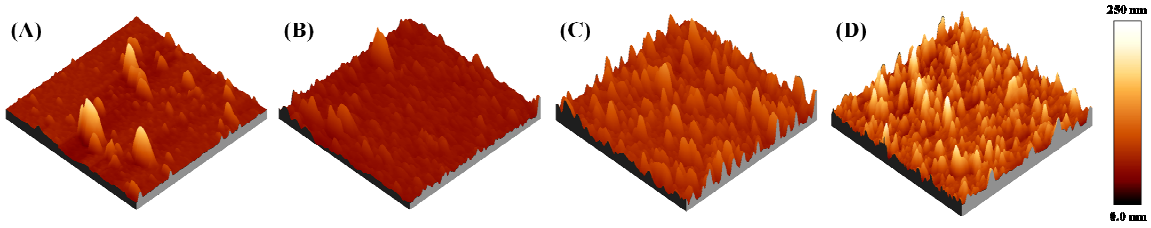


Fig. 3 AFM images (4.5 × 4.5 μm^2) of PDA/heparin coatings in PBS: (A) PDA-Hep(0), (B) PDA-Hep(2), (C) PDA-Hep(5), (D) PDA-Hep(10).

RSC Advances Accepted Manuscript

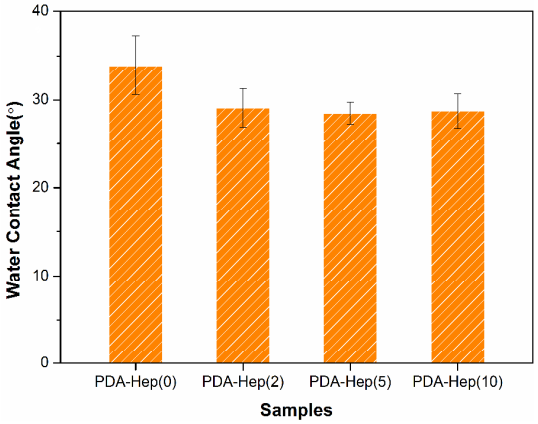


Fig. 4 Water contact angles of PDA/heparin coatings.

RSC Advances Accepted Manuscript

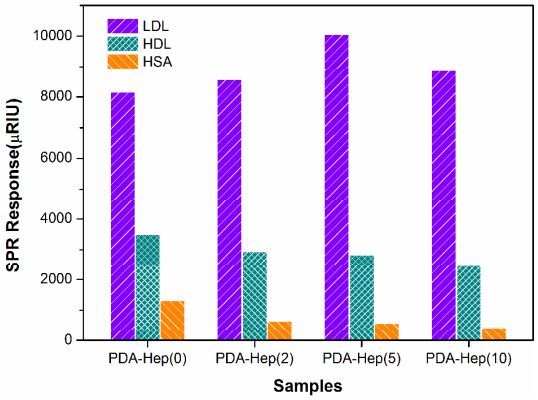


Fig. 5 Protein adsorption capacities of different PDA/heparin coatings.

RSC Advances Accepted Manuscript

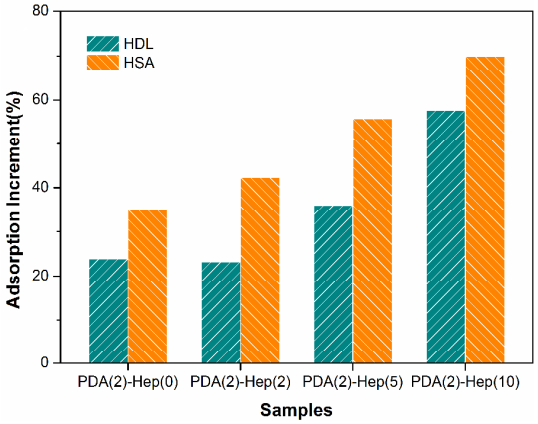


Fig. 6 Binary protein adsorption by PDA/heparin coatings.

RSC Advances Accepted Manuscript

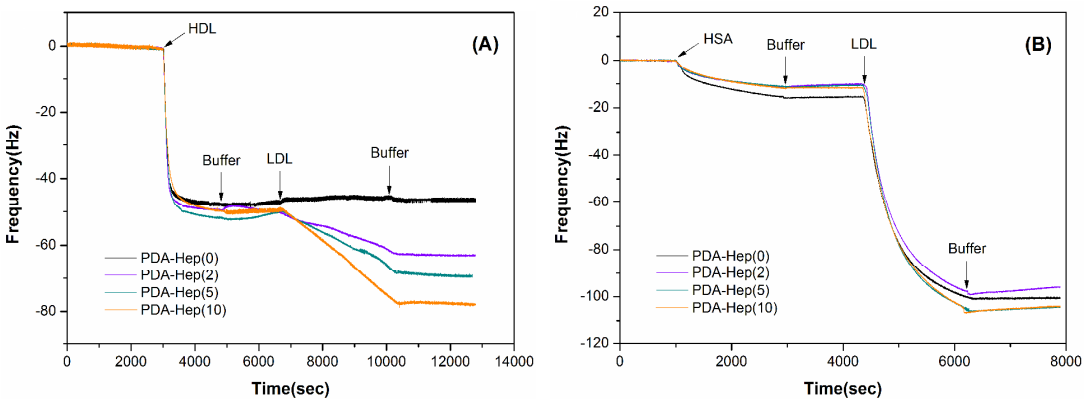


Fig. 7 QCM Frequency changes when HDL (A) or HSA (B) was used as competitive protein.

RSC Advances Accepted Manuscript

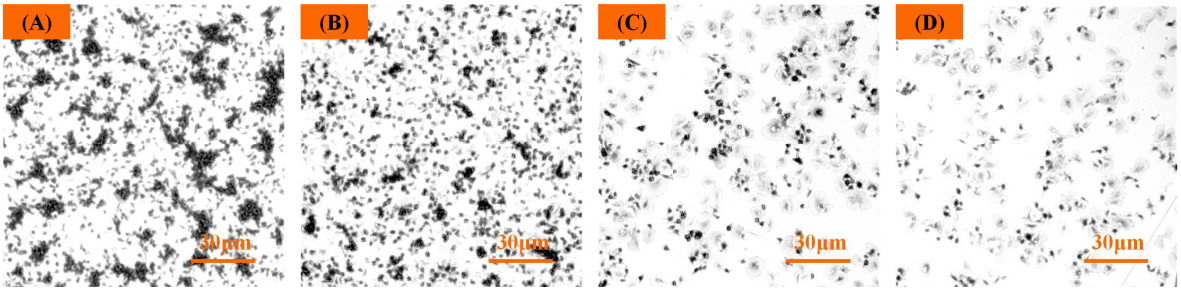


Fig. 8 SEM of platelet adhesion on PDA/heparin coatings: (A) PDA-Hep(0), (B) PDA-Hep(2), (C) PDA-Hep(5), (D) PDA-Hep(10).

RSC Advances Accepted Manuscript

Table Captions

Table 1 Surface roughness of PDA/heparin coatings determined by AFM.

Table 2 Frequency changes of PDA/heparin coatings at different stages in QCM tests.

RSC Advances Accepted Manuscript

Table 1 Surface roughness of PDA/heparin coatings determined by AFM.

	PDA-Hep(0)	PDA-Hep(2)	PDA-Hep(5)	PDA-Hep(10)
RMS(nm)-in air	9.3	5.9	8.4	9.1
RMS(nm)-in PBS	10.7	6.1	13.6	27.9

RSC Advances Accepted Manuscript

Table 2 Frequency changes of PDA/heparin coatings at different stages in QCM tests.

	PDA-Hep(0)	PDA-Hep(2)	PDA-Hep(5)	PDA-Hep(10)
HDL(Hz)	-47.8	-49.4	-49.9	-51.9
HDL-LDL(Hz)	2.4	-12.9	-17.6	-25.7
HSA(Hz)	-15.4	-11.9	-11.2	-11.1
HSA-LDL(Hz)	-84.9	-85.8	-94.7	-95.6

RSC Advances Accepted Manuscript