Construction of mussel-inspired coating via the direct reaction of catechol and polyethyleneimine for efficient heparin immobilization

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ABSTRACT

Dopamine could self-polymerize to form the coating on various substrates and the co-existence of catechols and amines was crucial in performing such polymerization process. In this work, a mimetic approach of coating formation was carried out based on the co-polymerization of catechol (CA) and polyethyleneimine (PEI). Mussel-inspired CA/PEI coating was deposited on 316L stainless steel (SS). Fourier transform infrared spectra (FTIR) and X-ray photoelectron spectroscopy (XPS) demonstrated the successful coating formation. QCM measurement showed good affinity of heparin immobilization on CA/PEI coating surface ascribed to the amine groups. Herein, vascular cell-material interactions like endothelial cells (ECs) and smooth muscle cells (SMCs) were also investigated. Interestingly, CA/PEI and heparin modified coatings presented no cytotoxicity to ECs, however to a certain extent, decreased SMCs proliferation. Moreover, heparin-binding surface presented significant anti-platelet adhesion and activation properties. These results effectively suggested that the mussel-inspired CA/PEI coating might be promising when served as a platform for biomolecule immobilization.

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1. Introduction

Cardiovascular diseases (CVDs) have been the leading causes of death and implants like vascular stents have been demonstrated its beneficial effects [1,2]. However, in response to acute vessel-wall injury caused by angioplasty, the increased incidence of thrombosis, restenosis, and intimal hyperplasia are still concerns [3–7]. Considerable efforts have been directed toward developing stents with properties of antithrombotic, anti-proliferation or aiming at rapid endothelialization [8–10]. Immobilization of biomolecules is an effective method for tailoring desired surface properties and has been widely utilized in current research, including molecular biology, analytical chemistry, bioprocess engineering, medical diagnostics, regenerative medicine, and tissue engineering [11–16].

Many vascular interventional devices, such as stents and venacava filters which are made of stainless steels (SS), titanium, sharp memory nitinol, and cobalt-based alloy, have been widely applied to clinical treatment [17–19]. However, the lack of sufficient reactive functional groups on the surface restricted their further application to obtain desired bio-functions. Pre-treatment of metal surface to obtain an ad-layer containing amines, carboxyls or quinones is effective for the further introduction of biomolecules. Methods like physical adsorption, self-assembly (including monolayer self-assembly and layer-by-layer (LBL) self-assembly [20,21], anchoring [22], and direct covalent immobilization [23–25] have made it available to immobilize different kinds of biomolecules onto the substrate surface [26]. Inspired by mussel adhesive chemistry, Lee and Messersmith had reported a facile and versatile method to modify various substrates via forming polydopamine (PDA) coating [27–29]. Numerous reports had demonstrated the effects of PDA to afford biomolecule like vascular endothelial growth factor (VEGF), Laminin and other peptides via Schiff based or Michael addition reactions [27,30–32]. In our institute, dopamine assisted surface modification was done to make a better platform for desired biological properties [33–35]. Moreover, many surface modification based on catechol moieties were performed to achieve desired properties were done [36–42]. These studies strongly demonstrated the effect of using catechol chemistry for en-rich surface modification techniques.

Though widely been investigated, the immobilization of biomolecules onto PDA was mainly based on the catechols/quinones, yet not on the amines groups. Does it mean that the exposed amine groups on PDA surface were in-sufficient for amine-induced immobilization (e.g. amine/carboxyl associated carbodiimide chemistry)? As reported, single catechol will not cause strong coating formation and the co-existence of catechols and amines was crucial in dopamine polymerization process [43]. Based on these findings, we would like to put forward a mimetic approach via the direct reactions of catechols and amines to form a dopamine-like polymerization process. In this work, catechol (CA) and polyethyleneimine (PEI) were chosen as the mimicry of catechol and amine moiety of dopamine. PEI was adopted to obtain a surface rich in amine groups. The CA/PEI coating was deposited on 316L stainless steel (SS). The reactive functional groups like catechols and amines retained on CA/PEI coating surface were also investigated. Heparin was subsequently immobilized onto CA/PEI surface and the in vitro hemocompatibility was tested. Considering the potential use of such coating in vascular grafts modifying, the interactions with endothelial cells (ECs) and smooth muscle cells (SMCs) were also tested.

2. Experimental details

2.1. Materials

The catechol, PEI (1300 MW) and heparin are all obtained from Sigma (Sigma-Aldrich Chemical Co.). Micro-BCA was obtained from Pierce Biotechnology Inc. (Rockford, USA). Various reagents used for the evaluation of hemocompatibility and cytocompatibility were provided from professional manufacturers which were mentioned in the experimental part. Other reagents were local products of analytical grade.

2.2. Preparation of CA/PEI films and immobilization of heparin

Mirror polished 316L stainless steel (SS) (Φ = 10 mm) was used to deposit CA/PEI coating. The films were fabricated on SS at room temperature in the mixture solution of catechol (4 mg/mL) and PEI (3.3 mg/mL) in Tris buffer solution (pH 8.5) overnight. After deposition, samples were ultrasonically cleaned with deionized (D.I.) water and labeled as CA/PEI. After that, heparin was further immobilized to CA/PEI surface using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) induced crosslinking between amine and carboxyl groups. Briefly, 30 mg heparin was dissolved in 0.05 M 2-Morpholinoethanesulfonic acid (MES) buffer (pH 5.5, 15 mL) and CA/PEI coated SS were immersed in such solution. EDC (0.1 M) was subsequently added to the heparin solution to activate the carboxylic acid groups of heparin molecules. The reaction was maintained at room temperature for 12 h and then the samples were ultrasonically cleaned with D.I water, and labeled as CA/PEI-Hep.

2.3. Chemical characterization of CA/PEI films

The chemical structures of the films were investigated by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, NICOLET 5700) at the range of 4000–500 cm^{-1}. The surface chemical compositions were characterized by X-ray photoelectron spectroscopy (XPS, Perkin-Elmer 16PC) with a monochromatic Al Kα excitation radiation (1486.6 eV). Binding energies were calibrated by using the containment carbon (C 1s = 284.7 eV). Peaks were fitted using Xpspeak 4.1 to obtain the high resolution information. Water contact angle was measured by static drops using DSA100 ((Krüss, Hamburg, Germany). Zeta potential test was performed as previously described [44].

2.4. Qualitative characterization of catechol and amine

For the amine content quantification, an acid orange II (AOII) method was adopted [45]. Briefly, 5×10^{-3} M AOII HCl solution (1 mM, pH 3) and a standard curve of AOII solution (pH 12) at 485 nm was first prepared to characterize the amine concentration. To determine of the amine concentration on CA/PEI surface, 50 μL AOII solution was added on the surface of each sample. The supernatant was removed from the plate after incubating for 12 h at 20 °C, and the samples were washed (3 times × 5 min) with HCl solution (1 mM, pH = 3). Then the samples were incubated for 15 min at 25 °C in NaOH solution (0.01 M, pH = 12) to elute the adsorbed AOII on the surfaces. Finally, 150 μL of the eluate from each sample well was transferred into a 96 well-plate and the absorbance was determined using microplate reader at 485 nm. For the catechol content quantification, a micro-BCA assay was adopted as described before [46,47]. Briefly, the linear absorbance at 562 nm could be seen after the interaction between catechol and BCA test solution. The amount of reactive catechol on CA/PEI surfaces was calculated according to the catechol-related standard calibration curve.

2.5. QCM for monitoring of heparin immobilization

After coating formation, surfaces could contain some functional groups which are useful for further functionalization. In this study, because of the retained amine groups on CA/PEI surface, the potential of such surface for biomolecule immobilization was tested using the quartz crystal microbalance (QCM) (Q-Sense AB, Sweden Company) measurements [48]. Due to the existence of catechol moiety, CA/PEI coatings could coat various metal substrates including gold (data not shown). Prior to the test, coatings were prepared on AT-cut 5 MHz Au-coated single crystal quartz (diameter of Au films: 10 mm). During test, 2 mg/mL heparin/MES buffer solution was injected at 50 μL/min continuously until the QCM traces did not vary. The frequency shift (Δf) was related to the adsorbed mass (Δm) according to the Sauerbrey relation [49].

$$\Delta m = \Delta f \times \frac{C}{n} \tag{1}$$

C (C = 17.7 ng/cm^2 Hz^{-1} at f_n = 5 MHz) was the mass-sensitivity constant and n (n = 1, 3, 5, . . .) was the overtone number.

2.6. In vitro hemocompatibility test

Freshly donated human whole blood was obtained legally from the Blood Center of Chengdu, China. The analysis was performed within 8 h after the blood donation. The amounts of the samples used for statistical count were no less than five. Platelet rich plasma (PRP) was prepared by centrifuging (1500 rpm, 15 min) fresh human whole blood. 50 μL of fresh PRP was added onto the sample surface and incubated for 2 h at 37 °C in humidified air. After washing with PBS solution, they were fixed using 2.5% glutaraldehyde solution for 12 h, and then were washed with distilled water for three times. The platelets adsorbed on the surfaces were dehydrated with 40, 50, 70, 90 and 100 vol% ethanol/water solution for 15 min each in sequence. After critical point drying, some samples were gold sputtered, and then examined by scanning electron microscopy (SEM, Quanta 200, FEI, Holland). Besides, platelet adhered on different samples were also fluorescence stained, and then examined by fluorescence microscope. Moreover a dynamic whole blood test was adopted as described before [50]. For the relatively activated platelets quantification, a P-selectin assay was taken. Briefly, 60 μL of PRP was added onto each sample surface