Biomaterials 35 (2014) 7679–7689

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Electropolymerization of dopamine for surface modification of complex-shaped cardiovascular stents

Jin-lei Wang [1], Bo-chao Li [1], Zi-jun Li, Ke-feng Ren*, Lie-jiang Jin, Shi-miao Zhang, Hao Chang, Yi-xin Sun, Jian Ji*

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

ARTICLE INFO

Article history:
Received 4 April 2014
Accepted 19 May 2014
Available online 12 June 2014

Keywords:
Electropolymerization
Poly(dopamine) coating
Surface functionalization
Cardiovascular stent
Vascular endothelial growth factor

ABSTRACT

Inspired by the adhesion strategy of marine mussels, self-polymerization of dopamine under alkaline condition has been proven to be a simple and effective method for surface modification of biomaterials. However, this method still has many drawbacks, such as the use of alkaline aqueous medium, low poly(dopamine) deposition rate, and inefficient utilization of dopamine, which greatly hinder its practical application. In the present study, we demonstrate that electropolymerization of dopamine is a facile and versatile approach to surface tailoring of metallic cardiovascular stents, such as small and complex-shaped coronary stent. Electropolymerization of dopamine leads to the formation of a continuous and smooth electropolymerized poly(dopamine) (ePDA) coating on the substrate surface. This electrochemical method exhibits a higher deposition rate and is more efficient in dopamine utilization compared with the typical self-polymerization method. The ePDA coating facilitates the immobilization of biomolecules onto substrates to engineer biomimetic microenvironments. *In vitro* and *in vivo* experiments demonstrate that ePDA coating functionalized with vascular endothelial growth factor can greatly enhance the desired cellular responses of endothelial cells and prevent the neointima formation after stent implantation. The proposed methodology may find applications in the area of metallic surface engineering, especially for the cardiovascular stents and potentially all biomedical devices with electroconductive surface as well.

1. Introduction

Cardiovascular disease is now the leading cause of death worldwide. Coronary heart disease alone, for instance, caused more than 7 million deaths per year worldwide [1]. Percutaneous transluminal angioplasty (PTA), which is based on metallic stent implantation, is primary and the most important treatment for cardiovascular diseases, including coronary vascular disease, disease of the blood vessels supplying the brain, and peripheral arterial disease [2]. However, clinical failures of the stents after the implantation occur frequently, such as in-stent restenosis and late stent thrombosis [3,4]. Surface modification of the cardiovascular stents is an extremely challenging while most effective strategy to resolve these clinical problems, since surface properties have a profound impact on the biological response [5,6]. Numerous surface modification strategies have been developed, such as plasma polymerization, polymer coating, sol–gel derived oxide coatings, chemical immobilization of bioactive molecules, and layer-by-layer self-assembly [7–12]. Nevertheless, these available methods have been used to a limited extent in practical applications due to the requirement of expensive equipment, multiple-step complicated processes, and lack of stability. Surface functionalization of the stents that are generally absent in reactive functional groups, especially for the small-sized coronary stents with complex shape, is still a great challenge [13].

Inspired by the bio-adhesion principle of marine mussels, Messersmith and his colleagues found that dopamine could spontaneously polymerized under alkaline conditions, leading to the formation a poly(dopamine) (PDA) coating with latent reactivity on a variety of substrates [14]. Due to its simplicity and good biocompatibility, PDA has recently attracted great interest and was intensively studied for surface modification of biomaterials [15–19]. Wang et al. reported that PDA formed on Ti foils could serve as anchors for immobilization of vascular endothelial growth

* Corresponding authors. Tel./fax: +86 571 87953729.
E-mail addresses: renkf@zju.edu.cn (K.-f. Ren), jijian@zju.edu.cn (J. Ji).
[1] Authors contribute equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2014.05.047
0142-9612/

factor (VEGF) [15]. The VEGF-functionalized surface could promote the growth of endothelial cells (ECs) and induce the differentiation of human mesenchymal stem cells into ECs. Despite extensive studies of self-polymerization of dopamine used for surface modification, there are still several shortcomings associated with this approach. First of all, spontaneous self-polymerization of dopamine occurs predominantly at alkaline solution (pH 8.5 is most often used), and it is thus not suitable for the alkaline-sensitive materials [20]. Secondly, the deposition rate of self-polymerized PDA is very low. At a dopamine concentration of 2 mg/mL, more than 10 h is needed to deposit a PDA coating with a thickness of 20 nm [21]. Last but not least, self-polymerization of dopamine under alkaline condition would result in the formation of numerous unavoidable nano-/microparticulate aggregates of PDA in solution. These aggregates not only tremendously decrease the efficiency of utilization of free dopamine molecules, but also give rise to a significant increment in surface roughness [22].

Although precise mechanistic details of PDA formation have not yet been fully disclosed, oxidation of dopamine to dopaminequinone is generally considered to be the initial and critical step [14,23,24]. In essence, this step is a typical redox reaction, which could be easily realized by an electrochemical method [25,26]. Considering that the electrochemical method possesses unique advantages for precise control over the redox reactions, we therefore hypothesize that, electrochemical oxidative polymerization of dopamine under neutral conditions may provide an alternative approach for tailoring the surface properties of metallic vascular stents. Herein, from a material's point of view, we investigated the electropolymerization of dopamine as a novel strategy for surface modification. The formation of electropolymerized poly(dopamine) (ePDA) was firstly monitored. The latent reactivity of ePDA coating towards VEGF was then studied. *In vitro* cellular responses of the ePDA-modified and VEGF-functionalized 316L stainless steel were systematically investigated to evaluate their cytocompatibility. After that, the ePDA-based approach was further extended to modify the coronary stent to testify the applicability of this approach. Finally, cell culture studies and animal experiments were carried out to evaluate the *in vitro* and *in vivo* biological responses of the VEGF-functionalized stent.

2. Materials and methods

2.1. Chemicals and materials

Dopamine hydrochloride was obtained from Sigma–Aldrich and used as received. Bovine serum albumin (BSA), tris-buffered saline (TBS), and phosphate buffered saline (PBS) were purchased from Shanghai Sangon Biotechnology (Shanghai, China). Cell culture reagents were obtained from Gibco (Invitrogen, USA). Triton X-100, phalloidin-tetramethyl-rhodamine B isothiocyanate (phalloidin-TRITC), and 4,6-diamidina-2-phenylin (DAPI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other reagents were of analytical grade and Milli-Q water was used throughout the whole experiment.

2.2. Electropolymerization of dopamine

All the electrochemical experiments were carried out in a standard three-electrode cell by using a CHI 660 electrochemical workstation (Chenhua, Shanghai, China) at room temperature. Saturated calomel electrode (SCE) was used as the reference electrode, while a platinum wire (Model CHI 115, CH Instruments, Inc.) or platinum plate (self-prepared) was used as the counter electrode. Gold electrode (Model CHI101, CH Instruments), gold-coated silicon wafer, 316L stainless steel disc (Antai Tech, Beijing, China), indium–tin oxide (ITO)-coated glass, or 316L stainless steel coronary stents (Sinomed, Tianjing, China) was used as the working electrode upon request for the specific experiment. Cyclic voltammetric method was employed to prepare ePDA coating on the working electrode surfaces. Typically, 20 mL of TBS (containing 25 mM Tris, 140 mM NaCl and 3 mM KCl, pH 7.4) was bubbled with high purified nitrogen for 10 min to remove oxygen. Then, 20 mg of dopamine was added to the TBS and electropolymerization of dopamine was carried out by successive cyclic voltammetric sweeps in the potential range from −0.5 to 0.5 V. After electropolymerization, the ePDA-modified working electrode was washed with Mill-Q water and used in the further experiments.

2.3. Electrochemical characterization

Cyclic voltammetry (CV) and impedance measurements were performed in 0.1 M KNO_3 solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. For electrochemical impedance spectroscopy (EIS) measurements, an alternating potential with amplitude of 5 mV was applied to the ePDA-modified gold electrode at open circuit potential and the frequency range was from 0.01 Hz to 100 kHz.

2.4. Surface characterization

The deposition kinetics of ePDA on gold-coated silicon wafers were studied by spectroscopic ellipsometry (M-2000, Woollam, USA). Thickness evolution of PDA coating formed through the classical approach, i.e., oxidative self-polymerization of dopamine (1 mg/mL) under alkaline conditions (10 mM Tris buffer, pH 8.5), were also measured by spectroscopic ellipsometry to compare the deposition rate of poly(dopamine) coatings prepared by different methods. Surface elemental compositions were analyzed with X-ray photoelectron spectroscopy (XPS, PHI 5000C ESCA System) with Mg K_α excitation radiation ($h\upsilon = 1253.6$ eV). Surface morphology of the 316L stainless steel coronary stent before and after being coated with ePDA were observed by a field-emitting scanning electron microscope (FE-SEM, Hitachi S-4800, Japan).

2.5. Immobilization of bioactive molecules

The ePDA-coated substrates were immersed into a solution of recombinant human $VEGF^{165}$ (PeproTech Inc, USA) (0.5 μg/mL in 10 mM Tris buffer, pH 8.5) for 12 h at 37 °C. The substrates were then washed with PBS to remove the unattached VEGF. For visualizing the immobilized VEGF on the ePDA coating, samples were blocked with 0.1% BSA solution for 1 h and incubated with polyclonal rabbit anti-human VEGF165 antibody (1:50 dilution, NRPB25, Hangzhou Neuropeptide Biological Science and Technology, China) for 2 h at 37 °C. The samples were then incubated with Alexa Fluor 568-conjugated goat anti-rabbit IgG antibody (1:400 dilution, A11011, Invitrogen, USA) for 1 h at 37 °C. After washing three times with TBS, the samples were mounted on glass slides with ProLong® gold antifade reagent (Invitrogen, USA). The immunofluorescence images were obtained via a Zeiss axio-vert 200 inverted microscope (Axio-vert 200M, Zeiss, Germany). To quantify the amounts of immobilized VEGF on the ePDA coating, the concentration of VEGF in the remaining solution after immobilization and in the combined washing solution was measured using an enzyme-linked immunosorbent assay (ELISA) test kit, as recommended by the manufacturer (NeoBioscience Technology, China). The surface density of immobilized VEGF could be calculated from the difference between the initial and remaining amounts of VEGF in the buffer solutions. SS-ePDA denotes the 316L stainless steel disc (SS) coated with eDPA, and SS-ePDA-VEGF represents SS-ePDA after immobilization of VEGF.

In order to demonstrate the uniform deposition and latent reactivity of ePDA coating, the 316L stainless steel coronary stent coated with ePDA (stent-ePDA) was immersed into a solution of fluorescein isothiocyanate-conjugated bovine serum albumin (BSA-FITC, Zhong Ke Chen Yu Trading Co., Ltd., China) (2 mg/mL in 10 mM Tris buffer, pH 8.5) for 24 h at 37 °C. The stents were then rinsed with Mill-Q water and observed by a Zeiss axio-vert 200 inverted microscope (Axio-vert 200M, Zeiss, Germany).

2.6. Cell culture

With local ethical committee approvals, primary human umbilical vein endothelial cells were isolated from newborn umbilical cords with collagenase (type I, GIBCO) as described previously [27,28]. Endothelial cells (ECs) were cultured in serum free medium (SFM, cat. no. 11111-044, Gibco, USA) supplemented with 10% FBS (fetal bovine serum, Gibco), 30 μg/mL ECGS (endothelial cell growth factor supplements, BD), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Culture medium was replenished every 3 days. After growing to approximately 80% confluence, cells were trypsinized to passage or seed onto the substrates. ECs at a population number between 2 and 8 were used in the study.

2.7. Cell adhesion and proliferation assays

For cell adhesion study, ECs were seeded on SS, SS-ePDA, and SS-ePDA-VEGF at a density of 5, 000 cells/cm. After 1 h or 4 h of incubation, cell-seeded samples were rinsed with PBS and fixed with 4% paraformaldehyde. The fixed cells were permeabilized with 0.2% Triton X-100 in TBS and blocked in TBS containing 0.1% BSA. Then, the cells blocked with BSA were incubated with phalloidin-TRITC, rinsed with TBS, stained with DAPI, and rinsed with PBS and Milli-Q water. Finally, the samples were mounted onto glass coverslips using ProLong® Gold antifade reagent (Invitrogen, USA), and visualized with a microscope (Axio-vert 200M, Zeiss, Germany). The number of adherent cells was measured by counting the number of cellular nuclei (stained with DAPI), and the projected area of an individual adherent cell was obtained by measuring the area of projected F-actin cytoskeleton (stained with phalloidin-TRITC) using ImageJ software (National Institutes of Health). More than 100 cells were measured for each group of samples.