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Immobilization of serum albumin and peptide aptamer for EPC on polydopamine coated titanium surface for enhanced *in-situ* self-endothelialization

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ABSTRACT

Restenosis and thrombosis are two major complications associated with vascular stents and grafts. The homing of circulating endothelial progenitor cells (EPCs) onto implant surfaces brings a new strategy to solve these problems by accelerating self -endothelialization *in situ*. Peptide aptamers with high affinity and specific recognition of EPCs can be immobilized to capture EPCs from the circulating blood. In this study, a biotinylated peptide aptamer (TPSLEQRTVYAK-GGGC-K-Biotin) for EPC, and bovine serum albumin (BSA) were co-immobilized onto titanium surface through avidin–biotin recognition to endow the surface with specific affinity for EPC and anti-platelet adhesion properties. Quartz crystal microbalance with dissipation (QCM-D), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and water contact angle measuring were adopted for coating characterization. EPC affinity and hemocompatibility of the coating were also investigated *in vitro*. The results demonstrated that aptamer and BSA co-immobilized surface significantly reduced platelet adhesion and fibrinogen adsorption/activation. Besides, such functional surface could remarkably enhance EPC adhesion, without affecting the behavior of endothelial cells (ECs) and smooth muscle cells (SMCs) obviously. The result shows the possibility of utilizing such a multifunctional surface in cardiovascular implants.

1. Introduction

Metallic biomaterials such as 316L stainless steel (SS), Co–Cr alloy and nitinol shape memory alloy have been widely used for vascular interventional devices due to their well-suited mechanical properties and excellent corrosion resistance [1]. Titanium (Ti) and its alloys also form an important class of biomedical materials because of their high strength-to-weight ratio, favorable mechanical properties and good biocompatibility [2]. However, the expansion of a vascular stent in the vessel could cause tissue damage around the implant. The tissue trauma is associated with thrombosis, inflammation, neointimal hyperplasia and matrix deposition/vessel remodeling [3], which can induce in-stent restenosis (re-narrowing inside the stent) within 3–6 months [4]. As known, blood can maintain a regular flow due to the antithrombogenic nature of endothelial cells (ECs) inside blood vessels. ECs are not only capable to prevent coagulation but also inhibit VSMC proliferation [5]. Thus, rapid re-endothelialization of implant surface to recover the physiological EC function is crucial for the success of vascular implants.

The discovery of EPCs in 1997 [6] brought new perspectives for endothelialization of blood contacting materials. EPCs mainly homed in the bone marrow and circulate at low concentrations in the peripheral blood of adults. EPCs with high proliferative potential can be fished on the position of injured intimae and differentiated into functional endotheliums which would maintain the optimal anti-thrombogenic properties and minimize the risk of restenosis [7].

The advanced strategy for EPC homing on a stent is coating capture molecules, such as antibodies [8–10], peptides [11,12], magnetic molecules [13,14], oligosaccharides [15,16], and aptamers [17–20] for fishing out EPCs directly from the bloodstream. However, only aptamer is selective for a single cell population [20,21]. Aptamers for fishing EPCs had been reported firstly by Hoffmann et al. who generated DNA aptamers with a high affinity to circulating porcine EPCs [22]. However, DNA aptamers rapidly lose their activity in the biological environment due to nuclease digestion. Recently, Veleva et al. [23] used phage display technique to isolate a peptide (TPSLEQRTVYAK), which could bind to human blood outgrowth endothelial cells (HBOECs) with high affinity. Blood outgrowth endothelial cells (BOECs) also known as "late-EPC" exhibit cobblestone morphology and long term proliferative potential [24,

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25]. In this work, peptide aptamers (TPSLEQRTVYAK-GGGC-K-Biotin) for capturing BOECs were designed and immobilized on implant surfaces.

As well accepted, a desired surface for vascular stent should not only show good affinity for rapid endothelialization, but also possessed good hemocompatibility. In relation to EPCs capturing model, modified surfaces should also present above properties. Immobilization of multifunctional biomolecules is a simple and effective method to obtain surfaces containing good hemocompatibility along with enhanced endothelialization properties. Bovine serum albumin (BSA) showed thromboresistivity and had been used to modify biomaterials surfaces. Immobilizing BSA onto the surface of blood-contacting materials has been shown to be a successful method to prevent blood platelet adhesion and improve the hemocompatibility [26,27].

In this study, we report a method to form a multifunctional surface based on the co-immobilization of BSA and peptide aptamer for EPCs on polydopamine surfaces which had been widely investigated as an ad-layer for biomolecule immobilization. The effect of the modified surface on EPCs, ECs and SMCs was also investigated. For a blood contacting material design, hemocompatibility test including platelet adhesion and fibrinogen adsorption/activation were elaborated.

2. Experimental

2.1. Chemicals and materials

Commercial pure Ti was purchased from Baoji Nor-ferrous Metal Co. Ltd. (Baoji, China). Avidin (A, Solarbio. Co.,Ltd., China) was dissolved in Phosphate-Buffered Saline (PBS, pH 7.4) to a final concentration of 0.1 mg/ml. The biotinylated peptide aptamer (TPSLEQRTVYAK-GGGC-K-Biotin) was designed (Fig. 1) and synthesized. The biotinylated peptide aptamer (BPA, Botai Biotech. Co., Ltd., Shanghai, China) was diluted to 0.1 mg/ml in ultrapurified-water. Modified Eagle's medium (α-MEM), and fetal bovine serum (FBS) were from Hyclone (Utah, USA). Vascular endothelial growth factor (VEGF, Purity ≥ 98.0%) was purchased from Peprotech (USA). And endothelial cell growth supplement (ECGS) was purchased from Millipore (Carlsbad, CA). All of the other reagents were local products with analytical grade.

2.2. Preparation of samples

Titanium was sliced into disks with 8 mm diameter as substrates. After polishing, the substrates were sonicated in acetone, ethanol and water in sequence for 3 times respectively. A layer of polydopamine was formed onto the titanium surface by immersing the substrates in a 0.5 ml solution of 2 mg/ml dopamine (DA, 10 mM Tris buffer, pH 8.5) at 37 °C for 24 h [28]. The substrates were sonicated in deionized water in order to remove the weakly bonded dopamine or polydopamine. After that, the samples were immersed in avidin solution at 37 °C for 30 min followed by being shaken for 3 times in PBS to remove the weakly bonded avidin. The samples were immersed in 1% bovine serum albumin (BSA) for 10 min. After that the specimens were immersed in the aptamer solution 37 °C for 30 min for grafting peptide aptamer. Then the specimens were shaken gently for 3 times in ultrapurified-water to remove nonspecifically adsorbed peptide aptamer (Fig. 2).

2.3. Characterization

The process of layer-by-layer self-assembly was monitored by QCM-D (Q-sense AB, Sweden) measurements and SEM (Quanta 200, FEI, Holland). The surface elemental compositions were determined by XPS (Perkin Elmer 16PC). Water contact angle was measured, using DSA 100 (Krüss, Hamburg, Germany). QCM-D is a lab-scale, fast analytical technique that can precisely monitor and quantify the amount of proteins adsorbed onto the surface of a quartz crystal in real time [29]. The resonant frequency of the crystal decreases when additional mass adsorbs on its surface. In detail, the quartz crystal was settled in the quartz crystal microbalance (QCM) chamber and PBS was injected for equilibration. The avidin solution was then injected at a flow rate of 50 μl/min continuously until the QCM traces did not change, followed by the buffer pump in the same speed. Then the BSA solution was injected at 50 μl/min, continuously until equilibrium, followed by the same buffer injection. After that, the biotinylated peptide aptamer solution was injected at 50 μl/min flow rate until reached equilibrium, again followed by the same buffer injection. The mass was recorded using DAQ (Data Acquisition) in a real time.

2.4. In vitro hemocompatibility test

Blood compatibility of the specimens was evaluated. To achieve this, fibrinogen adsorption, fibrinogen activation, platelet adhesion, and lactate dehydrogenase (LDH) were all characterized.

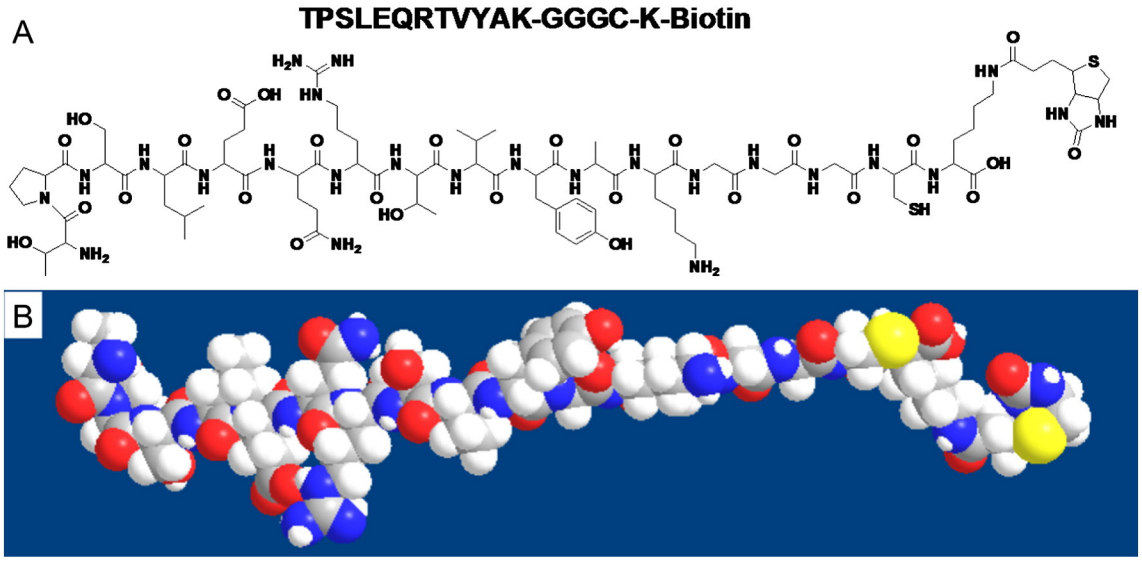

Fig. 1. Amino acid sequence of biotinylated peptide aptamer. (A) molecular structural formula (B)molecular structure mimetic graph, the N, O, H and S atoms are in blue, red, white and yellow respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)