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Heparin/DNA aptamer co-assembled multifunctional catecholamine coating for EPC capture and improved hemocompatibility of vascular devices

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ABSTRACT

Good hemocompatibility and rapid endothelialization are two key factors in the success of stent interventional therapy. In this study, aptamers with the ability to capture endothelial progenitors and anticoagulant molecular heparin were successfully immobilized on the surface of dopamine/polyethylenimine (PDA/PEI) copolymer coating *via* electrostatic interaction. The results of X-ray spectroscopy (XPS), water contact angle (WCA), and immunofluorescence staining tests confirmed the successful introduction of heparin and aptamers. Platelet adhesion and whole blood experiments demonstrated that the hemocompatibility of the co-modified surface was improved. Dynamic endothelial progenitor cell (EPC) capture experiments showed that the modified surfaces could effectively capture the endothelial progenitor in dynamic conditions. More importantly, *ex vivo* experiments revealed that the modified surfaces could regulate the distribution of CD34/vWF-positive cells on stent surfaces, and this was beneficial for the endothelialization of vascular stents. These results suggested that heparin and aptamer co-modified stents could capture EPCs and promote endothelialization. This surface co-modification strategy has great potential for enhancing stent development.

1. Introduction

Coronary artery disease (CAD) is a major cause of death in the developed world [1,2]. The main treatment strategy for obstructive CAD is implanting drug-eluting stents (DESs) in the lesion position. However, the drugs and polymer on the DES could cause a delay in endothelium healing, which may led to the formation of late stent thrombosis (LST) [3–5].

The endothelium is the protective barrier of vascular tissue; it reduces coagulation, prevents the leakage of plasma, and regulates inflammation response of the vessel [6–8]. Hence, complete re-endothelialization is very important for blood contact implants to reduce thrombosis and prevent implantation restenosis [8]. Endothelial progenitor cells (EPCs), one type of progenitor cell derived from bone marrow, have the potential to differentiate into mature endothelial cells (ECs) [9]. The EPCs can mobilize in response to vascular injury and repair endothelium, which can accelerate the re-endothelialization and healing of the blood vessel [10]. This is one of the major vascular repairing methods. Thus, EPCs have been utilized to achieve re-endothelialization on the stent surface. The EPC capture stent is an innovative device that makes use of the ability of bone marrow-derived EPCs to migrate to injured arterial segments to facilitate healing and proliferate into an endothelial layer [11]. There have been many design strategies utilized for EPC capture stents [12], such as anti-CD34 + antibody immobilization and DNA aptamer immobilization [13,14]. It was reported that the anti-CD34 + EPC-capture stent was not found to be superior to a bare metal stent (BMS) in preventing restenosis, since there were more than one type of CD34 + cells in circulation and these CD34 + cells had the ability to simultaneously differentiate into EPCs and smooth muscle progenitor cells (SMPCs) [15]. However, CD34 + antibodies combined with these CD34 + cells without specificity, and the combination of these antibodies with SMPCs may result in neointimal proliferation [15,16].

Aptamers are *in vitro* chemically synthesized oligonucleotides with high specificity and sensitivity towards a specific target [17,18]. Nucleic acid aptamers have higher affinity and specificity when combined with their ligand, comparable to the interactions between antibodies and antigens [19]. Hence, aptamers have been widely studied, and many kinds of aptamers have been introduced on different surfaces, which reveal strong specificity to their target cells [20,21]. One kind of DNA aptamer with the ability to specifically bind to EPCs in dynamic conditions was reported [22]. Later, this DNA aptamer was widely investigated since its low cost and high specificity. Above all, utilizing aptamers for cell

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homing is a fascinating strategy for rapid endothelialization in cardiovascular applications [23]. DNA aptamers have abundant phosphate groups that are electronegative in neutral conditions. Therefore, the DNA aptamer could be assembled on a surface that presents as electropositive in a neutral condition. Our earlier study showed that the DNA-EPC aptamer could be immobilized on the dopamine/polyethylenimine (PEI) copolymer-coated surface *via* electrostatic interaction [24]. The aptamer-functionalized surface exhibited a good EPC capture ability and was friendly to both EPC and EC growth, while not resulting in the excessive proliferation of smooth muscle cells (SMCs) [24]. This implied that the modified surface had the capacity to accelerate the realizing of re-endothelialization, which is crucial for the safety of the long-term implantation of stents. Nevertheless, the hemocompatibility of the aptamer-functionalized surface was not ideal for blood contact materials.

Hemocompatibility is critical for blood contact materials. The complex processes at the interface between the material surface and the blood influences the performance of the implant [25]. Hence, in the past few decades, much research has been done to improve the hemocompatibility of blood-contacting artificial devices. An effective method to improve hemocompatibility involves introducing anticoagulant molecules on the surface of blood contact devices [26]. Additionally, many kinds of anticoagulants have been employed to obtain good hemocompatibility, such as heparin, hirudin, peptides, and nitric oxide (NO). Heparin, an anticoagulant drug, is well known as a biologically important polysaccharide [27]. It was later found that the interaction between heparin and antithrombin accelerated the reaction with thrombin, thus avoiding the amplification of the anticoagulant cascade [28]. Many modification strategies have been applied to immobilize heparin on metal or polymer cardiovascular materials such as 316L SS, ePTFE, PET, polyurethanes, poly(lactic acid), polypyrrole, and polysulfone [29]. In addition, heparin on the surface has different effects on blood and vascular cells, including ECs and SMCs, and this is concentration-dependent [30]. However, as pure heparin-coating techniques mature, new designs and methods of using heparin have become of great interest to biomedical engineers (Scheme 1).

The aim of this work was to develop a surface with specific EPC capture properties and good hemocompatibility. Since stent materials usually lack functional groups for immobilizing biomolecules, our previous work reported a dopamine/PEI copolymer coating, which not only coated the surface of 316LSS but also provided multifunctional groups, such as the phenolic and amino groups [31]. The plentiful amino groups on the coating surface could present electropositivity in neutral conditions [32]. Hence, the DNA aptamer and heparin, which were negatively charged in the neutral pH range, could be assembled on the amino group-rich surface. In this paper, the chemical structure of modified surface was investigated, and the successful immobilized of aptamer was confirmed. The hemocompatibility and the capture ability of the aptamer/heparin functionalized surface were systematically evaluated.

2. Materials and methods

2.1. Materials

316L stainless steel (SS) discs (Φ = 10 mm), from New Material Co. Ltd. (Xi'an, China). Heparin, dopamine and polyethylenimine (PEI, MV = 25,000) were purchased from Sigma (Purity ≥ 99.0%). The EPC-aptamer, a 34 bases single strand DNA sequence (5′-CTT TAA TGC GGG GTA ATT TCT TTT CCA TAA TCG C-3′), were obtained from Sheng gong Bioengineering Ltd. Company (Shanghai, China). The cell counting kit-8 (CCK-8) was provided by BD Biosciences. All the other reagents used were analytical purity (>99%).

The stents were purchased from Kossel Medtech Co. Ltd. (Suzhou, China), made of 316L SS. The length of the stents was 18 mm, and the diameter was 2.75 mm.

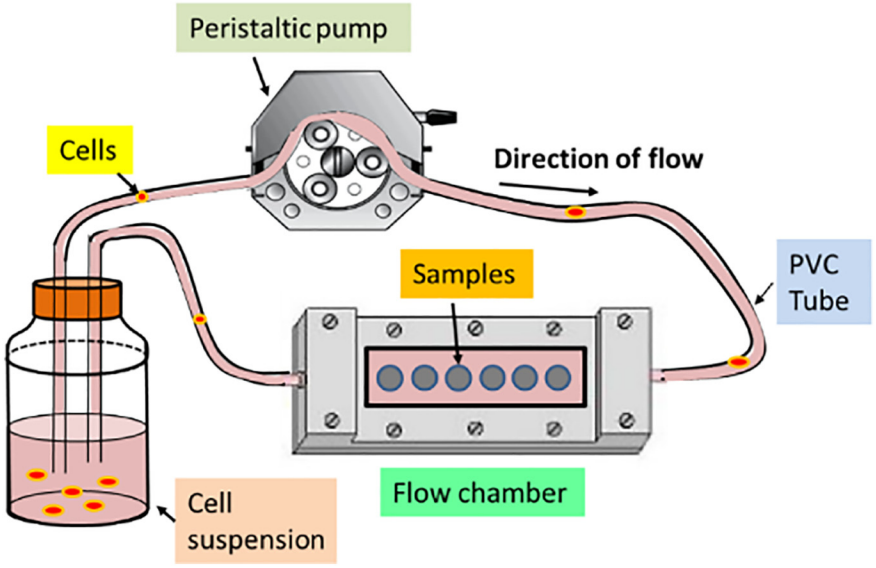
2.2. Film preparation

To obtain the dopamine/PEI copolymer film coated surface, the mirror-polished 316L SS specimens were immersed into 0.1 mg/ml dopamine solution (10 mM Tris buffer, pH 8.5) at room temperature. Forty-five minutes later, PEI was dissolved into the reaction solution, and the final concentration was 20 mg/ml. After 30 min, the specimens were sonicated in water to remove the weakly bonded molecules.

The samples were labeled as SS (316L SS) and DA/PEI (dopamine, 0.1 mg/ml; polyethylenimine, 20 mg/ml), respectively.

2.3. Immobilization of the EPC-aptamer and heparin

The heparin and EPC aptamers were immobilized on DA/PEI samples by dropping the 500 nM aptamer and 5 μg/ml heparin solution on the surface for 1 h, respectively. In addition, aptamer and heparin co-immobilized samples were obtained by dropping the mixed solution (500 nM aptamer, 5 μg/ml heparin) on the surface for 1 h. Later all samples were washed with reverse osmosis (RO) water. These modified



Scheme 1. The schematic diagram of dynamic cell culture.