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Hemocompatibility and anti-fouling behavior of multilayer biopolymers immobilized on gold-thiolized drug-eluting cardiovascular stents

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ABSTRACT

To solve the thrombosis and restenosis problem in cardiovascular stent implantation for cardiovascular artery disease, chondroitin 6-sulfate (ChS) with heparin (HEP) have been used as drug carrier layers and alternatively covalently bonded on gold (Au)-dimercaptosuccinic acid (DMSA)-thiolized cardiovascular metallic (SUS316 L stainless steel, SS) stents. Sirolimus, a model drug, was encapsulated in the ChS-HEP alternative layers. The behavior of the drug in releasing and suppressing the growth of smooth-muscle cells (SMCs) was evaluated with 5-layer CHS-HEP coating on the SS stents. Moreover, hemocompatibility of blood clotting time and platelet adhesion was performed. The results showed that the 5-layer ChS-HEP-modified SS stents displayed the greatest hemocompatibility, showing prolonged blood clotting time of the activated partial thrombin time (> 500 s) and less platelet adhesion to reduce thrombosis. Furthermore, sirolimus can be released continuously for more than 40 days with the 5-layer ChS-HEP coating and is beneficial for inhibiting the growth of SMCs; however, it does not affect the proliferation of endothelial cells, which can avoid restenosis formation. Therefore, the multilayers of ChS-HEP grafted onto the Au-DMSA-cardiovascular SS stents provide high potential for use as drug eluting stents.

1. Introduction

The first-generation of the drug (sirolimus)-eluting stent obtained CE Mark approval in Europe in 2002. Later, US Food and Drug Administration (FDA) approved in 2003. Compared with the pristine stent implantation, drug-eluting stent therapy significantly reduces restenosis and represents an important advance in percutaneous coronary interventions [1]. Durable thick polymers were used as drug carriers to store and elute drugs to the lesion site andincrease their antirestenotic efficacy, but the durable presence of these polymers is easy to be hypersensitivity and chronic inflammation reactions in the vessel wall. Some biodegradable polymers, such as poly(lactic acid) (PLA) and poly(lactic-coglycolic acid) (PLGA), have been developed for drug-eluting stents with respect to their biocompatibility, but the acidic products (lactic acid and glycolic acid) generated from polymer degradation still induce inflammatory reactions on the vessel wall. Furthermore, the hemocompatibility of the commercial coronary stents is insufficient to avoid the protein and platelets to adhere on the stents.

Many studies have been carried out on the use of the coating technique to improve in-stent restenosis; however, most of these only decrease the adsorption of platelet and albumin, which cannot completely improve it. For example, the self-assembled polyelectrolyte multilayer of hyaluronic acid (HA) and chitosan coated on the surface of NiTi stents loaded with sodium nitroprusside reduced platelet adhesion by 30%–40% [2], and the alginic acid layers coated on stainless-steel (SS) substrates can also decrease platelet adhesion by up to 70% [3]. Therefore, it is very important to find a material for encapsulating the drug into the stent. A polymer coating layer would be useful as a drug reservoir in the stent, and then the drug could be delivered by control release. Some characterizations of these polymer coating layers that make them suitable for drug-eluting stents include (1) biocompatibility, (2) sterilizability, (3) drug-compatibility, and (4) suitability for drug release. There are several natural biopolymers, such as chitosan, collagen, hyaluronic acid (HA), chondroitin 6-sulfate (ChS), and heparin (HEP) that are suitable as layers for the drug eluting stents. Additionally, the negative charge biopolymers of ChS and HEP display not only great biocompatibility, but also capability of anti-protein and platelet adhesion.

HEP, an anticoagulant and highly sulfated, anionic polysaccharide composed of repeating disaccharides of 1,4-linked glucosamine and uronic acid residues, can prevent thrombus formation by interacting with antithrombin III (AT III) after contact with blood. Thus, HEP is a

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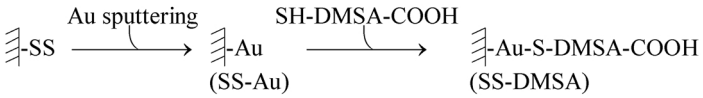
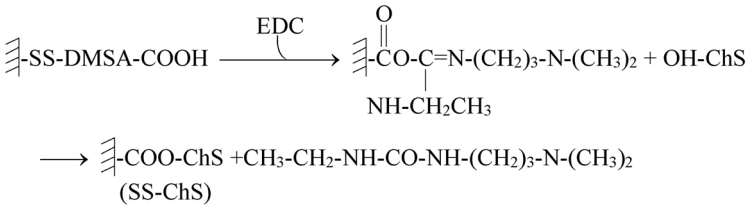
0927-7765/

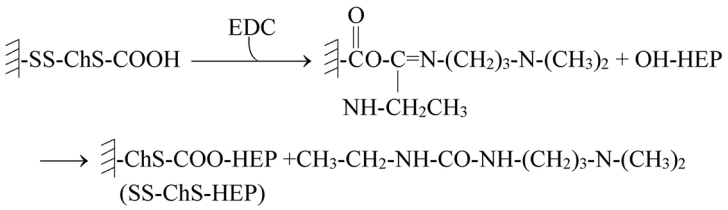
(1) Au coating of metallic stents and grafting of DMSA (SS-DMSA)

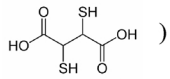


(2) Immobilization of chondroitin 6-sulfate (ChS) onto SS-DMSA by EDC



(3) Immobilization of heparin (HEP) onto SS-ChS by EDC



(=metallic SUS316 stainless-steel stents (SS), EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), DMSA = dimercaptosuccinic acid, 

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Scheme 1. The process of ChS and HEP immobilization onto the Au-DMSA cardiovascular metallic SS stents.

widely used anticoagulant and it exhibits improved hemocompatibility (anti-protein and platelet adhesion) after being grafted onto a biomedical surface [3–8]. Thus, many researchers have started to investigate the incorporation of sulfonate groups onto biomedical devices, called heparin-like or heparinoid surfaces–for example, blood vessels and cardiovascular stents. ChS is also a negatively charged polysaccharide found in connective tissue [9], which is the major glycosaminoglycan of the arterial wall, and has a reported anti-atherogenic effect in animal models [10]. Similar to the chemical structure of HEP, ChS displays both carboxyl and sulfonate negative charge groups, which can effectively decrease platelets activation to form thrombosis.

Rapamune® (sirolimus) is an immunosuppressive agent and macrocyclic lactone produced by *Streptomyces hygroscopicus*, which has been approved by the USA Food and Drug Administration [11]. It can suppress T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine stimulation by a mechanism that is distinct from that of other immunosuppressants. Furthermore, it is targeted to suppress the activation of T lymphocyte and the growth of smooth-muscle cells (SMCs), but without inhibiting the proliferation of endothelial cells (ECs). Thus, sirolimus can effectively reduce restenosis of the cardiovascular metallic stent and will be used as a model drug for the ChS-HEP eluting stents.

This study presents a multi-functional drug eluting stents, which alternately immobilized two kinds of excellent hemocompatible biopolymer (ChS and HEP) by covalent bonding to encapsulate the drug (sirolimus) and form anti-fouling layers at the same time. The drug release and biodegradeble rate could be manipulated by the degree of cross-linking and the layers of ChS/HEP biopolymers. Compared with the commercial layer-by-layer methods, it is beneficial to control. Furthermore, it could extend the life time after fabricating the more layers of ChS/HEP biopolymers, depended on the practical applications.

Based on our previous studies [12,13], a thin layer of gold (Au, 60 nm) was sputtered onto metallic SUS316 L SS sheets. Au is a great biocompatible material and is easy to be functionalized by thiolized chemical agents. Au-coated SS sheets were further thiolized by dimercaptosuccinic acid (DMSA), which is a biocompatible chemical agent used for the treatment of arsenic and mercury poisoning in humans and can be used as chelating agents to combine ZnO and proteins [14,15]. Finally, 1–5 layers of ChS and HEP with drugs (sirolimus) were covalently bonded on the Au-DMSA SS sheets to improve the thrombosis and restenosis of the pristine SS sheets [12]. In the present study, five layers of ChS and HEP biopolymers were covalently bonded onto the DMSA-modified SS metallic cardiovascular stents and then sirolimus as a cytostatic agent was encapsulated into the ChS-HEP biopolymers to avoid platelet adhesion and SMC proliferation. The hemocompatibility and drug releasing behaviors of sirolimus and the capability to suppress SMCs of the biopolymers-coated SS metallic cardiovascular stents were systematically investigated.

2. Experimental

2.1. Fabrication of Au and ChS-HEP modified cardiovascular SS stents [6–8,12,13,16]

The cardiovascular SS stents were sonicated in acetone three times and then immersed in nitric acid for 20 min for the pre-cleaning process. The cleaned SS stents were first sputtered with a nanolayer of Au (ca. 60 nm) by sputter deposition (JFC-1100E, JEOL, Japan) and then immersed in 0.5 wt% aqueous solution of DMSA (Aldrich, USA) and sonicated for 1 h. These are denoted as SS-Au and SS-DMSA stents, respectively. Furthermore, SS-DMSA stents were incubated in 0.01 M of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Sigma, USA) at