Colloids and Surfaces B: Biointerfaces 121 (2014) 238–247

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Atomic layer deposition enhanced grafting of phosphorylcholine on stainless steel for intravascular stents

Qi Zhong [a], Jin Yan [a], Xu Qian [a], Tao Zhang [a,b,*], Zhuo Zhang [c], Aidong Li [a]

[a] College of Engineering and Applied Sciences, Nanjing University, Nanjing 210093, China
[b] Nanjing Excellence Technology Center for Interventional Medical Devices, Nanjing 210093, China
[c] Department of Chemistry, Stony Brook University, Stony Brook, NY 11790, USA

ARTICLE INFO

Article history:
Received 22 February 2014
Received in revised form 5 June 2014
Accepted 9 June 2014
Available online 16 June 2014

Keywords:
Atomic layer deposition
Phosphorylcholine
Surface modification
Stainless steel
Intravascular stents

ABSTRACT

In-stent restenosis (ISR) and re-endothelialization delay are two major issues of intravascular stent in terms of clinical safety and effects. Construction of mimetic cell membrane surface on stents using phosphorylcholine have been regarded as one of the most powerful strategies to resolve these two issues and improve the performance of stents. In this study, atomic layer deposition (ALD) technology, which is widely used in semiconductor industry, was utilized to fabricate ultra-thin layer (10 nm) of alumina (Al_2O_3) on 316L stainless steel (SS), then the alumina covered surface was modified with 3-aminopropyltriethoxysilane (APS) and 2-methacryloyloxyethyl phosphorylcholine (MPC) sequentially in order to produce phosphorylcholine mimetic cell membrane surface. The pristine and modified surfaces were characterized using X-ray photoelectron spectroscopy, atomic force microscope and water contact angle measurement. Furthermore, the abilities of protein adsorption, platelet adhesion and cell proliferation on the surfaces were investigated. It was found that alumina layer can significantly enhance the surface grafting of APS and MPC on SS; and in turn efficiently inhibit protein adsorption and platelet adhesion, and promote the attachment and proliferation of human umbilical vein endothelial cells (HUVEC) on the surfaces. In association with the fact that the deposition of alumina layer is also beneficial to the improvement of adhesion and integrity of drug-carrying polymer coating on drug eluting stents, we expect that ALD technology can largely assist in the modifications on inert metallic surfaces and benefit implantable medical devices, especially intravascular stents.

1. Introduction

As a pioneer of implanted medical devices, intravascular stents have been subjected to clinical application such as coronary [1], renal [2], carotid [3], peripheral [4], and even intracranial arteries implantations [5]. Currently, most of the intravascular stents are made from metals such as stainless steel (SS), cobalt base alloy (e.g. Co–Cr alloy), titanium alloy (e.g. Nitinol) and platinum alloy (e.g. Pt–Cr alloy). Upon implantation, the small metal mesh tubes help support the inner wall of artery for months to years restoring or preserving the patency of the vessel. Unfortunately, in-stent restenosis (ISR) always impede the application of intravascular stents [6,7]. Approximately, one third of the patients who accept percutaneous transluminal angioplasty (PTA) with bare metal stents (BMS) have suffered from ISR [8], which resulted in recurrent angina and minor/major heart attack. Patients who developed severe restenosis even have to be subjected to repeat revascularizations. Although drug eluting stents (DES) have made a great progress in terms of reducing restenosis (ISR reduced to less than 10%) [9–13] and achieved huge commercial success in the past decade. However, a new problem, the late stent thrombosis (LAST) which is resulted from the proinflammatory properties of drug-carrying polymers is in urgent need of solutions [14]. Additionally, for intravascular stents (comparing to coronary artery), ISR is still an issue for the reason that restenosis rates are still high. A 2003 study of selective and systematic stenting for limb-threatening ischemia reported a restenosis rates of 32.3% for selective stenting patients and 34.7% for systematic stenting patient at one-year follow-up [15]. In a 2009 study, which compared bare nitinol stents with PTA in superficial femoral artery disease, restenosis was 34.4% for stented patients at one-year follow-up [16]. In 2013, a study of 206 cases in Iran reported a restenosis rate of 15.9% at 10.8 months (average time of 6–24 months) after stenting of symptomatic vertebral artery stenosis [17].

* Corresponding author at: College of Engineering and Applied Sciences, Nanjing University, Nanjing 210093, Jiangsu Province, China. Tel.: +86 25 83680009; fax: +86 25 83594668.
E-mail address: ztnj@nju.edu.cn (T. Zhang).

http://dx.doi.org/10.1016/j.colsurfb.2014.06.022
0927-7765/

Previous studies [6,7,18] have demonstrated that the delay of the re-endothelialization as well as the injury-induced migration and subsequent proliferation through smooth muscle cells are the major pathophysiological events that lead to neointima formation. The vessel endothelium plays an important role in the maintenance of the integrity of the vessel by preventing thrombosis and hyperplasia from happening. A rapid re-endothelialization has been proven to be a potential technique to prevent restenosis and late stent thrombotic [19–21]. Surface modification is believed to be one of the most effective ways to improve re-endothelialization and stent-vascular compatibility [22–24]. The modification methods have been seen to evolve from early simple physical polishing to current chemical modification, and even to the immobilization of biological molecules [23], such as coating or surface grafting of phosphorylcholine moieties. Phosphorylcholine is a zwitter ionic moiety, that can benefits from its reduced protein [25], platelet [26] and cell adhesion [27] in vitro [28]. Therefore, modification of intravascular stents with phosphorylcholine can lead to antithrombotic effects. DES with phosphorylcholine polymer-coated surfaces have been commercially available for over 5 years [29]. Significant differences in cardiac death/myocardial infarction and composite endpoints favored treatment with phosphorylcholine polymer-coated DES over comparator BMS have been observed in the 5-year clinical applications. Moreover, rates of clinical restenosis and safety events, including stent thrombosis beyond the first year of revascularization, have been proved to remain stable, which is a significant improvement comparing to first-generation DES [30]. The successes of phosphorylcholine-coated coronary stents inspired researches to explore other implantable materials. An increasing number of functionalized and complicated phosphorylcholine containing polymers [27,31,32], along with more other coated/grafted substrates [33–35] are under investigation right now.

Meanwhile simple routes to fabricate biocompatible surfaces are still being desired to avoid complicated evaluations and to prevent uncertain risks, especially by potential implantable medical devices manufacturer. Therefore, direct grafting of organic biofunctional molecules onto metal surface are still an attractive and cost-efficient method for intravascular stents development [36–38]. Unfortunately, organic reactions on an inert metallic surface are usually difficult, especially for those biomolecules which can only tolerate mild reaction conditions. Thus, how to introduce a reactive surface for intravascular implantable metals become a stepping stone to success. In the previous reports, surface-initiated atom transfer radical polymerization (ATRP) [39,40], RF-glow discharge plasma [41], and both air plasma and ATRP [42] processes were applied in order to enhance the stainless steel surface grafting of polymeric moieties. In this study, we creatively used atomic layer deposition (ALD) technology to achieve a biofunctional surface by enhancing the graft of phosphorylcholine onto stainless steel.

ALD was originally developed in the 1970's [43] to enable manufacturing of thin film electroluminescent displays, and then used in integrated circuit fabrications in the 1990's, normally worked at pressure of 0.01–1 kPa and temperatures of 100–400 °C. ALD is a self-limiting (the amount of film material deposited in each reaction cycle is constant), sequential surface chemistry that deposits conformal thin-films of materials onto substrates of varying compositions. Due to ALD's self-limiting as well as surface-reactive nature, ALD film growth makes atomic scale deposition control possible. In terms of the chemistry, ALD is similar but different to chemical vapor deposition (CVD). By pulsing a purge gas (typically N_2 or Ar) after each precursor pulse to remove excess precursor from the process chamber and prevent 'parasitic' CVD deposition on the substrate, ALD keeps the precursor materials separate during the reaction. This separation is maintained throughout the coating process, thus atomic layer control of film growth can be obtained as fine as about 0.1 Å (10 pm) per cycle. Currently, ALD can be used to deposit wide variety of thin films such as metals (e.g. Ru, Ir, Pt), metal nitrides (e.g. TiN, TaN, WN, NbN), metal sulfides (e.g. ZnS) and more important to us, various metal oxides (e.g. Al_2O_3, TiO_2, SnO_2, ZnO, HfO_2) [44,45]. It is the metal oxide that offers opportunity to fabricate intermediate layer between metallic substrate and organic biomolecules.

Narayan et al. [46] reported a ZnO and Pt coated alumina nanoporous membrane which was achieved by ALD. The surface was PEGylated to promote medical and environmental health applications. In 2011, the stainless steel with ALD mediated SiO_2 coating was found to be superior in terms(NY) of its performance in electrochemical testing. The novel coating that was achieved with ALD technology was also proved to be functionalized with biologically significant carbohydrates, implying that ALD has the potential of being adapted to the functionalization of metallic biomedical implants with other biologically relevant carbohydrates [47]. In this paper, we report a method to adopt ALD for the deposit of Al_2O_3 thin film onto model stainless steel sheets, which is followed by the direct connection of phosphorylcholine moiety onto the Al_2O_3 surface. The physical–chemical properties, hema-compatibilities and interactions with endothelial cells of the surfaces were investigated. Furthermore, the actual improvements of surface performance the ALD film have on intact coronary stents were studied.

2. Experimental

2.1. Materials

High-purity (99.9999%) trimethylaluminum (TMA, $Al(CH_3)_3$) was supplied by Jiangsu Nata Opto-electronic Material Co., Ltd. (Nanjing, China). Silane coupling agent, 3-aminopropyltriethoxysilane (APS) was purchased from Nanjing Shuguang Chemical Group Co. (Nanjing, China), and inhibitor-free 2-methacryloyloxyethyl phosphorylcholine (MPC, purity at 96%) was purchased from Joy-Nature Institute of Technology (Nanjing, China). Other chemicals were all purchased from Sinopharm Chemical Reagent Co. (Shanghai, China) and used as received.

Platelet-rich plasma (PRP), prepared on a Baxter CS-3000 Plus blood cell separator with 10^6 platelets/μL, was supplied by Jiangsu Province Blood Center (Nanjing, China) and used within 24 h after collection. Human umbilical vein endothelial cells (HUVEC) were supplied by Lifeline Cell Technology (Distributed by Beijing Qingyuanhao Biologics, Beijing, China). VEGF LS-1020 culture medium were purchased from Thermo Fisher Scientific China Branch (Shanghai, China). Fetal bovine serum (FBS) was purchased from Zhejiang Tianhang Biological Technology (Hangzhou, China), sterilized at 56 °C for 30 min and then stored at −20 °C. Carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) was purchased from Dojindo Molecular Technology (Shanghai, China).

2.2. Preparation of samples

Electropolished (kindly customized by Promed Medical Tech. Co., Ltd., Suzhou, China) or mechanical polished (only used in platelet adhesion experiments) 316L stainless steel (SS) sheets (20 × 20 mm) were ultrasonic cleaned in acetone, ethanol and distilled water for 3 times, respectively. Then 10 nm amorphous Al_2O_3 layer (100 cycles of ALD deposition) was deposited on SS surface by a Picosun SUNALE™ R-150B ALD reactor (Picosun, Finland), in which pulsed chemical precursors of $Al(CH_3)_3$ (Al precursor) and H_2O (oxygen precursor) were supplied alternately in a N_2 carrier gas at 150 °C. Then SS sheets and the SS sheets with Al_2O_3 film (SS–Al) were immersed in 1% APS ethanol solution for 30 min at room temperature and then washed with ethanol for 3 times. The