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Fabrication and characteristics of dual functionalized vascular stent by spatio-temporal coating

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ABSTRACT

Stent implantation with balloon angioplasty is a widely used treatment for coronary artery diseases. Stents have been developed from bare metal stent (BMS) to advanced forms such as drug-eluting stent (DES). However, modern DES still causes thrombosis and/or in-stent restenosis as long-term outcomes. For effective prevention of these problems, we fabricated a dual functionalized stent using spatio-temporal coating, which has two different surfaces, as a novel type of DES. Hyaluronic acid conjugated with dopamine (HA-DA) was applied to a bare cobalt-chromium (CC) stent prior to abluminal coating of sirolimus (SRL)-in-polymer such as poly(D,L-lactide). The SRL-in-polymer (P+S) coated on the abluminal surface of the HA-DA modified stent showed highly stable coating layer and prevented the crack formation after ballooning. In the blood- and cyto-compatibility tests, HA-DA coating displayed suppressive effects on adhesion and activation of platelets and maintained the cell viability and proliferation of human coronary artery endothelial cells even under the existence of SRL. In *in vivo* study using porcine restenosis model, the neointimal area and inflammation score of the dual functionalized stent with HA-DA and P+S were significantly reduced than those of BMS. It is expected that this novel type of DES can be effectively applied to utilize diverse anti-proliferative drugs and bioactive polymers.

Statement of Significance

Stents have been developed from bare metal stent to advanced forms such as drug-eluting stents (DESs). However, even DESs can still cause in-stent restenosis as long-term outcomes. This paper demonstrated a novel DES using spatio-temporal coating by dopamine-mediated hyaluronic acid coating (HA-DA) before asymmetric coating of sirolimus-in-poly(D,L-lactide) (P+S). It showed stable coating surface and prevented crack formation after ballooning. HA-DA coating also had an inhibitive effect on adhesion of platelets and maintained cell viability of endothelial cells even under the existence of sirolimus. Additionally, *in vivo* neointima area and inflammation score of HA-DA/P+S stent significantly decreased than those of BMS. We expected that this novel type of DES can be effectively applied to introduce diverse anti-proliferative drugs and bioactive molecules.

1. Introduction

Stent implantation is one of promising treatments for coronary artery diseases [1,2]. After bare metal stent (BMS) implantation, several adverse reactions have been reported such as neointimal hyperplasia and thereafter in-stent restenosis [3–5]. To settle these issues, drug-eluting stent (DES) has been developed by loading anti-proliferative drugs, such as sirolimus (SRL), paclitaxel, everolimus and others, on the BMS surface [6–8]. However, late- or very late-thrombosis is the long-term adverse event of using DES [9,10]. This occurs by delayed re-endothelialization due to its biologically poor environment for endothelial cells (ECs) [11–13]. The toxicity of anti-proliferative drugs in polymer layer was reported

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as one of causes for the delayed re-endothelialization. The polymer layer caused by physical stresses such as balloon expansion and blood flow could induce delamination and crack formation [14]. These might lead to promote thrombosis and uncontrolled drug release. Therefore, current DES is necessary to improve these instable polymer layer and biologically poor surfaces.

After stent implantation, stent forms two bio-interfaces, containing blood-faced luminal (inside) and vessel wall-faced abluminal (outside) interfaces [5]. During balloon angioplasty, the endothelium was denudated and a smooth muscle cell (SMC) layer is exposed to blood so that the abluminal surface of stent can encounter the hyper-proliferative SMC layer. For the successful recanalization, the endothelium should be tightly formed by either migration or proliferation of adjacent ECs as well as inhibition of the SMC proliferation. Therefore, the luminal surface of a stent should have biologically compatible surfaces for ECs, whereas the abluminal surface needs to possess anti-proliferative function against SMC proliferation. In this study, we immobilized hyaluronic acid (HA) as a bioactive molecule favored to allow ECs to adhere, on the luminal surface of the stent and introduced a SRL-in-polymer (P+S) layer only on the abluminal side of it.

HA is a linear polysaccharide made up of disaccharide units containing *N*-acetyl-D-glucosamine and glucuronic acid [15,16]. It has been reported to correlate with various biological processes, such as angiogenesis, wound healing, inflammation, and so on [17,18]. Especially, it is also expressed in blood vessel-associated cells such as SMCs and ECs [19]. Another physiological function of this is that it inhibits the platelet adhesion and thrombus formation [20,21]. Especially, native high molecular weight HAs are anti-angiogenic, while a low molecular weight of it stimulates migration and proliferation of ECs [22]. In this regard, we introduced the low molecular weight HA conjugated to dopamine (HA-DA) to form chemical bonds with metal surface.

Meanwhile, a P+S layer using an ultrasonic spray coating was applied to the HA-DA-coated stent equipped with a pre-designed jig, in order to confine the anti-proliferative function on only abluminal side of the stent. SRL is widely utilized for DES production which significantly reduced the occurrence of in-stent restenosis. Also, stents with other drugs appeared no improvement in clinical trials compared to the BMS [23]. In addition, the ultrasonic spray coater has been reported to be advantageous to obtain a polymer coating with a homogeneous and smooth surface as described in our previous works [24].

We demonstrate the consequence of the dual functionalization with the bioactive HA molecule and SRL-in-polymer dissimilarly on luminal and abluminal surfaces. Chemical and physical properties of both surfaces in the dual coated stent were characterized. In particular, the stability and crack formation of polymer layer after balloon expansion were evaluated. *In vitro* blood- and cyto-compatibilities of the dual coated stent were investigated in terms of biological properties. Moreover, the prepared dual functional stent was compared to BMS by *in vivo* study using a porcine coronary injury model.

2. Materials and methods

2.1. Materials

Cobalt-chromium plates (CC, 10 × 10 mm^2) and stents (diameter: 1.8 mm, length: 18 mm) were obtained from Minitube Co. (France) and BioAlpha Inc. (Seongnam, Korea), respectively. Poly

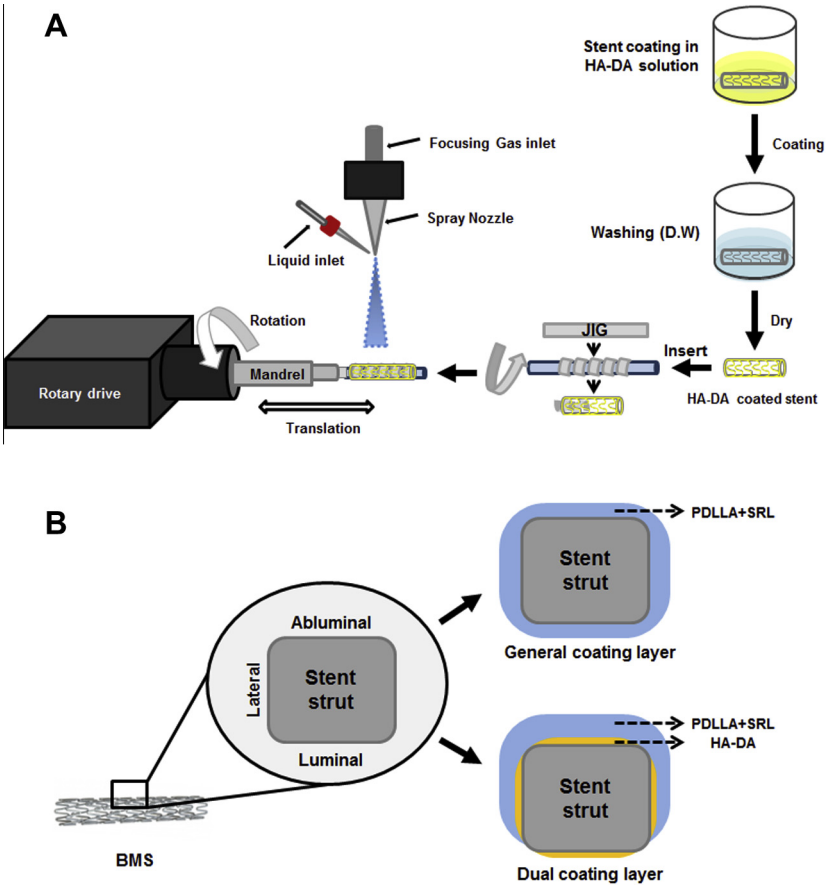

Fig. 1. (A) Coating procedure of the dual coated stent; a stent was dip-coated with HA-DA, inserted into a jig and ultrasonic spray coated with P+S solution. (B) Comparison of coating design of general coating type and dual coating type.