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Synergistic effect of anti-platelet and anti-inflammation of drug-coated Co–Cr substrates for prevention of initial in-stent restenosis

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ABSTRACT

Antiplatelet and antithrombotic therapies are systematically considered to prevent restenosis following coronary stent implantation. Currently, patients receiving medicated stents are prescribed to orally take anticoagulants and antiplatelet drugs such as aspirin (ASP) and prasugrel (PRAS). Propolis (PROP) known as a natural organic compound was recently evaluated for its antiplatelet activity, antibiotics and immunomodulatory activities. In this study, antiplatelet drug-coated Co–Cr substrates were prepared with biodegradable poly(D,L-lactide) (PDLLA) containing ASP, PRA, or PROP using electrospray and the blood compatibility of the different substrates was investigated by measuring protein adsorption and platelet adhesion. In addition, the anti-inflammatory properties of the modified Co–Cr surfaces were assessed by measuring IL-8 and IL-6 expression levels in human endothelial cell cultures. Drug-coated surfaces were found to resist the adsorption of fibrinogen when compared to bare Co–Cr or PDLLA-coated Co–Cr. Interestingly, ASP- and PROP-containing substrates not only showed reduced adhesion of platelets and delayed coagulation time, but also drastically reduced the expression level of IL-8 and IL-6. Such results are supported that ASP- or PROP-coated Co–Cr can be potentially used as a stent material to mitigate early stage of restenosis. The developed coating materials might be an interesting alternative to systemic anticoagulant therapies prescribed after stent implantation.

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1. Introduction

Coronary vascular disease is one of the leading causes of death globally, killing nearly 17.5 million people in 2012, representing 31% of all deaths [1]. This is caused mainly by the increasing level of cholesterol plaques in the blood that in turn narrows the luminal diameter and restricts the blood flow [2,3]. Metallic stent implantation is used extensively to restore the normal blood flow [4]. As a material for metallic stents, cobalt–chromium (Co–Cr) alloys have received considerable attention because of their superior properties, such as high abrasion resistance and fatigue strength along with good ductility [5,6]. In addition, stents made of Co–Cr enable the construction of more flexible and thinner struts [7]. Although Co–Cr has excellent physico-mechanical properties, the blood compatibility is always an issue as with other blood contacting materials [8,9]. In particular, the platelets interaction with biomaterial surfaces leads to significant platelet adhesion and consequently to risk of early thrombosis or in-stent restenosis [10].

Restenosis after coronary stent implantation is a complex process that an arterial injury induces triggers increasing platelet deposition and inflammatory response within the vessel wall that lead to the release of growth factors and cytokines activating smooth muscle cells and to neointimal hyperplasia [11,12]. It is necessary in most patients undergoing coronary stenting to continue the combination therapy of oral anticoagulation and antiplatelet drugs [13]. Aspirin (ASP), the most common antiplatelet medication, has been extensively evaluated for prevention of platelet aggregation through the inhibition of thromboxane production [14]. However, the lacking clinical outcomes with

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aspirin monotherapy recommended dual antiplatelet therapy with the association of thienopyridines whose active metabolites irreversibly bind to $P2Y_{12}$ receptor on platelet and thereby inhibit platelet function [15]. One such agent, prasugrel (PRAS), a third-generation thienopyridine, was recently approved for use in patients with atherosclerotic vascular disease to reduce the incidence of ischemic events as well as in-stent restenosis [16]. For patients with angioplasty or stents, 300 mg of aspirin and 60 mg of prasugrel were mostly administered and continued orally once daily. Propolis (PROP) is a natural extract produced by the honey bee and widely used in folk medicine as an antibiotic agent. Similarly, several studies have reported the potential of propolis to inhibit platelet aggregation and to be anti-inflammatory [17]. These antiplatelet drugs such as ASP, PRAS, and PROP might affect inflammatory responses as well as platelet function because thrombotic and inflammatory pathways share common signaling cascades [32].

The purpose of the present study is to enhance blood compatibility of Co–Cr alloy and to compare effectiveness of oral antiplatelet drugs on the stent surface. The hypothesis is that; (1) oral antiplatelet drug-coated stents are not only effective to directly suppress platelet adhesion and activation on the stent surface, but (2) they also have anti-inflammatory effect due to the shared signaling pathways of inflammation and thrombosis. Therefore, the drug-coated stent may have synergetic effect of anti-platelet and anti-inflammation on early stage of restenosis. In this study, antiplatelet drug-coated Co–Cr substrates were prepared with biodegradable polymer containing ASP, PRAS, or PROP by using electrospray (Fig. 1). Then, the blood compatibility of drug-coated Co–Cr substrates was evaluated in protein adsorption and platelet adhesion experiments. In addition, the anti-inflammatory properties of the modified Co–Cr surfaces were verified by measuring IL-8 and IL-6 expression levels in human endothelial cell cultures.

2. Materials and methods

2.1. Materials

Co–Cr alloy substrates ($10 \times 10\,mm^2$) were obtained from Han-Kook Vacuum Metallurgy (Gyunggi, South Korea). Poly(D-lactide-co-L-lactide) (PDLLA, molecular weight 115 K) was purchased from Lakeshore Biomaterials (Birmingham, AL, USA). Aspirin (ASP) and prasugrel (PRAS) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and propolis (PROP) was obtained from Seoul Propolis (Seoul, Korea). Human fibrinogen labelled with fluorescein isothiocyanate (FITC-FIB) was purchased from Invitrogen (Carlsbad, CA, USA). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from the Red Cross Blood Center (Seoul, Korea). All chemical reagents were used without further purification.

Human umbilical vein endothelial cells (HUVECs), endothelial growth medium-2 (EGM-2), human fibroblast growth factor-β (hFGF-b) and heparin were obtained from Lonza (Walkerscille, MD). Human interleukin-6 (IL-6) and human interleukin-8 (IL-8) immunoassay kit and cell counting kit-8 (CCK-8) were purchase from R&D systems (Minneapolis, MN, USA) and Dojindo (Kumamoto, Japan), respectively. Activated partial thromboplastin time (APTT) test kit was purchased from Wako (Osaka, Japan).

2.2. Preparation of drug loaded substrate

Co–Cr substrates were ground and polished with 1500 cw sandpaper and alumina using rotary equipment (GLP-S25, GPL Korea Co., Ltd., Korea). PDLLA coated Co–Cr (PDLLA), and ASP, PRAS or PROP-containing PDLLA coated Co–Cr substrates (P/ASP, P/PRAS, and P/PROP) were prepared by an electrospraying process [18]. Briefly, anti-platelet drugs and PDLLA were dissolved in acetonitrile (ACN) and methylene chloride (MC) co-solvents (ACN:MC = 7:3) as drug loaded polymer coating solution. The concentration of solutions was 0.3 wt% including 20 wt% drugs of total solute. The obtained solution was agitated at room temperature for 1 h. To cover Co–Cr substrates with the drug loaded polymer solutions, 10 mL of solution was electrosprayed from a PTEF syringe with 20 gauge needle. Electrospraying was utilized with flow rate of 0.5 mL/h and an applied voltage of 8 kV under humidity 15% (NNC-30K-2 mA Portable type, Nano NC, Korea). During spraying, the nozzle and substrate were fixed, and the amount of drug on Co–Cr substrates was controlled by varying the deposition time. PDLLA coated Co–Cr substrate without drug was prepared under the same conditions. After electrospraying, the drug/polymer coated substrates were dried in vacuum drying oven to remove the residual organic solvents from the substrates.

2.3. Surface characterizations

Surface structure and property of bare Co–Cr, PDLLA, P/ASP, P/PRAS and P/PROP substrates were characterized using various analytical tools. Elemental compositions of the surfaces were calculated using XPS (S-Probe, Surface Science Instruments, CA, USA). The XPS parameters include the power of analysis (25 W, 15 kV), monochromatic Al Kα (1486.6 eV) radiation, the takeoff angle (45°), and survey scans (0–1000 eV). The water contact angle was determined using optical bench-type contact angle goniometry (VCA Optima XE Video Contact Angle System, Crest Technology, Singapore). Droplets of distilled water (2 μL) were added onto the surface of the substrates, and the contact angle was measured

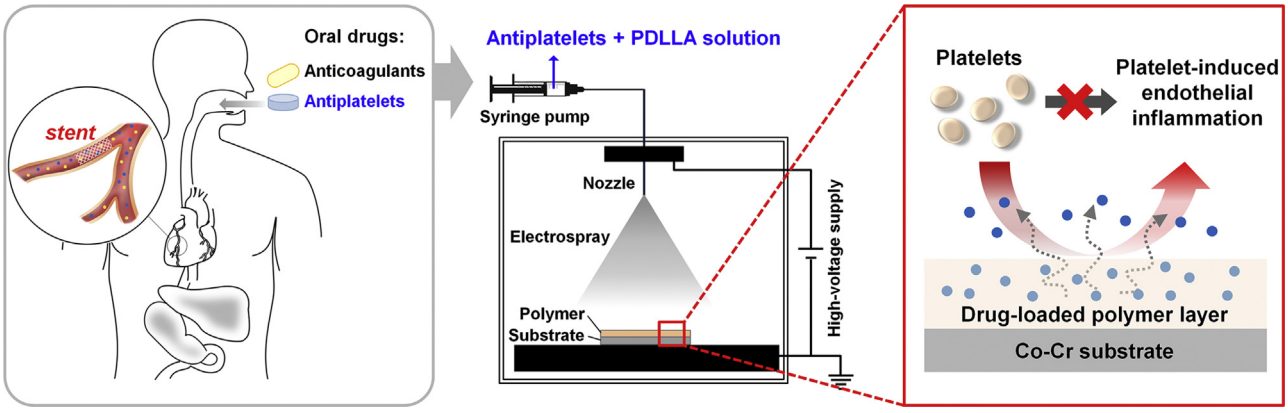

Fig. 1. Schematic illustration of proposed drug-eluting stent system and electrospraying process for stent coating.

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