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A novel polymer-free drug-eluting stent coated with everolimus using nitrogen-doped titanium dioxide film deposition in a porcine coronary restenosis model

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ABSTRACT

Background: Titanium dioxide (TiO_2) films have superior biocompatibility and may be effective as drug-binding matrices for drug-eluting stents (DESs). We sought to evaluate efficacy of a polymer-free DES coated with everolimus using nitrogen-doped TiO_2 film deposition in a porcine coronary restenosis model.
Methods: Forty coronary arteries in 20 pigs were randomly allocated to group 1 (bare-metal stents (BMSs), 3.0 × 18 mm, n = 10), group 2 (BMSs with nitrogen-doped TiO_2 film deposition, 3.0 × 18 mm, n = 10), group 3 [commercial everolimus-eluting stent, 3.0 × 18 mm, n = 10], and group 4 (polymer-free everolimus-eluting stent using nitrogen-doped TiO_2 film deposition, 3.0 × 18 mm, n = 10). Stents were randomly implanted in the left anterior descending coronary artery and left circumflex artery with stent:artery ratio of 1.3. Four weeks later, pigs underwent follow-up coronary angiography and were sacrificed for histopathologic analysis.
Results: Percent area stenosis was greater in group 1 compared to groups 3 and 4 (46.4 ± 13.8% vs. 30.2 ± 11.7% vs. 29.2 ± 8.9%, respectively, p = 0.005). Fibrin score was lower in groups 1 and 2, compared to groups 3 and 4: 0.87 ± 0.67 vs. 0.76 ± 0.61 vs. 2.27 ± 0.24 vs. 1.75 ± 0.31, respectively, p < 0.001). Injury score and inflammation score were not different. Comparison between DES showed a higher fibrin score in group 3 than group 4 (2.27 ± 0.24 vs. 1.75 ± 0.31, p = 0.023).
Conclusions: In a porcine model of coronary restenosis, a novel polymer-free DES using nitrogen-doped TiO_2 film deposition shows higher biocompatibility and compares favorably with a commercial DES.

1. Introduction

Drug-eluting stents (DESs) significantly reduced the rate of restenosis and subsequent repeat revascularization compared to bare-metal stents (BMSs) and have been preferentially used in the majority of percutaneous coronary intervention. The current DES treatment for coronary artery disease, however, has the limitation of delayed re-endothelialization and late stent thrombosis [1], which may be related to polymers used for drug coating on BMSs [2–4]. We developed a novel and efficient drug combining method onto titanium dioxide (TiO_2) film without polymers in order to avoid problems of polymer-based DESs [5,6]. This TiO_2 film deposition technology has been applied to design newer polymer-free DESs [7,8] using a new BMS, Chonnam National University Hospital (CNUH) stent we have recently developed [9,10].

In the present study, we aim to evaluate efficacy of a polymer-free DES coated with everolimus using nitrogen-doped TiO_2 film deposition in a porcine coronary restenosis model in comparison with commercial DESs.

2. Methods

2.1. Stent manufacture

Cobalt chromium (Co–Cr) (3.0 × 18.0 mm, L605) alloy was used as a stent material because many researchers have reported that Co–Cr alloy is the most appropriate material in terms of biocompatibility [11]. The design of stent was created at Chonnam National University Hospital (CNUH). Fabrication of BMSs with Co–Cr alloy was performed using a laser cutter (Rofin, Starcut, Hamburg, Germany), followed by ultrasonic cleaning, heat treatment, and polishing. The stent was coated with drug and polymer according to a previously reported method [12]. Briefly, everolimus and poly (lactic-co-glycolic acid) (PLGA, 50:50, MW 1.2 kDa, inherent viscosity of 0.16–0.24 dL/g) were mixed in tetrahydrofuran solvent. Drug was coated onto the BMSs using an ultrasonic stent-coating machine.

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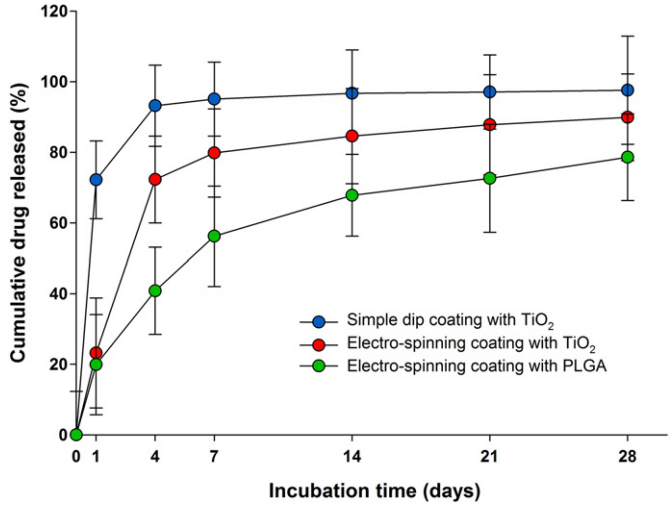

Fig. 1. In vitro cumulative release kinetics of everolimus from the stent. Stents were placed in the appropriate equipment designed for this study, and then phosphate-buffered saline was circulated through the lumen of the tube. The amount of everolimus released to media was measured at designated time point using ultraviolet–visible spectrophotometer. Indicating values are means ± SD (n = 10), *p < 0.05. PLGA = poly(lactic-co-glycolic acid); TiO_2 = titanium dioxide.

2.2. Preparation of TiO_2 film coated stent with everolimus

TiO_2 thin film was deposited onto BMSs by the plasma enhanced chemical vapor deposition process and its potential as a drug-combining matrix was investigated. When deposited at a discharge power of 5 W, the film showed a highly smooth surface, mechanical stability with good adhesion, and good blood compatibility. The film was surface-modified with water plasma to introduce hydroxyl groups on the TiO_2 surface. Then, drugs could be chemically grafted to the modified surface through the formation of ester bonds between hydroxyl groups on the modified TiO_2 film and carboxyl groups in the drugs. This technology has recently been granted a United States patent (No. US 8,999,456 B2): 'Method for manufacturing of drug-releasing stent coated with titanium-oxide thin film.' Everolimus was grafted onto the TiO_2-deposited and surface-modified stents, the grafted amount was measured and in vitro drug-release test was performed as described earlier [5,6].

2.3. Scanning electron microscopy

Surface morphology of the drug on the stent surface was investigated with scanning electron microscopy (SEM; TESCAN, MIRA3 LMU, Brno. Czech Republic). Samples were coated with a thin layer of gold by spattering (30 s at 45 mA, 50 mTorr) prior to analysis.

2.4. In vitro drug release kinetics

In vitro drug release kinetics was investigated with an ultraviolet–visible spectrophotometer. Unlike many studies that used a simple shaking procedure, specific equipment was designed for this study, mimicking the body's circulation system. A peristaltic pump (Jenie Well, Seoul, Korea) and various thicknesses of silicone tubing were used to function as the heart and vasculature of the body. Three stents with coating drugs were inserted and then expanded in the silicone tubing using a balloon (3.0 × 18 mm) with less than 10 mm Hg of pressure. Then, 5 mL of phosphate-buffered saline (PBS) was circulated through the tubing by dipping both open ends in the temperature-controlled reservoir. The circulating rpm was set at 150, and a unidirectional flow was used to simulate the body's circulatory system. PBS was sampled at every designated time point and analyzed using the spectrophotometer. The concentration of drugs released was calculated by comparing the value to a standard curve, and was expressed cumulatively.

2.5. Animal preparation and stent implantation

The animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-2014-34), and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the ARRIVE Guidelines for reporting animal research [13]. Study animals were Landrace pigs weighing 20–25 kg. Aspirin 100 mg and clopidogrel 75 mg per day were given for 5 days before the procedure. On the procedure day, pigs were anesthetized with zolazepam and tiletamine (2.5 mg/kg, Zoletil50®, Virvac, Caros, France), xylazine (3 mg/kg, Rompun®, Bayer AG, Leverkusen, Germany) and azaperone (6 mg/kg, Stresnil®, Janssen-Cilag, Neuss, Germany). Continuous oxygen was supplied through an oxygen mask. After subcutaneous 2% lidocaine injection, the left carotid artery was surgically exposed, and a 7 French sheath was inserted. Continuous hemodynamic and surface electrocardiographic monitoring was maintained throughout the procedure. Then 5000 units of heparin was administered intravenously as a bolus and the target coronary artery was engaged using standard 7 F guide catheters and baseline angiograms of both coronary arteries were performed using nonionic contrast agent in 2 orthogonal views. Stenting was performed by inflating the balloon with the resulting stent-to-artery ratio of 1.3 to 1. Coronary angiograms were obtained immediately after stent implantation. All pigs received 100 mg of aspirin and 75 mg of clopidogrel orally per day throughout the study period. Four weeks later, the pigs underwent repeat angiography in the same orthogonal views and were euthanized with intracoronary injection of potassium chloride (15%, 20 mL). The hearts were extracted, and the coronary arteries pressure-perfusion fixed at 70 mm Hg in 10% neutral buffered formalin overnight. The arteries were sectioned, and processed for histopathologic analysis.

2.6. Study groups

Forty coronary arteries in 20 pigs were randomly allocated to group 1 [CNUH (Tiger®) stent (CG Bio Inc., Jangseong, Republic of Korea), 3.0 × 18 mm, n = 10], group 2 [CNUH (Tiger®) stent with nitrogen-doped TiO_2 film deposition, 3.0 × 18 mm, n = 10], group 3 [commercial everolimus-eluting stent (Xience Prime®, Abbott Vascular, Santa Clara, CA, USA), 3.0 × 18 mm, n = 10], and group 4 [polymer-free everolimus-eluting stent using nitrogen-doped TiO_2 film deposition on CNUH (Tiger®) stent, 3.0 × 18 mm, n = 10]. Stenting was done randomly in the left anterior descending artery and left circumflex artery. Follow-up coronary angiography and histologic studies were performed 4 weeks later.

2.7. Histological analysis

Histopathologic evaluation was conducted by an experienced cardiovascular pathologist. The specimens were embedded in methylmethacrylate and sections were cut with a low-speed diamond wafer mounted to a Buehler Isomet saw (Buehler Ltd., Lake Bluff, IL,

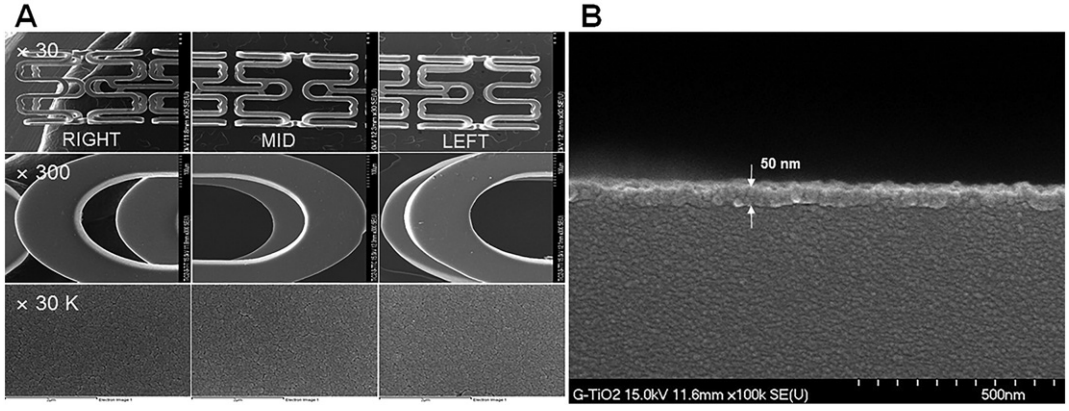

Fig. 2. Scanning electron microscopy images of the stent surface. Images of the stent according to longitudinal direction were represented (A). The coating thickness was measured in cross-sectional view (B).