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Original Article

Early thrombogenicity of coronary stents: comparison of bioresorbable polymer sirolimus-eluting and bare metal stents in an aortic rat model

Carole Verhaegen[1], Shakeel Kautbally[1,2], Diego Castanares Zapareto[1,3], Davide Brusa[4], Guillaume Courtoy[5], Selda Aydin[6], Caroline Bouzin[5], Cecile Oury[7], Luc Bertrand[1], Pascal J Jacques[8], Christophe Beauloye[1,2], Sandrine Horman[1], Joelle Kefer[1,2]

[1]Pole de Recherche Cardiovasculaire, Institut de Recherche Experimentale et Clinique (IREC), Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [2]Division of Cardiology, Cliniques Universitaires Saint-Luc, Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [3]Division of Intensive Care, Cliniques Universitaires Saint-Luc, Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [4]Plateforme de Cytometrie de Flux, Institut de Recherche Experimentale et Clinique (IREC), Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [5]IREC Imaging Platform (2IP), Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [6]Division of Anatomical Pathology, Cliniques Universitaires Saint-Luc, Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; [7]GIGA Cardiovascular Sciences, University of Liege, Liege, Belgium; [8]Institute of Mechanics, Materials and Civil Engineering, IMAP, Universite Catholique de Louvain (UCLouvain), Louvain-la-Neuve, Belgium

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Abstract: Background: Although 1-month dual antiplatelet therapy (DAPT) in patients treated with bare metal stents (BMS) is well established, the optimal duration of DAPT after implantation of a drug-eluting stent (DES) is still a matter of debate. The safety of shortened DAPT is under investigation due to concern about the risk of stent thrombosis. Data on platelet activation and prothrombotic response in vivo following bioresorbable polymer sirolimus-eluting stent (BP-SES) implantation are scarce. Objectives: The aim of our study was to compare the early thrombogenicity of BP-SES with that of BMS in an aortic rat model. Methods and Results: Overall, 30 rats underwent stent implantation in the abdominal aorta: BMS (Pro-Kinetic Energy; N=15) and BP-SES (Ultimaster Tansei; N=15) were compared in terms of their early thrombogenicity. CD62P exposure at the platelet surface and fibrinogen binding at the integrin receptor were not different between BMS and BP-SES over time. The thrombus coverage of the scaffold (0 vs. 0.1%, P=0.84) was similarly low in both groups at Day 28; thrombotic deposits had totally disappeared at Day 84. The endothelial strut coverage was similarly high at 1 month (90 vs. 95%, P=0.64) and 3 months (87 vs. 97%, P=0.99) following BMS and BP-SES implantation, respectively. Conclusions: This study demonstrates the low early thrombogenicity of a BP-SES implanted in an aortic rat model, which did not differ from a BMS. These data could be helpful to support the safety of a shortened 1-month DAPT duration following BP-SES implantation in the human coronary artery.

Keywords: Stent, thrombus, platelet, animal model

Introduction

Although 1-month dual antiplatelet therapy (DAPT) in patients treated with bare metal stents (BMS) is well established [1, 2], the optimal DAPT duration after implantation of a drug-eluting stent (DES) is still a matter of debate [3]. Prolonged DAPT, needed to compensate the delayed endothelialization process after DES implantation, has been shown to potentiate major bleeding [4], with an increased mortality risk [5]. Several clinical studies sought to investigate the safety of a shortened DAPT duration in patients with high bleeding risk receiving a new generation DES [6-8]. The guidelines [3] recommend 1-month DAPT after DES implantation in high bleeding risk patients in Class IIB (evidence C), based on limited clinical observations. No data are currently available concerning the extension of this one-month DAPT strategy following DES implantation in each case in daily practice.

Table 1. Stents characteristics

	BMS	BP-SES
Platform material	CoCr (L605)	CoCr (L605)
Strut thickness (μm)	60	80
Coating material	Silicon carbide	PDLLA-PCL
Polymer/coating thickness (μm)	1	15
Crimped stent profile (")	0.037	0.044
Duration of polymer/coating	permanent	3-4 months
Drug	NA	Sirolimus (0.8 μg/mm^2)
Duration of drug	NA	3-4 months

BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent; CoCr = cobalt-chromium; PDLLA-PCL = polyDL-lactide-co-caprolactone.

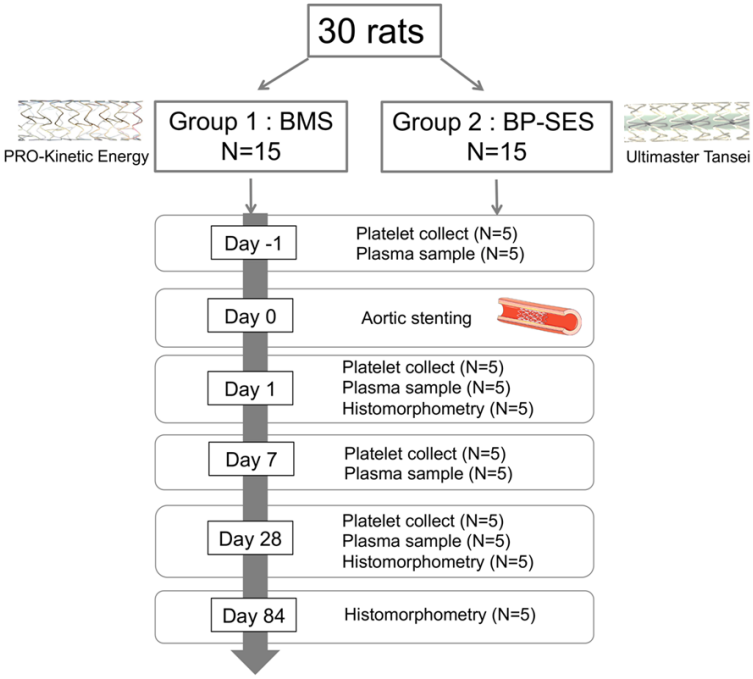

Figure 1. Study flow chart. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

The Ultimaster Tansei (Terumo Corporation, Tokyo, Japan) is a bioresorbable polymer sirolimus-eluting stent (BP-SES) with a very low rate of stent thrombosis and rapid strut coverage [9]. It was the first DES to obtain the CE mark for 1-month DAPT for patients who required drug discontinuation.

Early thrombogenicity of the stent is paramount in order to assess the device's safety profile, especially in the presence of abbreviated DAPT. Biological data on platelet activation and prothrombotic response in vivo following BP-SES implantation are scarce. Preclinical animal models have been used for understanding of the healing and inflammatory response after stenting, demonstrating their relevance to human coronary intervention. Aortic rat stenting has been shown to be a reliable model for histomorphometrical analysis and thrombogenicity assessment [10].

Our study sought to compare the early thrombogenicity of BP-SES with that of BMS (Pro-Kinetic Energy, Biotronik AG, Baar, Switzerland) in an aortic rat model.

Materials and methods

Animal models and study devices

Adult male wistar rats (body weight 500±50 g) were used as models. Of the total cohort of 30 rats, 15 underwent BMS implantation, while the other 15 underwent DES implantation.

The BMS was the Pro-Kinetic Energy, a silicon carbide passive coated cobalt chromium stent (Biotronik AG, Baar, Switzerland). The DES was the Ultimaster Tansei, a sirolimus-eluting stent with an abluminal bioresorbable polymer (Terumo Corporation, Tokyo, Japan). Details of the stents have been provided in **Table 1**.

Plasma and platelets were collected at Days -1, 1, 7, and 28, while histological sections of the stented areas were obtained at Days 1, 28, and 84 (**Figure 1**).

Animal procedures and protocols were approved by local authorities (*Comité d'éthique facultaire pour l'expérimentation animale*, 2016/UCL/MD/027) and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National

Institutes of Health (NIH Publication, N° 86-23, revised 2011).

Stent implantation procedure

During the whole procedure, rats were anesthetized with isoflurane. The left common carotid artery was surgically exposed, and the proximal portion of the vessel was ligated with 5-0 silk thread. The distal portion was temporally occluded using the same thread to block blood flow, with an incision performed at the mid-portion of the carotid artery. A 0.14-inch guide wire (Galeo Pro, Biotronik AG, Baar, Switzerland) was inserted at 12 cm from the puncture site and advanced up to reaching the infrarenal aorta. The stent (diameter 2.5 mm; length 9 mm) was deployed in the abdominal aorta using a balloon inflated for 20 seconds at 14 atm so as to reach a 10% oversize (target stent-to-aorta ratio of 1.1/1). Following stent deployment, animals were injected with buprenorphine (0.1 mg/Kg) and enabled to recover in their normal housing at the animal care facility. Analgesic injection was repeated every day for 1 week following the operation. The procedure was performed without heparin, and no antiplatelet therapy was administered before or following stenting. The animals were monitored daily until sacrifice.

Histomorphometrical analysis

Tissue processing: After rats were sacrificed by injecting a lethal Dolethal® dose, stented vessels were immediately perfusion-fixed *in situ* with 100 mL of normal saline followed by 50 mL of a solution of 4% formaldehyde, 0.65% di-sodium hydrogen phosphate (water free), and 0.4% sodium-dihydrogenphosphate monohydrate. Samples were harvested, fixed overnight in the same fixative solution, and incubated in 70% ethanol until processing. Stented vessels were embedded in methylmetacrylate resin and cut (10 µm thick) utilizing femtosecond laser technology with the TissueSurgeon by LLS ROWIAK (LaserLabSolutions GmbH, Hanover, Germany). Three sections per stent (proximal, central, distal) were performed and stained with hematoxylin and eosin (for samples of Days 1, 28, and 84) or with Sirius Red (for samples of Day 84). All sections were scanned using a Panoramic 250 Flash III Digital Slide Scanner (3DHISTECH) at × 40 magnification. Histological measurements were performed using Visiopharm software.

Definitions: Stent expansion was calculated as the area of the polygon drawn by linking the center of each strut. The injury score was determined for each strut using the following semi-quantitative score: 1= the strut contacts the tissue with no trace of damage; 2= the media is damaged by the strut; 3= the media is completely perforated, with the adventitia damaged by the strut.

The neointimal thickness was calculated as follows:

Neointimal thickness = neointima (NI) radius - lumen (L) radius

where NI radius = $\sqrt{(L\ area + NI\ area)/\pi}$ and lumen radius = $\sqrt{L\ area/\pi}$

The neointimal inflammation was determined around each strut using the semi-quantitative score described by Kornowski [11].

The neointimal maturity was evaluated around each strut using the following semi-quantitative score from Cheng [12].

The injury as well as inflammatory and maturity scores for each cross section were calculated as the sum of the individual scores divided by the number of struts in the examined section.

The degree of stenosis was calculated as the percentage of the lumen occupied by neointima as:

$$\frac{NI\ area}{NI\ area + L\ area} \times 100$$

In-stent restenosis was ≥50% lumen reduction in the stented area.

For fibrosis evaluation, red fiber area density was measured in 0.1 mm^2 neointimal areas drawn around each strut.

For each parameter, the average of the three sections per stent was calculated.

Struts with a distance from the vessel wall larger than 100 µm were considered malapposed and counted.

Thrombogenicity assessment

Thrombogenicity was assessed via the measurement of biological markers of platelet activation, coagulation, and systemic inflammation. Additional histological parameters were

investigated: thrombus formation, inflammatory cells, and endothelial strut coverage.

Platelet activation: Rat blood (0.5 mL) was collected from one of the sublingual venae into tubes containing 1/6 of acid citrate dextrose solution supplemented with apyrase 1 U/mL. Platelet-rich plasma (PRP) was obtained by centrifugation at 800 g for 5 seconds followed by 5 minutes at 100 g. PRP was washed by adding two volumes of acid citrate dextrose supplemented with apyrase 1 U/mL. Platelets were pelleted by centrifugation at 400 g for 5 minutes, then resuspended to a density of 2.5×10^5/μL in modified Tyrode's buffer (135 mM NaCl, 12 mM $NaHCO_3$, 2.9 mM KCl, 0.3 Na_2HPO_4, 1 mM $MgCl_2$, 5 mM D-glucose, 10 mM Hepes, and 1.5% BSA, pH 7.4 at 37°C). Platelets were counted with Cell-Dyn Emerald (Abbott Diagnostics, Abbott Park, Illinois, USA). After resting for 30 min at 37°C, platelets were incubated with an anti-P-selectin monoclonal antibody (ThermoFisher Scientific, Waltham, Massachusetts, USA, MA1-81-632) and an anti-Fibrinogen antibody (Abcam, Cambridge, United Kingdom, ab73925) at saturating concentrations (1:12.5). After adding 2 mM $CaCl_2$, the platelets were stimulated with thrombin (0.03, 0.1, or 0.3 U/mL) for 8 min at 37°C and then fixed with 4% paraformaldehyde. The samples were analyzed using the BD FACSCanto II flow cytometer, with at least 20,000 events recorded.

Systemic coagulation and inflammation: plasma analysis: Rat total blood was collected from one of the sublingual venae into tubes containing 1/10 volume of 3.8% citrate-dextrose solution. For the evaluation of the coagulation state, platelet-poor-plasma (PPP) was obtained by blood centrifugation at 1000 g for 15 min at 4°C. ELISA kits for human thrombin/antithrombin complex (Enzygnost®, Siemens, Munich, Germany, #OWMG15) and D-dimers (ASSERACHROM®, Stago, Asnières sur Seine Cedex, France, #00947) were used following the manufacturer's instructions.

For the evaluation of the systemic inflammation, PPP was obtained by blood centrifugation at 1000 g for 15 min at 4°C. Mouse RANTES Flex Set (BD™ Cytometric Bead Array, #5583-45), Mouse IL-1β (BD™ Cytometric Bead Array, #560232), and Mouse TNF Flex Set (BD™ Cytometric Bead Array, #558299) were employed following the manufacturer's instructions.

Thrombus: Thrombus was defined as an unstructured floconous matrix, made up of tangled mesh platelets, fibrin, degenerated leukocytes, and erythrocytes, visually detected with Visiopharm software.

The percentage of thrombus within lumen was calculated as follows:

$$\% \text{ Thrombus (T)} = \frac{\text{T area}}{\text{T area + L area}} \times 100\%$$

Inflammatory cells: At Day 1, the number of inflammatory cells around each individual strut was counted using the Euromex microscope with SL5510 LED light at magnification × 60. The average number of inflammatory cells around struts was calculated as the sum of the number of inflammatory cells divided by the number of struts in the examined section.

Endothelial strut coverage: The percentage of strut coverage was calculated as the percentage of the total length of the luminal sides of the struts covered by neointima as:

$$\% \text{ of strut coverage} = \frac{\text{length covered by neointima}}{\text{total length}} \times 100\%$$

Figure 2 illustrates the methodology applied for histological measurements.

Statistical analysis

Continuous variables are presented as mean ±1 standard deviation when normally distributed and as median and range when non-normally distributed. Normality was assessed using the Shapiro-Wilk test or Kolmogorov-Smirnov test when appropriate. Categorical variables are presented as counts and percentages. Continuous variables were compared using a paired t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Categorical variables were tested using chi-square or Fisher's exact test when appropriate. $P<0.05$ values were considered statistically significant. All statistical analyses were carried out using GraphPad Prism (GraphPad software).

Results

Overall, 32 rats were implanted, and 30 survived to the scheduled time point. Two animals in the BP-SES group experienced an unexpect-

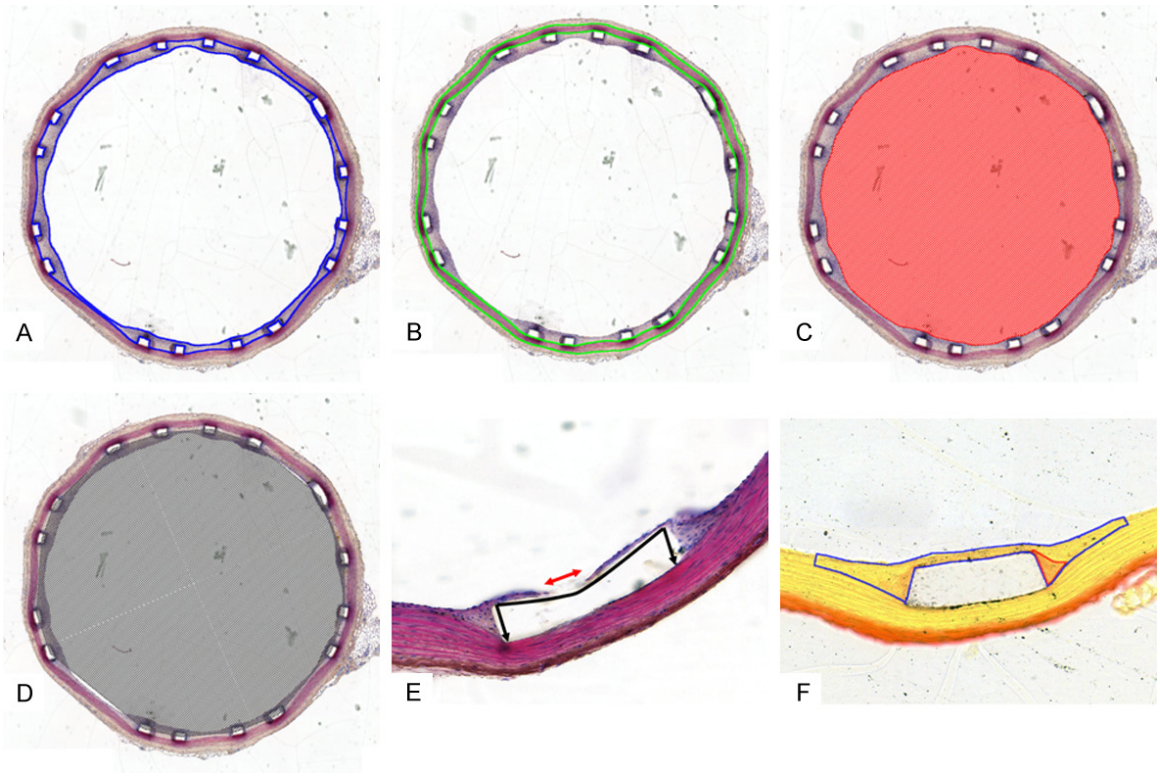

Figure 2. Method of histological measurements. A. Neointima area (blue); B. Media area (green); C. Lumen area (red); D. Stent expansion area (grey); E. Calculation of strut coverage in % (in black, total length of luminal struts sides; the red arrow delineates an uncovered strut segment); F. Percent of fibrosis within neointima (red).

ed death within four days from unexplained cause. Necropsy showed a partial obstruction of the stent lumen in the abdominal aorta with uncertainty about pre- or post-mortem material formation. These two animals were excluded from the rest of the analysis.

Histomorphometrical analysis

At Day 1, the achieved minimum lumen diameter (MLD) (2.43 vs. 2.41 mm), lumen area (5.19 vs. 5.07 mm^2), and platform expansion (5.05 vs. 4.74 mm^2) were similar between BMS and BP-SES (P=NS for all comparisons).

Incomplete strut apposition was observed in two experiments involving the BP-SES group representing 28 and 6% of their struts, respectively, while none of the BMS struts were malapposed.

After a more intensive healing process observed at Day 28 in the BP-SES group, reflected by a greater neointimal thickness and area, neointima/media ratio, and stenosis percentage (P<0.05 for all comparisons), the differences in intimal thickness between BMS and BP-SES dissipated at 3 months (**Table 2**).

The neointimal maturity score was not different between platforms at any time, though a smaller fibrotic area was observed in the neointima after BP-SES implantation versus BMS. The luminal stenosis remained low in the BMS and BP-SES groups (5 vs. 10% at 28 days; 5 vs. 7% at 84 days, respectively), without a single in-stent restenosis observed in the animals. The neointimal thickness did not change over time after BMS implantation, whereas this parameter was shown to be reduced at 3 months after reaching a peak level 28 days following BP-SES implantation (**Figure 3A**). At follow-up, MLD was not different between stents, with a luminal late loss of 0.06 mm at 3 months following BP-SES implantation (**Figure 3B**).

Thrombogenicity

Basal CD62P exposure at the platelet surface and fibrinogen binding at the integrin receptor were not different between BMS and BP-SES.

Table 2. Comparison of vascular healing after stenting

	BMS	BP-SES	*p*-value
DAY 1	*N=5*	*N=5*	
MLD (mm)	2.43 [2.43, 2.64]	2.41 [2.38, 2.51]	0.74
Lumen area (mm^2)	5.19 [5.19, 5.33]	5.07 [4.86, 5.14]	0.50
Stent expansion (mm^2)	5.05 [4.72, 5.08]	4.74 [4.45, 4.85]	0.37
Injury score	1.3 [1.3, 1.4]	1.2 [1.1, 1.2]	0.27
DAY 28	*N=5*	*N=5*	
Neointimal thickness (μm)	34.5 [31.6, 36.3]	68.1 [57.4, 71.5]	0.0003
Neointima area (mm^2)	0.29 [0.26, 0.29]	0.53 [0.44, 0.54]	0.0003
Neointima/Media area ratio	0.47 [0.43, 0.52]	0.81 [0.80, 0.82]	0.003
Inflammatory score	1.3 [1.2, 1.3]	1.6 [1.5, 1.6]	0.01
Neointimal maturity score	3.0 [3.0, 3.0]	2.9 [2.9, 2.9]	0.08
% stenosis	5.1 [4.7, 5.4]	10.4 [9.0, 11.1]	0.0003
In-stent restenosis	0	0	-
DAY 84	*N=5*	*N=5*	
Neointimal thickness (μm)	34.6 [23.0, 46.8]	47.5 [45.5, 51.8]	0.06
Neointima area (mm^2)	0.28 [0.19, 0.36]	0.40 [0.36, 0.40]	0.07
Neointima/Media area ratio	0.54 [0.34, 0.72]	0.71 [0.68, 0.78]	0.14
Inflammatory score	1.0 [0.9, 1.1]	1.4 [1.4, 1.5]	0.0009
Neointimal maturity score	3.0 [3.0, 3.0]	3.0 [3.0, 3.0]	0.08
% stenosis	5.2 [3.4, 7.0]	7.0 [6.8, 8.1]	0.05
In-stent restenosis	0	0	-
Fibrosis area in neointima (%)	3.0 [2.4, 3.4]	1.8 [1.7, 2.1]	0.02

Variables are expressed as median [interquartile range]. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent; MLD = minimum lumen diameter.

Following thrombin stimulation, BP-SES and BMS significantly decreased CD62P exposure at Day 1 compared to Day -1. The effect on CD62P exposure was alleviated for both stents at Day 7, whereas CD62P exposure was significantly increased at Day 28 for BMS but not for BP-SES. This is indicative of higher platelet reactivity following BMS implantation, which was not observed with BP-SES. The capacity of platelets to bind fibrinogen was similar over time for both stents (**Figure 4**).

Plasma concentrations of thrombin/antithrombin complexes, D-dimers, and RANTES did not change following the implantation of both stents. Neither TNF-α nor IL-1β was detected in rat plasma following implantation of either BMS or BP-SES (**Table 3**).

The thrombus coverage of the scaffold was similarly low in both groups at Day 1 (0.5 vs. 0.1%, P=0.84) and Day 28 (0 vs. 0.1%, P= 0.44), whereas thrombotic deposits had totally disappeared at Day 84 (**Table 4**). **Figure 5** illustrates the thrombus on struts observed for each stent type.

The endothelial strut coverage was similarly high at 1 month (90 vs. 95%, P=0.64) and 3 months (87 vs. 97%, P=0.99) following BMS and BP-SES implantation, respectively. **Figure 6** shows the healing and strut coverage of BMS and BP-SES at Days 28 and 84 of follow-up.

Discussion

The salient findings of this study are summarized below.

In an aortic rat model: 1) endothelial strut coverage at 1 month was very high (95%) after BP-SES implantation, not differing from than after BMS implantation; 2) thrombotic deposits were similarly low in both groups; 3) platelet activation was not different between BMS and BP-SES over time.

Endothelial strut coverage

The response to endothelium injury caused by stent implantation consists in a cellular cascade initiated by destroyed endothelial cells promoting platelet activation, leukocyte infiltration, as well as release of growth factors and cytokines. This process enables the formation of the neoendothelium and neointima covering the metallic stent struts. The antiproliferative effect of the drugs released by DES can result in poor endothelialization by slowing down endothelial cell re-growth and smooth muscle cell migration. Autopsy studies demonstrated that uncovered struts are the main predictor of stent thrombosis in patients receiving DES. By improving the drug's dosage and release kinetics, the second-generation DES allowed for a low rate of uncovered struts at around 3% in

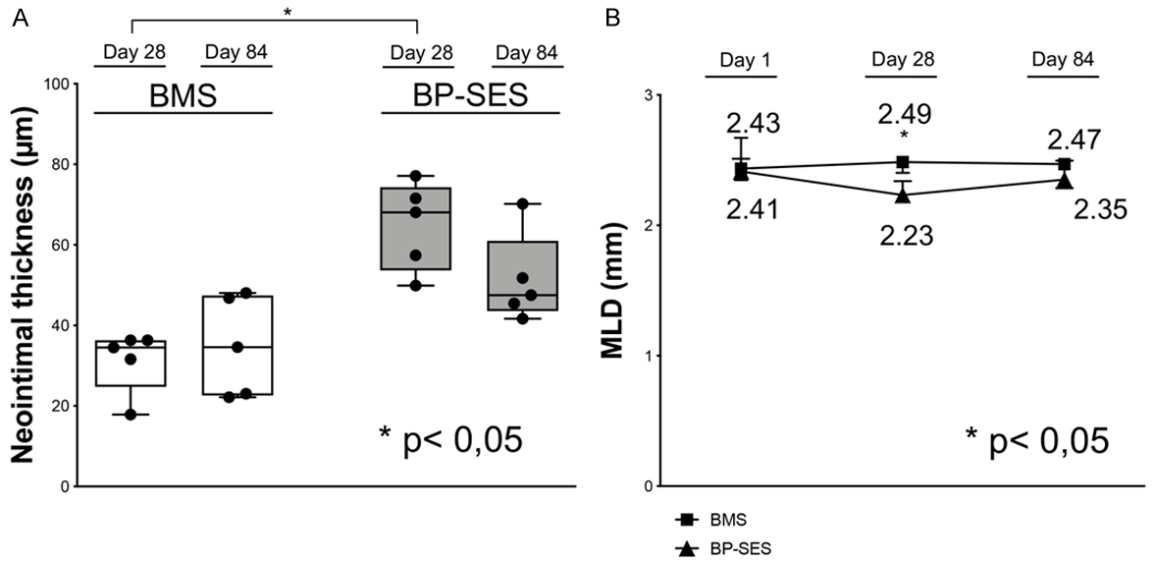

Figure 3. Histomorphometry parameters. A. Neointimal thickness upon follow-up following BMS and BP-SES implantation. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent. B. Time course of minimum lumen diameter during follow-up after BMS and BP-SES implantation. MLD = minimum lumen diameter; BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

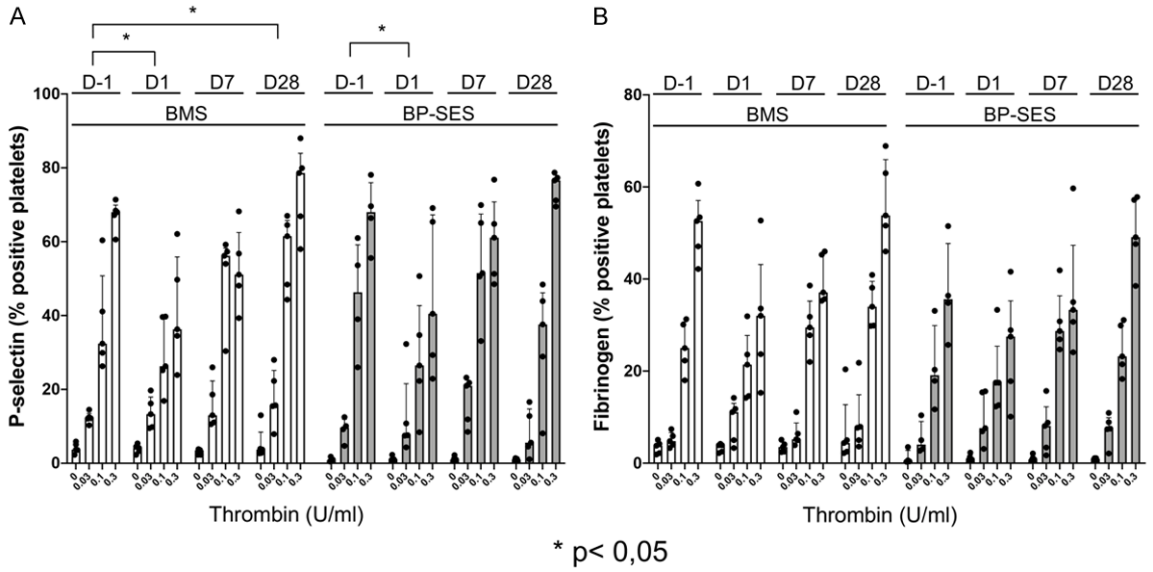

Figure 4. Platelet activation after stenting. A. Time course of CD62P exposure at platelet surface following thrombin stimulation after BMS and BP-SES implantation. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent. B. Time course of fibrinogen binding at platelet surface following thrombin stimulation after BMS and BP-SES implantation. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

animal models and humans to be reached at 9-month follow-up [9-14].

In addition to the drug, the polymeric DES component has been identified as a main cause of poor endothelialization by hypersensitivity reactions and persistent inflammation in the vessel wall [15]. Bioresorbable polymers have been developed in order to improve vascular healing and reduce the rate of stent thrombosis. The time course of vessel healing following BP-SES implantation was investigated by Chevalier et al. utilizing optical coherence tomography [16]. The reported rate of strut co-

Table 3. Time course of systemic coagulation and inflammatory markers

	Day -1	Day 1	Day 7	Day 28
BMS	*N=5*	*N=5*	*N=5*	*N=5*
Thrombin/antithrombin complex (µg/L)	9.07 [4.12, 19.82]	15.67 [11.24, 21.23]	6.16 [3.04, 8.72]	0.17 [0, 8.29]
D-dimers (ng/ml)	17.3 [7.25, 22.42]	21.0 [16.53, 25.34]	12.6 [11.03, 13.07]	3.9 [1.95, 6.75]
RANTES (µg/ml)	0.96 [0.88, 1.05]	0.27 [0.24, 1.88]	0.49 [0.45,0.54]	0.62 [0.39, 0.78]
IL-1β	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]
TNF-α	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]
BP-SES				
Thrombin/antithrombin complex (µg/L)	7.58 [0.62, 9.97]	5.58 [1.22, 9.77]	4.13 [2.22, 7.34]	5.93 [0, 6.84]
D-dimers (ng/ml)	15.9 [13.28, 30.81]	27.2 [23.97, 31.76]	18.91 [13.40, 23.32]	16.7 [14.5, 17.0]
RANTES (µg/ml)	0.26 [0.24, 0.95]	0.26 [0.09, 0.41]	0.33 [0.19, 0.89]	0.25 [0.22, 0.55]
IL-1β	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]
TNF-α	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]

Variables are expressed as median [interquartile range]. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

Table 4. Comparison of thrombogenic factors after stenting

	BMS	BP-SES	*p*-value
DAY 1	*N=5*	*N=5*	
% thrombus coverage of the total scaffold	0.5 [0.4, 0.7]	0.1 [0.1, 0.4]	0.84
Number of inflammatory cells around one strut	2.9 [2.5, 3.0]	0.9 [0.7, 1.3]	0.01
CD62P, % of positive platelets	36.3 [34.5, 49.7]	40.5 [29.3, 65.4]	0.99
Fibrinogen, % of positive platelets	32 [23.7, 33.6]	27.5 [17.8, 28.9]	0.82
DAY 28	*N=5*	*N=5*	
% thrombus coverage of the total scaffold	0	0.1 [0.0, 0.2]	0.44
Endothelial strut coverage (%)	90 [88, 91]	95 [93, 95]	0.64
CD62P, % of positive platelets	78.6 [66.9, 79.9]	76.6 [71.2, 77.3]	1
Fibrinogen, % of positive platelets	53.8 [51.6, 63.0]	49.1 [47.6, 57.2]	0.78
DAY 84	*N=5*	*N=5*	
% thrombus coverage of the total scaffold	0	0	-
Endothelial strut coverage (%)	87 [87, 92]	97 [92, 97]	0.99

Variables are expressed as median [interquartile range]. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

verage was 85.1, 87.9, and 95.2% at 1, 2, and 3 months, respectively.

Only few publications compared DES and BMS in terms of uncovered strut rates at 1 month, the crucial time when DAPT is stopped in studies investigating patients with high bleeding risk [6-8]. Our study performed a histological analysis of the abdominal rat aorta, stented with either BMS or BP-SES, resulting in a similarly high strut coverage rate (90 vs. 95% respectively, P=0.64) at a very short delay of 28 days. These early results were confirmed at 3 months, with only 3% of the BP-SES struts remaining uncovered. This rapid healing could explain the absence of stent thrombosis observed in our study, which was performed without either heparin or antiplatelet therapy.

The lack of DAPT administered to animals after stenting could explain the relatively large neointimal area at 1 month, given that platelets, which were not inhibited in our study, are the first agents to activate the vessel's healing response. Indeed, Buszman et al. showed that neointimal thickness at Day 28 was inferior after BP-SES implantation versus BMS in a porcine coronary restenosis model. In this study, the BMS, however, was Coflexus (Balton, Warsaw, Poland), the neointimal thickness was measured at 39 µm and lumen stenosis at 24%, which is much more than in our study [19]. Sojitra et al. [20] reported a lower neointimal area at Day 30 with BP-SES than BMS in porcine coronary arteries. Nevertheless, the BMS tested was Mitigator (Envision India), with heparin and DAPT administered in the experi-

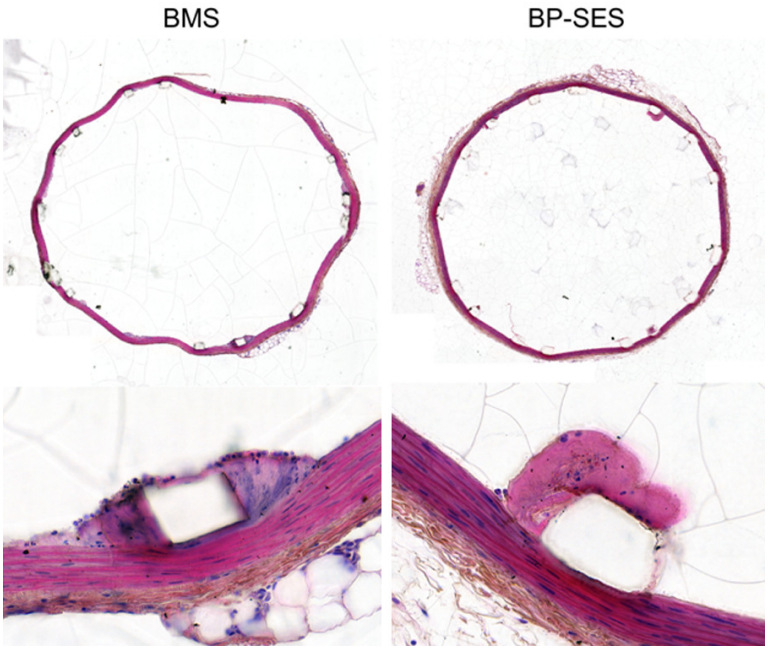

Figure 5. Thrombus deposit at Day 1 following implantation. Left: Histological BMS section at Day 1 (at the top: magnification 1.7 ×; at the bottom: magnification 20 ×). Right: Histological BP-SES section at Day 1 (at the top: magnification 1.7 ×; at the bottom: magnification 20 ×).

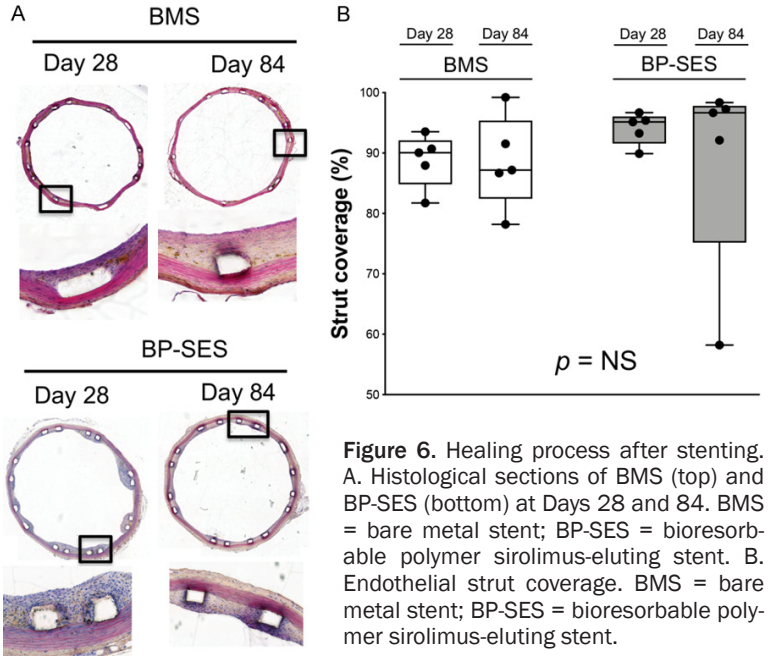

Figure 6. Healing process after stenting. A. Histological sections of BMS (top) and BP-SES (bottom) at Days 28 and 84. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent. B. Endothelial strut coverage. BMS = bare metal stent; BP-SES = bioresorbable polymer sirolimus-eluting stent.

ments. The Prokinetic Energy, with one of the lowest late loss after BMS implantation in humans [2] due to its specific design and passive coating with silicon carbide, provided excellent results in our study, with only few proliferation after implantation in the aortic rat model. Even if the healing process was more intense than expected at 1 month after BPSES implantation, the percentage of lumen stenosis remained low (10 and 7% at 1 and 3 months, respectively), without any restenosis, while differences between stents had dissipated at 3 months, after polymer resorption.

Thrombosis

The majority of stent thromboses, possibly leading to adverse events like myocardial infarction and death, occur in the acute or subacute phase (*i.e.,* within 30 days) after the procedure. Thrombus formation is a normal part of the initial reaction after stent-induced vascular damage, accompanied by inflammatory reaction, resulting in neointimal formation. In the event of an abnormal re-endothelialization process, the huge amount of thrombotic material could become obstructive within the vessel's lumen. Thrombus deposits were quantified in order to assess the thrombogenicity of new platforms in animal models. Magnesium and stainless steel sirolimus-eluting poly-L-lactic acid-coated stents were compared by Lipinski et al. [17] in a porcine ex-vivo arteriovenous shunt model. Thrombus deposition, which was quantified by scanning electron microscopy at 5.3% of the total scaffold area for the magnesium stent, turned out to be significantly less than with the stainless steel stent (8%, P=0.009). Ijichi et al. [18] reported a fibrin/internal elastic lamina ratio of 1.4% at Day 7 after BP-SES implantation in an in-vivo coronary pig model.

In our study, the thrombus was detected visually and quantified using Visiopharm software at 0.1 and 0.1% after BP-SES implantation vs. 0.5 and 0% after BMS implantation at Days 1 and 28, respectively. These deposits, observed in the early phase, had totally disappeared later. It is essential to emphasize that these very low thrombogenic results were obtained when stent implantation was performed without either anticoagulants or antiplatelet therapy.

Platelets

The initial and normal reaction to the mechanical injury of the vessel induced by stent implantation is platelet activation.

Otsuka et al. showed that in an ex-vivo porcine shunt model, platelet aggregation was inferior after durable polymer DES implantation versus four different biodegradable polymer DES implantations, without any BMS comparison performed [21]. Torii et al. reported that in a similar ex-vivo model, a durable fluoropolymer-coated everolimus-eluting stent imparted lower platelet adherence compared to BMS (Mulilink, Abbott Vascular, Santa Clara, CA, USA) and BP-SES, with CD42b/CD61 positive areas estimated at 4.1%, 25.1%, and 7.6%, respectively [22].

Our study is the first to evaluate the impact of BP-SES implantation on platelet activation in an animal model utilizing flow cytometry. We have shown that neither stent did lead to platelet activation over time after implantation. However, BMS induced increased CD62P exposure upon thrombin stimulation of platelets isolated 28 days after stent implantation. This was not observed with BP-SES, thereby supporting the poor platelet reactivity induced by the device.

Once again, it is paramount to mention that these observations have been made without using any antiplatelet agents with their associated platelet-inhibitory effect. To the best of our knowledge, this is the first report pertaining to the in-vivo vascular and thrombogenic reaction after stent implantation without DAPT and anticoagulants.

Limitations

Our study comprised a low number of experiments per group, while the stenting sites were not the coronary arteries but rather rat aortas. Moreover, our model did not include hypercholesterolemic animals, and the investigated vessel was free of atherosclerosis. While there was no randomization process for comparison between stents, we utilized two consecutive cohorts of rats implanted first with BMS and then with BP-SES.

Conclusions

This study demonstrates the low early thrombogenicity of a BP-SES implanted in an aortic rat model, which was not different from that observed after BMS implantation. All the thrombogenic parameters, such as endothelial strut coverage, platelet activation, and thrombus deposits, evaluated at 1 month were similar between the two stents without any stent thrombosis observed. These data could be helpful in supporting the safety of a shortened 1-month DAPT duration following BP-SES implantation in the human coronary artery.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Joelle Kefer, Pole de Recherche Cardiovasculaire, Institut de Recherche Experimentale et Clinique (IREC), Universite Catholique de Louvain (UCLouvain), Brussels, Belgium; Division of Cardiology, Cliniques Universitaires Saint-Luc, Universite Catholique de Louvain (UCLouvain), Brussels, Belgium. Tel: +3227642815; E-mail: joelle.kefer@uclouvain.be

References

[1] Wilson SH, Rihal CS, Bell MR, Velianou JL, Holmes DR and Berger PB. Timing of coronary stent thrombosis in patients treated with ticlopidine and aspirin. Am J Cardiol 1999; 83: 1006-1011.

[2] Dahm JB, Willems T, Wolpers HG, Nordbeck H, Becker J and Ruppert J. Clinical investigation into the observation that silicon carbide coating on cobalt chromium stents leads to early differentiating functional endothelial layer, increased safety and DES-like recurrent stenosis rates: results of the PRO-Heal Registry (PRO-Kinetic enhancing rapid in-stent endothelialisation). EuroIntervention 2009; 4: 502-508.

[3] Valgimigli M, Bueno H, Byrne RA, Collet JP, Costa F, Jeppsson A, Jüni P, Kastrati A, Kolh P, Mauri L, Montalescot G, Neumann FJ, Petricevic M, Roffi M, Steg PG, Windecker S, Zamorano JL and Levine GN; ESC Scientific Document Group; ESC Committee for Practice Guidelines (CPG); ESC National Cardiac Societies. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: The Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2018; 39: 213-260.

[4] Cassese S, Byrne RA, Tada T, King LA and Kastrati A. Clinical impact of extended dual antiplatelet therapy after percutaneous coronary interventions in the drug-eluting stent era: a meta-analysis of randomized trials. Eur Heart J 2012; 33: 3078-3087.

[5] Généreux P, Giustino G, Witzenbichler B, Weisz G, Stuckey TD, Rinaldi MJ, Neumann FJ, Metzger DC, Henry TD, Cox DA, Duffy PL, Mazzaferri E, Yadav M, Francese DP, Palmerini T, Kirtane AJ, Litherland C, Mehran R and Stone GW. Incidence, predictors, and impact of post-discharge bleeding after percutaneous coronary intervention. J Am Coll Cardiol 2015; 66: 1036-1045.

[6] Frigoli E, Smits P, Vranckx P, Ozaki Y, Tijssen J, Jüni P, Morice MC, Onuma Y, Windecker S, Frenk A, Spaulding C, Chevalier B, Barbato E, Tonino P, Hildick-Smith D, Roffi M, Kornowski R, Schultz C, Lesiak M, Iñiguez A, Colombo A, Alasnag M, Mullasari A, James S, Stankovic G, Ong PJL, Rodriguez AE, Mahfoud F, Bartunek J, Moschovitis A, Laanmets P, Leonardi S, Heg D, Sunnåker M and Valgimigli M. Design and rationale of the management of high bleeding risk patients post bioresorbable polymer coated stent implantation with an abbreviated versus standard DAPT regimen (MASTER DAPT) study. Am Heart J 2019; 209: 97-105.

[7] Watanabe H, Domei T, Morimoto T, Natsuaki M, Shiomi H, Toyota T, Ohya M, Suwa S, Takagi K, Nanasato M, Hata Y, Yagi M, Suematsu N, Yokomatsu T, Takamisawa I, Doi M, Noda T, Okayama H, Seino Y, Tada T, Sakamoto H, Hibi K, Abe M, Kawai K, Nakao K, Ando K, Tanabe K, Ikari Y, Hanaoka KI, Morino Y, Kozuma K, Kadota K, Furukawa Y, Nakagawa Y and Kimura T; STOPDAPT-2 Investigators. Effect of 1-month dual antiplatelet therapy followed by clopidogrel vs. 12-month dual antiplatelet therapy on cardiovascular and bleeding events in patients receiving PCI: the STOPDAPT-2 randomized clinical trial. JAMA 2019; 321: 2414-2427.

[8] Kedhi E, Latib A, Abizaid A, Kandzari D, Kirtane AJ, Mehran R, Price MJ, Simon D, Worthley S, Zaman A, Brar S, Liu M, Stone GW and Windecker S. Rationale and design of the Onyx ONE global randomized trial: a randomized controlled trial of high-bleeding risk patients after stent placement with 1 month of dual antiplatelet therapy. Am Heart J 2019; 214: 134-141.

[9] Wöhrle J, Markovic S, Rottbauer W, Muramatsu T, Kadota K, Vázquez-González N, Odenstedt J, Serra A, Antoniucci D, Varenne O, Saito S and Wijns W. Bioresorbable polymer sirolimus-eluting coronary stent compared with permanent polymer everolimus-eluting coronary stent implantation for treatment of small vessel coronary artery disease: CENTURY II trial. EuroIntervention 2016; 12: e167-174.

[10] Maurel B, Chai F, Maton M, Blanchemain N and Haulon S. In stent restenosis and thrombosis assessment after EP224283 injection in a rat model. Atherosclerosis 2013; 229: 462-8.

[11] Kornowski R, Hong MK, Tio FO, Bramwell O, Wu H and Leon MB. In-stent restenosis: contributions of inflammatory responses and arterial injury to neointimal hyperplasia. J Am Coll Cardiol 1998; 31: 224-30.

[12] Cheng Y, Shibuya M, McGregor J, Conditt GB, Yi GH, Kaluza GL and Granada JF. Biological effect on restenosis and vascular healing of encapsulated paclitaxel nanocrystals delivered via coated balloon technology in the familial hypercholesterolaemic swine model of in-stent restenosis. EuroIntervention 2016; 12: 1164-1173.

[13] Torii S, Nakazawa G, Ijichi T, Yoshikawa A, Ohno Y and Ikari Y. Comparison of the endothelial coverage in everolimus and zotarolimus-eluting stents in normal, atherosclerotic, and bifurcation rabbit iliac arteries. Cardiovasc Interv Ther 2018; 33: 55-61.

[14] Windecker S, Haude M, Neumann FJ, Stangl K, Witzenbichler B, Slagboom T, Sabaté M, Goicolea J, Barragan P, Cook S, Piot C, Richardt G,

Merkely B, Schneider H, Bilger J, Erne P, Waksman R, Zaugg S, Jüni P and Lefèvre T. Comparison of a novel biodegradable polymer sirolimus-eluting stent with a durable polymer everolimus-eluting stent: results of the randomized BIOFLOW-II trial. Circ Cardiovasc Interv 2015; 8: e001441.

[15] Koppara T, Joner M, Bayer G, Steigerwald K, Diener T and Wittchow E. Histopathological comparison of biodegradable polymer and permanent polymer based sirolimus eluting stents in a porcine model of coronary stent implantation. Thromb Haemost 2012; 107: 1161-1171.

[16] Chevalier B, Smits PC, Carrié D, Mehilli J, Van Boven AJ, Regar E, Sawaya FJ, Chamié D, Kraaijeveld AO, Hovasse T and Vlachojannis GJ. Serial assessment of strut coverage of biodegradable polymer drug-eluting stent at 1, 2, and 3 months after stent implantation by optical frequency domain imaging: the DISCOVERY 1TO3 study (evaluation with OFDI of strut coverage of terumo new drug eluting stent with biodegradable polymer at 1, 2, and 3 months). Circ Cardiovasc Interv 2017; 10: e004801.

[17] Lipinski MJ, Acampado E, Cheng Q, Adams L, Torii S, Gai J, Torguson R, Hellinga DG, Joner M, Harder C, Zumstein P, Finn AV, Kolodgie FD, Virmani R and Waksman R. Comparison of acute thrombogenicity for magnesium versus stainless steel stents in a porcine arteriovenous shunt model. EuroIntervention 2019; 14: 1420-1427.

[18] Ijichi T, Nakazawa G, Torii S, Nagamatsu H, Yoshikawa A, Souba J, Isobe A, Hagiwara H and Ikari Y. Comparisons of early vascular reactions in biodegradable and durable polymer-based drug-eluting stents in the porcine coronary artery. PLoS One 2019; 14: e0209841.

[19] Buszman PP, Michalak MJ, Pruski M, Fernandez C, Jelonek M, Janas A, Savard C, Gwiazdowska-Nowotka B, Żurakowski A, Wojakowski W, Buszman PE and Milewski K. Comparable vascular response of a new generation sirolimus eluting stents when compared to fluoropolymer everolimus eluting stents in the porcine coronary restenosis model. Cardiol J 2016; 23: 657-666.

[20] Sojitra P, Doshi M, Galloni M, Vignolini C, Vyas A, Chevli B and Sheiban I. Preclinical evaluation of a novel abluminal surface coated sirolimus eluting stent with biodegradable polymer matrix. Cardiovasc Diagn Ther 2015; 5: 254-263.

[21] Otsuka F, Cheng Q, Yahagi K, Acampado E, Sheehy A, Yazdani SK and Joner M. Acute thrombogenicity of a durable polymer everolimus-eluting stent relative to contemporary drug-eluting stents with biodegradable polymer coatings assessed Ex Vivo in a swine shunt model. JACC Cardiovasc Interv 2105; 8: 1248-1260.

[22] Torii S, Cheng Q, Mori H, Lipinski MJ, Acampado E, Perkins LEL, Hossainy SF, Pacetti SD, Kolodgie FD, Virmani R and Finn AV. Acute thrombogenicity of fluoropolymer-coated versus biodegradable and polymer-free stents. EuroIntervention 2019; 14: 1685-1693.