

Comparative preclinical evaluation of a polymer-free sirolimus-eluting stent in porcine coronary arteries

Christian Sperling, Matthias W. Waliszewski , Behrouz Kherad and Florian Krackhardt

Abstract

Background: Polymer-free drug-eluting stents (DES) without permanent-polymer coating may be associated with rapid vessel healing, providing a rationale to reduce dual-antiplatelet therapy (DAPT). The aim of the current study was to compare vessel healing of a polymer-free sirolimus-eluting stent (PF-SES), its bare metal stent (BMS) analogue to a permanent polymer-based sirolimus-eluting stent (SES) with proven effectiveness in porcine coronary arteries.

Material and methods: An ultrathin-strut cobalt–chromium PF-SES, its BMS analogue and an SES with a permanent polymer were used to study vessel healing and their antistenotic potential. Stents were implanted in porcine coronary arteries for histopathologic analysis at 7, 28 and 180 days.

In an additional *in vitro* study, the thrombogenicity of PF-SES was compared with a fluoropolymer-coated everolimus-eluting stent (EES) which demonstrated low stent thrombosis rates in numerous studies.

Results: In the animal study, neointimal growth and injury scores were minimal and inflammation scores were low in the neointima and adventitia in all study groups. After 28 days, neointimal area was lowest in PF-SES when compared with SES and BMS ($1.48 \pm 0.55 \text{ mm}^2$ versus $2.43 \pm 0.69 \text{ mm}^2$ versus $1.90 \pm 0.85 \text{ mm}^2$, respectively, $p < 0.05$) and endothelialization of luminal surfaces was nearly complete in all groups, though SES show the least coverage with occasional adherent luminal inflammatory cells ($p > 0.05$). At 180 days, neointimal area and thickness were most pronounced in SES ($p < 0.05$) and comparable with BMS implantations, which were characterized by nearly completed vessel healing. PF-SES and BMS had complete endothelialization, absence of fibrin and sustained low inflammatory reaction when compared with the permanent polymer-based SES (inflammation score: PF-SES 0.41 ± 0.74 versus SES 2.52 ± 1.72 versus BMS 0.30 ± 0.65 , respectively, $p < 0.05$ BMS versus SES). Granuloma formation and fibrin accumulation were most pronounced in SES but did not reach statistical significance, ($p > 0.05$). In the *in vitro* thrombogenicity study, the PF-SES confirmed comparable antithrombogenic properties with regard to the parameters fibrin and platelet binding, and platelet aggregation when compared with the EES.

Conclusions: As compared with BMS, the ultrathin-strut cobalt–chromium PF-SES showed similar endothelialization at 28 days and comparable healing characteristics at 180 days efficacious inhibition of neointimal proliferation in porcine coronary arteries with low inflammation responses and a BMS-like endothelialization at 180 days. In addition, in an *in vitro* model, the PF-SES also confirmed low thrombogenicity as compared with the EES.

Keywords: all-comers population, polymer-free, porcine model, preclinical, sirolimus, ultra-thin strut bare metal stent

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Introduction

Based on the latest recommendations by the European Cardiology,¹ drug-eluting stents (DES) are the gold standard for all indications. With the recent findings in the LEADERS FREE,² ZEUS³ and SENIOR trials,⁴ even the theoretical advantage of bare metal stent (BMS) implantations in terms of a shortened dual-antiplatelet therapy (DAPT) is greatly reduced by a proven safety record and the antisthenotic efficacy of latest-generation DES. Given the ethical dilemma of randomizing patients to receive either BMS or DES, the aforementioned clinical endpoint studies focused on challenged patient groups that are prone to higher bleeding risks. Nevertheless, preclinical work in the most preferred large animal model, that is, the porcine coronary artery model remains of vital interest during the earlier DES development process.^{5,6} In addition, today's intravascular imaging techniques do not allow a differentiation between endothelial cells and fibrin coating of the stent struts in the clinical setting. Therefore, the time course of endothelialization in the porcine model is a major indicator in preclinical DES research.⁷ Moreover, the preclinical reports by Finn *et al.*⁸ in early DES research revealed that endothelial coverage was the most important histological predictor of stent thrombosis in the porcine model. This fundamental relationship remains valid independent of optimized stent-coating technologies and stent architectures with ultrathin-stent struts that allow improved crossability. It is also noteworthy that the difference in lesion crossability between BMS and DES seem to be comparable with latest-generation DES. One of these latest device generations are those with polymer-free coatings on the abluminal stent surface only. In particular, the polymer-free stent coating consisting of sirolimus and probucol as an excipient which was extensively studied in randomized controlled trials⁹⁻¹¹ and large all-comer populations^{12,13} may hold the advantage of rapid stent coverage, thereby reducing the need for extended DAPT durations. Furthermore, delayed neointimal healing and incomplete endothelialization have gained attention as a cause for late stent thrombosis with first-generation permanent polymer-coated DES.¹⁴⁻¹⁶

The objective of this report is to document the *in vitro* thrombogenicity and the preclinical vascular healing characteristics of polymer-free sirolimus-eluting stents (PF-SES) with an ultrathin-strut bare metal backbone relative to

its vascular healing characteristics in the porcine overstretch model.

Methods

Animal study protocol

The present study was approved (IMTR42502-2-923 and IMTR42502-3-624) by the Animal Ethics Committee of Saxony-Anhalt, Germany, and conformed to the guidelines of the commission directive 86/609/EEC and the German Animal Protection Act.

Test devices for the implantation studies

An ultrathin-strut PF-SES (Coroflex® ISAR 3.0/3.5 × 13 mm, *n* = 24, B. Braun Melsungen AG, Berlin, Germany) was used in the treatment group. Its bare metal backbone is a cobalt-chromium stent with a strut thickness of 50/60 μm whose abluminal surface is surface modified to permit a microporous surface for the polymer-free matrix consisting of sirolimus and probucol. The concentration of sirolimus is 1.2 μg/mm² stent surface. Sirolimus is the active antiproliferative drug, probucol is an excipient controlling the release of the drug. Probucool mimics the function of a polymer by retarding the release of sirolimus. Sirolimus is eluted continuously from the stent throughout a period of 90 days with maximum local tissue levels at 1 day after implantation and more than 70% of the drug released at 28 days. The drug release profile of the PF-SES is therefore comparable with other SES, such as Cypher (Cordis, Johnson & Johnson, Warren, NJ, USA), Orsiro (Biotronik, Berlin, Germany), Supralimus (Sahajanand Medical Technologies, Mumbai, India) or Biomime (Meril Life Sciences, Vapi, India).

There were two control devices, one of which was its uncoated microporous stent platform [bare metal stent (BMS) 3.0/3.5 × 13 mm, *n* = 16], and an SES (Cypher Select Plus®, Cordis). The latter device uses a stainless-steel platform with a strut thickness of 140 μm coated with a sirolimus dose of 1.4 μg/mm² embedded in a permanent polymer made from polyethylene-covinyl acetate (PEVA) and poly-N-butyl methacrylate (PBMA), 3.0/3.5 × 13 mm, *n* = 24.

In vitro thrombogenicity study

An *in vitro* closed-loop system was used as previously described by Engels *et al.*¹⁷ Small amounts

of blood (30 ml per test) were obtained from a blood bank which was supplied by blood from healthy volunteers at the laboratory of Haemoscan (Groningen, The Netherlands). According to Dutch law, an ethics vote by the responsible ethics committee [Medical Ethics Toetsingscommissie (METC) of the University Medical Centre Groningen] was not necessary due to small blood volumes in the millilitre range. The exemption from ethics approval was based on Haemoscan's internal standard operating procedures (SOPs); that is, healthy adult donors, small amounts of blood and informed consent. The signed consent form, as part of Haemoscan's SOPs, detailed all uses of donor blood within the framework of the METC's exemption. Besides the consent form, we also informed the donors about the specific purpose of use their blood in a particular *in vitro* test.

In short, venous human blood was collected and anticoagulated using heparin. PF-SES (3.0 × 19 mm, *n* = 5) and everolimus-eluting stent (EES; Xience Pro, fluoropolymer-coated EES, Abbott Vascular, Santa Clara, California, USA, 3.0 × 18 mm, *n* = 5) were used. The stents and the reference materials, medical steel and low-density polyethylene (LDPE) were deployed into closed-loop systems made from medical grade PVC tubing. Circulation was performed for 1 h at 37°C. After incubation, blood was collected, and the materials were photographed and used to study binding of platelets and fibrin and for visualization by means of scanning electron microscopy (SEM). Adhesion of fibrin was studied by means of fibrin-specific labelled antibodies (American Diagnostica, Stamford, CT, USA). Platelet binding was determined by the activity of platelet-specific enzymes (platelet acid phosphatase) on the washed materials. Platelet aggregation was determined by means of impedance measurement in the presence of 5 mmol/l adenosine diphosphate (ADP).

Implantation and follow-up time points

The stents were implanted in the three main coronary arteries of 14 domestic pigs weighing 35.3 ± 2.3 kg and 12 Goettingen miniature pigs weighing 26.7 ± 2.2 kg. The pigs were sedated with ketamine (20 mg/kg) and Xylazin 2% (Riemser Arzneimittel GmbH, Greifswald, Germany) (0.2 mg/kg) before general anaesthesia was induced with intravenous propofol 1% (3 mg/kg Fresenius Kabi, Bad Homburg, Germany). The pigs were then intubated, and ventilation was

started using isoflurane (1–2 vol%). For postoperative pain management, Metacam (0.04 ml/kg) was administered subcutaneously; for prophylactic antibiotics, Ursocyclin® (0.2 ml/kg) was injected intramuscularly. An external carotid artery was surgically exposed and a 6F intra-arterial sheath introduced. Heparin (5000 IU) and acetylsalicylic acid (ASS; 250 mg) were administered intravenously as a bolus. Coronary angiograms were performed after administration of nitroglycerin (200 µg).

Appropriate segments of the left anterior descending (LAD), left circumflex (LCX) and right coronary artery were selected for stent implantation. The guiding catheter was used as a reference to obtain a 1.1:1 to 1.2:1 stent-to-artery ratio (10–20 % overstretch) compared with the baseline vessel diameter, and to induce moderate vessel injury (per method of Schwartz *et al.*)¹⁸ The stents were evenly distributed within the arteries resulting in two to three stents per animal.

Offline quantitative coronary angiography (QCA) was performed for the 7- and 28-day group for confirmation of the homogenous vessel diameters before and after stent implantation and calculation of the stent-to-artery ratio.

Oral ASS (100 mg) and clopidogrel (300 mg loading dose; 75 mg daily dose) were administered starting 2 days before the procedure and continued daily until euthanasia.

The animals were allowed to recover before they received appropriate postoperative care. After 7 days (6 domestic pigs), 28 days (8 domestic pigs), and 180 days (12 Goettingen miniature pigs) the pigs were again anaesthetized and re-angiography was conducted. Thereafter, the pigs were euthanized with intravenous bolus injection of saturated potassium chloride (10 ml).

Gross inspection of the major organs was done during necropsy and the hearts were removed for pressure fixation with formalin before they were sent to the CVPPath Institute, Gaithersburg, MD, USA for histology.

Histology

The stented arterial segments were dehydrated in a graded series of ethanol and embedded in methylmethacrylate (MMA) resin. After polymerization, 2–3 ml segments were sawed from the proximal,

mid and distal regions of each stent. Histologic segments from the stents were cut on a rotary microtome at 4–6 μm , and stained with haematoxylin and eosin (H&E) and modified Movat pentachrome (connective tissue stain).

Histomorphometric assessment

The cross-sectional areas [external elastic lamina (EEL), internal elastic lamina (IEL) and lumen] of each stented section were measured. Neointimal thickness was determined as the distance from the inner (abluminal) surface of each stent strut to the luminal border. Mean neointimal thickness was the average of each strut measurement. Area measurements were used to calculate vessel layer areas with the following formulas:

$$\text{Medial area} = \text{EEL area} - \text{IEL area}$$

$$\text{Neointimal area} = \text{IEL area} - \text{lumen area}$$

$$\% \text{ stenosis} = [1 - (\text{lumen area}/\text{IEL area})] \times 100$$

Ordinal data collected on each stent section included malapposition (defined as a strut distance $>50 \mu\text{m}$ away from the IEL), fibrin deposition, granuloma reactions, giant cells, and haemorrhage [% red blood cells (RBCs)] around stent struts and were expressed as a percentage of the total number of struts in each section. A vessel injury score was calculated according to the Schwartz method.¹⁸ An overall score for neointimal inflammation (0–4) and fibrin (0–3) was also determined for each section. Endothelial coverage was semiquantified and expressed as an estimated percentage of the lumen circumference.

Granulomas were further classified as unifocal, multifocal or circumferential. The primary reason for excluding granulomas from histomorphometric analysis is that their mere presence typically causes increased neointimal growth, which could potentially obscure any treatment effect.¹⁸ In the present study, exclusion criteria constituted a cumulative observation of more than one granuloma after examination of all three sections of an implant.

Neointimal inflammation was graded as: 0: $<25\%$ struts with fewer than 10 inflammatory cells; 1: $<25\%$ struts with greater than 10 inflammatory cells; 2: 25–50% struts with greater than 10

inflammatory cells; 3: $>50\%$ struts with greater than 10 inflammatory cells; and 4: 2 or more struts associated with granulomatous inflammatory reactions. Fibrin was graded on (a) a four-stage system (0: no fibrin is appreciated or only small strands; 1: at least 25% of struts involving confluent fibrin that surrounds up to 25% of the strut circumference; 2: at least 50% of struts involving confluent fibrin that surrounds $>25\%$ of strut circumference; 3: all struts with confluent fibrin surrounding $>50\%$ of strut circumference) or (b) confluent fibrin involving $>25\%$ strut circumference with involvement of $>50\%$ struts and extension between struts or bridging. Calcifications could only be defined qualitatively in the H&E stained slides without a prespecified grading scale or dedicated staining method.

Statistical analysis

Values were expressed as mean \pm standard deviation. Single-factor analysis of variance was used to compare continuous variables of all parametric data. The *post hoc* Tukey–Kramer was used to compare individual groups. Nonparametric score data, including injury, fibrin, and neointimal and adventitial inflammation were analysed with the Wilcoxon Kruskal–Wallis test. A value of $p < 0.05$ was considered statistically significant. If significance was reached with non-Gaussian distributions following the Wilcoxon Kruskal–Wallis test, a Bonferroni correction was done to yield a more stringent p value where $p < 0.05/n$ total comparisons.

Results

Animal study

The vessel sizes at stent implantation measured by offline QCA were comparable for all stents of the 7-day group (PF-SES: $2.3 \pm 0.3 \text{ mm}$, BMS: 2.6 ± 0.3 , SES: $2.4 \pm 0.2 \text{ mm}$; $p = 0.2829$), and of the 28-day group (PF-SES: $2.3 \pm 0.2 \text{ mm}$, BMS and SES: $2.4 \pm 0.2 \text{ mm}$). For the 180-day group the vessel diameters and stent: artery ratios were judged by an experienced interventionist to be comparable between the stents.

The mean diameters of the implanted stents were also comparable between the groups with $3.4 \pm 0.2 \text{ mm}$ for the PF-SES and SES, and $3.3 \pm 0.3 \text{ mm}$ for the BMS ($p = 0.7069$). The resulting

Table 1. Histological analysis of the 7-day group.

	BMS	PF-SES	SES	<i>p</i> value
Arteries (<i>n</i>)	3	5	5	–
EEL area (mm ²)	8.26 ± 0.73	7.33 ± 1.12	9.12 ± 0.59	0.027*
IEL area (mm ²)	7.36 ± 0.38	6.77 ± 1.00	8.64 ± 0.57	0.008*
Lumen area (mm ²)	6.85 ± 0.43	6.26 ± 1.00	8.04 ± 0.56	0.011*
Medial area (mm ²)	0.90 ± 0.36	0.56 ± 0.18	0.48 ± 0.08	0.050#
Neointimal area (mm ²)	0.50 ± 0.09	0.51 ± 0.21	0.60 ± 0.22	0.742
Stenosis (%)	6.88 ± 1.40	7.57 ± 3.02	6.90 ± 2.62	0.903
Neointimal thickness (mm)	0.017 ± 0.005	0.022 ± 0.020	0.008 ± 0.003	0.243
Struts with fibrin (%)	71.2 ± 32.2	83.8 ± 20.8	90.1 ± 19.0	0.548
Malapposition (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Struts with RBCs (%)	21.7 ± 16.4	24.9 ± 13.2	44.8 ± 23.4	0.183
Struts with giant cells (%)	33.5 ± 25.9	14.1 ± 16.6	41.9 ± 27.5	0.210
Struts with granulomas (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Endothelialization (%)	88.6 ± 9.6	58.5 ± 31.8	73.8 ± 22.3	0.291
Injury score (0–3)	0.36 ± 0.28	0.45 ± 0.32	0.70 ± 0.23	0.257
Fibrin score (0–3)	1.56 ± 0.69	2.07 ± 0.72	1.87 ± 0.30	0.577
Inflammation score (0–4)	0.33 ± 0.33	0.13 ± 0.30	0.53 ± 0.61	0.387

Tukey–Kramer comparison: *PF-SES versus SES; #SES versus BMS.
BMS, bare metal stent; EEL, external elastic lamina; IEL, internal elastic lamina; PF-SES, polymer-free sirolimus-eluting stent; RBC, red blood cell.

stent: artery ratios calculated by QCA were comparable ($p > 0.05$) for the PF-SES, BMS, and SES of the 7-day group (1.28 ± 1.14 , 1.19 ± 1.14 , 1.34 ± 1.12 , respectively), and of the 28-day group (1.21 ± 1.08 , 1.15 ± 1.05 , 1.27 ± 1.13 , respectively).

All animals reached the follow-up intervals. After 7 days, all stents were patent, with no evidence of occlusive luminal thrombosis or strut malapposition (Table 1). Vessel area measurements suggested a larger diameter in vessels treated with SES, whereas vessels implanted with PF-SES had the smallest reference diameters between all three groups. Neointimal growth in terms of stenosis and thickness was modest in all devices, consisted

mainly of thrombus composed of platelets/fibrin, and scattered inflammatory cells. Injury scores were considered minimal and cumulatively well below 1. Although inflammation scores were low for both neointima and adventitia, the percentage of struts with giant cells was relatively high, particularly for SES, while the lowest scores were observed in the PF-SES group.

No significant differences were observed in terms of endothelial coverage between the three groups.

After 28 days, all stents appear well expanded and are patent. Overall, IEL injury (Table 2) was minimal, although instances of focal to eccentric medial disruption were seen for all

Table 2. Histological analysis of the 28-day group.

	BMS	PF-SES	SES	<i>p</i> value
Arteries (n)	4	10	10	
EEL area (mm ²)	7.28 ± 0.73	7.08 ± 0.90	8.70 ± 0.92	0.001*
IEL area (mm ²)	6.23 ± 0.69	6.17 ± 0.76	7.78 ± 0.93	0.001*
Lumen area (mm ²)	4.33 ± 0.51	4.69 ± 1.02	5.35 ± 0.87	0.123
Medial area (mm ²)	1.05 ± 0.10	0.91 ± 0.21	0.92 ± 0.11	0.318
Neointimal area (mm ²)	1.90 ± 0.85	1.48 ± 0.55	2.43 ± 0.69	0.014#
Stenosis (%)	29.9 ± 10.8	24.4 ± 10.3	31.0 ± 8.2	0.290
Neointimal thickness (mm)	0.21 ± 0.13	0.17 ± 0.09	0.19 ± 0.07	0.668
Struts with fibrin (%)	19.8 ± 27.5	96.5 ± 5.4	96.3 ± 6.0	<0.001§
Malapposition (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Struts with RBCs (%)	6.5 ± 7.44	2.11 ± 2.05	5.91 ± 6.04	0.182
Struts with giant cells (%)	5.8 ± 3.98	7.4 ± 7.7	15.8 ± 14.1	0.154
Struts with granulomas (%)	0.9 ± 1.9	0.00 ± 0.00	0.00 ± 0.00	0.076
Endothelialization (%)	99.6 ± 0.5	98.8 ± 1.7	87.2 ± 32.8	0.517
Injury score (0–3)	0.34 ± 0.18	0.43 ± 0.25	0.61 ± 0.27	0.225
Fibrin score (0–3)	0.58 ± 0.96	2.10 ± 0.35	2.00 ± 0.27	0.023§
Inflammation score (0–4)	0.33 ± 0.67	0.00 ± 0.00	0.07 ± 0.21	0.292

Tukey–Kramer comparison: *PF-SES & BMS *versus* SES; #PF-SES *versus* SES; §PF-SES and SES *versus* BMS; Bonferroni correction: §PF-SES *versus* BMS.
BMS, bare metal stent; EEL, external elastic lamina; IEL, internal elastic lamina; PF-SES, polymer-free sirolimus-eluting stent; RBC, red blood cell.

stent groups. Comparison of cross-sectional areas showed significantly greater EEL, IEL and neointimal areas in SES *versus* PF-SES and BMS. Overall, diameter stenosis was least in PF-SES (mean stenosis: PF-SES = 24.4 ± 10.3%, SES = 31.01 ± 8.15%, BMS = 29.9 ± 10.8%, *p* value = 0.290). Fibrin deposition was moderate in PF-SES and SES and more pronounced as compared with the BMS group (*p* < 0.0001). Both PF-SES and SES have selected struts with focal calcification, and PF-SES have three sections with moderate calcification amid fibrin. Inflammation (Table 2) was minimal in all groups with PF-SES showing the least. Occasional strut-associated giant cells were

present in all groups. A single BMS section had unifocal granulomas. Endothelialization of luminal surfaces was nearly complete in all groups, though SES show the least coverage with occasional adherent luminal inflammatory cells.

At 180 days, the majority of implanted vessels had severe luminal narrowing (Table 3). The excessive luminal narrowing was suspected to be primarily a result of severe medial injury occurring at the time of deployment, considering the relatively high frequency of individual struts with injury scores ≥ 2 (reflecting involvement of the medial wall). Another contributor of increased stenosis and arterial injury was the

Table 3. Histological analysis of the 180-day group.

	BMS	PF-SES	SES	p value
Arteries (n)	9	9	9	
EEL area (mm ²)	6.21 ± 0.92	6.06 ± 1.12	7.90 ± 2.37	0.042
IEL area (mm ²)	5.55 ± 0.95	5.37 ± 0.97	6.44 ± 2.01	0.243
Lumen area (mm ²)	2.35 ± 1.08	2.47 ± 1.48	1.44 ± 0.95	0.155
Medial area (mm ²)	0.66 ± 0.28	0.69 ± 0.24	1.46 ± 0.80	0.004*
Neointimal area (mm ²)	3.19 ± 1.57	2.90 ± 1.05	5.00 ± 2.12	0.025 [#]
Stenosis (%)	56.0 ± 23.1	55.7 ± 21.8	76.2 ± 16.6	0.075
Neointimal thickness (mm)	0.48 ± 0.27	0.44 ± 0.21	0.74 ± 0.25	0.032 [#]
Struts with fibrin (%)	0.21 ± 0.62	0.00 ± 0.00	2.97 ± 4.81	0.061
Malapposition (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Struts with RBCs (%)	1.48 ± 1.87	0.39 ± 0.77	2.27 ± 5.90	0.548
Struts with giant cells (%)	26.0 ± 16.2	7.0 ± 7.7	18.3 ± 23.3	0.077
Struts with granulomas (%)	0.6 ± 1.9	1.6 ± 4.7	40.4 ± 45.9	0.006*
Endothelialization (%)	99.6 ± 0.3	99.8 ± 0.3	96.2 ± 10.6	0.384
Injury score (0–3)	1.24 ± 0.88	0.95 ± 0.68	1.54 ± 0.98	0.437
Fibrin score (0–3)	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.15	0.125
Inflammation score (0–4)	0.30 ± 0.65	0.41 ± 0.74	2.52 ± 1.72	0.017 [§]

Tukey–Kramer comparison: *PF-SES and BMS *versus* SES; [#]PF-SES *versus* SES; [§]BMS *versus* SES. BMS, bare metal stent; EEL, external elastic lamina; IEL, internal elastic lamina; PF-SES, polymer-free sirolimus-eluting stent; RBC, red blood cell.

excessive granulomatous inflammation; however, granulomas were primarily seen in SES-implanted vessels (in seven of nine total), and only one of nine PF-SES and one of nine BMS, and even after exclusion of these granulomatous sections, luminal narrowing remained moderate to severe.

Histopathology revealed that there is a pronounced intimal hyperplasia progression between 28 and 180 days in the BMS and the SES groups, whereas it was significantly less pronounced in the PF-SES group (Figure 1). Furthermore, the difference in strut thickness between SES and the other two treatment groups can be observed.

In vitro thrombogenicity study

As analysed by SEM, the EES stents showed some thrombus formation, the PF-SES also showed a few small spots with blood components (Figure 2). Fibrin and platelet binding was lowest on EES and almost similarly low on PF-SES stents (Table 2). Both types of stents had markedly lower fibrin and platelet binding than the reference materials. Platelet aggregation capacity was maintained in all loops and showed an increase in EES, medical steel and LDPE. This indicates platelet activation during the blood circulation procedure, which was moderate in the control and PF-SES (100% is baseline aggregation). Overall, no differences were observed between EES and PF-SES stent surface adhesion

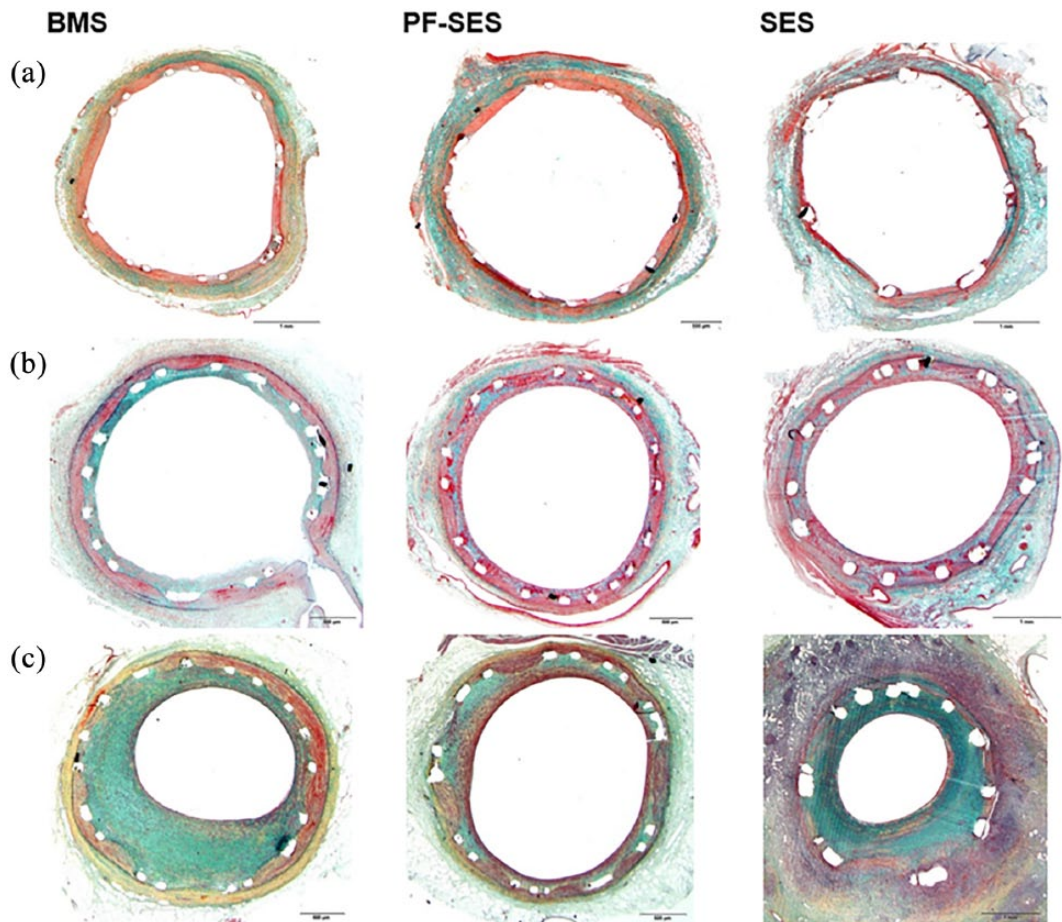


Figure 1. Panels stained by Movat, with twofold magnification. Panel (a) at 7 days; panel (b): 28 days, panel (c): 180 days. BMS, bare metal stent; PF-SES, polymer-free sirolimus-eluting stent.



Figure 2. Representative SEM images of EES and PF-SES, magnification $\times 350$. Representative SEM images of EES (left panel: EES with fibrin strands) and PF-SES (right panel: PF-SES with some small spots of fibrin), magnification $\times 350$. BMS, bare metal stent; EES, everolimus-eluting stent; PF-SES, polymer-free sirolimus-eluting stent; SEM, scanning electron microscopy.

Table 4. *In vitro* thrombogenicity study results.

	PF-SES	EES	MS	LDPE
Fibrin binding	0.15 ± 0.03	0.10 ± 0.03	1.54 ± 0.70	0.37 ± 0.03
Platelet binding	0.46 ± 0.40	0.16 ± 0.01	3.66 ± 0.53	0.17 ± 0.06
Platelet aggregation	135 ± 50	225 ± 112	244 ± 69	165 ± 49

EES, everolimus-eluting stent; LDPE, low-density polyethylene; MS, stainless steel; PF-SES, polymer-free sirolimus-eluting stent.
 $p > 0.05$ for platelet binding and aggregation and $p = 0.02$ for fibrin binding for PF-SES and EES.

of thrombotic material. After blood contact the EES and PF-SES stents showed almost no deposition in macroscopic or SEM images. The antithrombotic properties of the stents were further supported by the low levels of fibrin and platelet binding. Platelets were less activated during circulation of the PF-SES stents than of the EES and reference materials as indicated by the platelet aggregation (Table 4).

Discussion

To the best of our knowledge, this is the first report of preclinical histological long-term data up to 180 days after stent implantation of a PF-SES in direct comparison with a durable polymer-coated stent and a BMS with regard to vessel healing, inflammation and endothelialization. Despite the fact that the used SES in our control group has not been commercially available since 2012, it represents, nevertheless, an important reference to prior preclinical data. When discussing vascular healing in the porcine model and in humans, there are two criteria suspected to be instrumental in understanding thrombosis and restenosis. These are thrombogenicity and intimal hyperplasia.

Thrombogenicity

From our viewpoint, it appears to be oversimplistic to condemn all durable polymer stent coatings, even though the SES in our series (Cypher) was associated with a numerically higher strut fibrin load as compared with the device in the PF-SES group (2.97 ± 4.81 versus 0.00 ± 0.00 , $p_{\text{group}} = 0.061$) at 180 days.

It seems that it is not only the polymer *per se* but also the coating integrity that affects the thrombogenicity of the stent struts. Evidence for this

hypothesis can also be found in the preclinical work conducted by Otsuka *et al.*,¹⁹ who demonstrated in an *ex vivo* porcine shunt model that the durable polymer coating of an EES (Xience® Xpedition, Abbott Vascular) was superior in terms of platelet aggregation as compared with DES with bioabsorbable polymer-coating technologies (BioMatrix® Flex Biolimus-eluting stent, Biosensors, Singapore and Synergy® EES, Boston Scientific, Natick, Massachusetts, USA). Polymer-coating integrity most likely depends on stent architecture and on the manufacturing process, especially in early-generation DES. Guérin and colleagues,²⁰ who conducted bench deformation tests in bifurcation models demonstrated the lack of coating integrity when stent struts were plastically deformed.

In our *in vitro* study, the PF-SES showed no differences in acute thrombogenicity as compared with the fluoropolymer-coated EES. These findings can be furthermore correlated with clinical data showing also a low prevalence of early stent thrombosis in EES versus the first-generation DES^{21,22} and for the PF-SES.¹² For the PF-SES, Krackhardt and colleagues¹² reported an incidence of 0.4% acute stent thrombosis based on 2877 patients in an unselected largescale international, single-armed, multicentre, 'all comers' observational study. Consequently, it can be concluded that PF-SES and fluoropolymer-coated EES have comparably low thrombogenic potential in clinical practice.

Inflammation and neointimal proliferation

Vascular inflammation and neointimal proliferation are strongly correlated.^{23,24} Overall, results were comparable in terms of inflammation between the PF-SES and SES groups, except PF-SES had less inflammation and neointimal

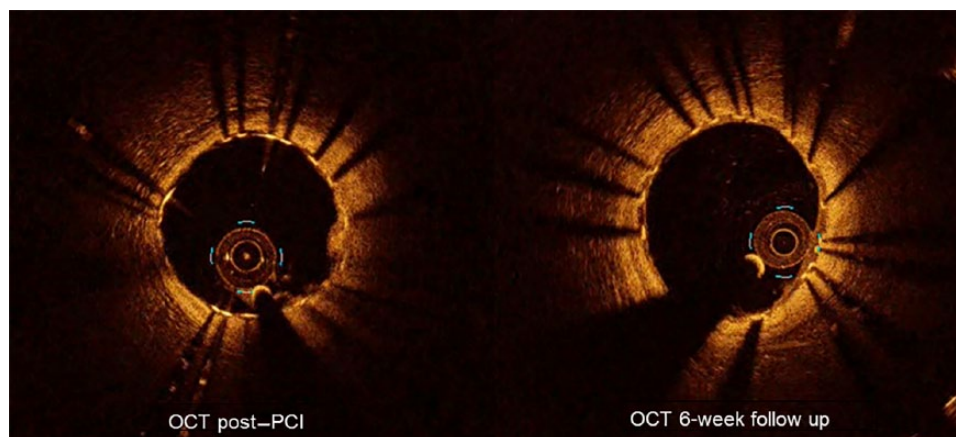


Figure 3. OCT images of PF-SES in the LAD-7 of a 75-year-old patient. The PF-SES used is the Coroflex® ISAR 2.75 mm. Left panel: OCT post-PCI; right panel: OCT follow up at 6 weeks. PF-SES, polymer-free sirolimus-eluting stent; OCT, optical coherence tomography; LAD-7, ; PCI, .

proliferation as compared with SES. In seven of nine arteries implanted with SES, granulomas were detected, which is a common finding in porcine-stent studies.¹⁸ The mere presence of granulomas typically causes increased neointimal growth, which could potentially obscure any treatment effect. When excluding these arteries from histomorphometric analysis, significant differences for PF-SES and BMS could be observed regarding endothelialization compared with SES (PF-SES 99.8 ± 0.3 , SES 84.0 ± 22.6 , BMS 99.7 ± 0.3 ; $p = 0.0096$). For all other parameters, no significant differences could be detected.

Vessel healing (fibrin deposition, inflammation, and endothelialization) with the permanent polymer SES tended to be delayed up to 180 days after stent implantation when compared with PF-SES but without reaching statistical significance.

The porcine model by Galon and coworkers²⁵ described a methodology to correlate intravascular imaging and histopathology. They demonstrated that indeed, the induced neointimal hyperplasia and the corresponding optical coherence tomography (OCT) images in their porcine overstretch model are remarkably humanoid. Unfortunately, we did not use OCT or intravascular ultrasound in our porcine model. However, it is our conviction that future preclinical research should consider OCT, since it did not increase animal mortality while significant information relative to neointimal hyperplasia of the complete stented artery could be obtained. Nevertheless, for every OCT, the animal requires additional

anaesthesia while the vascular access is accomplished. The associated risk must be properly balanced with the knowledge gained.

Endothelialization

In the BMS group, endothelial coverage was $88.6 \pm 9.6\%$ at 7 days, $99.6 \pm 0.5\%$ at 28 days and $99.6 \pm 0.3\%$ at 180 days, whereas the corresponding values in the SES group were $73.8 \pm 22.3\%$, $87.2 \pm 32.8\%$ and $96.2 \pm 10.6\%$, respectively, which seems to be in agreement with the early findings by Finn *et al.*,⁸ who used a rabbit model for various early-generation DES and their BMS backbones. However, in the PF-SES group, endothelial coverage at 7 days was only $58.5 \pm 31.8\%$, while it was greater than 70% for the remaining stents and most complete for BMS ($p > 0.05$). Even though, between 7 and 28 days stent coverage in the PF-SES group increased to $98.8 \pm 1.7\%$ and eventually reached $99.8 \pm 0.3\%$ at 180 days, we can only explain this low value with interanimal variability.

Clinical follow up using optical coherence tomography

A useful tool to evaluate stent strut coverage is OCT, which provides ‘a second pair of eyes’ to evaluate the postimplantation healing process. These intravascular images, however, can only be obtained as an adjunct to a justified angiography in the clinical setting. A time course of stent coverage in humans as observed by OCT is consequently not feasible, primarily due to ethical

concerns. Within the all-comers study conducted by Krackhardt *et al.*,¹² a number of patients underwent sequential OCT analysis between 4 and 6 weeks as part of their planned, staged procedures for multivessel interventions. Representative images are shown in Figure 3. Despite its anecdotal character, Figure 3 reveals that after 6 weeks post-PCI, all stent struts are covered. It remains speculative in nature if the struts are covered by endothelial cells or by fibrin. Moreover, there does not seem to be an agreement whether fibrin or endothelial cells are different relative to their thrombogenic potential.

Main outcomes

Preclinical evaluation of DES is still the most feasible methodology to distinguish the healing characteristics of newer DES. The ultrathin-strut cobalt–chromium PF-SES showed efficacious inhibition of neointimal proliferation in porcine coronary arteries with low inflammation and nearly complete endothelialization after 28 days. At 180 days, vessel healing was nearly completed for PF-SES, whereas the SES still showed high inflammatory reaction. Further clinical investigations are necessary to answer the pivotal question of how short a DAPT can be after implantation of the novel polymer-free DES used in this assessment. Preclinical animal work should include intravascular imaging, preferably OCT, to better describe the time course of vessel healing following stent implantations. These preclinical OCT images ought to be correlated with human OCT images to refine preclinical methodologies.

Conclusion

The ultrathin-strut cobalt–chromium PF-SES showed low inflammation and nearly complete endothelialization after 28 days. At 180 days, vessel healing was nearly completed for PF-SES, whereas the SES still showed a high inflammatory response. In addition, in an *in vitro* model, the PF-SES showed a similarly low thrombogenicity as compared with the EES.

Study limitations

The used animal model cannot reproduce the complexity of human disease conditions such as the presence of atherosclerotic lesions, and therefore, the findings have to be interpreted with

caution. The relatively small sample size and the use of two different species (domestic pigs and Goettingen minipigs) may also have affected the vascular response to the implants. Nevertheless, the porcine coronary artery model is currently considered the most appropriate for evaluating coronary DES and is one of the due diligence milestones before clinical assessments can commence. However, conclusions from these results relative to the duration of DAPT in the clinical setting cannot be drawn. In addition, it would have been ideal to have a more modern DES as a comparator. However, due to the wealth of available clinical data for the Cypher stent and due to the fact that this DES had an identical drug, the choice could be justified from a methodological standpoint. Finally, despite its anecdotal character, the presented OCT images with the PF-SES need to be clinically confirmed with ongoing OCT endpoint studies such as the FRIENDLY OCT trial [ClinicalTrials.gov identifier: NCT02785237].

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Conflict of interest statement

Except for FK (lecturing fees) and CS/MWW (full-time employment at Medical Scientific Affairs, B. Braun Melsungen AG) there are no conflicts of interest to declare.

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