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TRANSLATIONAL

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

Fumiyuki Otsuka, MD, PhD,* Qi Cheng, MD,* Kazuyuki Yahagi, MD,* Eduardo Acampado, DVM,* Alexander Sheehy, MS,† Saami K. Yazdani, PhD,* Kenichi Sakakura, MD,* Kristina Euller,* Laura E.L. Perkins, DVM, PhD,† Frank D. Kolodgie, PhD,* Renu Virmani, MD,* Michael Joner, MD*

ABSTRACT

OBJECTIVES This study sought to evaluate whether the permanent fluoropolymer-coated Xience Xpedition everolimus-eluting stent (Xience-EES) exhibits lower acute thrombogenicity compared with contemporary drug-eluting stents (DES) with biodegradable polymer coatings in an acute swine shunt model.

BACKGROUND Previous pre-clinical and clinical experience suggests that several factors may influence the predisposition for acute thrombus formation of polymer-coated DES, including stent design and the polymer coating technology. It remains unclear whether relevant differences exist with respect to acute thrombogenicity, particularly between current commercial stent designs using permanent polymers and those using biodegradable polymers.

METHODS An ex vivo carotid to jugular arteriovenous porcine shunt model involving a test circuit of 3 in-line stents, was used to test acute thrombogenicity, where Xience-EES ($n = 24$) was compared with 4 CE-marked DES with biodegradable polymer coatings (BioMatrix Flex, Synergy, Nobori, and Orsiro [$n = 6$ each]). After 1 h of circulation, platelet aggregation in whole mount stents was evaluated by confocal microscopy with immunofluorescent staining against dual platelet markers (CD61/CD42b) along with scanning electron microscopy.

RESULTS Xience-EES showed the least percentage of thrombus-occupied area as compared with the biodegradable polymer-coated DES, with a significant difference compared with BioMatrix Flex and Synergy (mean differences: [BioMatrix Flex: 15.54, 95% confidence interval [CI]: 11.34 to 19.75, $p < 0.001$; Synergy: 8.64, 95% CI: 4.43 to 12.84, $p < 0.001$; Nobori: 4.22, 95% CI: -0.06 to 8.49, $p = 0.055$; Orsiro: 2.95, 95% CI: -1.26 to 7.15, $p = 0.286$). The number of cell nuclei on strut surfaces was also the least in Xience-EES, with a significant difference relative to BioMatrix Flex, Nobori, and Orsiro (mean ratios: BioMatrix Flex: 4.73, 95% CI: 2.46 to 9.08, $p < 0.001$; Synergy: 1.44, 95% CI: 0.75 to 2.76, $p = 0.51$; Nobori: 5.97, 95% CI: 3.11 to 11.44, $p < 0.001$; Orsiro: 5.16, 95% CI: 2.69 to 9.91, $p < 0.001$).

CONCLUSIONS Xience-EES's overall design confers acute thromboresistance relative to contemporary DES with biodegradable coatings, with less platelet aggregation versus BioMatrix Flex and Synergy, and less inflammatory cell attachment versus BioMatrix Flex, Nobori, and Orsiro, in an ex vivo swine shunt model, which lends support to reported clinical findings of lower early stent thrombosis. (J Am Coll Cardiol Intv 2015;8:1248-60) © 2015 by the American College of Cardiology Foundation.

Early stent thrombosis (ST) remains a critical issue even with the use of contemporary drug-eluting stents (DES), where the indications for percutaneous coronary intervention have been expanded to include more complex lesions (1,2). Histomorphologic assessment of stents at autopsy from patients presenting with acute coronary syndrome from our laboratory has demonstrated an association of early ST with necrotic core prolapse, medial tear, and incomplete stent apposition (3). In addition to morphologic criteria of the underlying plaque, stent design, choice of polymer coating, its application (e.g., use of primer, coating integrity), and drugs may also influence acute thrombogenicity as experimental studies in modified Chandler loops have shown that durable polymer-coated stents with thin struts exhibit less acute thrombogenicity with respect to platelet aggregation as compared to uncoated or stents with thicker struts (4). Clinical studies have also demonstrated a lower prevalence of early ST in durable polymer-coated DES as compared with bare-metal stents (BMS), where the cobalt-chromium everolimus-eluting stents (EES) exhibit the least prevalence of early ST relative to BMS and the other durable polymer-coated DES (5-7). Considerable attention has been tied to the impact of polymer coatings on acute thrombogenicity and whether durable polymers exert differential effects in this regard compared with more recently introduced biodegradable stent coatings. In the absence of randomized clinical trials focused on acute thrombogenicity among comparable state-of-the-art DES, dedicated preclinical studies specifically designed to address the impact of stents with divergent configuration of individual components on acute thrombogenicity remain an important measure to reveal differential procoagulatory device effects.

Therefore, the current study was designed to compare acute thrombogenicity by assessing platelet aggregation and acute inflammation in Xience Xpedition EES (Xience-EES) (Abbott Vascular, Santa Clara, California) with durable polymer coating technology with 4 CE-marked DES with biodegradable polymer coatings in an ex vivo porcine arteriovenous shunt model.

ABBREVIATIONS AND ACRONYMS

BES = biolimus-eluting stent(s)
BMS = bare-metal stent(s)
CI = confidence interval
DAPI = 4′,6-diamindino-2-phenylindole
DES = drug-eluting stent(s)
EES = everolimus-eluting stent(s)
SEM = scanning electron microscopy
SES = sirolimus-eluting stent(s)
ST = stent thrombosis

METHODS

EXPERIMENTAL SET-UP AND TEST GROUPS. A porcine ex vivo arteriovenous shunt model was established to study the extent of platelet adherence, thrombus formation, and acute inflammation in Xience-EES (n = 24) compared with 4 contemporary DES with biodegradable coatings: 1) BioMatrix Flex biolimus-eluting stent (BES) (Biosensors, Newport Beach, California) (n = 6); 2) Nobori-BES (Terumo, Tokyo, Japan) (n = 6); 3) Synergy-EES (Boston Scientific, Natick, Massachusetts) (n = 6); and 4) Orsiro sirolimus-eluting stent (SES) (Biotronik AG, Bülach, Switzerland) (n = 6) (**Figure 1**, Online Figure 1).

Each shunt model had 3 stents and each animal had 2 shunt experiments. However, there was 1 animal that achieved only the first shunt experiment because of deterioration of general condition during the second shunt experiment. Therefore, 1 additional shunt experiment was performed in another animal. Thus, a total of 48 stents were deployed in 16 shunts from 9 swine for the assessment of acute thrombogenicity.

PORCINE ARTERIOVENOUS SHUNT MODEL. Three consecutive DES were deployed at nominal pressure in Sylgard (Dow Corning, Midland, Michigan) mock vascular phantoms (8). The Sylgard conduits (inner diameter: 2.70 mm × 11 cm length) were fabricated using 316L stainless steel tubing and commercial elastomer kit (Sylgard-184, Dow Corning). Before

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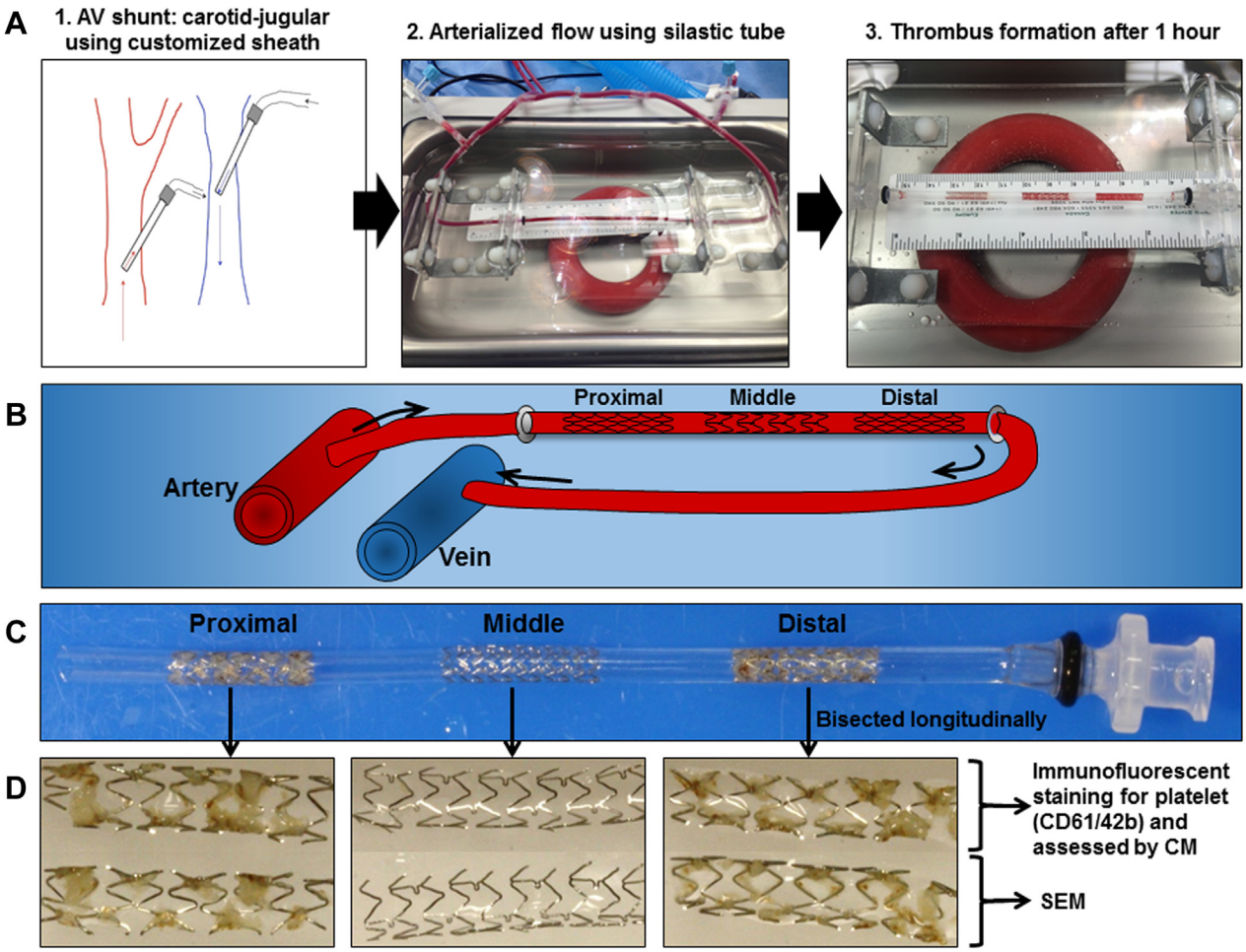
FIGURE 1 Acute Swine Shunt Model and Gross Photos of Mount Stents



(A) A porcine ex vivo carotid to jugular arteriovenous (AV) shunt model involving a test circuit of 3 in-line stents within a silastic tube was used to test thrombogenicity with 1 h of circulating blood. **(B)** Schematic illustration of the acute shunt model. **(C)** Gross photos of 3 in-line stents within a silastic tube. **(D)** The stents were bisected longitudinally; one-half for immunofluorescent staining against dual platelet markers (CD61/CD42b) with confocal microscopic (CM) assessment and the other for scanning electron microscopy (SEM), to identify the extent of platelet adherence in whole mount stents.

deploying stents, the tubing was mounted in a fixed apparatus. The conduit was then filled with autologous serum and stents were expanded to a 3.0-mm diameter at nominal pressure. The extent of platelet aggregation to struts was studied after exposure to circulating blood for 1 h through an established arteriovenous carotid to jugular shunt.

Target blood activated clotting times between 150 s and 190 s were achieved with intravenous heparin (100 IU/kg) dosing without antiplatelet agents such as clopidogrel or aspirin as the current study was specifically designed to examine inherent platelet-mediated thrombus formation induced by contemporary DES of differential design. Two shunts were studied in each animal where positioning of DES in each tube was defined to include either 2 flanking Xience-EES or biodegradable polymer DES, respectively, separated by their respective counterparts (Figure 1). Shunt positions were numerically noted as proximal, mid, and distal relative to the arterial side. Before establishing blood flow through the circuit, stents were primed with autologous platelet-poor plasma. During experiments, stented tubing was maintained in a 37°C water bath and flow rates were monitored continuously using an ultrasonic transducer (Transonic, Ithaca, New York). At the conclusion of each run, stents were gravity perfused with Ringer's lactate, fixed in 10% neutral buffered

formalin, and bisected longitudinally. One-half of each stent was immunostained using specific platelet markers relevant for aggregation of thrombocytes and examined en face by confocal microscopy, whereas the other one-half was processed for scanning electron microscopy (SEM).

ASSESSMENT OF PLATELET AGGREGATION. Confocal microscopy. Stent halves were incubated overnight in 4°C in an antibody cocktail directed against specific platelet markers: CD61 as a marker of platelet aggregation (Immunotech, IM0540, dilution 1:100, Beckman Coulter, Brea, California) and CD42b as a marker of platelet adhesion (sc-7070, dilution 1:40, Santa Cruz Biotechnology, Dallas, Texas) to capture both originating and propagated platelet thrombus. Positive staining was visualized using a secondary antibody conjugated to an Alexa Fluor 488 fluorophore (Life Technologies, Carlsbad, California). After immunostaining, nuclei were counterstained with 4′,6-diamindino-2-phenylindole (DAPI) and stents were mounted en face on glass slides and coverslipped in aqueous mounting media. The entire stent surface was scanned using pre-determined "fixed" parameters incorporating a tile feature with Z stack imaging (LSM 700, Zen 2011 software, Zeiss, Oberkochen, Germany). The positive area of platelet staining was analyzed by proprietary software (Zen image analysis tool) within defined regions of interest, which were maintained for all examined samples (40 mm^2) and reported as absolute positive area (mm^2) of the device and percentage of positive area, which was calculated by dividing the absolute positive area by a predefined region of interest spanning the entire bisected segment of the stented artery. In addition, the relative percentage of positive staining was also calculated by dividing the absolute positive area by total stent surface area measured by morphometry. The total number of platelet aggregate clots >0.1 mm^2 was also quantified from composite confocal and SEM images of stent halves.

Scanning electron microscopy. The remaining stent halves were processed for SEM. Specimens were dehydrated, critically point dried, and sputter-coated with gold. Digital images were acquired using a Hitachi SEM (model 3600N, Tokyo, Japan). Low power (15× magnification) images of the entire luminal surface were collected to assess the extent of thrombus attached to the strut surfaces. Stitched low power montage images of the entire luminal surface were then assembled into a single image. Higher power photographs of regions of interest were also taken at incremental magnifications of (50×, 200×, 600×, and 2,000×). The total number of platelet aggregate clots >0.1 mm^2 were also quantified from composite SEM images of stent halves.

QUANTIFICATION OF INFLAMMATORY CELLS. DAPI-positive nuclei were counted in 3 representative regions of maximal inflammatory cell adhesion within the proximal, mid, and distal regions of each stent. As both erythrocytes and thrombocytes lack nuclei, adherent cells were considered to be of leukocyte origin (i.e., inflammatory cells). The mean number of inflammatory cells was derived and cell numbers (density) were expressed as the mean total nuclei per mm^2.

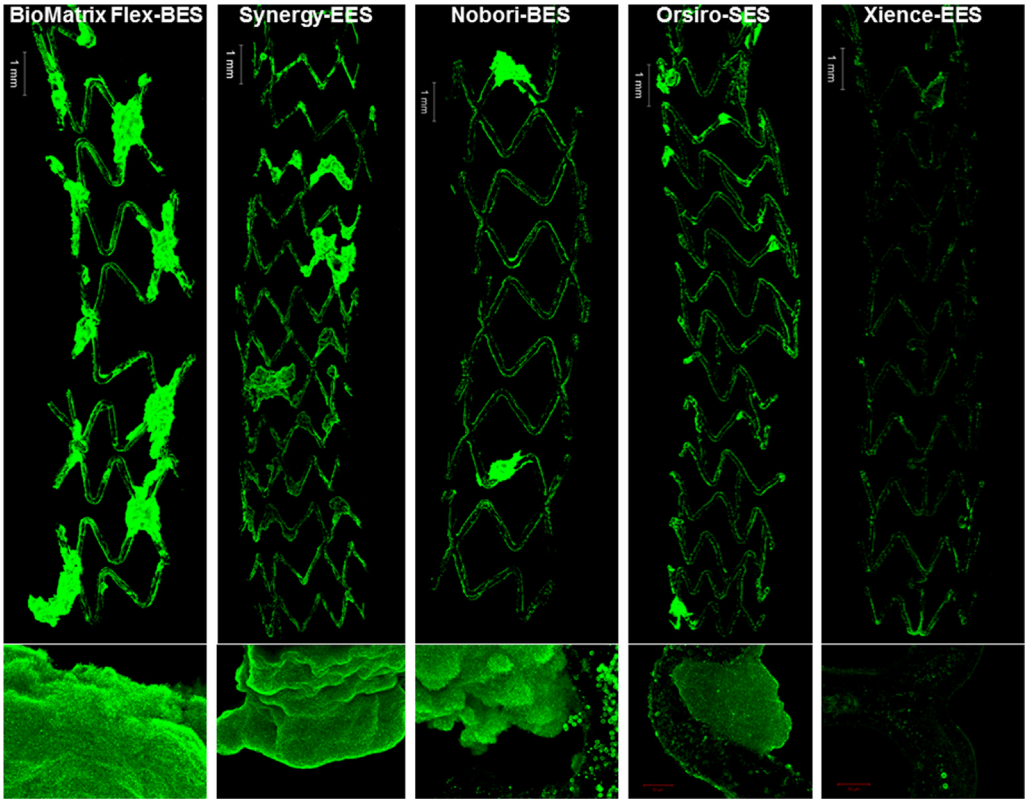
BLOOD COAGULATION AND PLATELET FUNCTION. Blood was serially drawn for coagulation and platelet function profiles pre-shunt (baseline) and after each experimental run to confirm the absence of coagulation and platelet function abnormalities. Indices of coagulation, platelet number, and function included platelet count, prothrombin time, partial thromboplastin time, and light transmission aggregometry. In addition, pig platelet factor 4 release was also assessed in collected plasma using a commercial ELISA kit (ab156530, Abcam, Cambridge, Massachusetts) (sensitivity = 0.1 ng/ml) according to the manufacturer's instruction.

STATISTICAL ANALYSIS. Nested generalized linear mixed models were used to investigate group differences in consideration of multiple measurements per individual. Within these models, the experimental factor variables animal, shunt number, and linear position were considered as nested random effects. The Akaike information criterion was evaluated for the goodness of model fit, and only number of cell nuclei on stent surface was logarithmically transformed before analysis. The analyses were performed by R software (version 3.1.0, R Core Team, Vienna, Austria) incorporating the multcomp R package (9). The statistical tests were 2-tailed and a value of $p < 0.05$ was considered to indicate statistical significance. Dunnett correction for many-to-1 comparisons was used to implement adjustment of p values and confidence interval lengths for multiple testing (10).

RESULTS

BLOOD COAGULATION AND PLATELET FUNCTION. There was no evidence of blood coagulation and platelet function abnormalities in any of the animals studied, and plasma pig platelet factor 4 release was below detection limit (Online Table 1). The mean activated clotting time was between 86 to 106 s at baseline, between 142 and 231 s during the first

FIGURE 2 Representative CM Images of BioMatrix Flex-BES, Synergy-EES, Nobori-BES, Orsiro-SES, and Xience-EES With Immunofluorescent Staining Against Dual Platelet Markers (CD61/CD42b) in a Swine Shunt Model



Low- and high-power confocal microscopic (CM) images showing least thrombus-occupied area in Xience Xpedition (Xience-EES) as compared with the other 4 CE-marked biodegradable polymer-coated drug-eluting stents. Note stent struts of Xience-EES are barely identified. BES = biolimus-eluting stent(s); SES = sirolimus-eluting stent(s).

shunt, and between 118 and 253 s during the second shunt (Online Table 1). Mean blood flow rates of shunts ranged from 93.22 to 164.62 ml/min and were similar among stent groups.

THROMBOGENICITY ASSESSED BY PLATELET IMMUNOFLUORESCENCE. Representative confocal microscopy images of Xience-EES and comparator biodegradable polymer-coated DES are shown in Figure 2. Xience-EES demonstrated the least absolute and percentage of positive fluorescence areas corresponding to aggregated platelets relative to DES with biodegradable polymer coatings (mean percent of fluorescence positive area: BioMatrix Flex-BES 27.2%, Synergy-EES 18.0%, Nobori-BES 21.1%, Orsiro-SES 17.6%, Xience-EES 13.2%) (Table 1). Linear mixed-effects model procedures demonstrated biologically relevant and statistically significant differences in percent of positive fluorescence area between Xience-EES versus BioMatrix Flex-BES (mean difference: 15.54, 95% confidence interval [CI]: 11.34 to 19.75, $p < 0.001$) and Synergy-EES (mean difference: 8.64, 95% CI: 4.43 to 12.84, $p < 0.001$) (Figure 3A). Overall, stent surface area measured by planimetry was similar between Xience-EES (mean stent surface area: 6.8 mm^2) and BioMatrix Flex-BES (6.9 mm^2, mean difference: 0.068, 95% CI: -0.63 to 0.77 versus Xience-EES, $p = 0.99$), Synergy-EES (6.3 mm^2, mean difference: -0.57, 95% CI: -1.27 to 0.13, $p = 0.16$ versus Xience-EES), and Orsiro-SES (6.6 mm^2, mean difference: -0.20, 95% CI: -0.90 to 0.51, $p = 0.93$ versus Xience-EES), whereas it was significantly greater for Nobori-BES (8.8 mm^2, mean difference: 1.96, 95% CI: 1.26 to 2.65, $p < 0.001$ versus Xience-EES) (Tables 1 and 2). When total platelet fluorescence area was normalized to stent surface area, differences remained significant among Xience-EES versus BioMatrix Flex-BES (mean difference: 80.57,

TABLE 1 Quantified Variables for the Assessment of Platelet Aggregation in Xience-EES and Biodegradable Polymer-Coated DES in a Swine Shunt Model

	BioMatrix Flex-BES	Synergy-EES	Nobori-BES	Orsiro-SES	Xience-EES
Absolute fluorescence positive area, mm^2	11.73 ± 1.76	8.94 ± 2.00	10.07 ± 2.23	7.89 ± 2.90	7.13 ± 2.92
Percentage of fluorescence positive area, %	27.19 ± 2.90	18.01 ± 4.42	21.07 ± 4.69	17.56 ± 6.71	13.18 ± 6.07
Stent surface area, mm^2	6.90 ± 0.35	6.29 ± 0.56	8.80 ± 0.63	6.58 ± 1.17	6.77 ± 0.47
Fluorescent positive area per stent surface area, %	169.48 ± 18.77	144.29 ± 40.71	115.93 ± 30.90	119.87 ± 40.54	105.02 ± 41.63
Number of platelet aggregation clots per stent by CM	7.50 ± 2.74	5.17 ± 4.36	0.50 ± 0.84	2.17 ± 1.47	0.37 ± 0.67
Number of platelet aggregation clots per stent by SEM	3.33 ± 2.25	5.50 ± 3.99	0.17 ± 0.41	2.17 ± 1.60	0.20 ± 0.55

Values are mean ± SD.

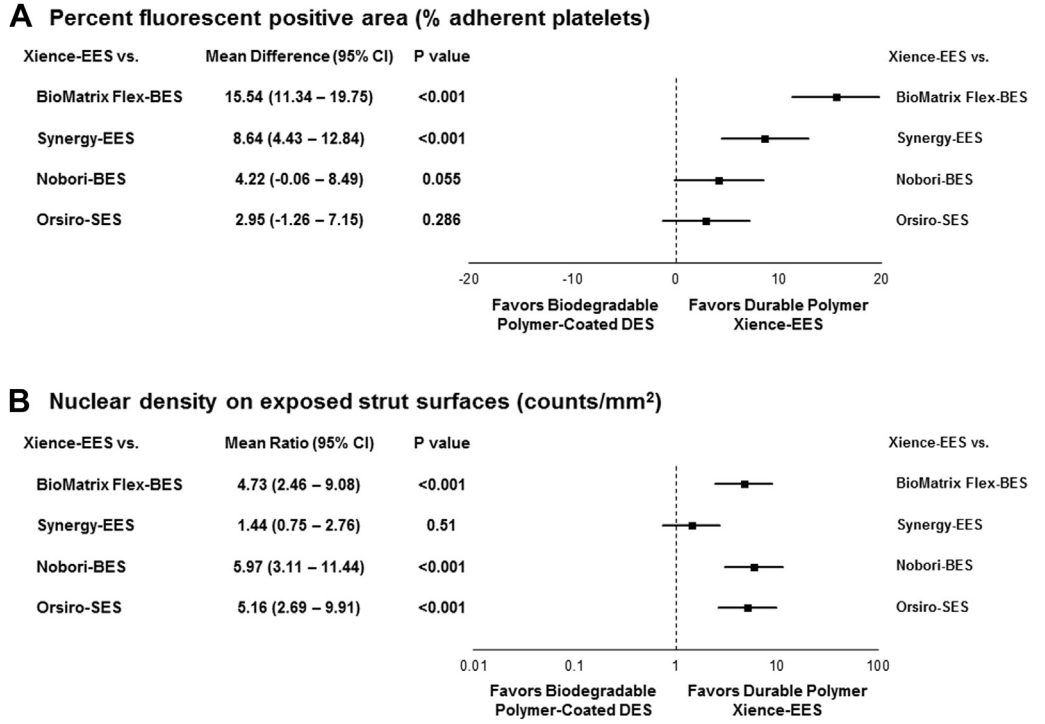
BES = biolimus-eluting stent(s); CM = confocal microscopy; EES = everolimus-eluting stent(s); SEM = scanning electron microscopy; SES = sirolimus-eluting stent(s); Xience-EES = Xience Xpedition everolimus-eluting stent.

95% CI: 47.51 to 113.64, $p < 0.001$) and Synergy-EES (mean difference: 63.16, 95% CI: 30.09 to 96.22, $p < 0.001$), whereas Nobori-BES and Orsiro-SES were similar to Xience-EES (Table 2).

The number of platelet aggregate clots >0.1 mm^2 as assessed by confocal microscopy was also least for Xience-EES (Table 1), where significant differences were consistently observed compared with BioMatrix Flex-BES (mean difference: 7.26, 95% CI: 5.10 to 9.41, $p < 0.001$) and Synergy-EES (mean difference: 4.94, 95% CI: 2.79 to 7.10, $p < 0.001$) (Table 2).

SCANNING ELECTRON MICROSCOPIC ASSESSMENT OF THROMBOGENICITY. The number of platelet

FIGURE 3 Comparisons of Acute Thrombogenicity and Inflammatory Cell Attachment on the Stent Surface Between Xience-EES Versus Biodegradable Polymer-Coated DES in a Swine Shunt Model



Linear mixed models showing comparisons of percentage of fluorescent positive area (percentage of area occupied by thrombus) **(A)** and number of cell nuclei on stent surface as assessed by confocal microscopy (4′,6-diamindino-2-phenylindole) **(B)** between Xience-EES versus biodegradable polymer-coated DES. A mean difference >0 denotes greater levels of the parameter in biodegradable polymer-coated DES as compared with Xience-EES, whereas for mean ratio, a value >1 denotes greater levels of the parameter in biodegradable polymer-coated DES versus Xience-EES. CI = confidence interval; DES = drug-eluting stent(s).

TABLE 2 Effect Estimates on the Basis of Nested Linear Mixed Models for Xience-EES Versus Biodegradable Polymer-Coated DES in a Swine Acute Shunt Model

	Mean Difference (95% CI)	p Value
Absolute fluorescence positive area, mm^2		
Xience-EES vs. BioMatrix Flex-BES	15.54 (11.34 to 19.75)	<0.001
Xience-EES vs. Synergy-EES	8.64 (4.43 to 12.84)	<0.001
Xience-EES vs. Nobori-BES	4.22 (-0.06 to 8.49)	0.055
Xience-EES vs. Orsiro-SES	2.95 (-1.26 to 7.15)	0.286
Stent surface area, mm^2		
Xience-EES vs. BioMatrix Flex-BES	0.068 (-0.63 to 0.77)	0.99
Xience-EES vs. Synergy-EES	-0.57 (-1.27 to 0.13)	0.16
Xience-EES vs. Nobori-BES	1.96 (1.26 to 2.65)	<0.001
Xience-EES vs. Orsiro-SES	-0.20 (-0.90 to 0.51)	0.93
Fluorescent positive area per stent surface area		
Xience-EES vs. BioMatrix Flex-BES	80.57 (47.51 to 113.64)	<0.001
Xience-EES vs. Synergy-EES	63.16 (30.09 to 96.22)	<0.001
Xience-EES vs. Nobori-BES	-13.03 (-46.36 to 20.31)	0.80
Xience-EES vs. Orsiro-SES	9.06 (-24.01 to 42.12)	0.94
Number of platelet aggregation clot per stent by CM		
Xience-EES vs. BioMatrix Flex-BES	7.26 (5.10 to 9.41)	<0.001
Xience-EES vs. Synergy-EES	4.94 (2.79 to 7.10)	<0.001
Xience-EES vs. Nobori-BES	0.26 (-1.90 to 2.41)	0.997
Xience-EES vs. Orsiro-SES	1.79 (-0.36 to 3.94)	0.144
Number of platelet aggregation clot per stent by SEM		
Xience-EES vs. BioMatrix Flex-BES	3.23 (1.26 to 5.19)	<0.001
Xience-EES vs. Synergy-EES	5.39 (3.42 to 7.36)	<0.001
Xience-EES vs. Nobori-BES	0.01 (-1.95 to 1.97)	>0.99
Xience-EES vs. Orsiro-SES	1.89 (-0.08 to 3.86)	0.066

A mean difference >0 denotes greater levels of the parameter in biodegradable polymer-coated DES as compared with Xience-EES.

CI = confidence interval; other abbreviations as in Table 1.

aggregate clots >0.1 mm^2 assessed by SEM was also least for Xience-EES compared with DES with biodegradable polymer coatings (Figure 4, Table 1), with significant differences achieved relative to BioMatrix Flex-BES (mean difference: 3.23, 95% CI: 1.26 to 5.19, $p < 0.001$) and Synergy-EES (mean difference: 5.39, 95% CI: 3.42 to 7.36, $p < 0.001$) (Table 2). Platelet aggregate clots were mostly observed at the point of the curved strut connectors in BioMatrix Flex-BES, whereas for the remaining stents they were typically found at the tip of the crown or near conjunctive sites involving the crown and the link (Figures 2 and 4).

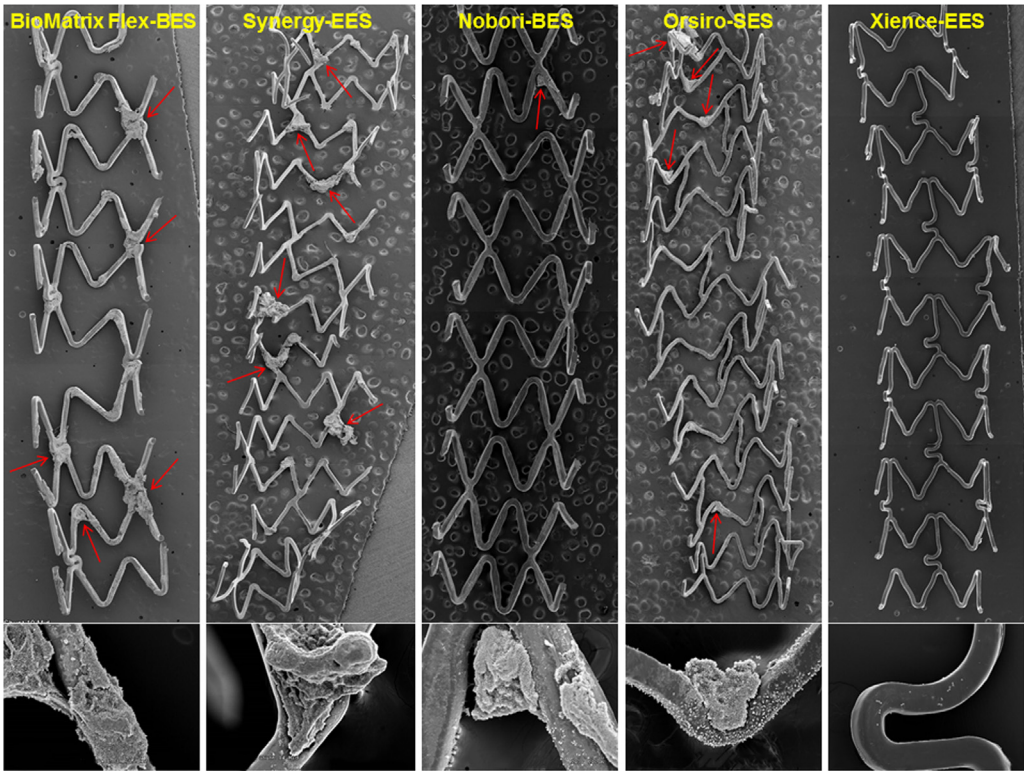
INFLAMMATORY CELL ATTACHMENT TO STRUT SURFACES. Whereas platelet thrombi generally included varying numbers of entrapped inflammatory cells, strut surfaces without thrombi also showed adherent inflammatory cells identified by both SEM and confocal microscopy using DAPI staining (Figures 5 and 6). The number of cell nuclei on strut surfaces was the least for Xience-EES as compared to biodegradable polymer-coated DES, where a significant difference was observed relative to BioMatrix Flex-BES (mean ratio: 4.73, 95% CI: 2.46 to 9.08, $p < 0.001$), Nobori-BES (mean ratio: 5.97, 95% CI: 3.11 to 11.44, $p < 0.001$), and Orsiro-SES (mean ratio: 5.16, 95% CI: 2.69 to 9.91, $p < 0.001$) (Figures 3B and 6).

DISCUSSION

In the present study, acute thrombogenicity with respect to platelet aggregation and inflammatory cell adhesion was compared among contemporary durable (Xience-EES) and biodegradable polymer-coated stents of variable geometry and different choice of rapamycin derivatives using an ex vivo arteriovenous shunt model in swine. With respect to the primary objective of this study, the most salient findings can be denoted as follows. 1) Platelet aggregation as assessed by specific platelet markers was significantly less in Xience-EES than in BioMatrix Flex-BES and Synergy-EES, whereas Nobori-BES and Orsiro-SES showed numerically greater platelet aggregation than Xience-EES without reaching statistical significance. 2) Deposition of acute inflammatory cells as important activators of pro-thrombotic pathways were also significantly less in Xience-EES than in BioMatrix Flex-BES, Nobori-BES, and Orsiro-SES, whereas no significant difference was observed in comparison to Synergy-EES. These findings indicate that the Xience-EES system design exhibits an antithrombotic effect relative to biodegradable polymer-coated DES used in the current study, with reduced acute platelet aggregation and inflammatory cell deposition.

RELEVANCE OF STENT GEOMETRY, POLYMER COATING, AND DRUG. The influence of polymer coating, strut dimensions, and positioning relative to the vessel wall was recently noted as a critical factor in an ex vivo "bench" stent thrombogenicity model (4). In study by Kolandaivelu et al. (4), polymer-coated stents were uniformly less thrombogenic as compared to their BMS counterparts. However, that study exclusively used DES with circumferential durable polymer coating adopted from first- and second-generation FDA-approved DES, and in this respect differs substantially from the current investigation in which both durable and biodegradable polymer DES, with circumferential or abluminal polymer coating were assessed. The circumferentially coated durable fluorinated Xience-EES copolymer (poly *n*-butyl methacrylate and vinylidene fluoride and hexafluoropropylene) exhibited substantially less platelet aggregation than did most contemporary

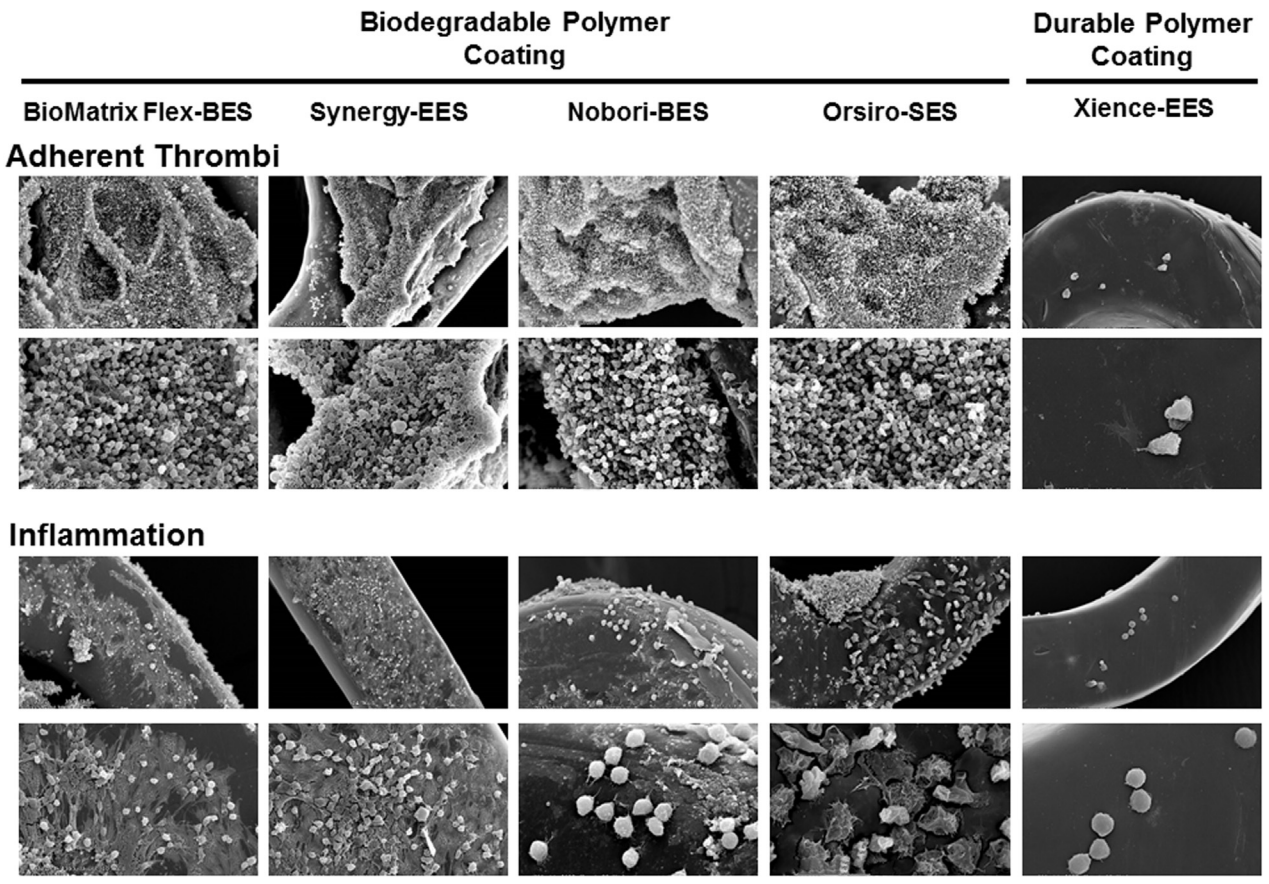
FIGURE 4 Representative SEM Images of BioMatrix Flex-BES, Synergy-EES, Nobori-BES, Orsiro-SES, and Xience-EES in a Swine Shunt Model



Low- (15×) and high- (200×) power images of scanning electron microscopy showing least platelet aggregation clot formation **(red arrows)** on Xience-EES as compared with the other 4 CE-marked biodegradable polymer-coated DES. Abbreviations as in Figures 1 and 2.

biodegradable stent coatings used in the current study. Furthermore, the current study used an arteriovenous porcine shunt model in contrast to the study by Kolandaivelu et al. (4), which implemented a modified Chandler loop system to study acute thrombogenicity and therefore is less likely to resemble flow conditions observed in clinical applications. However, a general statement that all durable polymers are less thrombogenic than biodegradable stent coatings are cannot be derived from the current study and should not be expected as the current study also showed substantial differences among the group of biodegradable stent coatings. To this extent, BioMatrix Flex-BES and Nobori-BES, which share similar metal composition, strut thickness, and polymer/drug coating technology, demonstrated differences in thrombogenicity, indicating factors other than the above-mentioned ones are critical to platelet aggregation and acute thrombosis. One remaining difference between Nobori-BES and BioMatrix Flex-BES pertains to an ultra-thin nondegradable parylene C coating in the former sandwiched between the stent and the abluminally coated biodegradable poly-DL lactic acid polymer, which may have contributed to the different outcomes of this study (11). The design intent of the parylene C is to improve coating integrity as in vitro study with SEM assessment showed poor coating integrity of BioMatrix Flex-BES (12), which may have also affected the difference in thrombogenicity between the 2 stents. The design platform is also substantially different, whereas the curved strut connector in BioMatrix Flex-BES to improve flexibility and trackability could present a nidus for platelet aggregation and thrombosis. The impact of differential stent design on thrombogenicity is likely attributable to the difference in flow condition and is supported by previous work in this field (4) and the observation of the current study that greater platelet aggregates were found within the link connectors of BioMatrix

FIGURE 5 Representative SEM Images of Durable and Biodegradable Polymer-Coated DES Implants From 1 H Ex Vivo Shunts in Swine

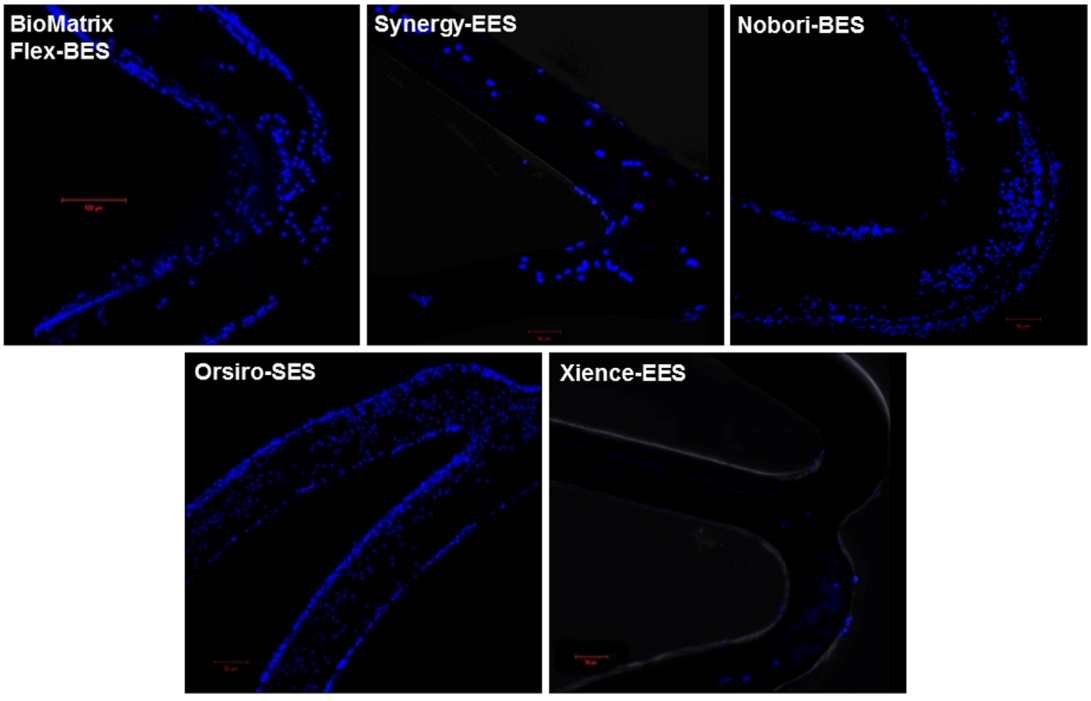


Upper pair of rows shows adherent thrombi whereas the **lower rows** represent inflammation (original magnification: upper and lower rows 600× and 2,000×, respectively).

Flex-BES. It has been shown in the past that strut thickness exerts an impact on pre-clinical and clinical outcomes of DES (13,14). As both BioMatrix Flex-BES and Nobori-BES share greater strut thickness than Xience-EES does, it seems likely that this highly relevant factor contributed to the overall study findings. Nevertheless, increased platelet aggregation in Synergy-EES (74 μm) and Orsiro-SES (60 μm) demonstrated that the advantages such as reduced strut thickness may be counterbalanced by other factors involved in acute thrombus formation. Rapamycin and its derivatives have been shown to inhibit platelet function via a mammalian target of rapamycin, or mTOR, inhibition pathway (15,16). A difference between circumferential polymer-coated DES, whether of durable or biodegradable polymers, versus abluminal polymer-coated DES is the active release of drug from the strut sidewall and luminal surfaces contacting the blood. This may be another factor influencing the platelet behavior with these circumferential versus abluminal polymer-coated DES.

PATHOLOGIC FINDINGS IN THE SETTING OF ACUTE STENT THROMBOSIS. ST manifesting within 30 days remains a clinically relevant issue complicated by increased mortality and morbidity, despite the introduction of newer-generation DES (1,2). Underlying clinical considerations of early ST include patient demographics, lesion characteristics, and procedural factors (17,18), whereas assessment of acute ST cases at autopsy from patients presenting with acute coronary syndromes indicate morphologic features such as necrotic core prolapse, medial tear, and incomplete stent apposition (3) as most relevant. Whereas a direct association with the main

FIGURE 6 Representative Confocal Images of Durable and Biodegradable Polymer-Coated DES Implants From 1 H Ex Vivo Shunts in Swine



Nuclei stained with 4′,6-diamindino-2-phenylindole represent adherent inflammatory cells. Abbreviations as in Figures 1 and 2.

findings of the current study is difficult to establish, the relevance of suboptimal stent implantation resulting in deteriorating flow conditions has been exemplified in our recent autopsy study and many previous clinical trials (19,20). An additional important factor contributing to early ST in autopsy cases of patients presenting with acute coronary syndromes pertains to the presence of a highly thrombogenic underlying necrotic core, which, in the case of sudden exposure to altered arterial blood flow, amplifies pro-thrombotic pathways irrespective of stent design or polymer choice. Therefore, it is important to understand that experimental studies such as the current one can only address individual components contributing to acute ST but cannot reproduce the complexity of human disease conditions.

COMPARISON WITH CLINICAL DATA. The low prevalence of early ST in Xience-EES versus the first-generation DES has recently been reported in clinical trials (5,6). Data from 13 randomized clinical trials demonstrated that EES had significantly reduced ST, target lesion revascularization, and myocardial infarction as compared to other stents (21). In a network meta-analysis by Palmerini et al. (5), Xience-EES showed significantly lower rates of definite ST than did BMS, as well as to first- and other second-generation DES at 1 and 2 years. Even in the setting of acute ST-segment elevation myocardial infarction, EES showed reduced rates of acute and subacute ST compared with those for the Multi-Link Vision BMS (Medtronic, Minneapolis, Minnesota). This reinforces the experimental findings that, in general, polymeric coatings may provide a protective barrier against acute thrombus formation. When directly compared with DES using biodegradable polymer coating, the COMPARE II (Comparison of the Everolimus Eluting With the Biolimus A9 Eluting Stent) and NEXT (Nobori Biolimus-Eluting Versus Xience/Promus Everolimus-Eluting Stent Trial) studies reported a substantial reduction in the risk of ST in Xience-EES as compared to Nobori-BES despite not reaching statistical significance at 1-year follow-up (22,23), which is in clear agreement with the findings of the current study. Considering that durable polymer coatings have been shown to reduce thrombogenicity as

compared with BMS (4), abluminal polymer coating (BioMatrix Flex-BES and Synergy-EES) may have a disadvantage in terms of platelet aggregation because of exposure of bare-metal on the luminal surface of the stents.

INFLAMMATION CONTRIBUTING TO ACUTE THROMBUS FORMATION. Platelet aggregation on the surface of stent struts is accompanied by recruitment of circulating leukocytes consisting mainly of neutrophils and monocytes, which can be regarded as an acute response to vascular injury following stent placement (24,25). Although arterial thrombosis is widely considered to arise from the exposure of atherosclerotic plaque-derived tissue factor, the role of leukocyte-born tissue factor in the initiation of this cascade has recently been demonstrated in an experimental study on human neutrophils and monocytes (26). In this study, human leukocytes were shown to be an important source of tissue factor, which may predispose atherosclerotic plaques or stented coronary arteries to arterial thrombosis in the absence of excessive tissue damage. Furthermore, it has elegantly been demonstrated that leukocyte-derived tissue factor can be transferred to platelets, thereby providing them with a source to propagate arterial thrombus formation (27). In addition to the pro-coagulatory pathways stimulated by release of tissue factor by monocytes, neutrophils have also been shown to contribute to arterial thrombosis in a cathepsin-dependent manner (28). These experimental observations give rise to the question whether polymeric durable coatings such as the one used on Xience-EES may also have important anti-inflammatory effects that amplify the acute protective function against clot formation.

Previous human autopsy studies have shown that inflammation characterized by macrophage infiltration is associated with increased neointimal thickness (29), whereas the involvement of neutrophils in the induction of smooth muscle cell proliferation has also been reported (30). In the current ex vivo shunt study, numerous inflammatory cells accompanied sites of thrombus formation, and they were also observed on the surface of stent struts in the absence of thrombus. Interestingly, not only abluminal polymer-coated BioMatrix Flex-BES, Synergy-EES, and Nobori-BES, but also circumferential polymer-coated Orsiro-SES showed significantly greater inflammatory cell attachment on the stent surface as compared to Xience-EES. Orsiro-SES has a hybrid coating with passive PROBIO amorphous silicon carbide (Biotronik) and active BIOlute high molecular weight poly-L-lactide acid coating (Biotronik), whereas fluorinated copolymer of Xience-EES consists of poly *n*-butyl methacrylate/vinylidene fluoride and hexafluoropropylene copolymer. Fluorinated surface coatings for Dacron grafts have been implied to reduce platelet activation and thrombogenicity, decrease inflammatory response, and promote faster endothelialization and healing, which are all desirable characteristics of stent coatings (31). Our results confirm the known benefits of fluoropolymer in reducing thrombogenicity and inflammatory cell attachment.

STUDY LIMITATIONS. The results from an ex vivo shunt model without antiplatelet agents cannot be directly applied to diseased arteries in living patients who are generally on dual antiplatelet therapy. Nevertheless, it should be noted that complex features of diseased arteries in humans generally do not allow us to directly evaluate an impact of individual stents on acute thrombogenicity, because multiple factors can influence thrombogenicity in humans, which cannot be fully adjusted. We believe that the current experiments with similar conditions across different conduits are appropriate models to compare acute thrombogenicity in individual stents. The predisposition toward blood coagulation may vary between animals, although we have carefully monitored and evaluated blood coagulation as well as platelet function during and after the experiment.

CONCLUSIONS

The permanent fluoropolymer-coated Xience-EES showed significantly lower acute thrombogenicity than did biodegradable polymer-coated BioMatrix Flex-BES and Synergy-EES, and Xience-EES also showed less inflammatory cell attachment than did BioMatrix Flex-BES, Nobori-BES, and Orsiro-SES, in a porcine arteriovenous shunt model. These findings highlight the existence of an acute protective effect of Xience-EES against thrombus formation.

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REPRINT REQUESTS AND CORRESPONDENCE: Dr. Michael Joner, CVPath Institute, Inc., 19 Firstfield Road, Gaithersburg, Maryland 20878. E-mail: mjoner@cvpath.org.

PERSPECTIVES

WHAT IS KNOWN? Early stent thrombogenicity is influenced by several factors including underlying plaque morphologies, stent design, choice of polymer coating, and drugs. Previous experimental studies in modified Chandler loops have shown that durable polymer-coated stents with thin struts exhibit less acute thrombogenicity with respect to platelet aggregation than do uncoated stents or stents with thicker struts. It remains unclear whether relevant differences exist with respect to acute thrombogenicity, particularly between current commercial stent designs using permanent polymers and those using biodegradable polymers.

WHAT IS NEW? We demonstrate that the permanent fluoropolymer-coated Xience-EES exhibits decreased acute thrombogenicity relative to contemporary DES with biodegradable polymer coatings with less platelet aggregation and inflammatory cell attachment in an ex vivo swine shunt model. Our data support the clinical findings of decreased thrombotic events in Xience-EES.

WHAT IS NEXT? Acute thrombogenicity in contemporary DES needs to be further evaluated in vivo. Furthermore, the prevalence of early stent thrombosis in contemporary DES needs to be investigated in large randomized clinical trials, especially in patients with complex coronary artery disease.

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KEY WORDS coronary disease, pathology, stents, thrombosis

APPENDIX For a supplemental table and figure, please see the online version of this paper.