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Comparison of Acute Thrombogenicity and Albumin Adsorption in Three Different Durable Polymer Coronary Drug-Eluting Stents

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Disclosure

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Abstract

Aims: This study sought to compare thromboresistance and albumin binding capacity of different durable polymer DESs using dedicated preclinical and in vitro models.

Methods and Results: In an *ex vivo* swine arterio-venous shunt model, fluoropolymer everolimus-eluting stent (FP-EES) (n=14) was compared with two durable polymer DES (BioLinx polymer coated zotarolimus-eluting stent (BL-ZES)(n=9) and a CarboSil® elastomer polymer coated ridaforolimus-eluting stent (EP-RES)(n=6)), and bare metal stents (BMS)(n=10). Stents underwent immunostaining using a cocktail of anti-platelet antibodies and a marker for inflammation and then were evaluated by confocal microscopy (CM). Albumin retention was assessed by using a flow loop model with labeled human serum albumin [FP-EES(n=8), BL-ZES(n=4), EP-RES(n=4), and BMS(n=7)] and scanned by CM. The area of platelet adherence (normalized to total stent surface area) was lower in order of FP-EES (9.8%), BL-ZES (32.7%),

EP-RES (87.6%) and BMS (202.0%) and inflammatory cell densities was least for FP-EES<BL-ZES<EP-RES<BMS. Although nearly full coverage by albumin binding was shown for all durable polymer DES, FP-EES showed significantly greater intensity of albumin as compared to BL-ZES, EP-RES and BMS (FP-EES;79.0%, BL-ZES;13.2%, EP-RES;6.1%, BMS;1.5%).

Conclusions: These results suggest that thromboresistance, and albumin retention vary by polymer type and that these differences might result in different suitability for short-term dual antiplatelet therapy.

Keywords: ACS/NSTE-ACS, Stable Angina, Stent thrombosis

Condensed abstract

This study compared both thromboresistance and albumin retention of three durable polymer DESs using an *ex vivo* swine arterio-venous shunt model and a flow loop model with fluorescently labeled human serum albumin, respectively. XIENCE FP-EES showed greater thromboresistance relative to other durable polymer DESs such as Resolute Onyx and EluNIR. All durable polymer DESs showed more albumin retention than BMS. However, FP-EES had greater intensity of albumin retention relative to other durable polymer DESs, suggesting a mechanism for its greater thromboresistance.

Abbreviations

BMS= bare metal stent

BL-ZES= BioLinx polymer coated zotarolimus-eluting stent

CM= confocal microscopy

EP-RES= CarboSil elastomer polymer coated ridaforolimus-eluting stent

FP-EES= fluoropolymer everolimus-eluting stent

DAPT= dual antiplatelet therapy

DES= drug-eluting stent

HAS= human albumin serum

PBMA= poly n-butyl methacrylate

ST= stent thrombosis

Introduction

Stent thrombosis (ST) continues to be one of the most feared complications after percutaneous coronary interventions[1]. Thus, thromboresistance is an ideal property for coronary stents. Previous data suggest polymeric coatings influence blood-materials interactions depending upon their adsorbing capacity with regards to specific plasma proteins which drive the stent's interaction with platelets and leukocytes, potentially leading to cell adhesion, activation and thrombosis[2-4]. For biomaterials, it has been proposed that surfaces with high levels of albumin adsorption are desired as albumin could passivate the polymer surface by preventing more reactive components, such as platelets and fibrinogen, from binding[5]. It is well known that fluorinated polymers such as poly(vinylidene fluoride-*co*-hexafluoropropylene) (PVDF-HFP) used in both the CoCr-everolimus-eluting stents (Abbott Vascular, Santa Clara, CA) and the platinum chromium everolimus-eluting stent (Boston Scientific, Natick, MA) possess this reaction[4]. While it is generally believed that polymeric coating, regardless of type, have some anti-thrombotic effect compared to bare metal surfaces, the relative effect of different durable polymers used in coronary

DESs have never been examined with respect to thrombogenicity and albumin adsorption and retention.

Here we compared the relative thromboresistance of three currently available durable polymer DES with distinct coatings using an *ex vivo* porcine shunt model and their albumin binding capacity to understand the association between albumin adsorption and thromboresistance for each polymeric DES (i.e. fluoropolymer everolimus-eluting stent (FP-EES, Abbott Vascular, Santa Clara, CA), BioLinx polymer coated zotarolimus-eluting stent (BL-ZES, Medtronic, Minneapolis, MN) and CarboSil® elastomer polymer coated ridaforolimus-eluting stent (EP-RES, Cordis, Milpitas, CA).

Methods

Detailed methods are described in Supplemental Materials.

Test devices

The test arm for 3 different studies was Xience Alpine (Abbott Vascular, Santa Clara, CA) (FP-EES). Comparators included two contemporary durable polymer DES: 1) BL-ZES which is coated with a mixture of hydrophilic C10 polymer, hydrophilic C19 polymer, and polyvinylpyrrolidone (PVP) (BioLinx polymer) and 2) EP-RES which is coated with a blend of CarboSil® and PBMA. Multi-Link 8 BMS (Abbott Vascular, Santa Clara, CA) served as the third comparator. Table 1 lists experimental models and devices, and Supplemental Table 1 shows the description of all devices.

Swine *ex vivo* arteriovenous shunt-model

For assessment of acute thrombogenicity and inflammation, platelet (CD61/CD42b), monocytes (CD14) and neutrophils (PM-1) were evaluated by confocal microscopy[3]. For quantification of platelet aggregation, the areas of positive staining within each stented segment were measured by ZEN software (Zeiss ZEN 2012, Carl-Zeiss Microscopy, Jena, Germany). The number of inflammatory cells were manually counted and expressed as cell density (cells/mm^2) relative to strut surface area.

Albumin

Preparation of stents

Stents were cut longitudinally to make uniform lengths (6-7 mm) and inserted into a bottomless channel slide used for perfusion application (sticky-Slide I Luer 0.8mm channel height, Cat. No. 80198, Ibidi, GmbH) (**Supplemental Figure 1A and B**).

Albumin *In vitro* Flow Loop

Labeled human albumin was circulated through silicone tubing in line with the Ibidi slide using a perfusion pump. The first 30 minutes under flow was for albumin binding (**Supplemental Figure 1C**), and the second 30 minutes under flow was for washing with FluoroBrite^{TM} DMEM (**Supplemental Figure 1D**). Albumin binding was imaged only during latter phases because of background noise generated by dissolved fluorescent proteins during the first 30 minutes.

Randomly selected regions of interest from one different area of each stent were scanned. In addition, the entire area of each stent was scanned by confocal microscopy and quantified by ZEN software. High and low signals of fluorescence were defined in each experiment to assess all stents in the same condition. Each image was recorded at 1-minute intervals.

Assessment of albumin adherence to varying stent surfaces

The positive area of albumin labeling (green channel) was quantitated within defined regions of interest. Intensity scale was defined as average of total light intensity of green signal from every pixel by the strut area. The analysis was performed every 1 min, and the covered strut area was quantified and expressed as percent albumin coverage (%) and intensity scale of albumin.

A real-time manner using human platelet *in vitro* flow loop

In addition, human platelet adherence to stent surfaces was evaluated in a real-time manner using an *in vitro* flow loop live-cell assay to determine the temporal distribution of platelet adherence.

Statistical analysis

In an *ex vivo* shunt model and an albumin flow loop model, nested generalized linear mixed models with Dunnett's correction for multiple testing were employed in order to investigate group differences in consideration of multiple measurements per individual. Within these models, stent type was considered as fixed effect, while the experimental factor variables animal or experiment, shunt number and linear position were considered as nested random effects. Values are expressed as estimated mean with 95% confidence interval. The analyses were performed with SPSS Advanced Statistics Version 25 (IBM, Armonk, New York). The statistical tests were 2-tailed and a value of $p<0.05$ was considered to indicate statistical significance

Results

Acute Thrombogenicity in a Porcine Arteriovenous Shunt model

There was no evidence of blood coagulation and platelet function abnormalities in any of the animals studied. Additionally, the measures of coagulation were similar in all shunt runs (**See Supplemental Table 2**).

Table 2 summarizes the results of platelet adhesion by CM. **Figure 1** shows representative CM images with immunofluorescent staining against dual platelet markers (CD61/42b) in FP-EES, BL-ZES, EP-RES and BMS. When total platelet fluorescence area was normalized to stent surface area, platelet adhesion was lower in order of FP-EES, BL-ZES, EP-RES and BMS. **Table 2** lists the results of immunofluorescent staining against neutrophil marker (PM-1) and monocyte marker (CD14) by CM and **Figure 2** showed the representative images from each group. Cell density of PM-1 was the least for FP-EES < BL-ZES < BMS < EP-RES. Similarly, cell density of CD14 was lower in order of FP-EES, BL-ZES, BMS and EP-RES.

Albumin absorption and retention in a flow loop model using HSA

Amount of fluorescent albumin on BMS surface increased over time and there were significant differences between times 0 and 30 min and between 30 min and 48 hours (Supplement Figure 2). Also, BMSs with 48-hour exposure to albumin showed significantly less amount of platelet attachment as compared to BMS with no exposure to albumin **(Supplement Figure 3 and Supplemental Table 3). Table 3** summarizes the results of albumin coverage by CM, and **Figure 3** shows representative CM images with immunofluorescent albumin in FP-EES, BL-ZES, EP-RES and BMS. **Figure 4** shows representative quantitated images in all stents as analyzed by ZEN software. When total albumin fluorescence area was normalized to stent surface area, an area of total albumin coverage was significantly greater in FP-EES, BL-ZES and EP-RES than in BMS,

whereas there were no significant differences in area between FP-EES, BL-ZES and EP-RES. However, the area of albumin coverage as determined by high signal intensity was higher in FP-EES versus all other stents. Although FP-EES, BL-ZES and EP-RES showed nearly complete albumin coverage on the stent struts, majority of sampled areas showed high albumin signal intensity in FP-EES, whereas it was low signal intensity in BL-ZES and EP-RES (**Figure 4**).

A real-time analysis over the 30-minute washing phase also showed that coverage of albumin was significantly higher in FP-EES, BL-ZES and EP-RES relative to BMS (**Supplemental Table 4, Figure 5**, **See the online video 1**). Intensity of albumin, indicative of a higher amount of adsorption, was higher in order of FP-EES, BL-ZES, EP-RES and BMS. All stents did not show a significant decrease in albumin retention over the 30min washing phase (**Supplemental Table 5**). Albumin intensity negatively correlated with platelet attachment in shunt model **(Supplemental Figure 4).**

Platelet adhesion in a real-time flow loop model using human platelets

A novel *in vitro* flow loop model using freshly isolated fluorescently labeled human platelets from healthy volunteers was constructed. Stents were exposed to circulating platelets, imaged and analyzed by CM over the course of 60 minutes. Consistent with the shunt study, the amount of platelet aggregation was numerically least in FP-EES, followed by BL-ZES and EP-RES and then BMS (n=3 per group), although there were non-significant differences between each **(See supplemental Table 6 and Supplemental Figure 5 and the online video 2).** Platelet adhesion in the flow model nicely correlated with that in the shunt model **(Supplemental Figure 6).**

Discussion

The current study evaluated acute thrombogenicity and albumin retention in currently available durable polymer DESs. The main findings of this preclinical study were as follows; (1) anti-thrombotic and anti-inflammatory reactions were greater in order of FP-EES, BL-ZES, EP-RES and BMS in an *ex vivo* porcine shunt model; (2) albumin coverage was greater in all durable polymer DES relative to BMS, and (3) albumin intensity indicating concentration of albumin was significantly the greatest in FP-EES, followed by BL-ZES, EP-RES and the least was BMS. Taken together, these data suggest durable polymer coatings provide some level of thromboresistance compared to BMS, which may be the mechanism by which polymeric coating to some extent have thromboresistant properties. From this standpoint, durable polymers used on DESs are not equivalent.

Different durable polymer coating DES in an *ex vivo* shunt model

This shunt study suggests polymeric coating provide a protective barrier against acute thrombus formation. Clinical data also suggest a protective effect of polymeric coated stents in patients with coronary artery diseases[6]. Among durable polymer DESs, fluoropolymer EES showed the strongest thromboresistance. BL-ZES showed some degree of thromboresistance when compared with BMS as the control. This suggests that the BioLinx polymer confers some ability to avoid platelet adhesion. The outer surface of the polymer consists of hydrophilic polyvinyl-pyrrolidinone which inhibited monocyte adhesion in one study[7] and, according to our study, allows for some degree of albumin adsorption. On the other hand, PBMA/CarboSil® coating showed comparable thrombogenicity to BMS. Previous *in vitro* testing showed initially good adsorption of albumin to PBMA polymer with very little retention after detergent washing and a relatively

low albumin/fibrinogen binding ratio compared to the PVDF-HPF fluoropolymer[4]. These data support the observation that even if durable polymers are applied, these polymers show differing individual potential with respect to thrombogenicity even in the current era.

Platelet aggregation on the surface of stent struts exists along with circulating leukocytes consisting mainly of neutrophils and monocytes[8]. Moreover, leukocyte tissue factors are transferred to platelets, promoting propagation of thrombus[9, 10]. In current study, density of inflammatory cells on the surface of struts is consistent with the amount of platelet adherence to the stent surfaces.

Albumin binding and retention in different polymer coating in flow loop models

Albumin has been traditionally used as a passivating protein. High albumin retention on the surface of the strut might be a beneficial mechanism to prevent a thrombogenic profile. The albumin-rich layer which tempers host-material interactions and competitively inhibits the adhesion of fibrinogen and von Willebrand factor [4, 11]. However, in an in-vivo setting, surface adsorption is a reversible process and the composition of adsorbed proteins may change over time, a phenomenon known as the Vroman effect [12]. Albumin can be replaced with other proteins leading to thrombosis such as von Willebrand factor and fibrinogen etc. However, absorptivity between albumin and stent surfaces may vary to a great extent based on materials. In fact, previous preclinical studies showed various capability of keeping albumin on stent surfaces by different materials after washing by sodium dodecyl sulfate [4]. Previously, Szott et al. reported that fluoropolymer can retain non-significantly more albumin on the stent surface relative to PBMA and polystyrene-b-polyisobutylene-b-polystyrene (Kaneka, Japan; SIBS-1 and SIBS-2) even after using sodium dodecyl sulfate, which is supposed to detach albumin from surface of struts. In

addition to differences in albumin retention, the superior thromboresistance of FP-EES was shown. Each stent provides different amount of albumin adsorption and level of affinity of albumin on the stent surface appears to affect thrombogenicity.

Application to short dual antiplatelet therapy (DAPT)

Differences in intrinsic thrombogenicity between stents is important when considering shortening DAPT therapy as stent struts may not be fully covered by endothelium at the time DAPT is discontinued. Bare struts, especially those which do not possess intrinsic thromboresistance, may become a nidus for thrombus formation. Superior thromboresistant features of polymers may contribute to short duration of DAPT (i.e., < 3 months).

In multicenter prospective trials using standard strategy for DAPT duration, the rate of ST was very low (0.1-0.6%) and comparable in different types of current DESs [6, 13-15]. However, information about the rate of ST after short DAPT strategy (i.e. 1-3 months) is limited. To date, a few studies are available where 1 month duration of DAPT duration was evaluated[16]. The LEADERS FREE trial was a landmark trial of 1-month DAPT whereby polymer-free DES was compared with BMS with the same 1-month DAPT protocol. However, this study showed high rate of ST in both group due to thicker thickness and bare surface although it also suggested it is feasible to perform short duration of DAPT after DES. More recently, the STOP DAPT II trial also showed feasibility of 1-month DAPT after implantation of FP-EES when comparing to 12-month DAPT [17]. The current preclinical study further supports these results. The potential differences in thromboresistance seen within this study for different DES suggests that one-month DAPT duration for different types of DESs may not standardized (regardless of DES type) and that each need to be tested in dedicated clinical trials.

Limitations

There are some limitations in this study. First, this study was performed with a swine *ex vivo* shunt-model without antiplatelet agents and with low dose heparin to evaluate the potential of the material itself. Therefore, this result cannot be simply applied to the clinical setting where patients are treated with mono- or dual-anti-platelet therapy. Second, the drug can affect inflammatory cell adhesion and platelet attachment of the adjacent stent in this model [18]. However, effect of drug on platelet attachment is minimal (**Supplemental Figure 7**). Third, an *in vitro* flow loop model was used in this study to assess human serum albumin adsorption and retention to different DES. Although this cannot completely mimic *in vivo* conditions, we believe the results of these studies simulate *in vivo* conditions since the concentration of albumin used is similar to that in human blood. Fourth, this study focused on albumin since albumin is considered as protein protecting against platelet attachment. Other type of proteins also may play important role in protecting against thrombogenicity. Previous data regarding the fact that fluoropolymer bound greater amounts of albumin as well as fibrinogen sounds counterintuitive given fibrinogen[4] is a knows promoter of clot formation through its conversion to fibrin by thrombin. Some emerging data suggest that albumin has an inhibitory property against fibrin polymerization and this may be one of the mechanisms by which fluoropolymers promote thromboresistance [19]. Further basic research is needed to further understand this relationship. Finally, studying platelet adherence using the in vitro flow loop model is different from that seen in the setting of vascular injury.

Conclusions

Overall, anti-thrombogenicity was better in order of FP-EES, BL-ZES, EP-RES and BMS. Although 3 durable polymer DESs showed greater albumin coverage as compared to BMS,

albumin intensity indicating concentration of albumin was not equivalent in 3 durable polymer DESs. The results of albumin adsorption might be the cause of differences in platelet adhesion seen in the 3 different types of durable polymer DES and BMS although other factors such as the adhesion of leukocytes also likely plays a role. Because we also showed that the anti-proliferative agent loaded onto DES have some thromboresisant properties, it is important to acknowledge the potential contribution of the anti-proliferative agents as contributing to the thromboresistant effects seen of the different DES studied. These experiments may lend insights into the suitability of different durable polymer DES for shortening of DAPT duration.

Impact on daily practice

FP-EES showed greater thromboresistance and concentration of superficial albumin on the stents as compared to other types of durable polymer DESs. These results suggest that there are variations among polymer type in terms of thromboresistance, and albumin binding and retention. These differences may result in different suitability for short-term DAPT.

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Figure Legends

Figure 1. Representative confocal microscopic images using immunofluorescent staining against dual platelet markers (CD61/CD42b) in an *ex vivo* shunt model.

A. Low (upper) and high (lower) power confocal microscopic images showed minimal platelet aggregation in FP-EES, whereas obvious platelet aggregation was observed in BL-ZES, EP-RES and BMS.

B. Graph shows fluorescent positive area per stent surface. Data are presented as mean±standard deviation for each. (∗, $p<0.05$ vs. FP-EES; †, $p<0.05$ vs. BL-ZES, ††, $p<0.05$ vs. EP-RES)

Figure 2. Representative confocal microscopic images using immunofluorescent staining against neutrophil marker (PM-1) and monocyte marker (CD14) in in an *ex vivo* shunt model.

A. The upper panels showed confocal microscopic images with PM-1 staining and the lower panels showed confocal microscopic images with CD-14 staining. FP-EES showed minimal neutrophil and monocyte attachment. However, BL-ZES, EP-RES and BMS showed many neutrophils and monocytes on the stent surfaces.

B. Graphs showed number of PM-1 and CD-14 positive cells on the stent struts, respectively. Number of PM-1 and CD-14 was significantly the least in FP-EES relative to BL-ZES, EP-RES and BMS. Data are presented as mean±standard deviation for each group. (∗, $p<0.05$ vs. FP-EES; †, $p<0.05$ vs. BL-ZES, ††, $p<0.05$ vs. EP-RES)

Figure 3. Representative confocal microscopic images using fluorescent human serum albumin in the flow loop model.

The low (upper) and high (lower) power images showed stent surface fully covered by obvious strong signal of fluorescent albumin in FP-EES. On the other hand, although BL-ZES and EP-RES also showed near complete coverage by albumin, the albumin signal (green) on the surface in BL-ZES and EP-RES were less intense relative to FP-EES. Minimal albumin signal was observed in BMS.

Figure 4. Quantitated images of confocal microscopy with fluorescent human albumin.

A. High signal of fluorescent albumin was colored by yellow, whereas low signal of albumin was colored by gray. FP-EES was fully covered by yellow color, whereas BL-ZES and EP-RES were covered mostly by gray. BMS showed minimal gray color.
B. C. and D. graphs showed high signal of albumin, low signal and total signal in each stent. Data are presented as mean±standard deviation for each group. (∗, $p<0.05$ vs. FP-EES; †, $p<0.05$ vs. BL-ZES, ††, $p<0.05$ vs. EP-RES)

Figure 5. Percent Coverage and Intensity of albumin in each group over 30 min during washing phases.

A. These images showed quantitated coverage and intensity of albumin at the end of washing phases. All durable polymer DES showed nearly full albumin coverage. However, albumin intensity was higher in order of FP-EES, BL-ZES and EP-RES. Coverage and intensity of albumin were minimal in BMS.
B. C. graphs showed estimated mean percent coverage and intensity over 30 min during washing phases, respectively. There were significant differences in FP-EES, BL-ZES, and EP-RES versus. BMS, although significant differences were not observed between FP-EES, BL-ZES and EP-RES. FP-EES the highest intensity, followed by BL-ZES, EP-RES and the least was BMS. Significant differences were seen in all comparisons. In percent coverage and intensity, minimal changes were observed over 30 min.

Table 1. Summary of Animal Model and Stents Tested

Type of study	Thrombogenicity study	Human Albumin study
Model	*Ex vivo* AV shunt model	*In vitro* Flow model
Animal	Porcine	NA
Period	1 Hour	30 min
Number of animals	7	NA
Type of stent		
1	FP-EES (n=14)	FP-EES (n=8)
2	BL-ZES (n=9)	BL-ZES (n=4)
3	EP-RES (n=6)	EP-RES (n=4)
4	BMS (n=10)	BMS (n=7)

AV:arteriovenous, BMS: bare metal stent, BP-ZES: BioLinx-polymer zotarolimus-eluting stent, EP-RES: elastomer polymer ridaforolimus-eluting stent, FP-EES: fluoropolymer everolimus-eluting stent, NA: not applicable.

Table 2. *Ex Vivo* Arteriovenous Shunt Model by Confocal Microscopy

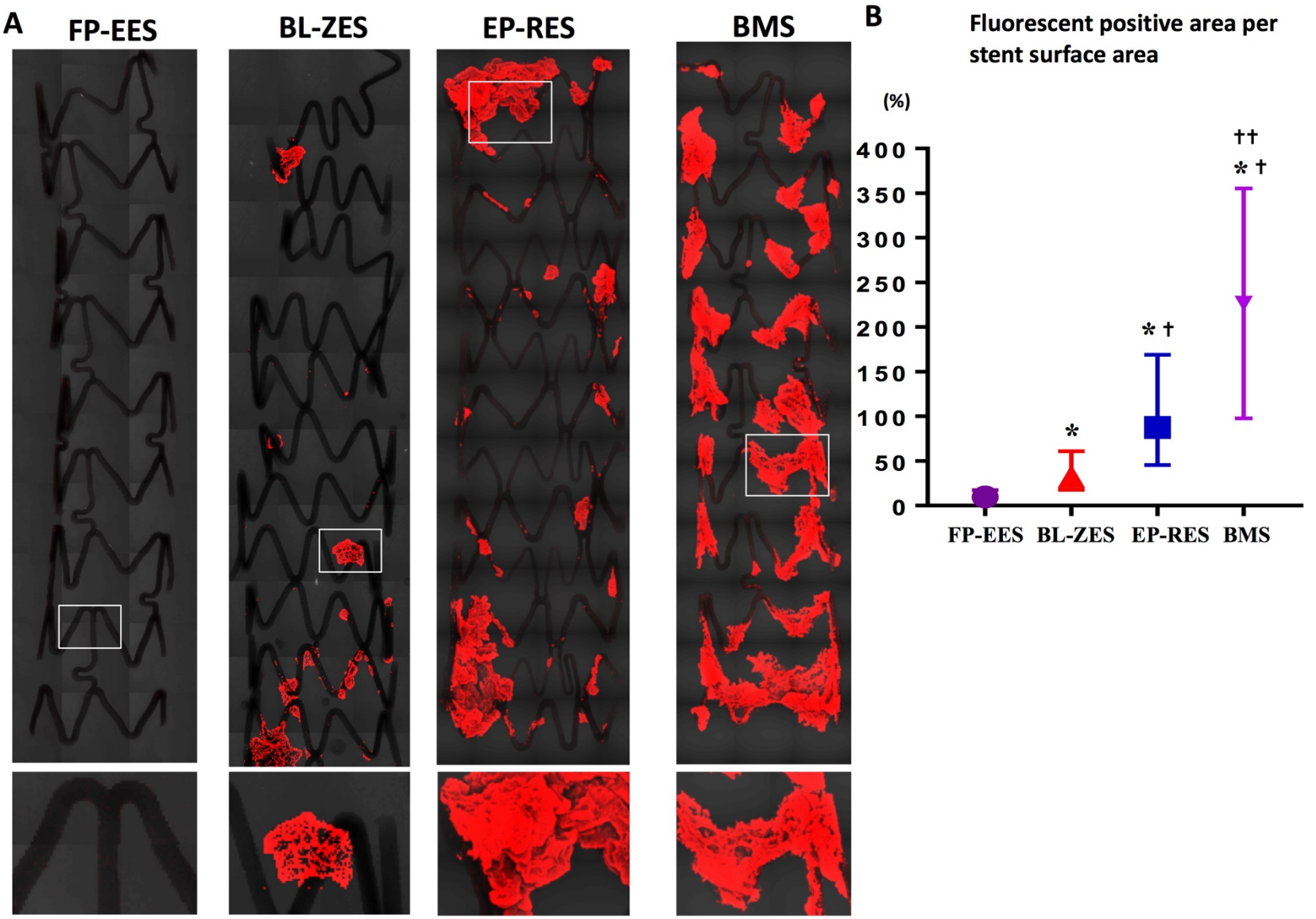
Percent CD42b/CD61 positive area of total stent strut (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	9.8 (8.5-17.3)	<0.01	<0.01	<0.01
BL-ZES	32.7 (17.6-60.8)	NA	0.03	<0.01
EP-RES	87.6 (45.4-168.9)	0.03	NA	0.01
BMS	202.0 (111.5-365.8)	<0.01	0.01	NA
PM-1 Positive cells density (number/mm^2)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	38.1 (29.6-49.0)	<0.01	<0.01	<0.01
BL-ZES	465.8 (340.0-638.1)	NA	0.23	0.64
EP-RES	637.7 (433.7-937.6)	0.23	NA	0.4
BMS	515.5 (382.4-694.9)	0.64	0.4	NA
CD14 Positive cells density (number/mm^2)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	34.9 (25.1-48.7)	<0.01	<0.01	<0.01
BL-ZES	286.9 (189.6-433.7)	NA	0.25	0.83
EP-RES	428.0 (257.9-70.3)	0.25	NA	0.32
BMS	305.2 (206.1-451.9)	0.83	0.32	NA

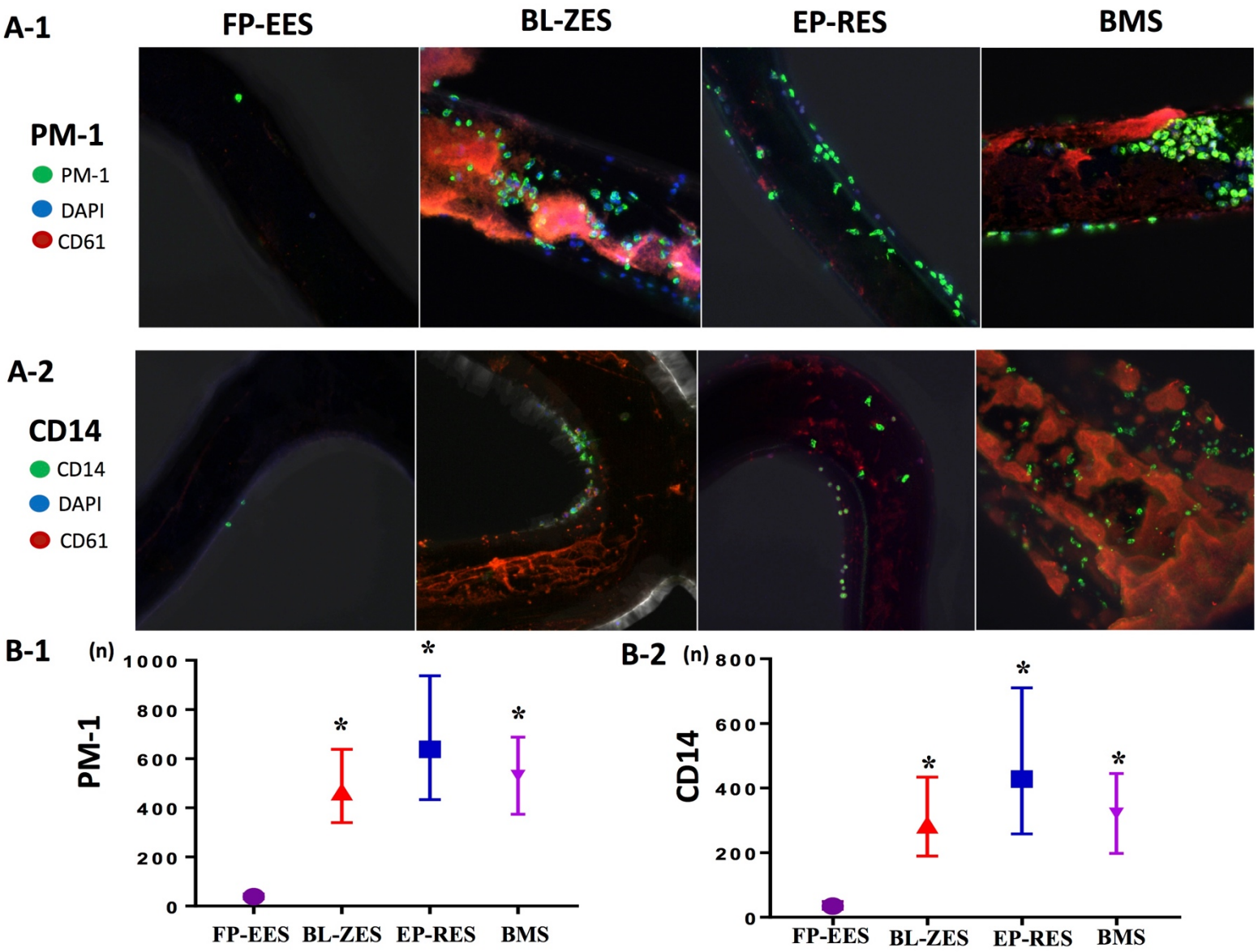
CI: confidential interval. Other abbreviations are same as Table 1.

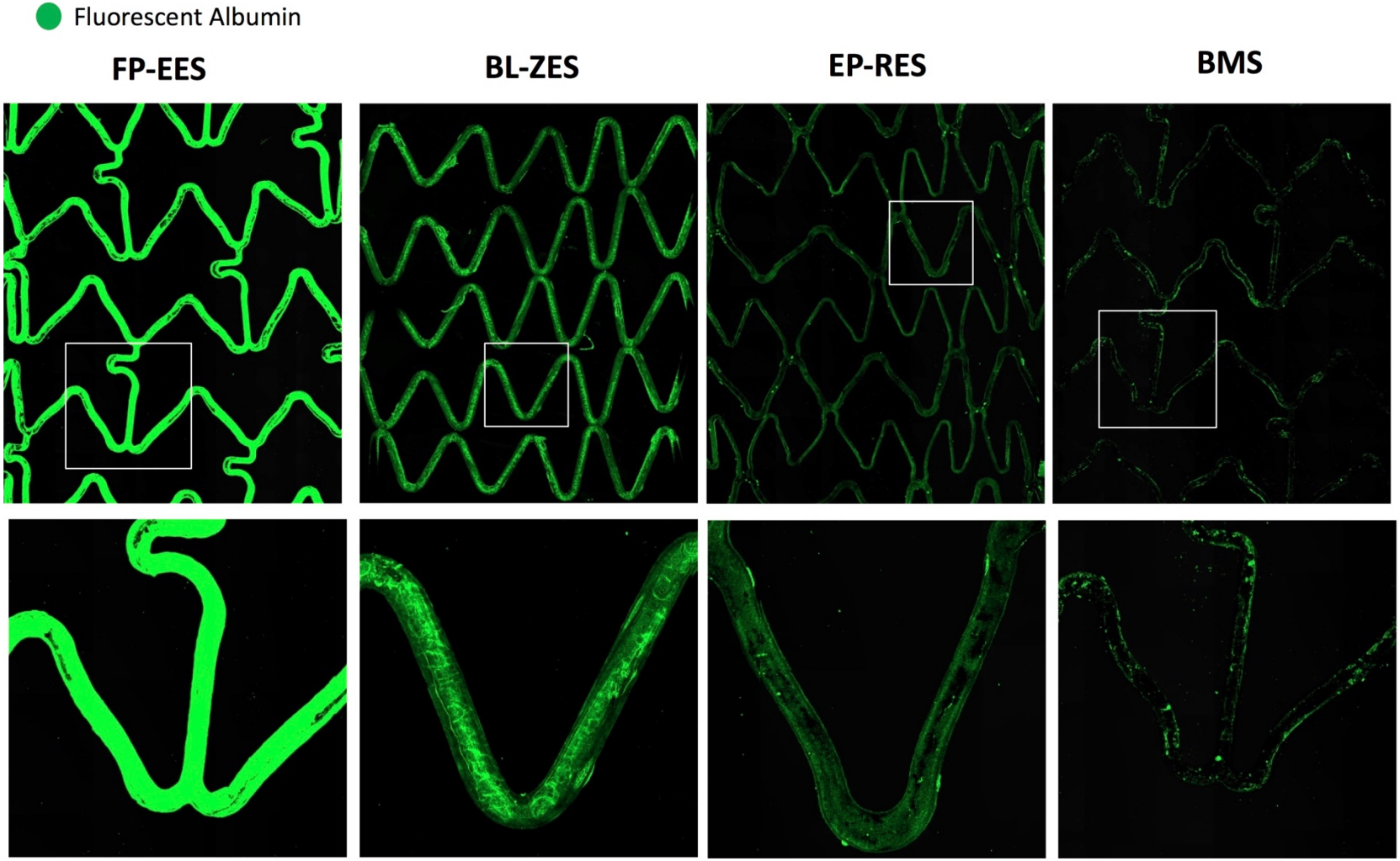
Table 3. Human Albumin Absorption in *In Vitro* Flow Model

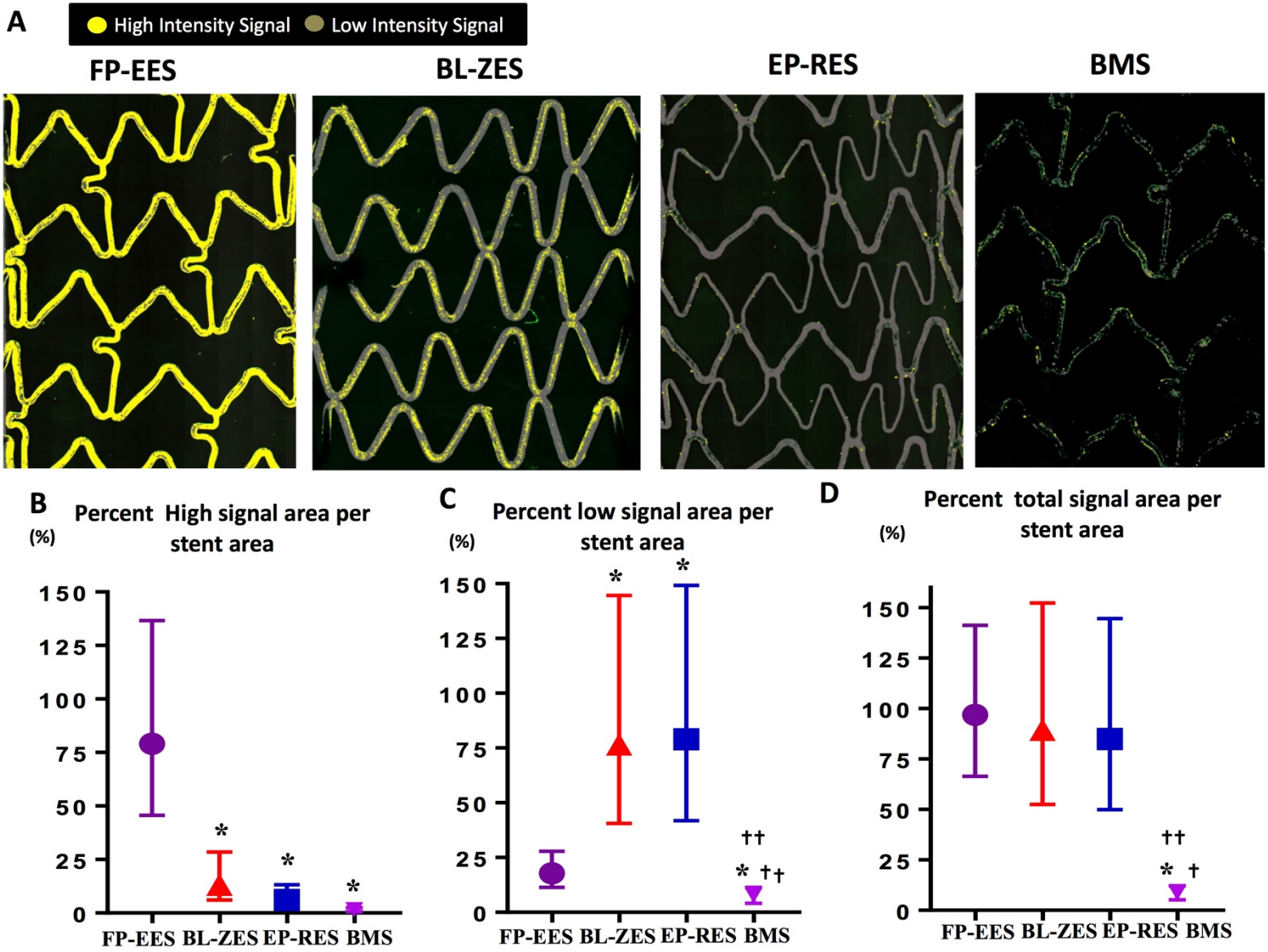
Percent low signal area of total stent strut (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	17.8 (11.3-27.8)	0.02	0.02	0.02
BL-ZES	76.6 (40.6-144.6)	NA	0.94	<0.01
EP-RES	79.0 (41.9-149.3)	0.94	NA	<0.01
BMS	7.2 (4.5-11.7)	<0.01	<0.01	NA
Percent high signal area of total stent strut (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	79.0 (45.7-136.6)	<0.01	<0.01	<0.01
BL-ZES	13.2 (6.1-28.5)	NA	0.20	0.03
EP-RES	6.1 (2.8-13.2)	0.20	NA	0.06
BMS	1.5 (0.8-2.6)	0.03	0.06	NA
Percent total signal area of total stent strut (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	96.9 (66.5-141.3)	0.79	0.67	<0.01
BL-ZES	89.5 (52.5-152.3)	NA	0.89	<0.01
EP-RES	85.0 (49.9-144.7)	0.89	NA	<0.01
BMS	8.3 (5.6-12.4)	<0.01	<0.01	NA

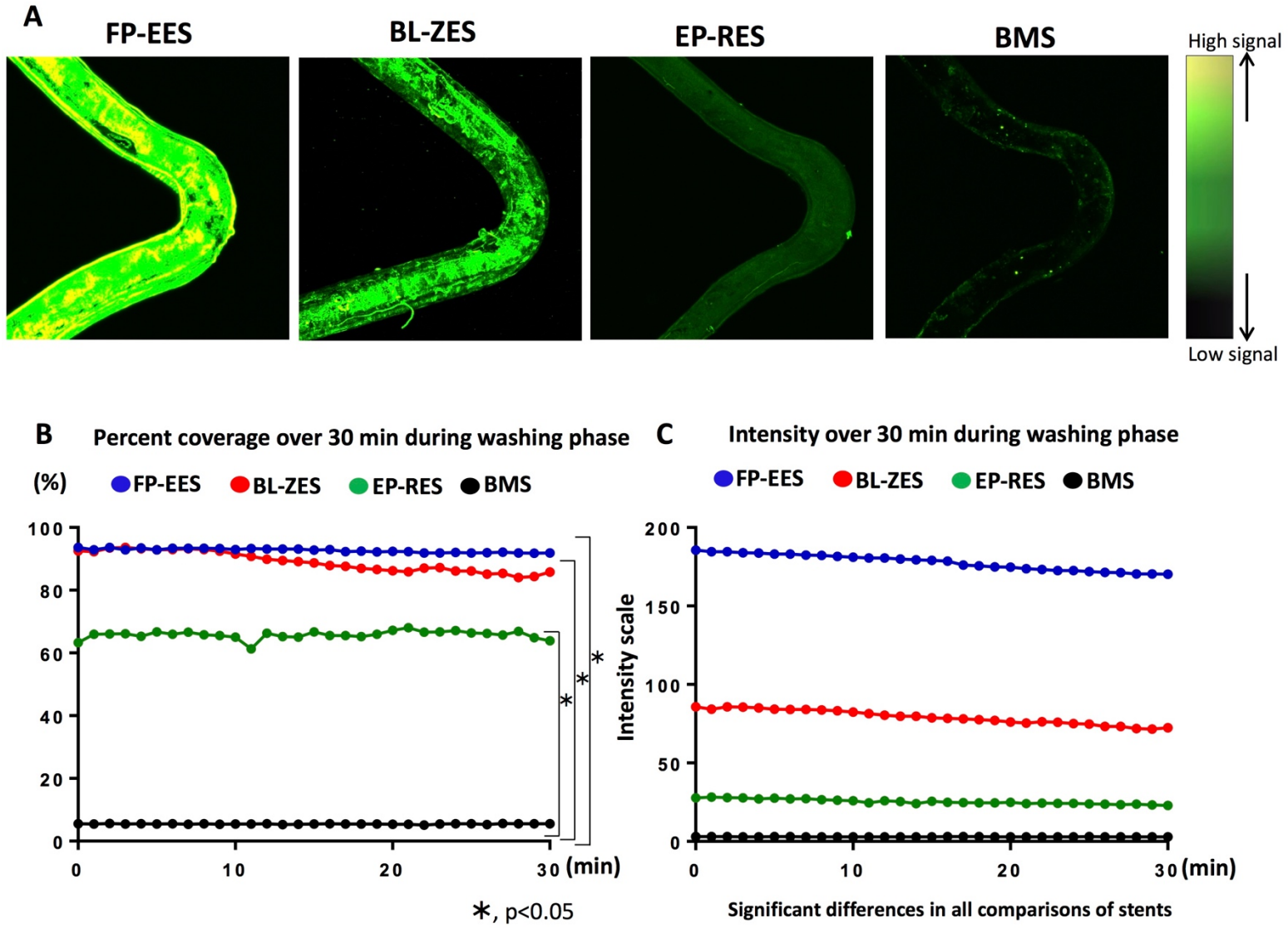
Abbreviations are same as Table 2.











Supplemental Materials

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Supplemental Methods

Supplemental Figure and Movie Legend

Supplemental Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the MedStar Health Research Institute.

Test devices

Using non-commercial stent backbones is ideal to test specific blood material interactions. However, stent designs including polymer, drug, strut thickness and strut platform comprehensively affect thromboresistance. Because using commercial DESs can provide practical information to help in real world practice, commercial DESs were used in this study. Four stents were compared for assessment of acute thrombogenicity and inflammation in an *ex vivo* arteriovenous shunt model and albumin retention and platelet adhesion in an *in vitro* flow loop models using fluorescent human albumin serum (HSA) and human platelets, respectively. In an *ex vivo* shunt study, 3.0 x 12 mm stents for each shunt run were used. In the flow loop models, stents with diameter of 3.0 mm were sectioned to put into slides.

Swine *ex vivo* arteriovenous shunt-model

A total of 42 stents were deployed in 14 shunts from 7 swine for the assessment of acute thrombogenicity and inflammation. A porcine *ex vivo* arteriovenous shunt model involving a test circuit of 3 different in-line test stents was performed for 60 minutes to evaluate platelet adherence, thrombus formation, and acute inflammation on the devices. Each shunt model had 3 different stents and each animal had 2 shunt experiments for a total of n=6 stents per animal.

Low dose intravenous heparin (100 IU/kg) was used to minimize effect of platelet aggregation and thrombus formation on surface of stents in order to examine inherent platelet-mediated thrombus formation induced by each stent type. Target blood activated clotting times

was measured every 20 minutes and blood activated clotting time was kept between 150 and 190s using bolus and maintenance dosing. At the end of each shunt run, stents were gravity perfused with Ringer's Lactate until cleared of blood, fixed in 10% neutral buffered formalin, and longitudinally bisected for dual immunofluorescent staining.

1. Assessment of platelet aggregation and inflammation

In the shunt model, one-half of the stent was stained with dual immunofluorescence of the anti-platelet makers (anti-CD61/CD42b) (anti-CD61: Immunotech, Commerce, CA, and anti-CD42b: sc-7070, Santa Cruz, Dallas, TX) and monocyte maker (CD14, Novus Biological, Littleton, CO). The other stent half was immunostained using the platelet makers (anti-CD61/CD42b) and neutrophil maker (PM-1, BMA Biomedicals, Switzerland; dilution 1:800). For quantification of inflammatory cells, images with regions relatively free of platelet thrombus were obtained.

A real-time manner using human platelet *in vitro* flow loop

1. Preparation of stents

Xience Alpine (FP-EES) (3.0 mm x 12mm, Abbott Vascular, Santa Clara, CA) was compared to Resolute Onyx (BL-ZES) (3.0 mm x 12 mm, Medtronic, Inc., Minneapolis, MN), EluNir (EP-RES) (3.0 mm x 12mm, Cordis, Milpitas, CA) and Vision (3.0 mm x 12 mm, Abbott Vascular, Santa Clara, CA). One stent from each group was used. Stents were expanded at nominal pressure on the bench under sterile conditions, cut longitudinally and transversely to make all of uniform length. Stents were carefully cut in order to fit into sticky slide. If stent tips did not fit into lumen of slide, those tips were discarded and then new stent tips were cut. Stent tips were inserted into a bottomless channel slide used for perfusion applications (sticky-Slide I Luer 0.8mm channel

height, Cat No. 80198, ibidi, GmbH). Each stent was primed with human serum at 37°C before initiating the flow.

2. Preparation of human platelets

Whole blood (60ml) was collected [final concentration: 0.32% sodium citrate (M/V)] by venipuncture from healthy volunteers, none of whom was on any anti-platelet therapy or other regular prescription. The blood was centrifuged for 10 min at 200×g and RT without brake. The supernatant was collected with a plastic pasteur pipette. After collection of the supernatant, the supernatant was centrifuged again for 17 min at 700×g without brake. The lower 1/3rd was platelet rich plasma (PRP) and upper 2/3rd was platelet-poor plasma (PPP). Platelet pellets were formed from the PRP and suspended the in PRP. CMFDA (5-chloromethylfluorescein diacetate) (Molecular Probes, Eugene, OR) at final concentrations of 15μM, was used to label PRP through incubation in the dark for 45 min at 37°C. After labeling PRP, it was centrifuge again for 10 min at 1300×g without brake. At the bottom of the tube, platelet pellets formed. Plasma with exception of platelet pellets was removed and the platelet pellets were suspended in fresh plasma from PPP by gently shaking the tube to a volume of 25-30 ml.

3. *In-Vitro* flow loop and confocal microscopy

The temporal distribution of fluorescent platelets to various stent designs was studied by serial acquisition of confocal images after placement of the ibidi chamber slide/stent positioned on a microscope stage (Zeiss LSM700) equipped with a live-cell incubation chamber and Plan-Apochromat 10×/0.45 objective. Labeled platelets were circulated through silicon tubing (Cole-Palmer, Tygon Silicone Tubing, 1/8"ID x 3/16"OD) at a rate of 8 mL/min (estimated shear stress:

2.0 dyn/cm^2) in line with the ibid slide using a perfusion pump (ISMATIC, |*IPC* Series, Model No. SM931A, Wertheim, Germany). Randomly selected regions of interest (ROIs) from each stent were scanned and exported in tagged image file format (TIFF) micrographs from Zen original images files using software (LSM 700, Zen 2011, Zeiss, Oberkochen, Germany). Each image was recorded at 1-1.5 min intervals, which are dependent on Z-stacks, for 60 mins to create a movie file.

4. Assessment of platelet adherence to varying stent surfaces

The positive area of platelet labeling was analyzed by Nikon NIS-Elements AR 5.02 64-bit within defined regions of interest for first 60 min. The analysis was run on each frame of the time lapse photography (MK). The covered strut area was quantified and expressed as percent of platelet coverage (%). See the methods in the manuscript.

A real-time manner using human albumin *in vitro* flow loop

Albumin is considered as be passivating to platelets. Thromboresistance of fluoropolymer surfaces is believed to be related to their strong affinity for albumin, so called "fluoropassivation". Therefore, this study focused on relative albumin absorption and thromboresistance of FP-EES to other durable polymer DESs.

1. Labeling methods for fluorescent albumin

The FITC variety averaged 1.6 fluoresceins per molecule. Fluorescent molecules react mostly through primary amines, so most likely they were attached to the lysines. Fluorescein was added using isothiocyanate chemistry.

2. Preparation of albumin

HSA was used in 8 experiments (Product code: HSF, Protein Mods, Madison, WI). FluoroBrite^{TM} DMEM (Gibco, Waltham, Massachusetts) was added to HSA of 750 mg to make it 20 ml final volume consistent with the concentration of albumin in human blood (3.5-5.0 g/dL).

3. Albumin *In vitro* Flow Loop

Labeled albumin was circulated through silicone tubing (Cole-Palmer, Tygon Silicone Tubing, 1/8"ID x 3/16"OD) at a rate of 8 mL/min in line with the Ibidi slide using a perfusion pump (ISMATIC, |IPC Series, Model No. SM931A, Wertheim, Germany).

The temporal distribution of fluorescent albumin binding to various stent designs was studied by serial acquisition of confocal images after placement of the Ibidi chamber slide/stent positioned on a microscope stage (Zeiss LSM800) equipped with Plan-Apochromat 10x/0.45 objective.

4. Assessment

Scanned images were exported in tagged image file format micrographs from ZEN original images files using software (LSM 700, Zen 2011, Zeiss, Oberkochen, Germany). Analysis was performed by Nikon NIS-Elements AR 5.02 64-bit. For assessment of the washing phases of albumin, one area was randomly selected from each type of stent. Therefore, selection bias cannot be excluded. However, the selected high-power areas are considered as the representative areas from each type of stents since the assessment using the entire stents showed the same tendency as the assessment using the selected high-power areas.

2. Statistical Analysis

A power calculation

Based upon previous shunt study (*Torii et al. EuroIntervention 2019;14:1685-1693*), we estimated that minimal sample size of 6 stents per group with an expected difference of roughly 30% in terms of endpoint (Percentage of CD42b/CD61 immunofluorescence relative to total scanned stent surface area) with a standard deviation of 20 % would provide 80% power to detect differences between groups using a two-sided alpha of 0.05. Also, based upon preliminary data for albumin study, we estimated that minimal sample size of 4 stents per group with an expected difference of roughly 25% in terms of endpoint (Percent low and higher signal area per stent area) with a standard deviation of 10 % would provide 80% power to detect differences between groups using a two-sided alpha of 0.05.

Supplemental Figure Legend

Supplemental Figure 1. A shows that FP-EES (left), EP-RES (middle) and BMS (right) are inserted into Sticky-Slide I Luer (Ibidi, Germany). B is cross-sectional images at yellow line in A. C shows the model set for imaging by confocal microscopy during an albumin binding phase, whereas D shows the model set during a washing phase.

Supplemental Figure 2. Relationship between exposure-time and amount of albumin on BMS.

A. BMS was exposed to fluorescent albumin to confirm whether exposure-time could play a role in increasing amount of albumin surface of stents. Three different time-points were evaluated (0, 30 min and 48 hours) and each time-point group had 3 BMSs. Two pre-defined areas from each stent were analyzed (n=6 for each time-point group). Upper panels show low-power images of BMSs with albumin signal, while lower panels show high-power images. B. Amount of fluorescent albumin significantly increased over time. (between 0 min vs. 30 min; p=0.013, between 30 min vs. 48 hours; p=0.009, between 0 min vs. 48 hours; p=0.0004). Data was analyzed by Turkey's multiple comparisons test taking into account each pair.

Supplemental Figure 3. Protection of Albumin against Platelet Attachment on Surface of BMS.

A. Labeled human platelets were measured by confocal microscopy in BMS with 48-hour exposure to fluorescent albumin versus BMS not exposed to albumin. Each group had 3 BMSs and 2 pre-defined areas of high-power images were analyzed (n=6 high power regions per group). Upper panels show low-power images of BMSs with platelet signals, while lower panels are high-power images on a representative individual strut. B. BMS with 48 hour exposure to albumin showed significantly lower platelet attachment than those with no exposure ($p<0.0001$). Upper graph shows percent coverage of platelet from low-power images and data were expressed as

estimated mean and 95% confidential interval. Lower graph shows percent coverage of platelet from high-power images and data were expressed as mean and interquartile.

Supplemental Figure 4. Relationship between platelet adhesion, albumin retention and intensity.

Albumin intensity negatively correlated with platelet adhesion in the shunt model. BMS: bare metal stent, BP-ZES: BioLinx-polymer zotarolimus-eluting stent, EP-RES: elastomer polymer ridaforolimus-eluting stent, FP-EES: fluoropolymer everolimus-eluting stent. Data were expressed as median and interquartile range.

Supplemental Figure 5. Percent of platelet coverage in the flow model.

Platelet aggregation in the flow model was tested in FP-EES, BL-ZES, EP-RES and BMS (n=3 each). Using generalized linear mixed models, FP-EES had numerically less platelet aggregation amongst the four stents tested, followed by BL-ZES, BMS and the greatest platelet aggregation was seen in in EP-RES, although there was not a significant different between each group. BMS: bare metal stent, BP-ZES: BioLinx-polymer zotarolimus-eluting stent, EP-RES: elastomer polymer ridaforolimus-eluting stent, FP-EES: fluoropolymer everolimus-eluting stent.

Supplemental Figure 6. Percent of platelet positive area in the shunt and flow loop models.

Two studies using the shunt and flow loop models (60 min) consistently showed the least platelet aggregation in FP-EES followed by BL-ZES. EP-RES or BMS had the most attachment of platelets in both types of experiments. Data was expressed as median and interquartile range.

Supplemental Figure 7. Comparison of platelet attachment between BMS without drug and with drug (100nM) in the flow model.

We compared everolimus 0nM with 100 nM (n=3 for each) in order to investigate effect of drug on platelet attachment. The drug dose of everolimus was determined based on previous our study (Harari E, et al. ATVB 2018 38, 2217-2224). There were significant differences between both groups at 10, 20, 30, 40 min however, percent of platelet coverage did not differ between both at 50 and 60 min. BMS, bare metal stent; NS, not significant. Data was expressed as median and interquartile range.

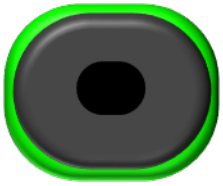
Supplemental Movie Legend

Online Video Legends

Online Video 1. Real-time albumin retention by percent coverage and intensity over 30 minutes. The top movies show percent coverage and intensity of albumin analyzed by Nikon NIS-Elements AR 5.02 64-bit within defined regions of interest in each group. The bottom movies show graph of percent coverage and intensity corresponding to each group one-to-one.

Online Video 2. Real-time platelet adhesion over 60 minutes. The top movies show positive area of platelet analyzed by Nikon NIS-Elements AR 5.02 64-bit within defined regions of interest in each group. The bottom movies show graph of strut coverage by platelets corresponding to each group one-to-one.

Supplemental Table 1. All device descriptions.

Device	Xience Xpedition	Resolute Onyx	EluNIR	Vison
Shape				
Platform	MULTI-LINK 8	Onyx	-	MULTI-LINK 8
Material	CoCr	CoNi / Ptlr Core	CoCr	CoCr
Strut thickness	81 µm	81 µm	87 µm	81 µm
Drug type	Everolimus	Zotarolimus	Ridaforolimus	-
Drug dose	100 µg/cm^2	1.6 µg/cm^2	1.1 µg/mm2	-
Materials of the polymer	PBMA/PVDF-HFP	BioLink polymer	PBMA/CarboSl® 20 55D	-
Coating type	Circumferential	Circumferential	Circumferential	-
Coating thickness	7-8 µm / side	6 µm / slide	7 µm	-

Supplemental Table 2. Summary of the Mean Values from All Animal's Blood Coagulation (PT, PPT), Platelet Quantification (platelet counts, platelet EST), Platelet Function (LTA), and Activated Clotting Time (ACT) in *Ex vivo* Swine Acute Shunt Model

Test	Mean (Min - Max)			Fold Change (%)		
	Baseline	After 1st Shunt	After 2nd Shunt	1st Shunt vs. Baseline	2nd Shunt vs. Baseline	2nd Shunt vs. 1st Shunt
Prothrombin Time (seconds)	9.2 (8.0 – 10.7)	10.1 (9.9 – 13.8)	11.9 (10.71 – 15.40)	1.22	1.30	1.07
PPT (seconds)	11.4 (9.1 – 13.2)	69.6 (33.6 – 100)	84.6 (50 – 100)	6.11	7.42	1.22
Platelet Count (x1000/µL)	231.8 (103– 472)	253.2 (87 – 297)	233.9 (83 – 401)	1.09	1.01	0.92
Platelet EST	Adequate	Adequate	Adequate	N/A	N/A	N/A
LTA (ADP = 20uM) (% platelet aggregation)	58.7 (33 – 99)	33.0 (12 – 51)	23.2 (4 – 44)	0.56	0.39	0.70
LTA (ADP = 5µM) % Platelet Aggregation	32.7 (11 – 41)	12.8 (3 – 31)	10.6 (4 – 39)	0.39	0.32	0.83
ACT (seconds)	102.14 (97 – 114)	165.20 (143 – 189)	172.42 (131 – 204)	1.62	1.69	1.04

Platelet EST is considered to be adequate when the platelet count was estimated to be within the reference interval.

ADP = adenosine diphosphate; LTA = light transmission aggregometry; PPT = partial thromboplastin time.

Supplemental Table 3. Percent of platelet coverage on bare meteal stent surface at 60 minutes in *In Vitro* Flow Model.

Group	48-Hour exposure to albumin	No exposure to albumin	P value
Low power (%)	0.45 (0.27-0.77)	51.89 (43.08-62.50)	<0.0001
High power (%)	4.07 (1.93-8.58)	37.73 (34.74-40.98)	<0.0001

Analyzed by GLMM (generalized linear mixed models). Data was expressed as estimated mean and 95% confidential interval.

Supplemental Table 4. Human Albumin Retention over 30 min during Washing Phase in *In Vitro* Flow Model.

Percent coverage of albumin at 0 min (%)				
		P value		
Stent types	Estimated mean (95% CI)	vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	93.6 (70.8-123.7)	0.96	0.09	<0.01
BL-ZES	92.6 (62.4-137.3)	NA	0.18	<0.01
EP-RES	63.3 (42.7-93.9)	0.18	NA	<0.01
BMS	5.5 (4.1-7.5)	<0.01	<0.01	NA
Percent coverage of albumin at 30 min (%)				
		P value		
Stent types	Estimated mean (95% CI)	vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	91.9 (68.3-123.6)	0.78	0.14	<0.01
BL-ZES	85.8 (56.4-130.5)	NA	0.32	<0.01
EP-RES	63.9 (42.0-97.2)	0.32	NA	<0.01
BMS	5.5 (4.0-7.6)	<0.01	<0.01	NA
Intensity scale of albumin at 0 min				
		P value		
Stent types	Estimated mean (95% CI)	vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	185.5 (137.5-250.4)	<0.01	<0.01	<0.01
BL-ZES	85.7 (56.2-130.7)	NA	<0.01	<0.01
EP-RES	27.7 (18.2-42.2)	<0.01	NA	<0.01
BMS	3.1 (2.2-4.2)	<0.01	<0.01	NA
Intensity scale of albumin at 30 min				
		P value		
Stent types	Estimated mean (95% CI)	vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	170.2 (122.3-236.9)	<0.01	<0.01	<0.01
BL-ZES	72.5 (45.4-115.7)	NA	<0.01	<0.01
EP-RES	22.9 (14.3-36.5)	<0.01	NA	<0.01
BMS	3.0 (2.1-4.3)	<0.01	<0.01	NA

BMS: bare metal stent, BP-ZES: BioLinx-polymer zotarolimus-eluting stent, CI: confidencial interval, EP-RES: elastomer polymer ridaforolimus-eluting stent, FP-EES: fluoropolymer everolimus-eluting stent, NA: not applicable.

Supplemental Table 5. Human Albumin Retention over 30 min during Washing Phase in *In Vitro* Flow Model.

Percent difference of coverage between 0 to 30 min (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	3.2 (1.35-7.66)	0.24	0.35	0.10
BL-ZES	14.1 (3.58-55.4)	NA	0.57	0.94
EP-RES	8.3 (2.1-32.5)	0.57	NA	0.43
BMS	15.0 (5.7-39.4)	0.94	0.43	NA
Percent difference of Intensity scale between 0 to 30 min (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	10.4 (6.3-17.2)	0.27	0.24	0.79
BL-ZES	17.2 (8.9-33.5)	NA	0.79	0.21
EP-RES	19.7 (9.1-42.5)	0.79	NA	0.19
BMS	9.4 (5.5-16.3)	0.21	0.19	NA

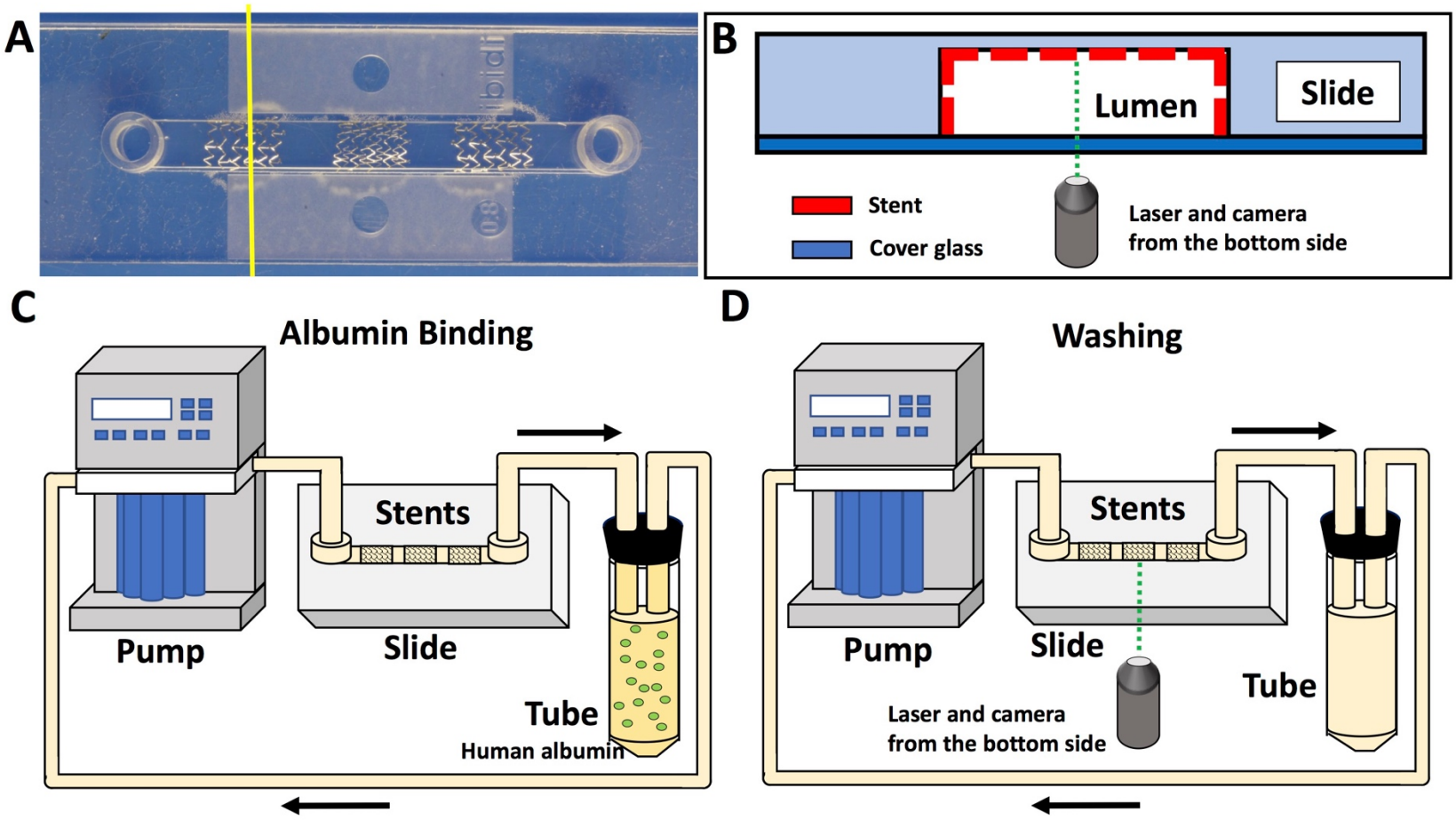
Abbreviations are same as Supplemental Table 4.

Supplemental Table 6. Percent of platelet coverage at 60 minutes in *In Vitro* Flow Model.

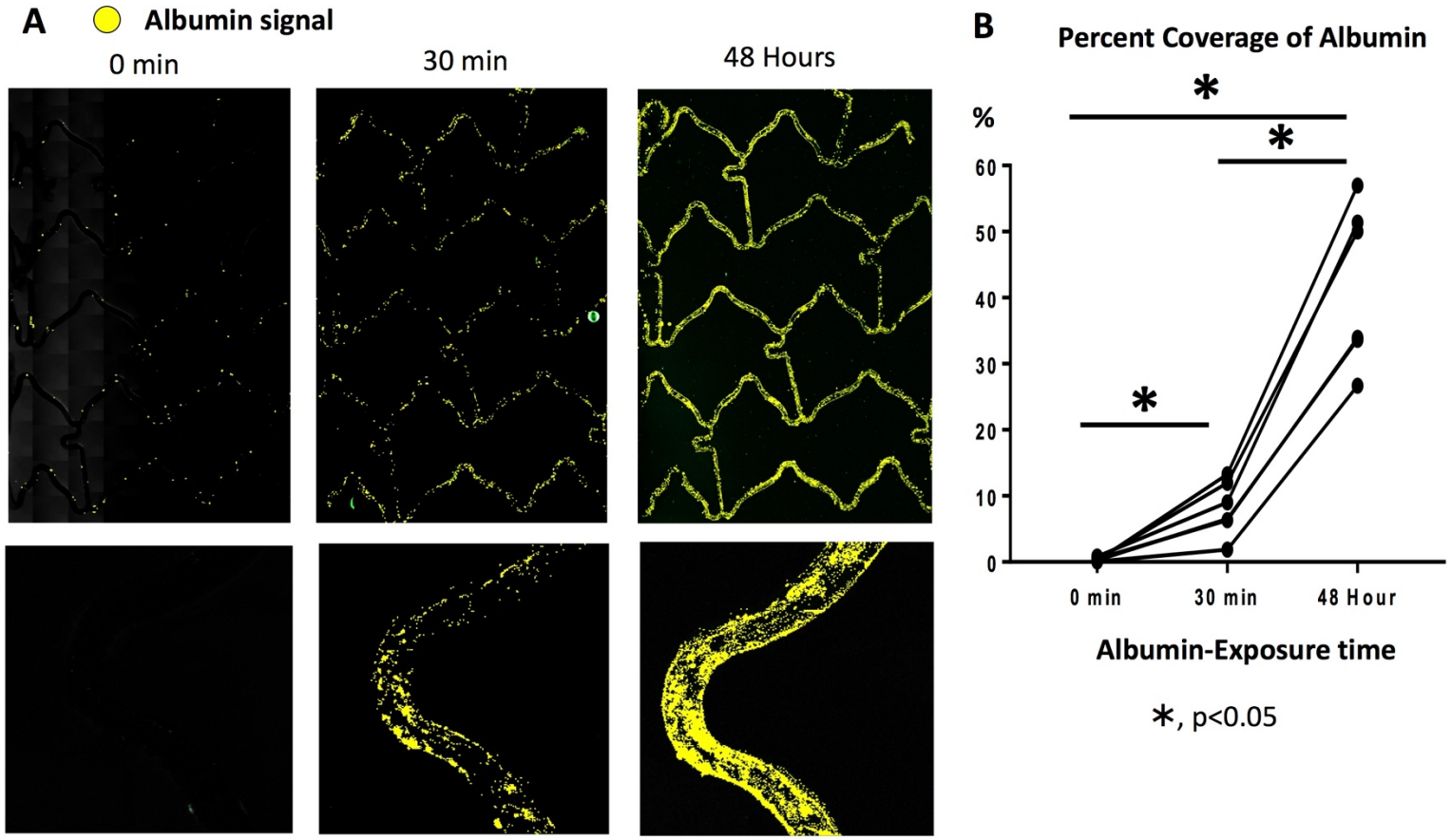
Percent of platelet coverage at 60 min (%)				
Stent types	Estimated mean (95% CI)	P value		
		vs. BL-ZES	vs. EP-RES	vs. BMS
FP-EES	6.76 (2.49-18.37)	0.12	0.11	0.11
BL-ZES	25.63 (8.99-73.10)	NA	0.95	0.97
EP-RES	26.42 (9.630-72.48)	0.95	NA	0.91
BMS	25.24 (9.20-69.25)	0.97	0.91	NA

Abbreviations are same as Supplemental Table 4. Analyzed by GLMM (generalized linear mixed models)

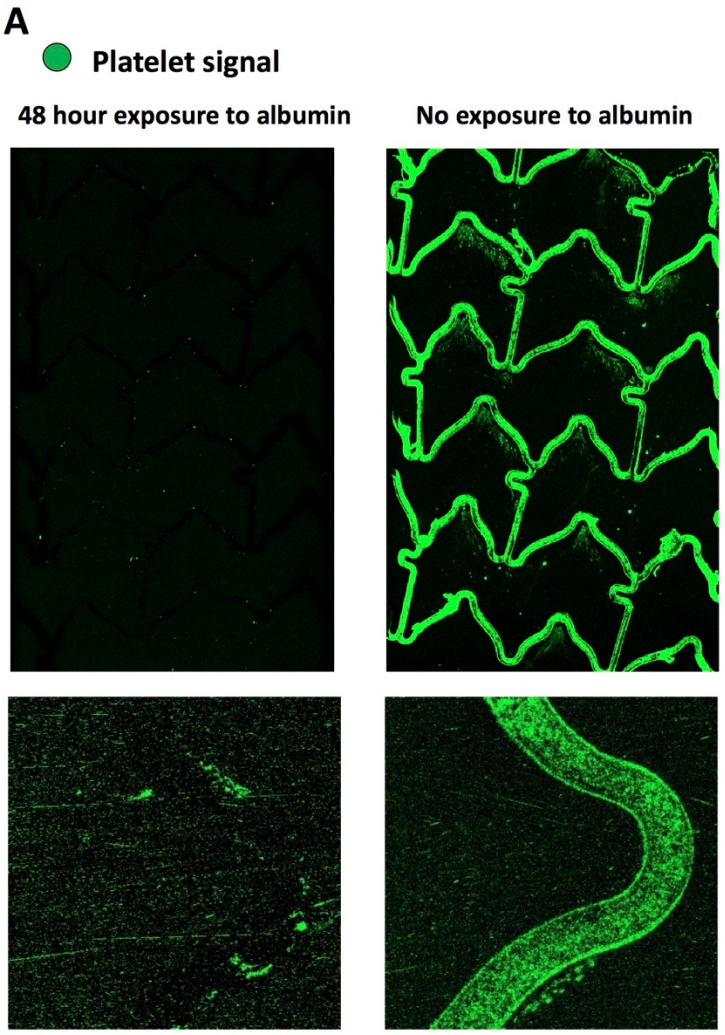
Supplemental Figure 1

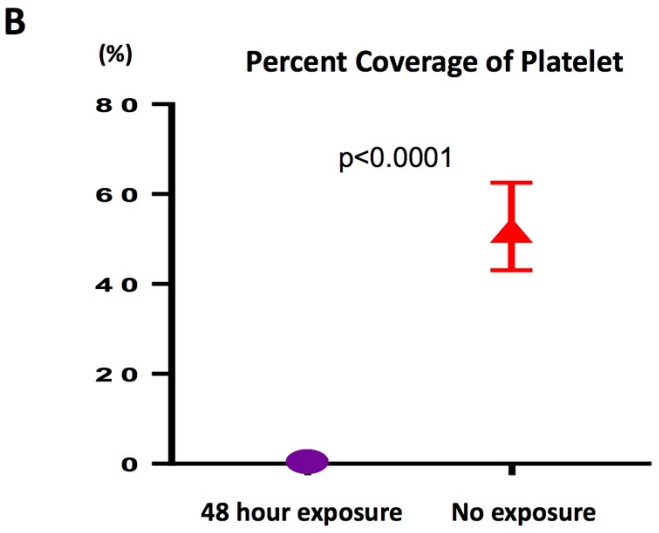


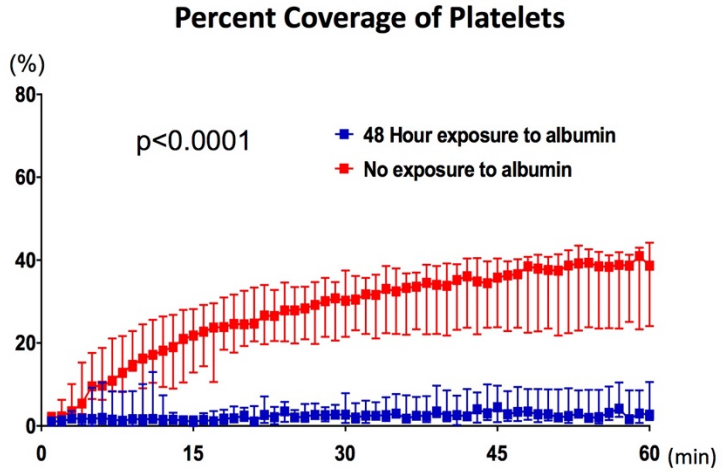
Supplemental Figure 2



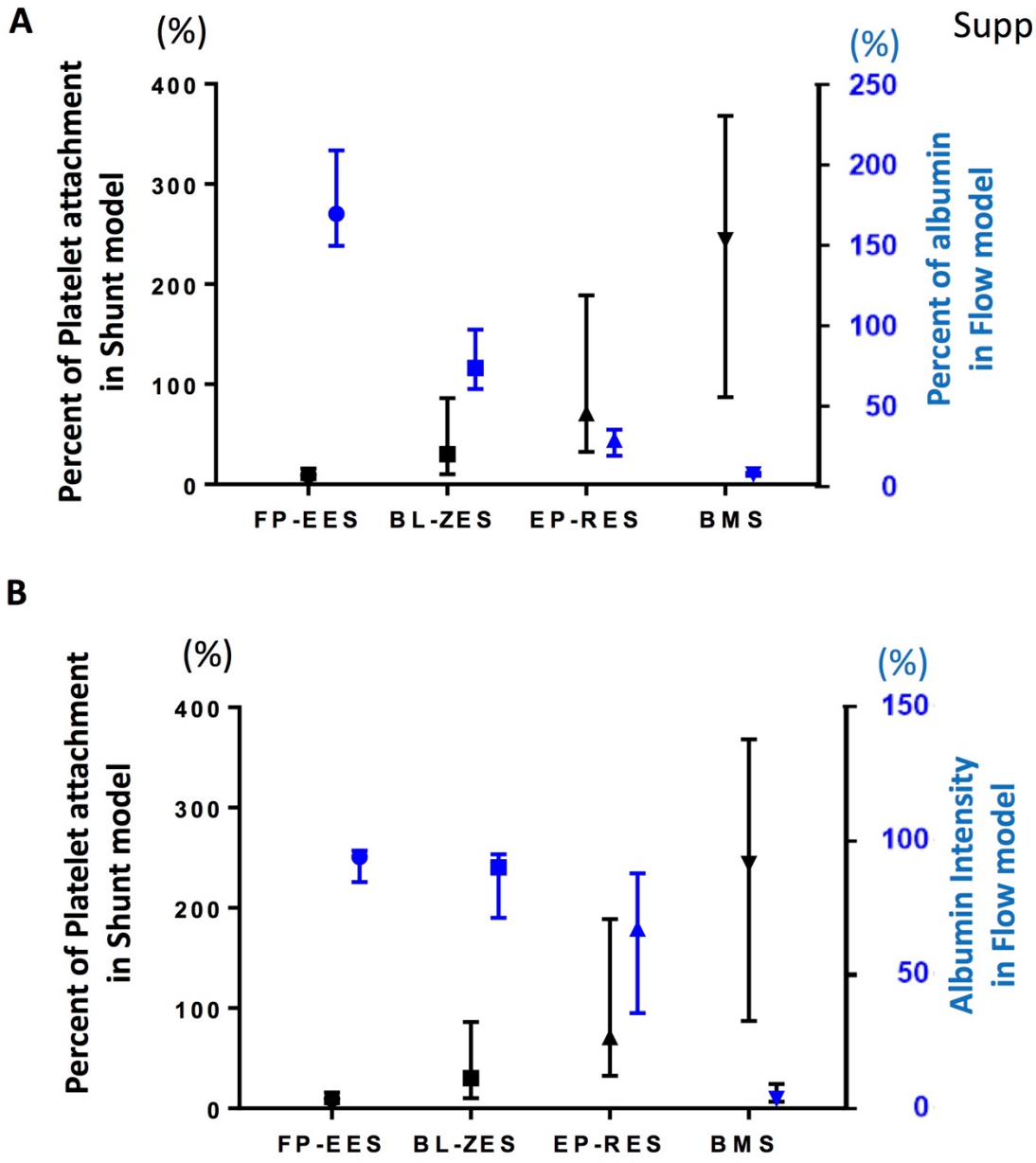
Supplemental Figure 3



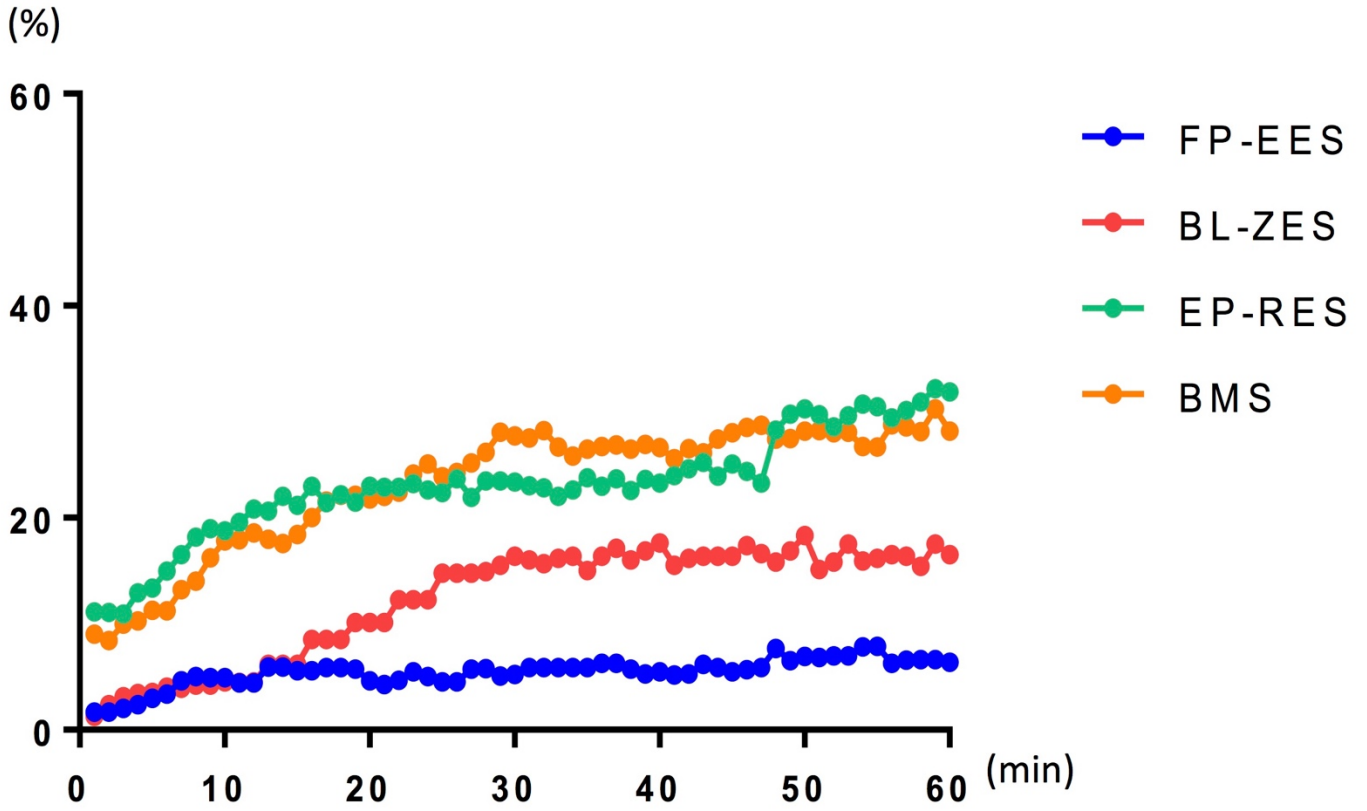




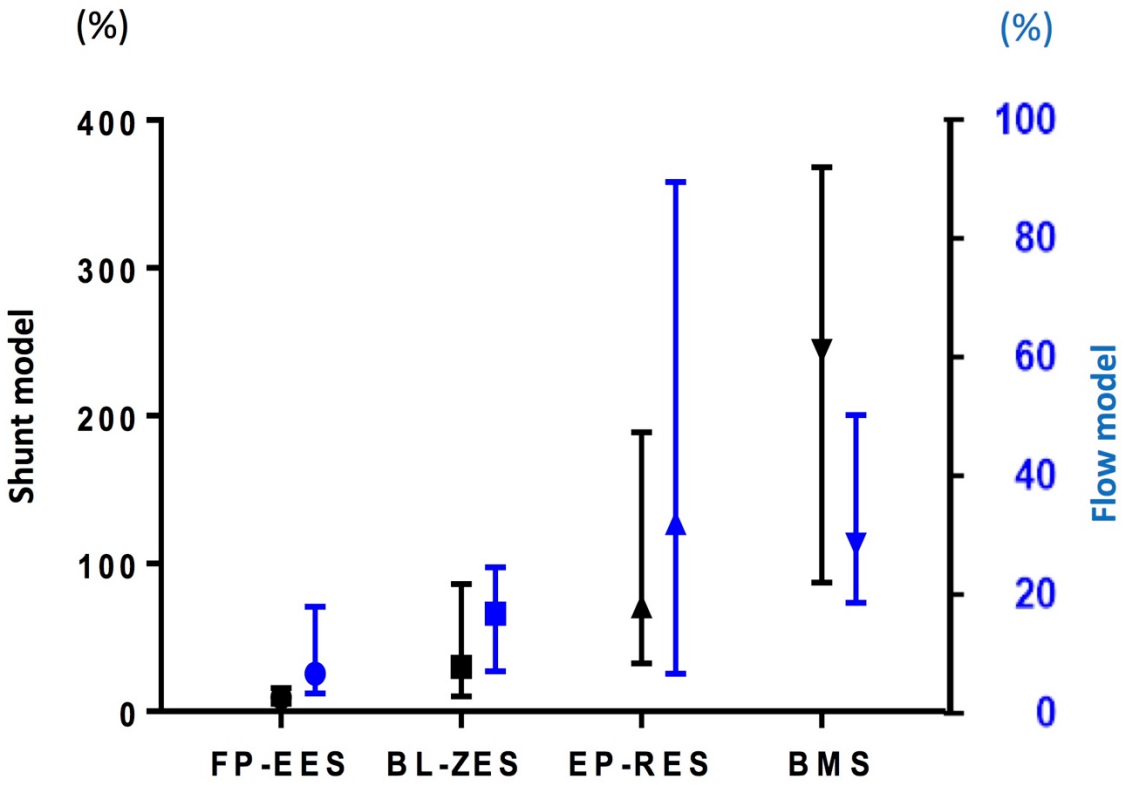
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7