

Thrombogenicity assessment of Pipeline, Pipeline Shield, Derivo and P64 flow diverters in an in vitro pulsatile flow human blood loop model

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ABSTRACT

Flow diversion is a disruptive technology for the treatment of intracranial aneurysms. However, these intraluminal devices pose a risk for thromboembolic complications despite dual antiplatelet therapy. We report the thrombogenic potential of the following flow diversion devices measured experimentally in a novel human blood in-vitro pulsatile flow loop model: Pipeline™ Flex Embolization Device (Pipeline), Pipeline™ Flex Embolization Device with Shield Technology™ (Pipeline Shield), Derivo Embolization Device (Derivo), and P64 Flow Modulation Device (P64). Thrombin generation (Mean $\pm$ SD; μ g/mL) was measured as: Derivo (28 ± 11), P64 (21 ± 4.5), Pipeline (21 ± 6.2), Pipeline Shield (0.6 ± 0.1) and Negative Control (1.5 ± 1.1). Platelet activation (IU/ μ L) was measured as: Derivo (4.9 ± 0.7), P64 (5.2 ± 0.7), Pipeline (5.5 ± 0.4), Pipeline Shield (0.3 ± 0.1), and Negative Control (0.9 ± 0.7). We found that Pipeline Shield had significantly lower platelet activation and thrombin generation than the other devices tested ($p < .05$) and this was comparable to the Negative Control (no device, $p > .05$). High resolution scanning electron microscopy performed on the intraluminal and cross-sectional surfaces of each device showed the lowest accumulation of platelets and fibrin on Pipeline Shield relative to Derivo, P64, and Pipeline. Derivo and P64 also had higher thrombus accumulation at the flared ends. Pipeline device with Phosphorylcholine surface treatment (Pipeline Shield) could mitigate device material related thromboembolic complications.

1. Introduction

Flow diversion has been approved as a next generation therapy for treatment of large and giant intracranial aneurysm treatment [1,2]. Clinical data has progressively demonstrated superior aneurysm occlusion outcomes with flow diversion [3] compared to coiling and stent assisted coiling which has reports of incomplete occlusion in some cases at 12 month follow-up [4]. Flow diverters consist of intraluminal cylindrical porous meshes that divert the blood flow away from the aneurysm sac leading to gradual thrombosis and healing of the sac while also providing a scaffold for endothelial growth across the aneurysm neck [5]. Thromboembolic events are known to occur with both coils [6] and flow diverters [7]. In the latter case dual antiplatelet therapy is mandated for at least 3 months post implantation, and therefore has limitations for treatment in patients, particularly with ruptured aneurysms [2]. Therefore, improving hemocompatibility of flow diverters is of significant interest. There has been recent development in the biomimetic surface treatment of flow diverters. One such approach

involves covalent attachment of phosphorylcholine to the implant wires (Shield Technology™). This has been shown to reduce thrombus formation in vitro [8], ex vivo [9] and in vivo [10] studies.

In-vitro models offer a reproducible and cost-effective method to compare thrombogenic potential of different devices and surface treatments. One type of such model consists of closed loops, i.e., short lengths of tubular-shaped materials connected end-to-end to form small volume torus-shaped test vehicles, and has been used in a number of variations over the years to simulate thrombosis or investigate blood-device interactions for vascular devices, in particular stents [11–15]. Common to all these models has been some type of rotational motion (usually with a stepper motor) imparted to the test loop to simulate physiological blood flow and/or shear rates. Platelet activation and thrombin generation can be measured as end points with clinical assays [12], in addition to visual and microscopic analysis of thrombus on the devices.

Several flow diversion devices are commercially available including Pipeline (Medtronic), Pipeline Shield (Medtronic), Derivo (Acandis),

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Surpass (Stryker), FRED (Microvention), P64 (Phenox), Silk (Balt Extrusion), and Tubridge (MicroPort). The Pipeline device consists of a braid of 36 Cobalt-Chromium alloy wires together with 12 Platinum wires for radiopacity. The Pipeline Shield device has a 3 nm phosphorylcholine-based surface modification (Shield Technology™) that is covalently bound to the Pipeline (Co–Cr–Pt) braid surface. This surface treatment imparts a low-thrombogenic and biomimetic surface that has also been shown to reduce intimal hyperplasia [16] and increase early neointimal growth in preclinical studies [17]. The P64 flow diverter consists of a 64-wire nitinol braid (wires are proximally bundled into 8 groups of 8 wires) with 2 platinum wires and a flared end with 8 Platinum-Iridium (Pt–Ir) markers for fluoroscopic visibility. The Derivo device consists of 24 Nitinol wires with a radiopaque platinum core and is looped at the distal end resulting in a braid of 48 wires with flared ends and 3 radiopaque markers at each end [18]. The Derivo braid has a 50 nm thick blue oxide surface finish (BlueXide™) which – according to the manufacturer – imparts easier delivery, better corrosion resistance and may impart lower thrombogenicity. We have previously measured the in vitro material thrombogenicity of FRED [19] and Silk [8] devices relative to Pipeline and Pipeline Shield. We found that the thrombogenicity of Silk was comparable to Pipeline [8] and the thrombogenicity of FRED was significantly higher than Pipeline [19]. Thrombogenicity of Pipeline Shield was significantly lower than both Silk and FRED [8, 19]. We note that phosphorylcholine surface treatment only reduces material thrombogenicity of the device and may not impact coagulation due to other factors that are unrelated to device material (such as vessel damage during deployment and any inherent coagulation pathologies) that may manifest during challenging clinical use conditions for the device.

In this study, we quantitatively assessed the material thrombogenicity of four commercially available flow diverters: Pipeline™ Flex Embolization Device (Pipeline), Pipeline™ Flex Embolization Device with Shield Technology™ (Pipeline Shield), P64 Flow Modulation Device (P64), and Derivo Embolization Device (Derivo) in the presence of freshly drawn human blood in a clinically representative pulsatile flow model.

2. Materials and methods

2.1. Devices

The following four flow diversion devices were tested: (a) Pipeline™ Flex Embolization Device (Pipeline, N = 3, 5 mm × 30 mm, Medtronic), (b) Pipeline™ Flex Embolization Device with Shield Technology™ (Pipeline Shield, N = 3, 5 mm × 30 mm, Medtronic); (c) Derivo Embolization Device (Derivo, N = 3, 5 mm × 30 mm, Acandis); and (d) P64 Flow Modulation Device (P64, n = 3, 5 mm × 30 mm, Phenox). Devices were deployed in medical grade PVC tubing (4.76 mm internal diameter, Medtronic). All devices tested were final sterilized products. A summary of devices evaluated is shown in Table 1.

2.2. Flow-loop model and experiment

Our in-vitro flow loop model [19] consists of tubing segments connected with a one-way check valve. The loop is initially filled with heparinized buffer and contains the deployed device implant to be

evaluated. Fresh human blood from the antecubital vein of the donor is then delivered directly into this loop via saline displacement. This has several advantages including: (a) any blood-air interface is avoided, (b) time delay between the blood draw and exposure of the test device to blood is minimized and (c) blood contact with intermediary storage and transfer materials that could cause artifacts in coagulation response, is minimized. This flow loop model and experimental conditions are described in detail below.

Each closed loop (approximate volume 6.4 mL) consisted of a hollow torus-shaped assembly of plastic tubing containing two blood injection and withdrawal ports and a single one-way check valve. These components are solvent bonded using tetrahydrofuran to assure a leak-free construction. The test devices are placed into the lumen of the tubing as shown (Fig. 1). Each loop is prefilled with a concentration of heparin that results in a clinically representative concentration of heparin in the loop blood. This is achieved by dilution of stock solution of heparin (10 U/mL) in PlasmaLyte A (Baxter) buffer solution such that the final desired heparin concentration in blood is 0.5 U/mL (80% whole blood and 20% heparin and PlasmaLyte A; by volume). Institutional Review Board approved protocols have been established for this method and blood is collected from healthy adult human volunteers after informed consent. Blood is drawn from the antecubital vein of the human donor directly into each loop. The filling is achieved by PlasmaLyte A displacement (withdraw) into a 10 mL syringe until each loop has 5.0 mL of blood. The filled loops are immediately mounted on a drum which is connected to a programmable computer driven hollow rotary actuator (Pulsatile Drive System) that applies a defined and repeating motion profile to the 10-cm diameter drum (i.e., the acceleration, deceleration, velocity, and pulsatility of the profile is precisely defined). For Neurovascular applications, this motion corresponds to pulsatile flow (pulse rate of 60 min⁻¹) profile of the blood inside the loop with an average flow rate of 100 mL/min. This flow rate is representative of the lower end of reported average cranial artery blood flow rates estimated by Magnetic Resonance phase contrast imaging [20]. The order of filling of loops and thereby placement on the drum was randomized to eliminate bias during the fill process, such as a slow increase or decrease in blood activation. The whole system was placed inside a 37 °C chamber for the duration of the experiment.

At 90 ± 1 min from filling the first loop with blood, the Pulsatile Drive System was stopped, and the drum was removed, inverted, and re-attached to the system with motion started in counterclockwise direction. Loops were therefore removed from the drum in the order of filling with blood. This ensured that the blood exposure time for each loop remained at approximately 90 ± 2–3 min.

A total of 5 loops were used per experiment (N = 4 Test Devices; N = 1 Negative Control – empty loop), for a total of 3 experiments performed on different days with the same blood donor. After each experiment, blood was withdrawn from each loop into syringes pre-filled with CTAD (citrate, theophylline, adenosine, and dipyridamole) solution (1:10 by volume) and put on ice. This was done to immediately arrest any further coagulation and platelet activation, post experiment. This blood was tested for differential cell counts and then centrifuged (2500 × g @ 20 min) and the supernatant plasma frozen at –80 °C until analysis with commercial ELISA kits for Thrombin-Anti-thrombin (TAT) complex generation (Enzygnost TAT micro, Siemens) or Platelet Activation (beta-thromboglobulin, Asserachrom). Each loop was rinsed

Table 1

Name and brief description of flow diversion devices tested in the study. The abbreviated form of the device name is used throughout the manuscript.

Device name (Abbreviation)	Description (Implant section of each device)
Pipeline™ Flex Embolization Device (Pipeline)	A self-expanding mesh cylinder braided from Cobalt-Chromium alloy wires
Pipeline™ Flex Embolization Device with Shield Technology™ (Pipeline Shield)	A self-expanding mesh cylinder braided from Cobalt-Chromium alloy wires, with a novel surface treatment of the implant
Derivo Embolization Device (Derivo)	A self-expanding mesh cylinder from Nitinol wires with BlueXide™ surface finishing technology
P64 Flow Modulation Device (P64)	A self-expanding mesh cylinder braided from Nitinol wires

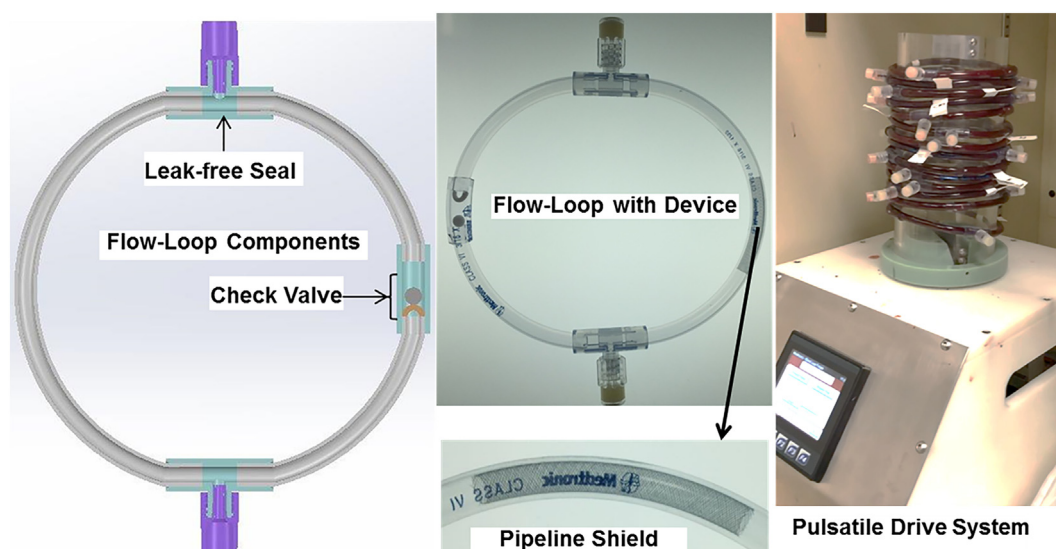


Fig. 1. (left) Flow-loop construction with leak-free seals and one-way flow check valve (middle) flow loop with Pipeline Shield device; (right) Pulsatile Drive System with flow-loops (with devices and blood) mounted.

with PlasmaLyte A to remove non-adherent blood, photographed for gross thrombus, and then filled with Karnovsky's fixative for SEM (Scanning Electron Microscopy) analysis.

2.3. SEM analysis of thrombus

Following fixation in Karnovsky's reagent, an approximate 1 cm length of each flow diverter was cut out of loops mid-section leaving the tubing sheath present. Two sections were made: (a) Longitudinal section to image the intraluminal surface of each device, and (b) circumferential section to image any entrapment of thrombus at the mid-section of the devices. These cut samples were fixed in 2% osmium tetroxide for one hour and dehydrated in graded ethanol from 40 to 100% prior to being subjected to critical point drying using a critical point dryer (Tousimis Autosamdri-814). The devices were then carefully removed from the PVC (longitudinal sections) or left in PVC sheaths (circumferential sections), mounted, and sputter coated with Au/Pd for 60 s using a sputter coater (Denton Vacuum Desk II). A Scanning Electron Microscope (JEOL 6700F) operated with an accelerating voltage of 3 keV (large thrombus accumulation) or 10 keV (small thrombus accumulation) was then used to take representative 30–2000 $\times$ micrographs of the implant luminal and circumferential sections.

2.4. Optical microscopy of thrombus at device ends

Following fixation in Karnovsky's reagent, an approximate 0.2 cm length of each flow diverter tubing end-section was cut out of loops leaving the tubing sheath present. Digital microscopy images were acquired for the ends ($N = 6$ per device for all 3 experiments) of the 4 devices (still in PVC tubing) to compare thrombus accumulation specifically in this region. The images were acquired at 50 $\times$ magnification (Keyence VHX-5000 Digital Microscope; VHZ20-UR Ultra-Small High Performance Zoom Lens).

2.5. Statistical analysis

ANOVA was performed for Platelet Activation and Thrombin Generation measurements for the 5 samples per test ($N = 4$ Test Devices; $N = 1$ Negative control) and 3 experimental runs. Post-hoc Fisher's LSD (Least Square Difference) was used to identify differences between the 4 test articles with a significance value of 0.05. For non-

normal datasets, a Johnson transformation was performed to normalize the data and statistics were performed and reported on the transformed data. Note that the data for the Negative Control (No device) from one experiment was not available and is excluded from analysis.

3. Results

ANOVA showed that there were significant differences between test articles both for platelet activation and thrombin generation ($p < .05$). Additionally, there was no significant effect of individual experimental runs on these measurements and overall trends were similar for all the measurements ($p > .05$).

3.1. Gross thrombus analysis

Significant accumulation of thrombus was observed on Pipeline, Derivo and P64 devices. The loops with Pipeline Shield and empty loops (Negative control) did not have significant attachment of thrombus (Fig. 2).

3.2. Thrombin generation

Mean thrombin generation was measured as (Mean $\pm$ SD; $\mu\text{g/mL}$): Baseline (0.0 ± 0.0), Derivo (28 ± 11), P64 (21 ± 4.5), Pipeline (21 ± 6.2), Pipeline Shield (0.6 ± 0.1) and Negative Control (1.5 ± 1.1). The results are shown in Fig. 3A. Thrombin generation was significantly lower for the Pipeline Shield compared to Pipeline, P64 and Derivo devices. Additionally, thrombin generation was comparable between the Negative Control (empty loop) and Pipeline Shield. The raw data is provided in Table 2 and a summary of p -values for post-hoc Fisher's LSD is shown in Table 3.

3.3. Platelet activation

Mean platelet activation was measured as (Mean $\pm$ SD; IU/ μL): Baseline (0.0 ± 0.0), Derivo (4.9 ± 0.7), P64 (5.2 ± 0.7), Pipeline (5.5 ± 0.4), Pipeline Shield (0.3 ± 0.1), and Negative Control (0.9 ± 0.7). The results are shown in Fig. 3B. Platelet activation was significantly lower for the Pipeline Shield compared to Pipeline, P64 and Derivo. Additionally, platelet activation was comparable between the Negative Control (empty loop) and Pipeline Shield. The raw data is provided in Table 2 and a summary of p -values for post-hoc Fisher's LSD

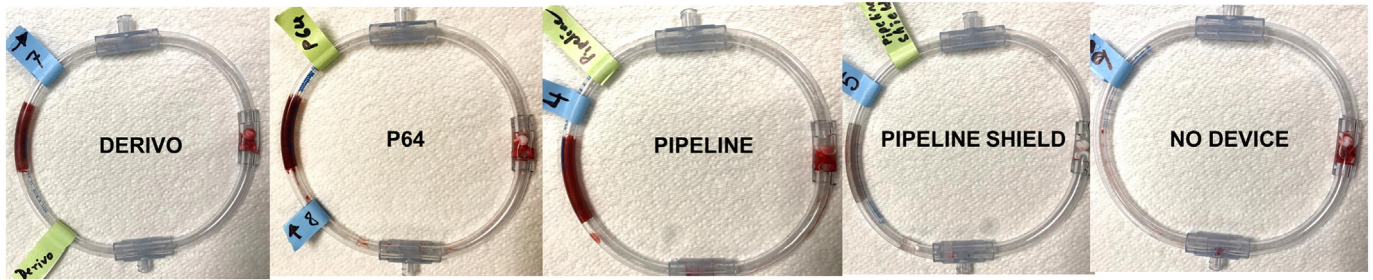


Fig. 2. Images of the flow-loops post-experiment: Gross observation of thrombus on the Derivo, P64, Pipeline, Pipeline Shield, and No device (Negative Control); note minimal thrombus accumulation on Pipeline Shield device.

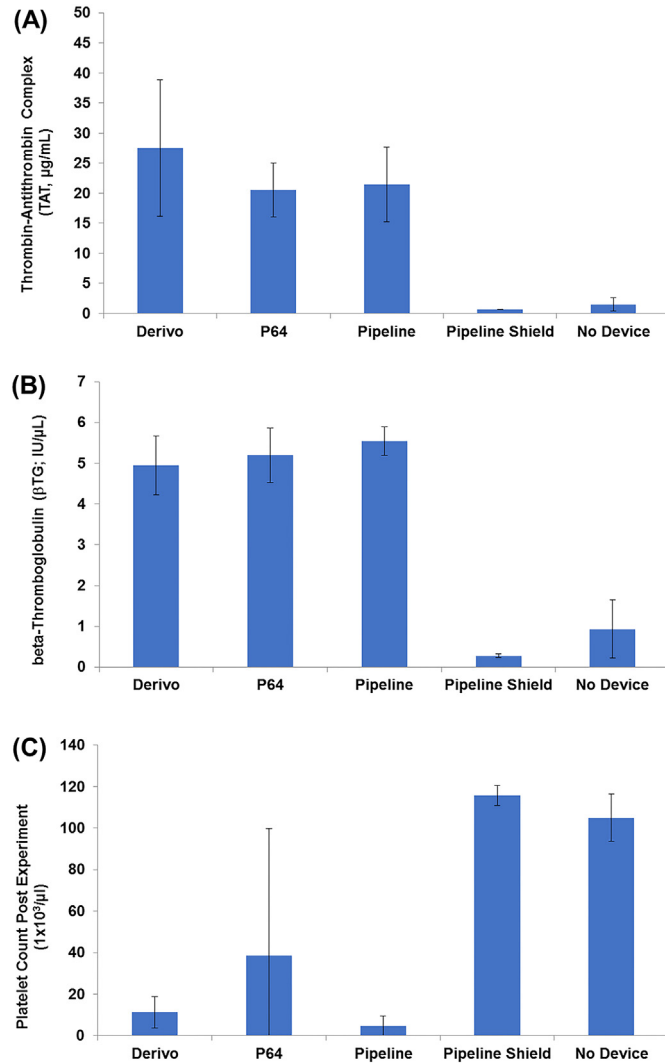


Fig. 3. A Thrombin-Antithrombin (TAT) complex formation (Mean $\pm$ SD; $\mu\text{g}/\text{mL}$) measured post-experiment for Derivo, P64, Pipeline, Pipeline Shield and No device (Negative Control). B beta-thromboglobulin release (Mean $\pm$ SD; $\text{IU}/\mu\text{L}$) measured post-experiment for Derivo, P64, Pipeline, Pipeline Shield and No device (Negative Control). C Platelet counts (Mean $\pm$ SD; $1 \times 10^3/\mu\text{L}$) measured post-experiment for Derivo, P64, Pipeline, Pipeline Shield and No device (Negative Control).

is shown in Table 3.

3.4. Platelet counts

Mean platelet counts post experiment was measured as ($1 \times 10^3/\mu\text{L}$;

Table 2

TAT ($\mu\text{g}/\text{mL}$), βTG ($\text{IU}/\mu\text{L}$), and platelet counts ($1 \times 10^3/\mu\text{L}$) measured post-experiment for all devices tested in each of the 3 experiments. Note the “No device” control measures were lost for experiment 3 due to sampling error.

Device	Experiment number	TAT	βTG	Platelet counts
Baseline (Immediately Post Blood Collection)	1	0.011	0.068	151
	2	0.014	0.024	108
	3	0.013	0.042	151
Pipeline	1	21.39	5.134	7
	2	27.69	5.753	7
	3	15.31	5.737	0
Pipeline Shield	1	0.610	0.270	119
	2	0.590	0.239	110
	3	0.704	0.329	118
Derivo	1	40.67	4.669	6
	2	20.98	4.403	20
	3	20.94	5.772	8
P64	1	23.28	4.927	109
	2	22.92	5.965	7
	3	15.33	4.712	0
No device (Empty Loop)	1	2.265	1.435	97
	2	0.712	0.425	113
	3	–	–	–

Table 3

Post-hoc Fisher's LSD was conducted to distinguish between test devices. The p -values show that thrombin generation (TAT), platelet activation (βTG), and post experiment platelet counts, for Pipeline Shield is comparable to Negative Control.

Device Pair	p -value (TAT)	p -value (βTG)	p -value (Platelet counts)
Pipeline Shield and Derivo	0.001	0.002	0.017
Pipeline Shield and P64	0.004	0.001	0.013
Pipeline Shield and No device	0.888	0.130	0.315
P64 and Derivo	0.217	0.357	0.881
P64 and No device	0.011	0.017	0.125
Derivo and No device	0.002	0.068	0.155
Pipeline and Pipeline Shield	0.003	0.001	0.005
Pipeline and No device	0.008	0.034	0.049
Pipeline and Derivo	0.281	0.640	0.443
Pipeline and P64	0.860	0.639	0.532

Mean $\pm$ SD): Baseline (137 ± 25), Derivo (11 ± 7.6), P64 (39 ± 61), Pipeline (4.7 ± 4.7), Pipeline Shield (115 ± 4.9), and Negative Control (105 ± 11). The results are shown in Fig. 3C. Platelet counts post experiment were significantly higher for Pipeline Shield indicating less aggregation of activated platelets relative to Pipeline, Derivo and P64. This corresponds to the trend of TAT and βTG values reported above for these devices. The raw data is summarized in Table 2 and a summary of p -values for post-hoc Fisher's LSD is shown in Table 3.

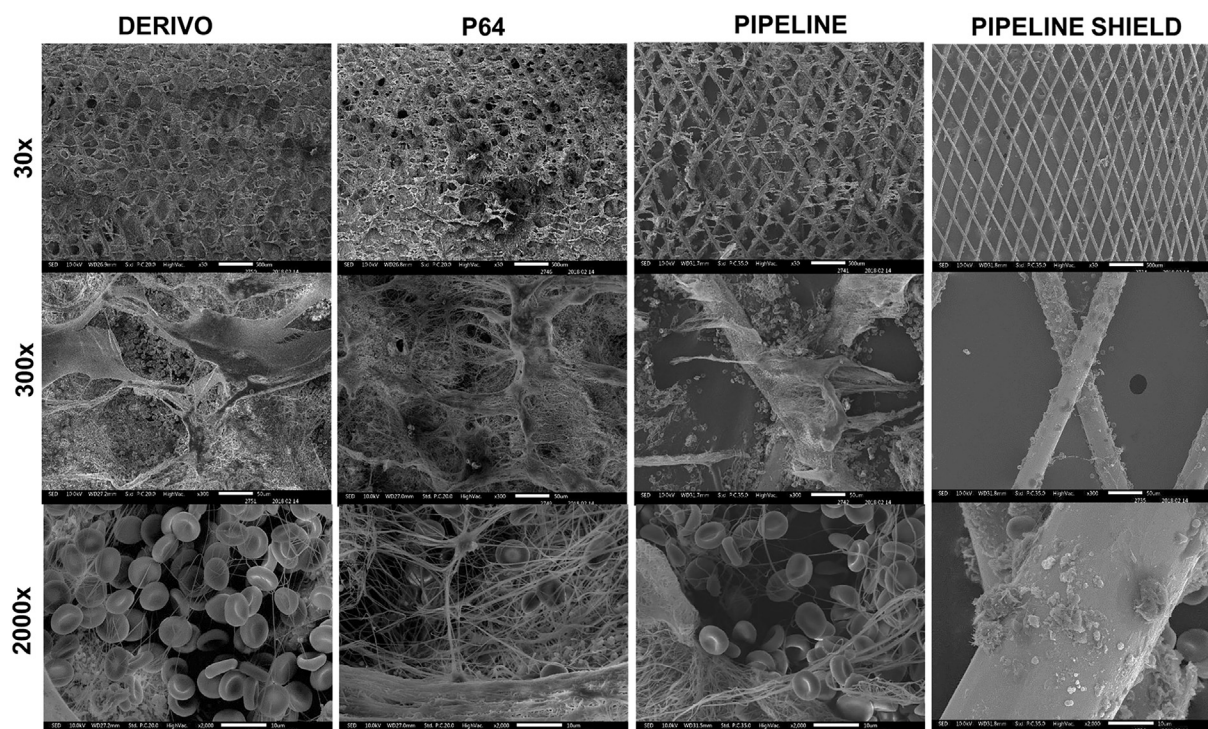


Fig. 4. High resolution Scanning Electron Microscopy (SEM) representative images of thrombus accumulation on the intraluminal surfaces of Derivo, P64, Pipeline and Pipeline Shield devices (30 $\times$, 300 $\times$, 2000 $\times$, magnification).

3.5. Intraluminal SEM analysis of thrombus

High resolution scanning electron microscopy (SEM) images were obtained for each device intraluminal surface at three magnifications (30 $\times$, 300 $\times$, and 2000 $\times$). Significant cellular and proteinaceous accumulation was observed on Pipeline, P64 and Derivo devices (Fig. 4). In some instances, the struts of the devices (Pipeline, P64 and Derivo) were completely covered with thrombus. The thrombus appears to be an intercalated network of cross-linked fibrin with entrapped activated platelets and red blood cells. On the other hand, insignificant accumulation was observed on Pipeline Shield for both cellular and acellular blood components with the absence of intercalated fibrin network. The wires of the Pipeline Shield device were clearly visible even at higher magnification (2000 $\times$) without a significant accumulation of thrombus. These images show strong correspondence with the gross images and the measurements for thrombin generation and platelet activation.

3.6. Cross-sectional SEM analysis of thrombus

High resolution scanning electron microscopy (SEM) images were obtained for each device cross-sections at the middle-section at four magnifications (30 $\times$, 100 $\times$, 300 $\times$, and 2000 $\times$). Thrombus accumulation in the mid-sections was similar between Pipeline, P64, and Derivo (Fig. 5). In contrast, Pipeline Shield devices presented noticeably less surface thrombus.

3.7. Optical microscopy of thrombus at device ends

High resolution digital microscopy of the ends of the devices was performed at 50 $\times$ magnification (Fig. 6). Accumulation of thrombus at the ends appeared to be significantly higher relative to the mid-section of the devices, particularly for Derivo and P64. In particular, thrombus accumulation at the ends was significant for: (a) 4 out of 6 ends for Derivo, (b) 3 out of 6 ends for P64, (c) 2 out of 6 ends for Pipeline, and (d) 0 out of 6 ends for Pipeline Shield. As noted before for the

intraluminal and mid-section SEM images, insignificant accumulation was observed on Pipeline Shield for both cellular and acellular blood components for all 3 of the Pipeline Shield devices at their mid-section, and proximal or distal ends (Figs. 5, 6).

4. Discussion

Flow diverters – being intraluminal devices – mandate the use of dual antiplatelet therapy for a significant period of time post implantation. The issue of thromboembolic events post implant remains a concern for these devices. One method to improve hemocompatibility is to confer biomimetic properties to the surface (Shield Technology™). This has been shown to reduce both platelet and fibrin adhesion to the surface ex vivo [9] and in vivo [10] as well as reduce thrombin generation in vitro [8]. However, the thrombogenic response of Derivo and P64 relative to Pipeline and Pipeline Shield in freshly drawn human blood under pulsatile flow conditions has not been evaluated before. We found that both platelet activation and thrombin generation was significantly elevated for Pipeline, Derivo and P64 relative to Pipeline Shield and the Negative Control, with marked deposition of cellular and acellular blood components on the Pipeline, Derivo and P64 devices. Thrombogenicity of the Pipeline device – as measured with TAT and β TG levels post experiment – was statistically similar to Derivo and P64 devices. Recent clinical data with the Pipeline Shield device shows no aneurysm recurrence or retreatment and no major strokes or neurological death at one year follow-up [21]. Additionally, Pipeline Shield has been shown to not adversely impact aneurysm occlusion at long term follow-up [21]. The aneurysm occlusion due to flow diversion is not a consequence of higher thrombogenicity of the intraluminal device (flow diverter) but rather the level of flow stagnation that is achieved in the aneurysm that leads to gradual thrombosis and occlusion of the aneurysm sac over time. Several single center clinical studies additionally support the safety profile of Pipeline Shield [22,23] including studies in subsets of patients that may benefit from a low thrombogenicity flow diverter [24].

The Derivo embolization device has a surface finish known as

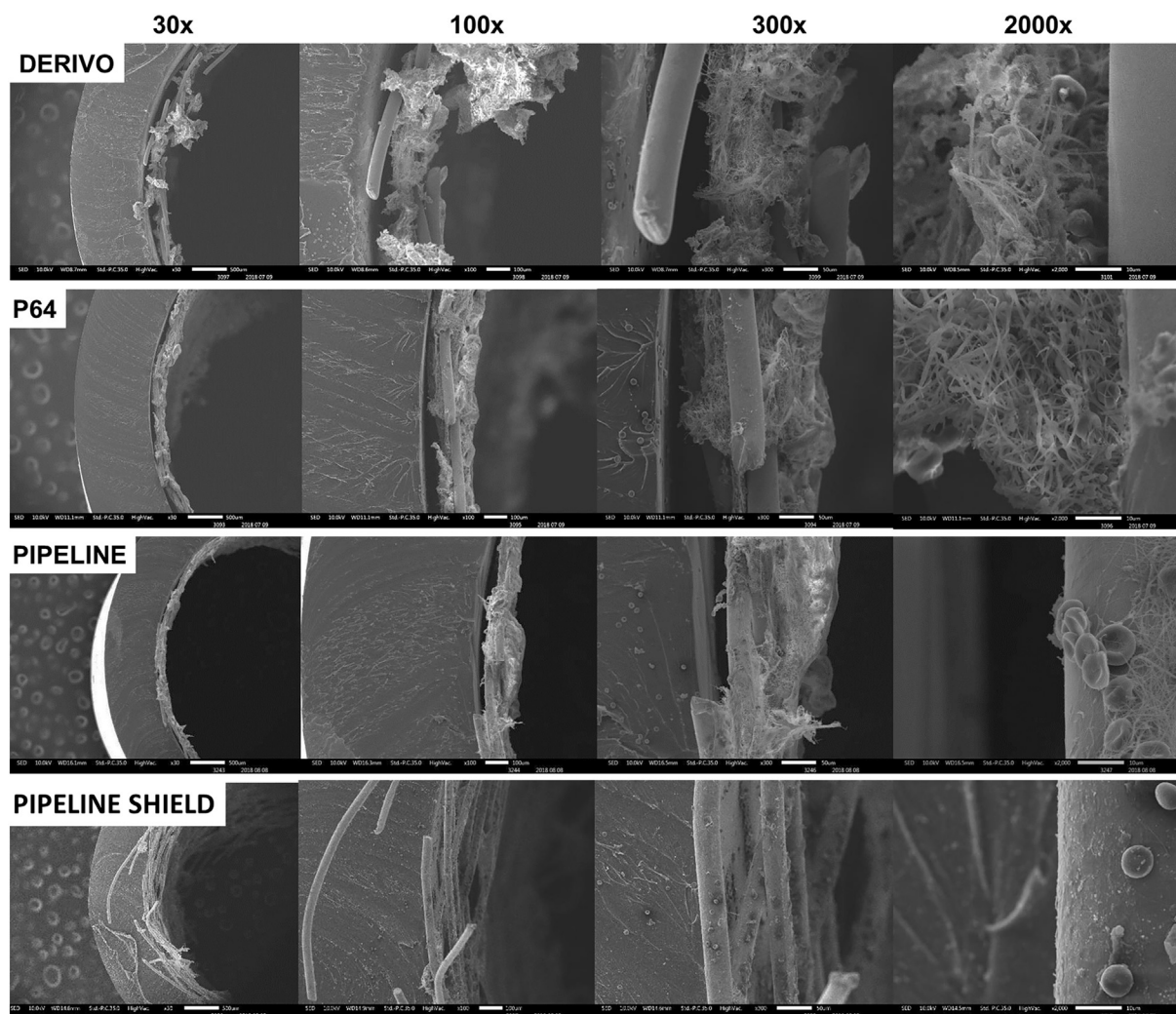


Fig. 5. High resolution Scanning Electron Microscopy (SEM) representative images of thrombus accumulation on the cross-section surfaces of the mid-sections of Derivo, P64, Pipeline and Pipeline Shield devices (30 $\times$, 100 $\times$, 300 $\times$, 2000 $\times$, magnification).

BlueXide that is manufactured by an electropolishing and annealing process. However, this surface treatment did not appear to confer a low-thrombogenic benefit to the device in the current study. Additionally, the two flared ends of the Derivo device have 3 fluoroscopic markers each and seem to accumulate more thrombus relative to the mid-section of the device. Recent clinical data with the Derivo embolization device shows high incidence of thromboembolic events including in-stent thrombosis [25,26]. Similarly, P64 – a braided Nitinol flow diverter – was found to have higher platelet activation, thrombin generation, and thrombus deposition on the devices intraluminal surfaces post experiment. P64 also has one flared end with 8 fluoroscopic markers and this end was found to have significant higher thrombus accumulation relative to the mid-section of the device. In-stent thrombosis has also been reported clinically for P64 device in addition to reduction in flow in covered branch arteries [27]. In preclinical studies with Pipeline Shield, thrombus formation on the intraluminal surface of the device and at the ostium of perforating vessels was minimized relative to Pipeline [10,28].

Both TAT [29,30] and β TG [31] are widely accepted as indicators of clinical thrombosis and were therefore used as end-points for thrombogenicity measurements in this study. TAT and β TG measures post experiment for Pipeline Shield were statistically similar to the Negative Control. We utilized a well-established closed-loop system [12,19] to expose the devices to human blood and note the following model limitations: (a) The flow-loops require a small check valve to support

pulsatile flow generation which may confer some local flow induced thrombogenicity to the baseline Negative Control, (b) There is some blood dilution (20%) due to the saline displacement fill method and heparin anticoagulation concentration requirements, (c) An expensive micro-stepper motor is required to generate the precise pulsatile flow profile, (d) There is no aneurysm in the loop and only material induced thrombogenicity is measured under physiological flow conditions, (e) The sample sizes of the devices investigated here is low, and does not account for variability between blood donors, (f) All commercially available flow diverters were not evaluated in this study, (g) The addition of antiplatelets to the blood were not considered in the current thrombogenicity evaluations to be able to evaluate the baseline thrombogenic profile of the device material, and (h) The devices were deployed in a hollow tube with uniform radius of curvature as opposed to a tortuous Internal Carotid Artery (ICA) segment – which could be more representative of clinical use anatomy. We acknowledge that these limitations are significant departures from clinical use conditions of flow diversion devices.

We note that despite the limitations, the thrombogenicity differences observed here between Pipeline Shield and other flow diverters are very significant, with Pipeline Shield being consistently similar to Negative Control and therefore these responses are unlikely to be significantly affected by blood donor variabilities or sample sizes. This additionally confirms prior findings in non-human primate and rabbit elastase models [8,10] where Pipeline Shield had the lowest measured

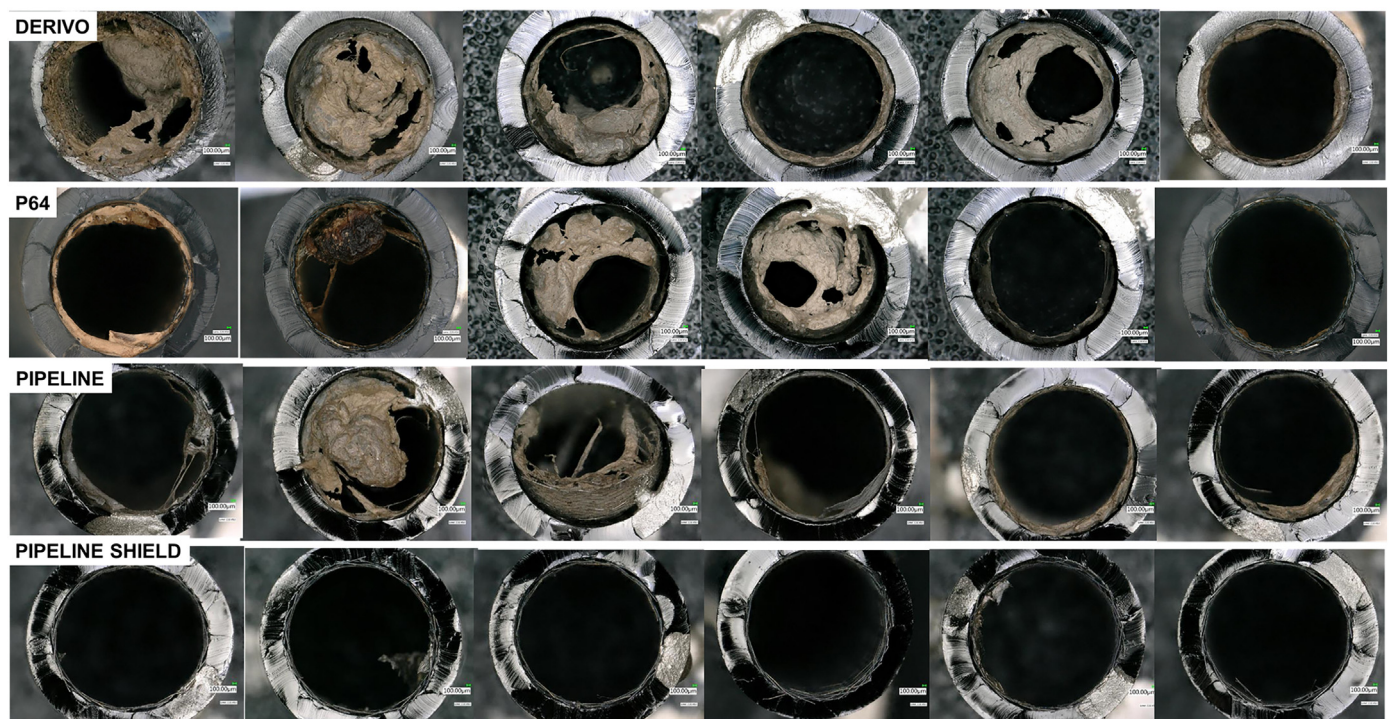


Fig. 6. High resolution optical microscopy images of thrombus accumulation on the cross-section surfaces of the ends of Derivo, P64, Pipeline and Pipeline Shield devices for all 3 experiments (both ends of each of the 4 devices is shown at 50× magnification).

thrombogenicity despite known species-wide coagulation profile differences [32]. The current study therefore demonstrates that under clinically low levels of anticoagulation and in a closed recirculating flow-loop, Pipeline Shield may mitigate device material related thromboembolic events. Future in-vitro studies in this flow model will incorporate the presence of perforating vessels, aneurysms, patient specific ICA anatomy, and antiplatelet agents, to assess the effect of these clinically relevant factors on the thrombogenicity of flow diversion devices.

5. Conclusions

This in vitro study demonstrates the significantly lower thrombogenicity of Pipeline Flex Embolization device with Shield Technology™ in a clinically relevant human blood flow loop model relative to Pipeline Flex Embolization Device, Derivo Embolization Device and P64 Flow Modulation Device. Thrombogenicity of Pipeline was comparable to P64 and Derivo flow diverters in this in vitro study.

References

- [1] S.L. Zuckerman, I.M. Eli, P.J. Morone, et al., Novel technologies in the treatment of intracranial aneurysms, *Neurol. Res.* 36 (4) (2014) 368–382.
- [2] Y.J. Alderazi, D. Shastri, T. Kass-Hout, et al., Flow diverters for intracranial aneurysms, *Stroke Res. Treat.* 2014 (2014) 415653.
- [3] T. Beckske, W. Brinjikji, M.B. Potts, et al., Long-term clinical and angiographic outcomes following pipeline embolization device treatment of complex internal carotid artery aneurysms: five-year results of the pipeline for uncoilable or failed aneurysms trial, *Neurosurgery* 80 (1) (2017) 40–48.
- [4] J. Raymond, F. Guilbert, A. Weill, et al., Long-term angiographic recurrences after selective endovascular treatment of aneurysms with detachable coils, *Stroke* 34 (6) (2003) 1398–1403.
- [5] R.M. Starke, A. Turk, D. Ding, et al., Technology developments in endovascular treatment of intracranial aneurysms, *J. Neurointerventional Surg.* 8 (2) (2016) 135–144.
- [6] R.J. Rahme, S.G. Zammar, T.Y. El Ahmadieh, et al., The role of antiplatelet therapy in aneurysm coiling, *Neurol. Res.* 36 (4) (2014) 383–388.
- [7] P.K. Nelson, P. Lylyk, I. Szikora, et al., The pipeline embolization device for the intracranial treatment of aneurysms trial, *AJNR Am. J. Neuroradiol.* 32 (1) (2011) 34–40.
- [8] G. Girdhar, J. Li, L. Kostousov, et al., In-vitro thrombogenicity assessment of flow diversion and aneurysm bridging devices, *J. Thromb. Thrombolysis* 40 (4) (2015) 437–443.
- [9] M.W. Hagen, G. Girdhar, J. Wainwright, et al., Thrombogenicity of flow diverters in an ex vivo shunt model: effect of phosphorylcholine surface modification, *J. Neurointerventional Surg.* 9 (10) (2017) 1006–1011.
- [10] N. Strelnikov, V. Berestov, A. Gorbatykh, et al., Acute thrombus formation on phosphorilcholine surface modified flow diverters, *Interv. Neuroradiol.* 10 (4) (2018) 406–411.
- [11] W. van Oeveren, I.F. Tiellu, J. de Hart, Comparison of modified chandler, roller pump, and ball valve circulation models for in vitro testing in high blood flow conditions: application in thrombogenicity testing of different materials for vascular applications, *Int. J. Biomater.* 2012 (2012) 673163.
- [12] K. Munch, M.F. Wolf, P. Gruffaz, et al., Use of simple and complex in vitro models for multiparameter characterization of human blood-material/device interactions, *J. Biomater. Sci. Polym. Ed.* 11 (11) (2000) 1147–1163.
- [13] R.A. Gardner, An examination of the fluid mechanics and thrombus formation time parameters in a Chandler rotating loop system, *J. Lab. Clin. Med.* 84 (4) (1974) 494–508.
- [14] S. Sinn, T. Scheuermann, S. Deichelbohrer, et al., A novel in vitro model for pre-clinical testing of the hemocompatibility of intravascular stents according to ISO 10993-4, *J. Mater. Sci. Mater. Med.* 22 (6) (2011) 1521–1528.
- [15] G. Tepe, H.P. Wendel, S. Khorchidi, et al., Thrombogenicity of various endovascular stent types: an in vitro evaluation, *J. Vasc. Interventional Radiol.* 13 (10) (2002) 1029–1035.
- [16] J. Caroff, T. Tamura, R.M. King, et al., Phosphorylcholine surface modified flow diverter associated with reduced intimal hyperplasia, *J. Neurointerventional Surg.* 10 (11) (2018) 1097–1101.
- [17] Y. Matsuda, J. Chung, D.K. Lopes, Analysis of neointima development in flow diverters using optical coherence tomography imaging, *J. Neurointerventional Surg.* 10 (2) (2018) 162–167.
- [18] E. Akgul, H.B. Onan, S. Akpınar, et al., The DERIVO embolization device in the treatment of intracranial aneurysms: short- and midterm results, *AJNR Am. J. Neuroradiol.* 95 (2016) 229–240.
- [19] G. Girdhar, A. Andersen, E. Pangerl, et al., Thrombogenicity assessment of Pipeline Flex, Pipeline Shield, and FRED flow diverters in an in vitro human blood physiological flow loop model, *J. Biomed. Mater. Res. A* 106 (12) (2018) 3195–3202.
- [20] S.O. Oktar, C. Yucel, D. Karaosmanoglu, et al., Blood-flow volume quantification in internal carotid and vertebral arteries: comparison of 3 different ultrasound techniques with phase-contrast MR imaging, *AJNR Am. J. Neuroradiol.* 27 (2) (2006) 363–369.
- [21] M. Martinez-Galdamez, S.M. Lamin, K.G. Lagios, et al., Treatment of intracranial aneurysms using the pipeline flex embolization device with shield technology: angiographic and safety outcomes at 1-year follow-up, *J. Neurointerventional Surg.* (2018), <https://doi.org/10.1136/neurintsurg-2018-014204>.
- [22] G.J. Agnoletto, P. Aguilar-Salinas, R. Santos, et al., PED Flex with Shield Technology: a feasible alternative for fusiform MCA aneurysms, *Stroke Vasc. Neurol.* (2018), <https://doi.org/10.1136/svn-2017-000132>.

- [23] K. Orlov, D. Kisilitsin, N. Strelnikov, et al., Experience using pipeline embolization device with Shield Technology in a patient lacking a full postoperative dual anti-platelet therapy regimen, *Interv. Neuroradiol.* 24 (3) (2018) 270–273.
- [24] N.W. Manning, A. Cheung, T.J. Phillips, et al., Pipeline shield with single anti-platelet therapy in aneurysmal subarachnoid haemorrhage: multicentre experience, *J. Neurointerventional Surg.* (2018), <https://doi.org/10.1136/neurintsurg-2018-014363>.
- [25] L. Goertz, F. Dorn, B. Kraus, et al., Safety and efficacy of the Derivo Embolization device for the treatment of ruptured intracranial aneurysms, *J. Neurointerventional Surg.* (2018), <https://doi.org/10.1136/neurintsurg-2018-014166>.
- [26] B. Kraus, L. Goertz, B. Turowski, et al., Safety and efficacy of the Derivo Embolization device for the treatment of unruptured intracranial aneurysms: a multicentric study, *J. Neurointerventional Surg.* 11 (2019) 68–73.
- [27] F. Briganti, G. Leone, L. Ugga, et al., Mid-term and long-term follow-up of intracranial aneurysms treated by the p64 flow modulation device: a multicenter experience, *J. Neurointerventional Surg.* 9 (1) (2017) 70–76.
- [28] M. Marosfoi, F. Clarencon, E.T. Langan, et al., Acute thrombus formation on phosphorilcholine surface modified flow diverters, *J. Neurointerventional Surg.* 10 (4) (2018) 406–411.
- [29] I. Fernandez-Cadenas, M. Mendioroz, J. Munuera, et al., Lower concentrations of thrombin-antithrombin complex (TAT) correlate to higher recanalisation rates among ischaemic stroke patients treated with t-PA, *Thromb. Haemost.* 102 (4) (2009) 759–764.
- [30] A. Sekiya, T. Hayashi, Y. Kadohira, et al., Thrombosis prediction based on reference ranges of coagulation-related markers in different stages of pregnancy, *Clin. Appl. Thromb. Hemost.* 23 (7) (2017) 844–850.
- [31] B. Walkowiak, C.S. Cierniewski, Kinetics of the beta-thromboglobulin release from alpha-granules of blood platelets activated by ADP, *Thromb. Res.* 46 (5) (1987) 727–736.
- [32] J.M. Siller-Matula, R. Plasenzotti, A. Spiel, et al., Interspecies differences in coagulation profile, *Thromb. Haemost.* 100 (3) (2008) 397–404.